Dipartimento del Farmaco
Istituto Superiore di Sanità - Roma

SmartDrugs

English Edition

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Introduction

The terminology “Smart-Drugs“ includes all legal compounds of natural or synthetic origin, not prohibited by various drug laws in different countries. The drugs may contain active compounds with known or presumed psychoactive properties [(1)]. The definition of “Smart Drugs“ is changing continuously, not only by including different types of substances that fall into this category, but also from a conceptual and cultural point of view.

In the 90s’ the term “Smart Drugs“ spread in the United States referring to some drugs used as adjuvants in diseases such as senility. In 1991, the book “Smart Drugs and Nutrients”, written by the American gerontologist Ward Dean and by the journalist John Morgenthaler described dozens of substances with “brain action” or “nootropics” which reportedly increased mental performance and sexual potency [(2)]. Only a few of the products of plant origin were mentioned in the book. Indeed, the “American” definition of “Smart Drugs“did not change over time: it still deals with a series of pharmacologically active substances including steroids, which have an impact on the general “performance” of the individual.

On the other hand, at the end of the 90’s a tendency spread in Europe among high school and university students to use natural or synthetic substances which could be legally purchased and were considered helpful in improving concentration and memory due to real or perceived psychoactive properties.

Currently, there is no unique terminology for the term “Smart Drugs” because they can refer simultaneously to plant drugs, ethnic drugs, ethnobotanical drugs, natural drugs, biodrugs, etc. For some people, the term “Smart Drug” indicates a series of energy drinks or stimulating pills (which attempt to simulate the effect of “ecstasy”) which guarantee stimulating effects while remaining within legality (caffeine, ginseng, etc..): they are proposed and consumed especially in youth circles (clubs, raves etc...).

As above-reported, the “Smart Drugs” are generally not included in the list of illicit drugs as per the Laws banning the sale of such substances (in Italy, the Article 14 of Republic Presidential Decree 309/90 and subsequent updates) [(3)]. However, the active compound/compounds contained in the fresh or dry parts of the plants marketed as “Smart Drugs“ may be included in this list, while, neither the plant, nor parts of it are, making them automatically legal. In fact, for the past fifteen years there have been legal “Smart Shops”, in different European countries specializing in the sale of herbal products of different origin or formulation. There are about one hundred “Smart Shops”, in Italy which sell not only natural and synthetic “Smart Drugs” (the latter such as amino acid containing capsules, GABA type neurotransmitters , etc) with the seal of the European Council, but they also sell products for the cultivation of the plants (mostly mushrooms and hemp) and accessories used to optimize the effect of the smoked substances (cigarette papers, filters, pipes, water pipes, vaporizers). In addition, these products are considered “smart” because they can be acquired through the internet web sites as incense and home scents with precise indications: “not for human consumption”, although there are specific websites which explain in great detail different types of administration routes (ingestion, smoking of the dry plant etc) of these products.

The heterogeneity of “Smart Drugs” is reflected by a multitude of classification criteria: method of use, chemical class to which the active principles belong, purpose of use etc. Most of these substances, extracted from herbs, were originally consumed in the alternative ethnic medicine, being at the centre of traditional commemorative rites and customs.

The so called primitive peoples knew very well the dangers associated with the use of these plants and considered them sacred. “Sacred” derives in fact from the Latin ***sacer*** and indicates “from which you may stay far away.”

Starting from 2003, on behalf of the Minister of Health, the “Drug of abuse and doping” Unit of the Italian Institute of Health carried out more than 500 quali-quantititative analyses and pharmaco-toxicological evaluation on more than 200 products seized by the Italian Carabinieri police force and by the public prosecutors’ offices in different cities. These products come from “Smart Shops”, herbalist’s shops, ethnobotany shops, e-commerce and they usually are packages containing dried plant extracts with different names, labels and casings. The analysis and classification of these substances is ongoing.

In 2005, the Italian Institute of Health, asked by the Department for Anti-drug Policies, prepared a book of monographs for the most common 25 “Smart Drugs” considered at risk of drug-dependence and intoxication for the reported pharmacological and subjective effects[(4)].

In each monograph, the taxonomic characteristics of the plant were given including a list of the active compounds, in which parts of the plant the active compounds are concentrated and the geographical origin of the plant. The physico-chemical characteristics of the active compounds as well as the historical and current use were also highlighted. Information about the legislation for possession and use of each of these plants as well as of their active compounds in different countries was also given. In addition, the pharmacological and toxicological properties of the active compounds were listed. Finally, in each monograph it was also possible to obtain information on the operating procedure to follow for setting up the analytical determination of active components in biological fluids of consumers or in plant parts.
The book was neither listing nor organizing all the plant products available in "Smart Shops", but aimed to alert the interested people and law enforcement agencies about some plants containing real or presumed psychoactive compounds (stimulants, hallucinogens,"aphrodisiacs, etc.) whose consumption could be harmful in one way or other.

The book on "Smart Drugs" published by the Italian Institute of health had a large circulation in the scientific world as well as in the mass media. But, as mentioned, in the recent years the trend in use and consumption of these substances has changed, as some websites have revealed. Moreover, new "Smart Drugs" have been introduced and new scientific information about their use and toxicity appeared in the international literature.
Therefore, in this new edition, six new "Smart Drugs" have been added and the monographs of the old ones have been updated with new information concerning legislation, pharmacological properties and analytical methodologies for determination of active principles in biological fluids of eventual consumers, or in the plant parts. Special attention has been paid to "Spice", mixtures of different "Smart Drugs", which have aroused the interest for the pharmacological and toxicological actions due to the presence of several products of plant origin and synthetic substances with effects similar to those of cannabis.
Although not exhaustive, the data presented in this second Edition of the "Smart Drugs" book originates from the most recent international literature and investigations from our group and provide useful information for investigators, legislators and law enforcement agencies.

References

1. BAKER LS. “Smart drugs”: a caution to everybody. Am J Psychiatry. 1996; 153: 844-845.
2. DEAN W, MORGENTHALER J. Smart Drugs and Nutrients: how to improve your memory and increase your intelligence using the latest discoveries in neuroscience. Smart Publications - Petaluma, CA USA 1990.
3. Decreto del Presidente della Repubblica (D.P.R.) n. 309 del 9 ottobre 1990 e suo testo aggiornato nel 2006 e presente nella Gazzetta Ufficiale n. 62 del 15 marzo 2006.
4. PICHINI S, PALMI I, MARCHEI E, PELLEGRINI M, PACIFICI R, ZUCCARO P. Smart Drugs. Osservatorio Fumo, Alcol e Droga, Dipartimento del Farmaco, Istituto Superiore di Sanità; ottobre 2006.

Symbols and abbreviations

CAS: Chemical Abstract Service, a division of the American Chemical Society, which assigns a numerical identifier that individualizes in an unequivocal way each chemical substance described in literature.
ED50: the amount of active ingredient efficacious in 50% of the participants in an experiment.
LD: the amount of active ingredient lethal in the participants in an experiment.
LD50: the amount of active ingredient lethal in 50% of the participants in an experiment.
LDLo: the minimum amount of lethal active ingredient in the participants in an experiment.
TDLo: the minimum amount of active ingredient toxic for the participants in an experiment.
UVmax: the wavelength corresponding to the maximum absorbance of a chemical compound.
IC50: the concentration of an inhibitor where the response (or binding) is riduced by half.

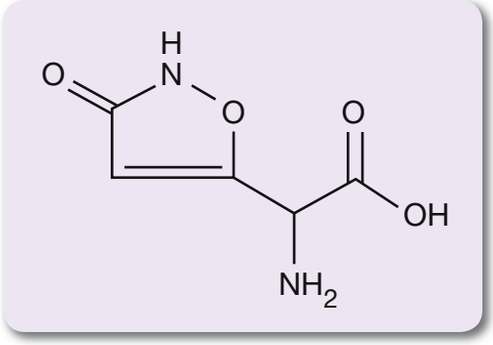
Amanita muscaria

(fly agaric)

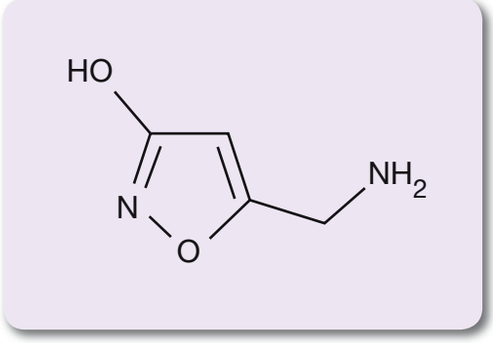
Name: *Amanita muscaria*
Family: *Amanitaceae*
Genus: *Amanita*
Species: *Amanita muscaria* L. (Hooker)
Synonims: *Agaricus pseudoaurianticus Buillard*; Segnabrise, Fly agaric, Fausse oronge
Origin: Ubiquitous: it grows in the fall in the coniferous woods and in hardwood
Active compounds: muscimol, ibotenic acid, muscazone. muscarine [1]

Amanita muscaria belongs to a poisonous mushroom family. Its name can relate it erroneously to a toxin, the muscarine, which in fact is contained in the mushroom only in minimal amount. However, it is worthwhile to mention that historically muscarine has been isolated for the first time from this mushroom. Conversely, the biologically active compounds in the *Amanita muscaria* are derived from isoxazol: ibotenic acid, muscimol and muscazone [2]. These molecules are psychoactive, being able to induce a state of euphoria similar to that produced by ethyl alcohol with phenomena of excitement, sedation, hallucinations and spasmodic movements. According to the literature, 100 g of dried mushroom contain 180 mg of a mixture of active compounds (ibotenic acid, muscimol and muscazone), only 25 mg being ibotenic acid [2]. Probably, the mushroom as a whole contains toxins still unknown since nor the pure extract of ibotenic acid or the muscimol extract are known to produce the nausea frequently observed after the ingestion of *Amanita muscaria* [3].

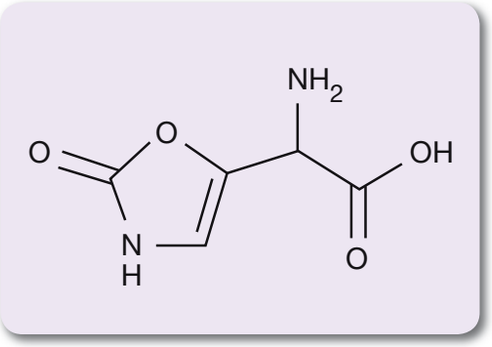
Chemical formula and physico-chemical properties of the active compounds:[1]



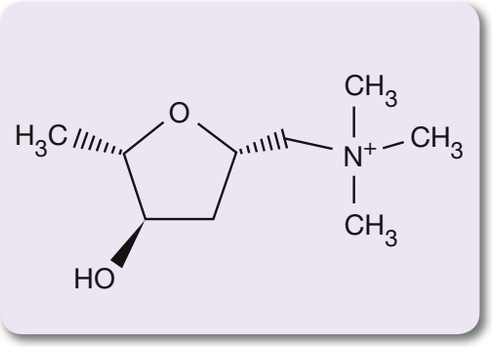
Name: Ibotenic acid.
Molecular formula: $C_5H_6N_2O_4$ (molecular weight = 158.1).
Systematic Name: alpha-amino-2,3-dihydro-3-oxo-5 isoxazol acetic acid.
CAS Registry Number: 2552-55-8.
Melting point: 151-152°C (anhydrous), 144-146°C (monohydrate).
UVmax: 230 nm.
Solubility: water, methyl alcohol and dimethyl sulphoxide.



Name: Muscimol.
Molecular formula: $C_4H_6N_2O_2$ (molecular weight = 114.1).
Systematic Name: 5-(aminomethyl)-3-isoxazolol.
CAS Registry Number: 2763-96-4.
Melting point: 175°C.
UVmax: 230 nm.
Solubility: water.



Name: Muscazone.
Molecular formula: $C_5H_6N_2O_4$ (molecular weight = 158.1).
Systematic name: amino (2-oxo-2,3-dihydro-1,3-oxazol-5-yl) acetic acid.
CAS registry number: 2255-39-2.
Melting point: 175°C.
UVmax: (pH 2-7) = 212 nm, (pH 12) = 220 nm.
Solubility: no data in the literature.



Name: Muscarine.
Molecular formula: $C_9H_{20}NO_2$ (weight = 174.2).
Systematic name: 2S-(2α,4β, 5α)-(tetrahydro-4-hydroxy-5-methyl-furfuryl) trimethylammonium.
CAS registry number: 300-54-9.
Melting point: 180-181°C
UVmax: no data in the literature.
Solubility: water and ethyl alcohol.

Historical use

From the literature, it results that some arctic populations and people from Western Siberia (Khanty, Chukchi, Koryak and others), have traditionally used *Amanita muscaria* for religious purposes to improve the psychophysical performances of the individuals. It seems that the Vikings consumed the mushroom before battles to obtain a state of "frenzy" due to the muscimol. In certain arctic populations, individuals who had particular connections with the religion used the mushroom, without confining the use to any specific social class. *Amanita muscaria* has been used in religious - magic field in order to establish contact with the realm of the dead, to communicate with the spirits, to treat sicknesses, to interpret dreams, to see in the past, to foresee the future, to visit new worlds. According to some scholars, in certain populations, the mushroom has been regarded as s supernatural being. *Amanita muscaria* was used to improve the psychophysical performance in the context of hard work or intense physical exercise (during the hunt, the run etc.). The mushroom has been also used in particular situations of social life by certain groups in different communities. The users were in search of the sensation of happiness, fun, readiness of spirit, euphoric state, pleasant visual and auditory hallucinations that derived from the use of the mushroom. *Amanita muscaria* is consumed raw, cooked, dried or in form of decoction [(4)].

Current use

Some arctic populations keep on using the mushroom in their ceremonials. Beyond the "traditional" or "historical" use of the mushroom, today many individuals culturally far from these populations use the *Amanita muscaria* in search of the hallucinations (euphoria, psychedelic effects) produced by the ingestion of the fructiferous body of the mushroom itself. The dry mushroom is sold in fact through internet web sites and smart shops, that promise effects of visual and auditory hallucinations.

Legislation

In many European countries (Sweden, Norway, Holland, Finland, Denmark, England) *Amanita muscaria* can be bought, sold and possessed legally. In Canada, the use of the mushroom is not controlled. In the United States, particularly in the state of Louisiana, the human consumption of *Amanita muscaria* is not legal, while the law allows its possession and cultivation for decorative and aesthetic purposes. In Italy, neither the ibotenic acid, nor the muscimol, the muscazone, the whole mushroom or parts of it are included in tables containing narcotic or psychotropic substances subject to the

supervision and control under Article 14 of Republic Presidential Decree 309/90 and subsequent updates.

Pharmaco-toxicological properties

The *Amanita muscaria* is used for its hallucinogenic properties. The mushroom is usually eaten fresh or partially dried. Within 30 minutes - 1 hour after the ingestion of the drug, a state of excitement occurs, similar to the one observed with excessive doses of alcohol, followed by drowsiness, muscular contractions, bradycardia, delirium and loss of consciousness. The hallucinations produced by the mushroom are both auditive and visual [5].

The psychotropic effect of the *Amanita muscaria* is due to the presence of ibotenic acid and muscimol, which is the decarboxilation product of ibotenic acid, a step occurring when the mushroom is dried. [6]. Both compounds exercise their neurotoxic effect, after having crossed the blood-brain barrier probably through an intermediate transporter [7-8]. It has been observed that the ibotenic acid, structurally correlated to the glutamic acid, causes excitement, while the muscimol, being similar to the gamma-amino butyric acid (GABA), exercises a depressing effect [9]. The hallucinogenic effect of the muscimol is about 5 times higher compared to that of the ibotenic acid.

About a third of the muscimol is excreted unchanged in the urine. This would explain the fact that in some shamanist rites, the urine of the people who consumed the mushroom is drunk in order to initiate the divine visions[2-4,9].

The pharmacological effects of the muscazone are similar, but minor, when compared to those from the other compounds [10]. Muscarine has a powerful cholinergic effect, but it does not have any psychotropic effect. In any case, its content in the mushroom is very low and it cannot be considered responsible for the symptoms associated with the intoxication[10].

Toxicity

In rats, the intraperitoneal administration of an aqueous extract of *Amanita muscaria*, induces alterations of some hemato-chemical parameters. In particular, there is reduced activity of acetylcholinesterase , reduction of the hepatic levels of glycogen with subsequent increase of glycemia and reduction of azotemia. The activity of the serum transaminase remains unchanged and vital organs such as liver and kidney are not compromised. Within 6 hours the values modified by the ingestion of the mushroom tend to get back to normal [11].

In mice and rats, the intraperitoneal administration of ibotenic acid and muscimol produces an increase in the cerebral levels of serotonin and dopamine as a consequence of a reduced turnover of neurotransmitters [12-13]. Such an increase seems to be responsible for some of the central effects induced by the mushroom (for example the anorectic effect and the mydriasis). In man the toxic dose of muscimol is 6 mg, while that one of ibotenic acid is 30-60 mg.

Data regarding the acute toxicity of ibotenic acid [14]

In mouse: LD50 following intravenous administration: 15 mg/kg.
In mouse: LD50 following oral administration: 38 mg/kg.
In rat: LD50 following intravenous administration 42 mg/kg.
In rat: LD50 following oral administration: 129 mg/kg.

Data regarding the acute toxicity of muscimol [14]

In mouse: LD50 following subcutaneous administration: 3.8 mg/kg.
In mouse: LD50 following intraperitoneal administration: 2.5 mg/kg.
In rat: LD50 following intravenous administration: 4.5 mg/kg.
In rat: LD50 following oral administration: 45 mg/kg.

Adverse Effects

Amanita muscaria, like other kinds of Amanita, although not containing tropane alkaloids, can induce a definite poisoning " micoatropinic syndrome" characterised by symptoms similar to those observed with atropinic plants such as *Atropa belladonna*, *Datura stramonium* and *Hyosciamus niger*. The first manifestations of the poisoning include dizziness, difficulty in maintaining balance and movement coordination and drowsiness. This is followed by psychomotor excitement

accompanied by euphoria and anxiety. In this phase, hallucinations may occur as well [5]. Manifestations of excitement and drowsiness can alternate several times. In addition to dry skin and mucous membranes, tachycardia, reduction of intestinal motility, sweating, salivation, gastrointestinal problems such as nausea and diarrhoea may occur as well. In severe poisonings tremors, convulsions, seizures and coma can be observed. The exitus, rare after the doses which cause hallucinations, can happen in consequence of the ingestion of more than 10 mushrooms. Sporadically, sweating and hypersalivation occur. Finally, anterograde amnesia can be also observed [15].
The treatment of the syndrome is symptomatic using gastric lavage and administration of active charcoal to induce vomiting with the purpose of taking the toxic substances away from the gastrointestinal stream before they are absorbed [16]. Benzodiazepines and / or atropine can be used to control respectively, the state of agitation and the delirium [17].
Poisonings in the grown-ups are usually not severe, but it can happen that the user in the state of maniacal agitation damages himself or others. In children, complex manifestations of neurological type (for example, convulsions and coma) can persist up to 12 hours after the ingestion of the mushroom. Generally, there is no need for therapy, apart from the cases in which anticonvulsive treatment and respiratory assistance is warranted [18].

Pharmacological interactions

There are no reports of pharmacological interactions.

Effects in pregnancy

There is no data regarding use in pregnancy or during breastfeeding.

Analytical determinations

No methodologies have been reported in the international literature concerning the analysis of *Amanita muscaria* active principles in biological fluids of eventual consumers. However, analytical methods have been published regarding the determination of these active principles in commercial products and in cap and stem of both fresh and dry mushroom [19-21]. The first method, rather obsolete, involves liquid chromatography coupled to ultraviolet spectrophotometric detection [19], the second uses liquid chromatography with diode array spectrophotometric detection or tandem mass spectrometry [20], the third a gas chromatography instrument coupled to mass spectrometry [21].

The method below reported is a brief outline useful to the investigator to set up the analysis. For specific details, it is advisable to refer to the original text.

Analysis of hallucinogenic constituents in *Amanita* mushrooms

(From: TSUJIKAWA K, MOHRI H, KUWAYAMA K, MIYAGUCHI H, IWATA Y, GOHDA A, FUKUSHIMA S, INOUE H, KISHI T. Analysis of hallucinogenic constituents in Amanita mushrooms circulated in Japan. Forensic Sci Int. 2006; 164: 172-178) [21].

The analysis is carried out on dry mushrooms of *Amanita muscaria* and *Amanita panterina* and in commercial products containing *Amanita muscaria* by gas chromatography-mass spectrometry (GC-MS).

Extraction of the compounds

For the determination of ibotenic acid and muscimol on dry mushrooms: dry mushrooms are divided in the cap and the stem. Cuticle and flesh of caps are further divided. Each section is pulverised in a mortar. 50 mg powder are extracted two times with 2 ml aqueous solution of 70% methyl alcohol, mixed during 1 minute and sonicated during 5 minutes. After centrifugation at 3000 rpm for 3 minutes, 200 µl supernatant are transferred in a glass tube and evaporated under nitrogen. The dried extract, added with 20 µg/ml n-pentadecane as internal standard, is derivatised with a mixture containing 50 µl N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 10% Trimethylchlorosilane (TMCS) and 50 µl ethyl acetate at

80°C for 30 minutes. A volume of 1 µl is injected in the gas chromatograph.
For the screening of commercial products containing *Amanita muscaria*: 20 mg of commercial product are dissolved in 2 ml water, mixed for 1 minute and sonicated for 10 minutes. After adjusting pH at 2 with drops of 3 M hydrochloric acid, the aqueous solution is extracted two times with 2 ml diethylester. The organic phase is dried under nitrogen and redissolved in 500 µl methyl alcohol (acidic fraction). Aqueous phase is alkalinised at pH 12 with drops of 1 M sodium hydroxide and extracted two times with 2 ml chloroform. The organic phase is dried under nitrogen and in 500 µl methyl alcohol (basic fraction). The acqueous phase is finally neutralised at pH 6-7 with 0.5 M hydrochloric acid, alkalinised at pH 9.6 with 2.8% ammonium hydroxide and extracted with 2 ml chloroform/isopropyl alcohol (3:1, v/v). The organic phase is dried under nitrogen and redissolved in 500 µl methyl alcohol (weak basic fraction).
The dried extracts, added with 20 µg/ml n-pentadecane as internal standard, are derivatised with a mixture containing 50 µl BSTFA with 10% TMCS and 50 µl ethylacetate at 80°C for 30 minutes. A volume of 1 µl each fraction is injected in the gas chromatograph.

Analytical conditions

Chromatographic column: DB-5 ms (0.25 mm x 30 m x 0.25 µm)
Injector temperature: 250°C
Carrier gas: Helium at 72.3 kPa for ibotenic acid and muscimol and at 67.5 kPa for the screening of commercial products
Injection mode: splitless
Temperature program: starting temperature at 100°C for ibotenic acid and muscimol and 50°C for the screening of commercial products for 1 minute, then 100°C-300°C a 15°C/min
Detector: mass spectrometer with electron impact interface

Retention times of the tested compounds

Muscimol: 7 minutes
Ibotenic acid: 9.1 minutes
n-pentadecane (internal standard): 7.3 minutes

Characteristic fragments for the tested compounds

Muscimol: m/z 243, 169, 73
Ibotenic acid: m/z 257, 359, 73
n-pentadecane (internal standard): m/z 57

Standards

The standard of ibotenic acid used in the analyses of the samples was obtained from Biosearch Technologies (Novato, CA, USA) and muscimol standard was purchased from Sigma (St. Louis, MI, USA)

Calibration curves

The calibration standards of ibotenic acid (10, 25, 50, 150 and 400 ppm) and of muscimol (25, 50, 150, 400 e 2000 ppm) and standards used for quality control tests: 300 ppm for ibotenic acid and 1500 ppm for muscimol (high control), 80 ppm for ibotenic acid and 300 ppm for muscimol (medium control) and 20 ppm for ibotenic acid and 40 ppm for muscimol (low control)) were prepared daily adding selected concentration of standard solutions to samples of *Amanita* mushrooms containing very low amounts of analytes under investigations.

Results

Table 1 shows ibotenic acid and muscimol concentration in commercial products containing *Amanita muscaria* and screening analysis of eventual hallucinogenic constituents contained in those products. It can be observed that the content of ibotenic acid and muscimol is very low (below the limit of quantification) to produce dissociative effects. The screening analysis revealed the presence of other psychoactive substances such as hallucinogenic tryptamine deriva-

tives (5-methoxy-N, N-diisopropyltryptamine , 5-MeO-DIPT, and 5-methoxy-N, N-dimethyltryptamine, 5-MeO-DMT), reversible monoamine oxidase inhibitors (harmine and harmaline) and tropane alkaloids (atropine and scopolamine). These compounds have been artificially added since they are not naturally present in fungi of the genus Amanita. Table 2 shows the concentrations of ibotenic acid and muscimol found in analyzed dried mushrooms of *Amanita muscaria* and *Amanita panterina*. Analysis evidenced a higher alkaloids concentration in the cap with respect to the stem. Finally, Table 3 reports the concentration of ibotenic acid and muscimol found in the cuticle and into the flesh of the head of the analyzed mushrooms.

Table 1. Determination of hallucinogenic constituents in commercial products containing *Amanita muscaria* sold in Japan [20]

Sample	Concentration (ppm)		Other constituents
	Ibotenic acid	Muscimol	
1	nd	<25	5-MeO-DIPT
2	<10	<25	5-MeO-DIPT
3	< 10	<25	5-MeO-DIPT, harmaline, harmine, atropine
4	nd	<25	5-MeO-DIPT, 5-MeO-DMT, harmaline, harmine, atropine, scopolamine, caffeine

(nd) not detected
5-MeO-DIPT: 5-methoxy-diisopropyltryptamine,5-MeO-DMT:5-methoxy-N,N-dimethyltriptamine

Table 2. Determination of ibotenic acid and muscimol in the cap and the stem of dry mushrooms of *Amanita muscaria* and *Amanita panterina* species [20]

Sample	Ibotenic acid (ppm)		Muscimol (ppm)	
	Cap	Stem	Cap	Stem
Amanita muscaria				
1	612	nd	286	nd
2	97	-	472	-
3	342	-	254	-
4	<10	-	46	-
5	2845	-	1052	-
Amanita panterina				
1	188	<10	1880	64
2	269	-	1554	-

(nd) not detected ; (-) no sample

Table 3. Determination of ibotenic acid and muscimol in the cuticle and the flesh of dry mushrooms of *Amanita muscaria* and *Amanita panterina* species [20]

Sample	Ibotenic acid (ppm)		Muscimol (ppm)	
	cuticle	flesh	cuticle	flesh
Amanita muscaria				
1	84	527	239	425
2	54	1366	35	558
3	58	322	54	202
4	<10	<10	<25	125
5	187	732	297	774
Amanita panterina				
1	508	985	1304	3544
2	491	377	929	1242

References

1. THE MERCK INDEX An Enciclopedia of chemicals, drugs, and biologicals. 10th Ed. Merck & Co., Inc. 1983.
2. HALPERN JH, Hallucinogens and dissociative agents naturally growing in the United states. Pharmacol Ther. 2004; 102: 131-138.
3. http.//www.emedicine.com/ped/topic1505.htm
4. SAAR M. Ethnomycological data from Siberia and North-East Asia on the effect of *Amanita muscaria*. J Ethnopharmacol. 1991; 31: 157-173.
5. DAVIS DP, WILLIAMS SR. *Amanita muscaria*. Emerg Med. 1999; 17: 739.
6. MICHELOT D, MELENDEZ-HOWELL LM. *Amanita muscaria*: chemistry, biology, toxicology and ethnomycology. Mycol Res. 2003; 107: 131-146.
7. OLPE HR, KOELLA WP. The action of muscimol on neurones of the substatia nigra of the rat. Experientia. 1978; 34: 325.
8. CURTIS DR, LODGE D, McLENNEN H. The excitation and depression of spinal neurones by ibotenic acid. J. Physiol. 1979; 291: 19-28.
9. WALKER RJ, WOODRUFF GN, KERKUT GA. The effect of ibotenic acid and muscimol on single neurons of the snail, *Helix aspersa*. Comp Gen Pharmacol. 1971; 2: 168-174.
10. GUNNAR SAMUELSSON. Farmacognosia. Farmaci di origine naturale. Ed. EMSI.
11. YAMAHURA Y, KOMIYMA S, FUKUHARA M, TAKABATAKE E, HASIMOTA T. Biochemical effects of *Amanita muscaria* extract in mice. Journal of Food and Hygiene Society of Japan (Shokuhin Eiseigaku Zasshi) 1983; 24: 459-464.
12. KONIG-BERSIN P, WASER PG, LANGEMANN H, LICHTENSTEIGER W. Monoamines in the brain under the influence of muscimol and ibotenic acid, two psychoactive principles of *Amanita muscaria*. Psychopharmacologia. 1970; 18: 1-10.
13. GUNDLACH AL, BEART PM. Effect of muscimol on dopamine metabolism of the rat hypothalamus. Experientia. 1980; 36: 1312-1313.
14. http://toxnet.nlm.nih.gov/
15. SATORA L, PACH D, BUTRYN B, HYDZIK P, BALICKA-SLUSARCZYK B. Fly agaric (*Amanita muscaria*) poisoning, case report and review. Toxicon. 2005; 45: 941-943.
16. BENJAMIN DR. Mushroom poisoning in infants and children: the Amanita pantherina/muscaria group. J Toxicol Clin Toxicol. 1992; 30: 13-22.
17. GOODMAN & GILMAN'S - The pharmacological basis of therapeutics. McGraw-Hill Medical Publishing Division. Tenth Edition 2001: 237-238.
18. DOULL J, KLASSEN CD, AMDUR MD. Casarett and Dull's toxicology. 3rd Ed. New York: Macmillan Co., Inc. 1986: 764.
19. GENNARO MC, GIACOSA D, GIOANNINI E, ANGELINO S. Hallucinogenic species in Amanita muscaria. Determination of muscimol and ibotenic acid by ion-interaction HPLC. J Liq Chrom & Rel Technol. 1997; 20: 413-424.
20. TSUJIKAWA K, KUWAYAMA K, MIYAGUCHI H, KANAMORI T, IWATA Y, INOUE H, YOSHIDA T, KISHI T. Determination of muscimol and ibotenic acid in Amanita mushrooms by high-performance liquid chromatography and liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2007; 852: 430-435.
21. TSUJIKAWA K, MOHRI H, KUWAYAMA K, MIYAGUCHI H, IWATA Y, GOHDA A, FUKUSHIMA S, INOUE H, KISHI T. Analysis of hallucinogenic constituents in Amanita mushrooms circulated in Japan. Forensic Sci Int. 2006; 164: 172-178.

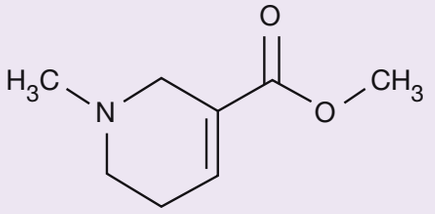
Areca catechu

(areca-nut)

Name: *Areca catechu* Linn
Family: *Arecaceae (Palmae)*
Genus: *Areca* L.
Species: *Areca catechu* Linn
Synonims: Betel nut, betel palm, pinang, bing, lang, areca-nut
Origin: originally from Ceylon (Sri Lanka) Malaysia, it is cultivated in South-East Asia, in India and in some regions of central Africa
Active compounds: arecoline, arecaidine, guvacine and guvacoline

Arecoline is the principal alkaloid of the *Areca catechu*, the other three substances are present in smaller amounts [(1)]. According to the international literature, the concentration of the pharmacologically active compounds in the areca-nut is the following: 7.5 mg/g arecoline, 1.5 mg/g arecaidine, 2 mg/g guvacoline, and 2.9 mg/g guvacine [(2)].
The *Areca catechu* nut is chopped into little pieces and together with lime (calcium hydroxide) and the leaves of the *Piper betle* (betel pepper), it forms a little ball (betel quid) that is chewed or held in the mouth in order to release slowly the contained substances. In addition, it is possible to mix the ground nut with tobacco to make cigarettes rolled up in a leaf of betel pepper that are smoked and commonly called betel nuts [(3)].

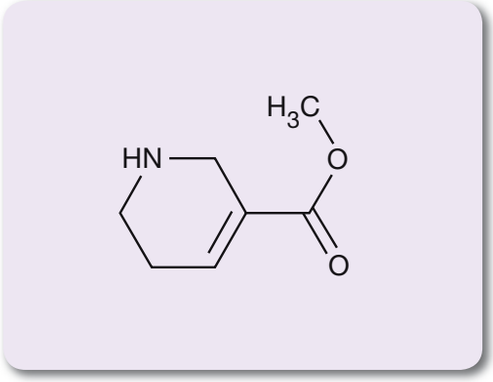
Chemical formula and physico-chemical properties of the active compounds



Name: Arecoline.
Molecular formula: $C_8H_{13}NO_2$ (molecular weight = 155.1).
Systematic name: 1,2,5,6,tetrahydro-1-methyl-3- piridincarboxilic acid methyl ester.
CAS registry number: 63-75-2.
Melting point: <25°C.
UVmax: the coumpound does not show significant absorbance, it absorbs between 230 and 360 nm.
Solubility: water, chloroform and ether.

Name: Arecaidine.
Molecular formula: $C_7H_{11}NO_2$ (molecular weight = 141.1).
Systematic Name: 1,2,5,6,tetrahydro-1-methyl-nicotinic acid.
CAS registry number: 499-04-7.
Melting point: 232°C.
UVmax: no data in the literature.
Solubility: water.

Name: Guvacine.
Molecular formula: $C_6H_9NO_2$ (molecular weight = 127.1).
Systematic name: 1,2,5,6-Tetrahydronicotinic acid.
CAS registry number: 498-96-4.
Melting point: no data in the literature.
UVmax: no data in the literature.
Solubility: water.



Name: guvacoline (norarecoline).
Molecular formula: $C_7H_{11}NO_2$ (molecular weight = 141.1).
Systematic name: 3-Pyridinecarboxylic acid, 1,2,5,6-tetrahydro-, methyl ester.
CAS registry number: 495-19-2.
Melting point: no data in the literature.
UVmax: no data in the literature.
Solubility: no data in the literature.

Historical use

The origin of the habit to chew the nut of *Areca* (areca nut or betel nut are used like synonyms, although the betel nut here refers to the nut smoked with tobacco) is to be found in South-East Asia, probably in Malaysia, where the name of the province of Penang actually means areca-nut. Ancient oriental writers have left testimony that the practice of chewing Betel in China and India was already quite deep-rooted two thousand years ago [3]. It has been reported that the plant had many effects, such as: anthelmintic, appetite stimulant, breath freshener, diuretic, laxative, neurostimulant, therefore finding application in the ayurvedic medicine [3]

Current use

The areca-nut (the fruit or nut of the *Areca catechu*) is consumed commonly by the Asiatic populations and by the immigrant Asiatic communities in Europe and in North America. In the immigrant communities, the consumption of the Areca-nut has also some religious connotations. Members of the Hindu community in the United Kingdom are the greatest betel consumers (up to 80% of the adolescents and grown-ups of the community), while the Sikh communities consume less (50% of adolescents and grown-ups) and the Indian and Pakistani Moslems, even less [4]. Typically, after being chopped, the nut is chewed combined with a variety of other natural products (such as tobacco), wrapped in a pepper leaf (*Piper betle*) (Table 1) [5].

In the Indian traditional medicine, the betel (the "cigar" which is made of areca-nut alone), is recommended especially for his laxative and carminative effects [3]. In addition, the areca nut is used because of the plant's effects: a sense of well-being, palpitations (aphrodisiac, stimulating effect), quick reflexes, resistance to hunger. Besides, the Asiatic women in pregnancy chew the areca nut because it is believed to prevent morning sickness [6].

About 200 million people chew regularly betel in the Western Pacific and in South Asia, and about 600 millions people in the entire world (about 10-20% of the world population) chew areca-nut [3,7]. Only three other psychoactive molecules (nicotine, ethyl alcohol and coffee) are consumed more frequently than the areca nut [7].

Table 1. Areca nut Preparations [5]

Name	Content	Consumption patterns
Mawa	A thin slice of areca nut with tobacco and slaked lime, sold in cellophane papers.	Before consumption, the cellophane pouches are rubbed to mix the contents, which are kept in the mouth in the vestibule and chewed slowly.
Paan	Also known as betel quid, has 4 main ingredients: tobacco, areca nut slices and slaked lime wrapped in betel leaf. May also contain cardamom, coconut, cloves and sugar.	All ingredients are chewed slowly. The contents with the juices are either swallowed or spat out of the mouth.
Gutkha	A powdered mixture of tobacco, areca nut slices and slaked lime with spices and flavoring agents.	The powder is placed in the mouth and slowly chewed. Contents are usually swallowed.
Paan masala	A powdered mixture of areca nut slices and slaked lime with spices and flavoring agents.	The powder is placed in the mouth and slowly chewed. Contents are usually swallowed.
Khaini	Tobacco leaves and areca nut mixed with lime.	The dried tobacco leaves are hand-mixed with lime and made into a bolus that is placed in the mouth, either in the vestibule or below the tongue.
Betel Nut	Chopped tobacco, areca nut slices, slaked lime wrapped in betel leaf.	The “cigarette” is smoked

Legislation

In the countries belonging to the European community as well as in the USA there are no restrictive legislative measures with respect to the use of the areca-nut or its active compounds. In Italy neither arecoline, nor arecaidine, the whole plant or parts of it can be found in the in tables containing narcotic or psychotropic substances subject to the supervision and control under Article 14 of Republic Presidential Decree 309/90 and subsequent updates.

The Italian Ministry of Health included the *Areca catechu* nut in the list of vegetal extracts not admitted in dietary supplements [8].

Pharmaco-toxicological properties

The greatest part of the pharmaco-toxicological properties of the betel nut is attributed to arecoline, the principal alkaloid of the *Areca catechu*.

The arecoline is able to interact either with the muscarinic or nicotinic receptors, producing effects such as bradycardia, hypotension, bronchospasm,, miosis, increase of the muscular tone and increase of salivary, gastric, pancreatic, bronchial and lacrimal secretion [9].

In addition to being a competitive inhibitor of the neurotransmitter gamma aminobutyric acid (GABA), the arecoline is

able to bind to its receptors, hampering the GABAergic neurotransmission and its inhibitory effect on neurotransmission. Thus, while the benzodiazepines (for example: the diazepam) strengthen the inhibitory effect of GABA on the bioelectrical activity of the brain and its tranquilizing effect, arecoline acting as an inhibitor of GABA, produces stimulating effects or euphoria that are the opposite of the sedating effect produced by the benzodiazepines [10].

Arecaidine produces the same parasympathomimetic effects such as those from arecoline: in the mouse, it interferes with its behaviour, reducing the motility of the animal and its wish to explore the environment. *In vitro* studies on the brain of rats, have shown that both, arecaidine and guvacine could act as competitive substrate inhibitors of the GABA uptake [2].

It has been demonstrated also that, in rats, arecoline and arecaidine increase the levels of cerebral acetylcholine [11].

The betel nut extracts have antidepressant effects in animal models (mouse), due to monoamine oxidase (MAO) inhibition [12], or due to the antihypertensive effects of the tannins through the inhibition of the angiotensin converting enzyme (ACE) [13].

Toxicity

In mice, the oral administration of an anhydrous alcoholic extract of betel nut, in doses of 0.5, 1 and 3 g/kg has shown little acute toxicity. The observed effects have been: absence of corneal reflex, ataxia and an increase in the respiratory rhythm [14].

The chronic oral administration of 100 mg/kg of an anhydrous alcoholic extract for 3 months produced an increase in mortality. The animals receiving such an extract had a higher number of white cells and higher haemoglobin. The higher activity of the bone marrow cells has been attributed to the tannins and to the alkaloids contained in the betel nut [14].

Data regarding the acute toxicity of arecoline [15]

In dog - LD50 following subcutaneous administration: 5 mg/kg
In mouse - LD 50 following intraperitoneal administration: 190 mg/kg
In mouse - LD 50 following intravenous administration: 36 mg/kg
In mouse - LD 50 following oral administration: 550 mg/kg
In rat - LD 50 following intraperitoneal administration: 40 mg/kg
In rat - LD 50 following oral administration: 2500 mg/kg

In the rat, it has been observed that a diet enriched with betel in a percentage equal or higher than 15% produces toxic effects such as: necrosis of the oral and intestinal mucosa, larger spleen, fat deposits in the liver and a delay in the skeletal development [16]. There are no toxicity data with respect to arecaidine.

Adverse effects

The betel nut has been associated with the development of cardiovascular pathologies. Elevated levels of homocisteine, associated with an increased ischemic cardiopathy risk, have been observed in the people who chew betel nuts chronically. Literature describes a case of a patient suffering from previous cardiac pathology, who experienced a myocardial infarction due to the betel nut. The arecoline contained in the nuts of betel can cause a spasm of the coronary arteries with a pathogenetic mechanism traceable to a parasympathomimetic effect that explains the endothelial damage of the vessels [17].

In another case, a cardiac patient died after having chewed betel nut, in spite of the repeated defibrillation attempts, because of an acute myocardial infarction with ventricular fibrillation [18].

The use of betel nut can cause tachycardia and increase in the blood pressure. More than half of the chronic users report having palpitations. The heart rate increases within 2 minutes of the mastication, it reaches a peak within 4-6 minutes and ends on average within 18.8 minutes. The average increase of the heart rate (expressed as beats / minute) is equal to 17 for the new users, 16.2 for the casual users and 13.3 for the chronic users. The duration of the effects is greater in the new users compared to the chronic ones, with a possible hypothesis of developing a certain tolerance towards these effects [2] as a result of repeated exposure to the betel nut.

The betel nuts contain significant quantities of copper that can increase the activity of the lysyl oxidase, a copper dependent enzyme which is involved in the formation of the cross-links between collagen and elastin, making the collagen chains more resistant to degradation. This might favour the aterogenesis in the large blood vessels [19,20].

The chronic use of betel nuts can cause the depletion of vitamin B_{12}. Laboratory tests on 11 subjects using betel nut for at least 35 years have shown a remarkable reduction of the B_{12} vitamin blood levels [21].

At the central nervous system, arecoline produces muscarinic effects (miosis, reduced sense of fatigue , increased attention and concentration, agitation followed by a phase of depression and prostration) mediated by an increase of acetylcholine levels and a sense of wellbeing [21-23]. In subjects who use betel nut for the first time dizziness can be observed [3].

The betel nut use has been associated with the development of diabetes [19]. It has been demonstrated particularly in the mouse that about 8.5% of the animals subjected to administration of such a drug develops non- insulin dependent diabetes [24].

A case of two people who have shown a reversible syndrome named “milk alkali”, characterised by hyperglycemia, metabolic alkalosis and renal insufficiency, as a consequence of the betel nut use together with an alkaline paste composed mainly of oyster shells has been reported [25].

At gastrointestinal level, the use of the betel nut can cause intestinal irritation and cramps. The arecoline, given orally, can produce the start of nausea and vomiting causing at the same time an increase in peristalsis [23,26].

The mouth and the teeth of the subjects that chew continuously betel nut, together with calcium hydroxide, get stained quickly assuming a variable colour of red to brown, in addition to numbness of the tongue and dryness of the jaws. The new users can experience a sense of throat and esophagus constriction [8,3,27].

About 0.5% of the chronic users of betel nut develop an oral submucous fibrosis, a pathology characterised by epithelial cell atrophy and collagen accumulation in the oral mucosa. This adverse effect is believed to be the result of the catechines and tannins in the plant, which, by stabilising the collagen, makes it resistant to the human or bacterial collagenases [28]. Subsequently, 15% of these subjects develop tissue anomalies (atypias) while in 7% of the cases the beginning of squamous cell carcinoma was observed [29].

There are descriptions of a series of clinical cases of oral submucous fibrosis and a case of lichen planus associated with the use of betel nut [30-32].

In the mice, chronic betel nut administration caused an increase in the blood levels of the cytochrome B5 and P450 enzymes, of the malondialdehyde and of the glutatione S-trasferase (GST) [33].

Because of its cholinergic effects, arecoline produces bronchoconstriction and can increase the asthmatic manifestations [9,34]. There were two cases described in the literature, of asthmatic patients who have been hospitalised because of a severe asthma attack as a consequence of betel nut chewing [22].

Epidemiological data from China show that the use of betel nut can be a risk factor for the development of chronic renal insufficiency [35].

The betel chewers report a sensation of heat; in fact it has been possible to record an 0.5-2°C increase of the face temperature at the moment of chewing the nut.

Dependence and tolerance

To this date it is not completely clear if the *Areca nut* consumption is able to induce true dependence phenomena. Still, as a consequence of the chronic betel use, especially if associated to tobacco [36], a tolerance very similar to the one observed with cigarette smoking can be developed [21]. Besides, people who stop chewing the betel can show episodes of reversible toxic psychoses characterised by hallucinations and maniacal ideas [9].

Other symptoms frequently reported as a consequence of stopping the use of betel are: alterations of the sense of humour, reduced power of concentration, sleeping problems and increase of the appetite [21].

Carcinogenicity

The use of betel nut can be associated with higher risk of developing squamous cell carcinoma as well as other types of tumour. In chronic betel nut users, leucoplasia- a pre-cancerous lesion that develops subsequently into cancer of the

mouth- has been described [37].

The carcinogenic effect seems to be due to the elevated levels of nitrosamines (originating from the metabolism of the alkaloids) present in the saliva of users [9]. The cytotoxic and genotoxic effect of 3-(N-nitrosomethylamine) propionitrile on the cells of the buccal epithelium, in particular, has been described [38].

The aqueous and acidic betel extracts are able to induce cancer of the esophagus, of larynx and gastrointestinal tumours; the incidence of cancer of the esophagus is comparable with the one described in the tobacco users. These extracts could be responsible for DNA degradation in the hepatocites of the experimental animals [39]. *In vitro* the alkalinity of calcium hydroxide increases the production of oxygen free radical responsible for the toxic effects on the DNA [40]. The habit to chew together calcium hydroxide and betel might be the culprit in the carcinogenic mechanism.

In mice, betel nut in conjunction with calcium hydroxide induces the development of papillomas of the vaginal epithelium, thickening of the vaginal mucosa and alterations of the epithelium and of the inner vaginal mucosa. This type of tumour can metastasize at pulmonary, renal and intraperitoneal level [41].

Teratogenicity

There is no data available on the teratogenicity in the humans, but there is data available in animals [42].A study on chicken embryos has demonstrated that the alcoholic extract causes a dose dependent mortality in these embryos. Malformations have been observed in the bowels, in the limbs as well as reduction of body weight [43].

Pharmacological interactions

The pharmacological interactions of the betel nut are attributable to the cholinergic properties of the contained alkaloids. The betel nut consumption therefore will act as an inhibitor of the anti-muscarinic drugs and will enhance the effects of cholinergic agonists [42].

The arecoline, acting as an inhibitor of GABA, can inhibit the sedative effect of benzodiazepine [2].

Pharmacological interactions can manifest particularly with:

- triciclic antidepressants: reduction of the antidepressant activity;
- amantadine, fenotiazine, olanzapine, molindone, loxapine, haloperidol: increase of the incidence of extrapyramidal effects;
- anticholinergics: reduction of the pharmacological efficiency.

Effects in pregnancy

Numerous cases indicate that the *Areca nut* has a potential genotoxic, mitogenic and clastogenic (it causes breaking of the chromosomes) effect when combined with tobacco [44,45].

The arecoline is able to cross the placenta. Analysis of the meconium of 32 newborn babies, children of Asiatic mothers who had consumed betel nut during the pregnancy, has revealed the presence of arecoline in 6 of these children in a concentration range of 0.006 and 0.012 µg/g [46]. Two of these newborn babies were presenting with low birth weight, hypotonia, intrauterine growth retardation and one of them suffered of neonatal abstinence syndrome [46]. In general, the average weight of the newborns from mothers using betel in pregnancy was lower. The frequency of neonatal jaundice was lower in these children, too [47].

A more recent study confirms the influence of betel nut use in pregnancy on some parameters such as newborns weight and length, which result below the population average values [48].

Analytical determinations

Scientific literature reports a series of analytical methodologies to measure active principles of *Areca catechu* in different biological matrices [46,49,50]. An assay to determine active principles in the plant nut uses capillary electrophoresis coupled to ultraviolet spectrophotometric detection [1].

The method below reported is a brief outline useful to the investigator to set up the analysis. For specific details, it is advisable to refer to the original text.

Analysis of arecoline in meconium, cord blood, fetal urine, placenta and hair

(From: PICHINI S, PELLEGRINI M, PACIFICI R, MARCHEI E, MURILLO J, PUIG C, VALL O, GARCÍA-ALGAR O. Quantification of arecoline (Areca Nut Alkaloids) in neonatal biological matrices by high-performance liquid chromatography/electrospray quadrupole mass spectrometry. Rapid Commun Mass Spectrom. 2003; 17: 1958-1964. GARCÍA-ALGAR O, VALL O, ALAMEDA F, PUIG C, PELLEGRINI M, PACIFICI R, PICHINI S. Prenatal exposure to arecoline (areca nut alkaloid) and birth outcomes Arch Dis Child Fetal Neonatal Ed. 2005; 90; 276-277. MARCHEI E, DURGBANSHI A, ROSSI S, GARCÍA-ALGAR O, ZUCCARO P, PICHINI S. Determination of arecoline (areca nut alkaloid) and nicotine in hair by high performance liquid chromatography/ electrospray quadrupole mass spectrometry. Rapid Commun Mass Spectrom. 2005; 19: 3416-3418) [46,49,50].

The analysis is carried out on different biological matrices by liquid-chromatography coupled to mass spectrometry.

Extraction of the compounds

To 1 g meconium, 1 ml cord blood, 1 ml urine and 500 mg placenta, 1 ml of ammonium chloride at pH 9.5 and 5 ml of 95% chloroform and 5% of isopropanol mixture are added. With respect to hair, 50 mg sample is digested by 2 ml sodium hydroxide at 40°C for 18 hours. Then the solution, at ambient temperature is added with 1 ml of ammonium chloride at pH 9.5 and 5 ml of 95% chloroform and 5% of isopropanol mixture. The tubes are placed on a horizontal shaker for 5 minutes and then centrifuged at 2000 rpm for 5 minutes. After centrifugation, the organic phase is transferred to clean test tubes and 2.5 ml of 0.5 M hydrochloric acid is added. Once again, the samples are centrifuged at 2000 rpm for 5 minutes and subsequently the organic phase is neutralised with 1 ml of 1M sodium hydroxide and 2 ml of ammonium chloride at pH 9.5. Finally, a second extraction with 5ml of a mixture of 95% chloroform and 5% of isopropanol is performed. The organic phase is evaporated under a stream of nitrogen and the residue is dissolved in 100 µl of 10 mM ammonium acetate solution at pH 4.3. A volume of 20 µl is injected into the liquid cromatograph.

Analytical conditions

Chromatographic column: Phenomenex Luna C18 (150 x 4.6mm x 3 µm)
Mobile phase: 90% ammonium acetate 10 mM (pH 3) and 10% acetonitrile
Separation: isocratic
Flow rate: 0.5 ml/min
Detector: mass spectrometer with positive mode electrospray (ESI) interface
Evaporation gas temperature: 350°C
Nebulization gas pressure: 40 psi
Capillary voltage: 1550 V
Fragmentor voltage: 110V

Retention times of the tested compounds

Arecoline: 4.5 minutes
Pilocarpine (internal standard) : 8.1 minutes

Characteristic fragments for the tested compounds

Arecoline: m/z 156, 140, 118

Pilocarpine (internal standard) : m/z 209, 96, 95

Standards

The arecoline and pilocarpine (internal standard) standards used in the analyses have been purchased from Sigma -Aldrich (St. Luis, MI, USA).

Calibration curve

The stock standard solutions at 1 mg/ml were prepared in methyl alcohol. The working standard solutions of 10, 1 and 0.1 µg/ml were prepared by diluting the stock solutions with methyl alcohol and were kept at -20°C. The working standard solutions and calibrators (range of concentrations: 0.005-1 µg of arecoline per gram of meconium and placenta; 0.005 -1 µg of arecoline per millilitre of cord blood or urine and 0.3 - 10 ng of arecoline per milligram of hair) were prepared daily, diluting the stock solutions with methyl alcohol and adding them to samples of meconium, cord blood, urine, placenta and hair samples previously tested as drug-free. The internal standard concentration was 10 µg/ml.

Similar to the working standards, the quality controls : 0.85 µg/g or 0.85 µg/ml, 0.12 µg/g or 0.12 µg/ml and 0.012 µg/g or 0.012 µg/ml were prepared adding the methyl alcoholic solutions to samples of meconium, placenta, cord blood or urine, previously tested as drug-free. For the hair analysis, quality control samples of 8 ng/mg, 3.2 ng/mg and 0.5 ng/mg were prepared adding the methyl alcoholic solutions to hair samples previously tested as drug-free. The quality control samples were included in each batch to check calibration, precision, accuracy and the stability of the previously frozen samples. The quantitative analysis was achieved by comparing the peaks of samples and standards identified by the ion m/z 156 for arecoline and the ion m/z 209 for pilocarpine used as internal standard.

Results

The analysis of the arecoline in several biological matrices listed above showed an average amount of arecoline such as:

Meconium:	0.008 µg/g
Urine:	0.01 µg/g
Cord blood:	traces
Placenta:	0.01 µg/g
Hair:	1.71 ng/mg when the areca nut is smoked
Hair:	1.18 ng/mg when the areca nut is chewed

References

1. LORD GA, LIM CK, WARNAKULASURIYA S, PETERS TJ. Chemical and analytical aspects of areca nut. Addict Biol. 2002; 7: 99-102.
2. CHU NS. Effects of betel chewing on the central and autonomic nervous systems. J Biomed Sci. 2001; 8: 229-236.
3. NORTON SA. Betel: consumption and consequences. J Am Acad Dermatol. 1998; 38: 81-88.
4. WARNAKULASURIYA S. Areca nut use following migration and its consequences. Addict Biol. 2002; 7: 127-132.
5. AULUCK A, HISLOP G, POH C, ZHANG L, ROSIN MP. Areca nut and betel quid chewing among South Asian immigrants to Western countries and its implications for oral cancer screening. Rural Remote Health. 2009; 9: 1118. Disponibile on-line sul sito: http://www.rrhorg.au
6. YANG MS, CHANG FT, CHEN SS, LEE CH, KO YC. Betel quid chewing and risk of adverse pregnancy outcomes among aborigines in southern Taiwan. Public Health. 1999; 113: 89-92.
7. GUPTA PC, WARNAKULASURIYA S. Global epidemiology of areca nut usage. Addict. Biol. 2002; 7: 77-83.
8. The list of vegetal extracts not admitted in dietary supplements in Italy can be found at http://www.salute.gov.it
9. PICKWELL SM, SCHIMELPFENING S, PALINKAS LA. 'Betelmania'. Betel quid chewing by Cambodian women in the United States and its potential health effects. West J Med 1994; 160: 326-330.
10. BOUCHER BJ, MANNAN N. Metabolic effects of the consumption of Areca catechu. Addict Biol. 2002; 7: 103-110.
11. MOLINENGO L, FUNDARO AM, CASSONE MC. Action of a chronic arecoline administration on mouse motility and on acetylcholine concentrations in the CNS. J Pharm Pharmacol. 1988; 40: 821-822.

12. DAR A & KHATOON S. Antidepressant effects of ethanol extract of Areca catechu in rodents. Phytotherapy Res. 1997; 11: 174-176.
13. INOKUCHI J-I, OKABE H, YAMAUCHI T NAGAMATZU A, NONAKA G, NISHIOKA I. Antihypertensive substance in seeds of Areca catechu L. Life Sci. 1986; 38: 1375-1382.
14. SHAH AH, QUERESHI S, TARIQ M. Toxicity studies on six plants used in the traditional Arab system of medicine. Phytother Res. 1989; 3: 25-29.
15. http://toxnet.nlm.nih.gov/
16. SAIKIA M & VAIDEHI MP. Studies on the pathological effects of feeding betel nut meal in albino rats. Br J Exp Path. 1983; 64: 515-517.
17. HUNG DZ, DENG JF. Acute myocardial infarction temporally related to betel nut chewing. Vet Hum Toxicol. 1998; 40: 25-28.
18. DENG JF, GER J, TSAI WJ, KAO WF, YANG CC. Acute toxicities of betel nut: rare but probably overlooked events. J Toxicol Clin Toxicol. 2001; 39: 355-360.
19. TRIVEDY C & WARNAKULASURIYA S. Areca nuts can have deleterious effects. BMJ 1999; 318: 1287.
20. TRIVEDY C, BALDWIN D, WARNAKULASURIYA S JOHNSON N PETERS T. Copper content in Areca catechu (betel nut) products and oral submucous fibrosis. Lancet 1997; 349: 1447.
21. WINSTOCK AR, TRIVEDY CR, WARNAKULASURIYA KAAS PETERS TJ. A dependency syndrome related to areca nut use: some medical and psychological aspects among areca nut users in the Gujarat community in the UK. Addiction Biol 2000; 5: 173-179.
22. TAYLOR RF, AL-JARAD ,N JOHN LM, CONROY DM, BARNES NC. Betel nut and chewing and asthma. Lancet 1992; 339: 1134-1136.
23. MUJUMDAR AM, KAPADI AH, PENDSE GS. Chemistry and pharmacology of betel nut Areca catechu Linn. J Plantation Crops. 1979; 7: 69-92.
24. MANNAN N, BOUCHER BJ, EVANS SJW: Increased waist size and weight in relation to consumption of Areca catechu (betel nut); a risk factor for increased glycaemia in Asians in East London. Brit J Nutrition. 2000; 83: 267-275.
25. WU KD, CHUANG RB, WU FL, HSU WA, JAN IS, TSAI KS. The milk alkali syndrome caused by betel nuts in oyster shell paste. Clin Toxicol. 1996; 34: 741-745.
26. ARJUNGI VKN. Areca nut. Arzneim-Forsch (Drug Res) 1976; 26: 951-956.
27. FARNSWORTH ER. Betel nut--its composition, chemistry, and uses. Sci in New Quinea. 1976; 4: 85-90.
28. SCUTT A, MEGHJI S, CANNIFF JP HARVEY W. Stabilisation of collagen by betel nut polyphenols as a mechanism in oral submucous fibrosis. Experimentia 1987; 43: 391-393.
29. CANNIFF JP & HARVEY W. The aetiology of oral submucous fibrosis: the stimulation of collagen synthesis by extracts of areca nut. Int J Oral Surg. 1981; 10: 163-167.
30. ANIL S, BEENA VT. Oral submucous fibrosis in a 12-year-old girl: case report. Pediatr Dent. 1993; 15: 120-122.
31. SHAH B, LEWIS MA, BEDI R. Oral submucous fibrosis in a 11-year-old Bangladeshi girl living in the United Kingdom. Br Dent J. 2001; 191: 130-132.
32. STOOPLER ET, PARISI E, SOLLECITO TP. Betel quid-induced oral lichen planus: a case report. Cutis. 2003; 71: 307-311.
33. SINGH A & RAO AR. Modulatory influence of areca nut on the mouse hepatic xenobiotic detoxication system and skin papillomagenesis. Teratog Carcinog Mutagen. 1995; 15: 135-146.
34. FUGH-BERMAN A. Herb-drug interactions. Lancet 2000; 355: 134-138.
35. CHOU CY, CHENG SY, LIU JH, CHENG WC, KANG IM, TSENG YH, SHIH CM, CHEN W. Association between betel-nut chewing and chronic kidney disease in men. Public Health Nutr. 2009;12: 723-727.
36. BENEGAL V, RAJKUMAR RP, MURALIDHARAN K. Does areca nut use lead to dependence? Drug Alcohol Depend. 2008; 97: 114-121.
37. SHIU MN, CHEN THH, CHANG SH HAHN LJ. Risk factors for leukoplakia and malignant transformation to oral carcinoma: a leukoplakia cohort in Taiwan. Br J Cancer. 2000; 82: 1871-1874.
38. SUNDQVIST K, LIU Y, NAIR J BARTSCH H, ARDVIDSON K, GRAFSTROM RC. Cytotoxic and genotoxic effects of Areca nut-related compounds in cultured human buccal epithelial cells. Cancer Res. 1989; 49: 5294-5298.
39. SHARAN RN & WARY KK. Study of unscheduled DNA synthesis following exposure of human cells to arecoline and extracts of betel nut in vitro. Mutat Res. 1992; 278: 271-276.
40. NAIR UJ, FRIESEN M, RICHARD I MacLENNAN R, THOMAS S, BARTSCH H. Effect of lime composition on the formation of reactive oxygen species from areca nut extract in vitro. Carcinogenesis. 1990; 11: 2145-2148.
41. KAPADIA GJ, CHUNG EB, GHOSH B SHUKLA YN, BASAK SP, MORTON JF, PRADHAN SN. Carcinogenicity of some folk medicinal herbs in rats. J Natl Cancer Inst. 1978; 60: 683-686.
42. SING A., RAO AR. Effect of arecanut, a masticatory, on hepatic drug metabolising enzymes-SH content and lipid peroxidation in lactating mothers and their sucking neonates. Cancer Lett. 1995; 92: 175-180.
43. PAUL K, MOITRA PK, MAITY CR GHOSAL SK. Teratogenicity of crude areca nut extract in chick embryos. Ind J Physiol Allied Sci. 1996; 50: 182-187.
44. LEE CH, LIN SH, LIU SH LIN-SHIAU SY. Mutual interactions among ingredients of betel quid in inducing genotoxicity on Chinese hamster ovary cells. Mutat Res. 1996; 367: 99-104.
45. SEN S, TALUKDER G & SHARMA A. Betel cytotoxicity: further evidence from mouse bone marrow cells. Int J Pharmacognosy 1991; 29: 130-140.
46. GARCIA-ALGAR O, VALL O, ALAMEDA F, PUIG C, PELLEGRINI M, PACIFICI R, PICHINI S. Prenatal exposure to arecoline (areca nut alkaloid) and birth outcomes. Arch Dis Child Fetal Neonatal Ed. 2005; 90: F276-F277.
47. DE COSTA C., GRIEW AR. Effects of betel chewing on pregnancy outcome. Aust N Z J Obstet Gynaecol. 1982; 22: 22-24.

48. YANG MS, LEE CH, CHANG SJ, CHUNG TC, TSAI EM, KO AM, KO YC. The effect of maternal betel quid exposure during pregnancy on adverse birth outcomes among aborigines in Taiwan. Drug Alcohol Depend. 2008; 95: 134-139.
49. PICHINI S, PELLEGRINI M, PACIFICI R, MARCHEI E, MURILLO J, PUIG C, VALL O, GARCÍA-ALGAR O. Quantification of arecoline (Areca Nut Alkaloids) in neonatal biological matrices by high-performance liquid chromatography/electrospray quadrupole mass spectrometry. Rapid Commun Mass Spectrom. 2003; 17: 1958-1964.
50. MARCHEI E, DURGBANSHI A, ROSSI S, GARCÍA-ALGAR O, ZUCCARO P, PICHINI S. Determination of arecoline (areca nut alkaloid) and nicotine in hair by high performance liquid chromatography/ electrospray quadrupole mass spectrometry. Rapid Commun Mass Spectrom. 2005; 19: 3416-3418.

Argemone mexicana
(prickly poppy)

Name: *Argemone mexicana*
Family: *Papaveraceae*
Genus: *Argemone* L.
Species: *Argemone mexicana* L.
Synonims: mexican prickly poppy
Origin: South America, in particular Mexico
Active compounds: Berberine, Protopine, Sanguinarine, Dihydrosanguinarine, Allocryptopine, Chelerythrine, Coptisine

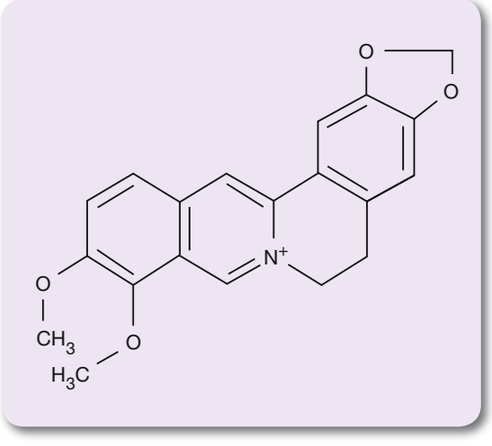
The *Argemone mexicana* or prickly poppy as this plant is commonly known, originates from South America, Mexico and India [1]. The first studies on the chemical composition of the *Argemone mexicana* reported the presence of berberine and protopine in the seeds of the plant [1,2].

The seed produces 22-36 % of a yellow-brownish oil, called argemone oil or katkar oil. This unedible oil, contains two toxic alkaloids: sanguinarine and dihydrosanguinarine [3]. Studies on the *Argemone mexicana* report the presence of other alkaloids such as allocryptopine in the root and in the airy parts of the plant, chelerythrine in the root and the coptisine in the stalk, in the leaves, in the capsule, in the seeds and in the seedlings [4]. The chemical composition of the seeds of the *Argemone mexicana* is reported in Table 1.

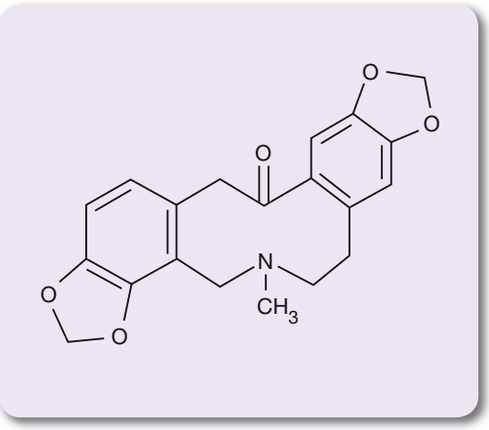
Table 1. Alkaloid content of the *Argemone mexicana* seeds [5]

Alcaloids	Percentage
Total alkaloids	0.13
Dihydrosanguinarine	87.00
Sanguinarine	5.00
Berberine	0.57
Protopine	0.34
Chelerythrine	0.12
Coptisine	0.03

Chemical formula and physico-chemical properties of the active compounds [6-8]



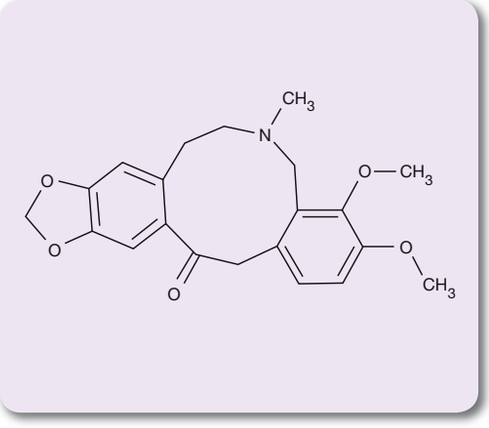
Name: Berberine.
Molecular formula: $[C_{20}H_{18}NO_4]^+$ (molecular weight = 336.4).
Systematic name: 5,6-dihydro-9,10-dimethoxybenzo(g)-1,3-benzodioxol(5,6-a)quinolizinium.
CAS registry number: 2086-83-1.
Melting point: 145°C.
UVmax: 265, 343 nm.
Solubility: slightly soluble in water.



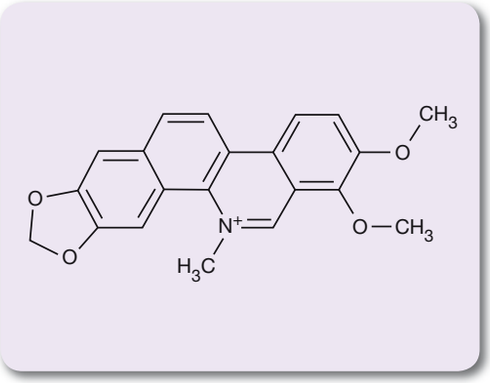
Name: Protopine.
Molecular formula: $C_{20}H_{19}NO_5$ (molecular weight = 353.4).
Systematic name: 4,6,7,14-tetrahydro-5-methyl-bis(1,3)-benzodioxol(4,5-c-5',6'-g)azecin-13(5H)-one.
CAS registry number: 130-86-9.
Melting point: 208°C.
UVmax: 239, 291, in 95% ethyl alcohol = 293 nm.
Solubility: ethyl acetate, carbon bisulphide, benzene, petrolium ether. Practically insoluble in water.

Name: Sanguinarine.
Molecular formula: $C_{20}H_{14}NO_4$ (molecular weight = 332.3).
Systematic name: 13-methyl(1,3)benzodioxol(5,6-c)-1,3-dioxol(4,5-i)phenantridine.
CAS registry number: 2447-54-3.
Melting point: 273-274°C.
UVmax: 234, 283, 325 nm (methyl alcohol).
Solubility: alcohol, chloroform, acetone, ethyl acetate.

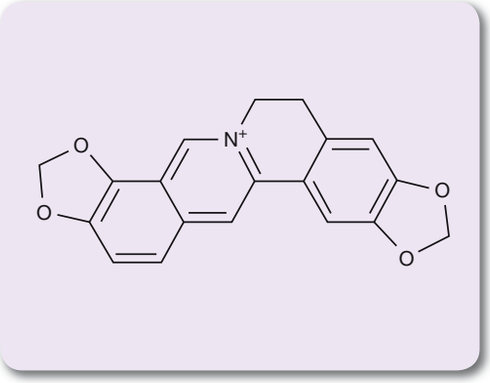
Name: Dihydrosanguinarine.
Formula Molecolare: $C_{20}H_{15}NO_4$ (molecular weight = 333.3).
Systematic name: 13,14-dihydro-13-methyl(1,3) benzodioxol(5,6-c)-1,3-dioxol(4,5-i)phenantridine.
CAS registry number: 3606-45-9.
Melting point: 188-189°C.
UVmax: 237, 284, 322 nm (ethyl alcohol).
Solubility: no data in the literature.



Name: Allocryptopine
Molecular formula: $C_{21}H_{23}NO_5$ (molecular weight = 369.4).
Systematic name: 5,7,8,15-tetrahydro-3,4-dimetoxy-6-methyl(1,3) benzodioxol(5,6-e)(2)benzazecin-14(6H)-one.
CAS registry number: 485-91-6.
Melting point: 160-161°C.
UVmax: 232, 284 nm.
Solubility: alcohol, chloroform, ether, ethyl acetate and dilute acids.



Name: Chelerythrine.
Molecular formula: $[C_{21}H_{18}NO_4]^+$ (molecular weight = 348.4).
Systematic name: 1,2-dimethoxy-12-methyl(1,3)benzodioxol(5,6-c) phenanthridinium.
CAS registry number: 34316-15-9.
Melting point: 200-206°C.
UVmax: 226, 283, 320 nm.
Solubility: dimethylsulphoxide (DMSO), ethyl alcohol, petroleum ether. Not soluble in water.



Nome: Coptisine.
Molecular formula: $[C_{19}H_{14}NO_4]^+$ (molecular weight = 320.3).
Systematic name: 6,7-dihydrobis(1,3)benzodioxol(5,6-a:4',5'-g) quinolizinium.
CAS registry number: 3486-66-6.
Melting point: 218°C.
UVmax: 229, 244, 267, 353.
Solubility: slightly soluble in water, partially soluble in alcohol, soluble in alkalies.

Historical use

According to certain Internet sources, the *Argemone mexicana* was used by the Aztecs as "food for the dead", to be offered to gods during the sacrifices. The Aztecs used to do a mixture with the seeds similar to bread which they modelled in an image of the god known as Huitzilopochtli [9]. In the Florentine Code (the name given to 12 books created under the supervision of Bernardino de Sahagún between 1540 and 1585, approximately) it is described that during the sacrificial rites, a highest priest was "killing" the image of god, and using it as food for the sacrificed. As confirmed in the Florentine Code, Prickly Poppy was always one of three plants used by Aztecs [9].

The juice of the leaves was used in the treatment of cataracts, headaches and inflammations of the eyes [1]. It was believed that the plant possessed narcotic properties due to the presence of morphine. In 1868, Charbonnier reported that he isolated morphine from the leaves and the capsule of *Argemone Mexicana*, but later studies demonstrated that the plant did not contain morphine, but alkaloids such as berberine and the protopine [1].

Current use

The *Argemone mexicana* is used as a medicinal plant in different countries. In Mexico, the seeds are considered an antidote for snake poison. In India, the fumes of the seeds are used to alleviate teeth ailments. The fresh extract of the seeds contains certain substances that can solubilize the proteins and it is effective in the treatment of warts, labial herpes, cutaneous infections, skin diseases, itching and also for the treatment of hydropsy and jaundice [10]. In India the plant is used for the treatment of a wide range of diseases in the ayurvedic and unani medicine, one of them being malaria. It is used as antimalarial also in different African countries, such as Benin, Mali and Sudan [11].

There is no documentation regarding the psychoactive properties of *Argemone Mexicana*. According to the Internet sources, the leaves are smoked as substitutes for marijuana or used for preparing tea as an accompaniment for cigarette. The *Argemone mexicana* is sold in a resin form to be mixed with other herbs. It is suggested to mix it with tobacco and smoking it to experience a relaxing, and aromatized tobacco effect. For a stronger effect, the pure resin can be used in pipes or it can be vaporized.

Legislation

There are no restrictive measures in Europe for the use of the plant or its active compounds.The use of *Argemone mexicana* is not regulated in the United States of America. In Italy, neither the alkaloids of *Argemone mexicana* nor the whole plant or parts of it are included in tables containing narcotic or psychotropic substances subject to the supervision and control under Article 14 of Republic Presidential Decree 309/90 and subsequent updates. The Italian Ministry of Health included the flower, the leaf, the oil, the root and the seeds of *Argemone mexicana* to the list of the vegetable extracts not admitted in dietary supplements [12].

Pharmaco-toxicological properties

Bose et al. have reported that most of the pharmacological activities of *Argemone mexicana* extracts are related to the total fraction of the alkaloids [13].

The sanguinarine and the berberine present a wide range of biological and/or pharmacological effects, such as anti-inflammatory properties, respiratory stimulation, transitory hypotension, convulsions, uterine contraction, antiarrhythmic properties, positive inotropic effects, adrenocorticotropic effect and analgesic activity [14]. Because of the quaternary nitrogen of the planar and polycyclic structure, the sanguinarine and the berberine can react with the nucleophylic groups and anionic amino acids of different biomolecules, receptors and enzymes. For example, these alkaloids become attached to microtubules, inhibit different enzymes such as the sodium potassium-ATPase, favor the oxidative phosphorilation and are able to intercalate in the DNA rich regions of guanine-citosine [14].

Given orally to mice at a dose of 100 mg/kg, berberine has similar effects to the anxiolytic effects of 1 mg/kg diazepam and 2 mg/kg buspirone in humans. The anxiolytic mechanism of berberine might be related to the increase in the percentage of monoamine turnover in the cerebral cortex and to the reduced activity of the serotoninergic system. It has been reported that berberine reduces the activity of the serotoninergic system through the activation of the somatodendritic autoreceptors 5-HT_{1A} and the inhibition of the postsynaptic somatodendritic autoreceptors 5-HT_{1A} and 5-HT_2 [15].

In rats, protopine, cryptopine and allocryptopine have the ability to increase the connection of gamma aminobutyric acid to the central receptors, having a sedative and anxiolytic action similar to benzodiazepines [16]. Cryptopine has a stimulating *in vitro* effect (at a dilution 1:1000000) on the uterus of the guinea pig. It reduces the respiratory rhythm through a central action and acts directly on the myocardium slowing down all its functions [17]. Allocryptopine presents similar action, as well as antifibrillatory action and local antitumoral and anaesthetic activity [17].

Chelerythrine has a wide range of biological activities such as anti-aggregating piastrinic, anti-inflammatory and antibacterial activities as well as antitumoral effects [18].

The sensitivity of two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram-negative ones (*Escherichia coli* and *Pseudomonas aeruginosa*) to the extracts (cold aqueous, warm aqueous and methyl alcoholic) of *Argemone mexicana* leaves and seeds was tested. Although all the extracts have been found effective, the methyl alcoholic extract, either from the seeds or the leaves, had a greater activity against the microorganisms compared to the aqueous extracts [10].

Other studies indicate that protopine and the allocryptopine have been able to reduce the opiate abstinence in *in vitro* experiments [19]. These compounds are able to produce their effects at the level of the μ and κ opioid receptors [20].

Toxicity

The seeds of *Argemone mexicana* are similar in size and aspect to those of black mustard (*Brassica nigra*). The mustard seeds oil is used very much in the Indian cuisine and an accidental ingestion of oil adulterated with oil extracted from the seeds of *Argemone mexicana* causes epidemic hydropsy characterized by the pathological accumulation of liquids in tissues and body cavities. Focuses of the disease have been reported in the Mauritius, in the Fiji islands, in the Madagascar and in the South Africa [21,22]. Studies on epidemic hydropsy indicate that 1 % of argemone oil adulteration in mustard oil is sufficient to produce clinical symptoms [22].

The toxicity of *Argemone mexicana* is due mostly to sanguinarine, which seems to be 2.5 times more toxic compared to another toxic alkaloid, the dihydrosanguinarine, even if they are interconvertible through a simple oxidation-reduction [21].

The values for the acute toxicity in an animal model for sanguinarine, berberine, protopine and chelerythrine are presented in Table 2.

Table 2. Accute toxicity values of *Argemone mexicana* alkaloids in an animal model [24,25]

Mode of administration	Specie	LD50/LC50/LoD/LD
sanguinarine		
s.c.	cat	LD50 = 120 mg/kg
	dog	LD50 = 70 mg/kg
	frog	LD50= 70 mg/kg
	rabbit	LD50 = 125 mg/kg
s.c.	mouse	LD50 = 80 mg/kg
i.p.		LD50 = 18 mg/kg
i.v.		LD50 = 19400 µg/kg
inhalation	rat	LC50 = 2200 g/m^3
i.p.		LD50 = 18 mg/kg
i.v.		LD50 = 28700 µg/kg
orale		LD50 = 1660 mg/kg
berberine		
s.c	mouse	LD50 = 18 mg/kg
oral		LD50 = 329 mg/kg
s.c	rabbit	LDLo = 100 mg/kg
i.p.	rat	LD = >500 mg/kg
protopine		
i.p.	Guinea pig	LD50 = 116 mg/kg
oral		LD50 = 237 mg/kg
i.p.	mouse	LD50 = 482 mg/kg
i.v.		LD50 = 31 mg/kg
i.p.	rat	LDLo = 100 mg/kg
chelerythrine		
i.v.	mouse	LD50 = 18,5 mg/kg

s.c.= subcutaneous; i.p. = intraperitoneal; i.v.=intravenous; LD50 = lethal dose in 50% of the tested animals; LC50 = lethal concentration in 50% of the tested animals after a specific time interval; LDLo = minimum lethal dose; LD = lethal dose.

There is no published data on the acute toxicity of dihydrosanguinarine, allocriptopine and to the coptisine. The ethyl alcoholic extract of the *Argemone mexicana* leaves, following intraperitoneal administration in the mouse, has a LD50 of 400 mg/kg of body weight [26].

Adverse Effects

Studies on the toxicity of *Argemone mexicana* seeds in the rat have shown a significant reduction of the body weight, significant increase in glycemia, azotemia and of the glutamic oxaloacetic-transaminase and lesions indicative of hepato nephropathy [27]. It has been demonstrated that the hepatic microsomal enzymes as well as the mitochondrial membranes are susceptible to the peroxidative action of the seed oil of *Argemone mexicana* which, in turn, could be the cause of hepatotoxic symptoms observed in victims of *Argemone mexicana* poisoning [28]. The diarrhoea can be attributed to gastroenteritis or to the cholinergic effect of the plant's compounds [29]. The involvement of the nervous system in the poisoning,

above all in the epidemic hydropsy, is controversial although paresthesia and muscular spasms have been reported in the literature [30].

Pharmacological interactions

There are no reports on the pharmacological interactions.

Effects in pregnancy

Due to the presence of toxic alkaloids, it is not advisable to administer *Argemone mexicana* to pregnant women or during breastfeeding [31].

Analytical determinations

No methodologies have been reported in the international literature concerning the analysis of *Argemone mexicana* active principles in biological fluids of eventual consumers. However, analytical methods have been published regarding the determination of these active principles in seeds oil of the plant [22,32]. Of these methods, the first consists of a separation on high performance thin layer chromatography (HPTLC) coupled to ultraviolet spectrophotometric detection and subsequent mass spectrometric analysis of active principles isolated by thin layer chromatography, whereas the second uses liquid chromatography coupled with diode array spectrophotometric detection [32].

The method below reported is a brief outline useful to the investigator to set up the analysis. For specific details, it is advisable to refer to the original text.

Quantitative determination of sanguinarine and dihydrosanguinarine in the oil of *Argemone mexicana* seeds

(From: GHOSH P, REDDY MMK, SASHIDHAR RB. Quantitative evaluation of sanguinarine as an index of argemone oil adulteration in edile mustard oil by high performance thin layer chromatography. Food Chem. 2005; 91: 757-764) [22].

The analysis is carried out on *Argemone mexicana* seeds oil and mustard seed oil using high performance thin layer chromatography (HPTLC) with CAMAG ultraviolet detection (SelectScience Ltd, CorstonBath, UK). The extracts isolated from the chromatographic plates are subsequently analyzed by mass spectrometry.

Extraction of the compounds

About 50 g of seeds are pulverised mechanically and the oil is extracted with n-hexane for six hours. Subsequently, the extracted oil is passed through anhydrous sodium sulphate in order to remove any trace of moisture. A volume of 20 µl of argemone oil is diluted in one milliliter of chloroform and 5 µl are used for HPTLC. A volume of 50 µl of mustard seed oil is diluted in one milliliter of chloroform and 5 µl are used for HPTLC.

Analytical conditions

Chromatographic plate: aluminum covered with silica gel 60 (20 cm. x 10 cm.)
Mobile phase: hexane:acetone: methyl alcohol (80:15:5, v/v/v)
Detector 1: mercury lamp (366 nm)
Detector 2: mass spectrometer interfaced with chemical ionization in positive mode with direct infusion

Retention Factor (RF) of the tested compounds

Sanguinarine: 0.36
Dihydrosanguinarine: 0.82

Characteristic fragments of the tested compounds

Sanguinarine: m/z 332, 291, 241, 169

Dihydrosanguinarine: m/z 291, 275, 241, 169

Standard

The sanguinarine standard use in the analysis was obtained from Sigma (St. Luis, MI, USA).

Calibration curves

The standard solution of sanguinarine (1 mg/ml) was prepared in methyl alcohol and kept at - 20 °C. The calibration solutions (range of concentrations: 1 - 60 µg/ml) were prepared daily diluting the standard solutions with methyl alcohol. To evaluate the percentage of adulteration of the samples, different concentrations (range 1% – 30%, v/v) of pure argemone were been added to the mustard seed oil. A volume of 100 µl of each adulterated oil was diluted to one milliliter with chloroform.

Results

The concentration of sanguinarine determined in the samples is presented in Table 3. In fifteen analyzed samples, only four were void of sanguinarine. The percentage of argemone oil present in the samples varied from 1.2 % to 8.8 %.

Table 3. Sanguinarine concentration and percentage of argemone oil found in the mustard seed oil samples.

Sample	Sanguinarine (µg/ml ± SD)	% adulterant
1	nd	-
2	48.3 ± 2.0	1.3
3	45.5 ± 4.0	1.3
4	58.0 ± 2.0	1.6
5	nd	-
6	70.0 ± 2.0	1.9
7	60.0 ± 2.0	1.6
8	45.0 ± 2.0	1.3
9	46.0 ± 2.0	1.3
10	44.0 ± 3.0	1.2
11	nd	-
12	345.0 ± 11.0	8.7
13	300.0 ± 10.0	7.6
14	103.0 ± 17.0	2.7
15	nd	-

nd = not determined; SD = standard deviation

References

1. SCHLOTTERBERCK JO. Does “Argemone mexicana” contain morphine? J Am Chem Soc. 1902; 24: 238-242.
2. SANTOS AC, ADKIN PJ. The alkaloids of Argemone mexicana. Am Chem Soc. 1932; 54: 2923.
3. SHUKLA AK, DIXIT AK, SINGH RP. Detection of Argemone Oil in Mustard Oil. J Oleo Sci. 2005; 54: 81-83.
4. KAPOOR LD. Handbook of ayurvedic medicinal plants. 2001; p. 47
5. DAS M, KHANNA SK. Clinicoepidemiological, toxicological ans safety evaluation studies on Argemone oil. Clin Rev toxicol. 1997; 27: 273-297.
6. THE MERCK INDEX An Enciclopedia of chemicals, drugs, and biologicals. 10th Ed. Merck & Co., Inc. 1983.

7. VOLOSHCHUK TP, PATSKOVSKY YV, ZAYIKA LA. Study of Ukrain composition using HPLC and UV-spectroscopy methods. Ukr Bioorg Acta. 2006; 2: 27-32.
8. KRANE BD, FAGBULE MO, SHAMMA M. The benzophenanthridine alkaloids. J Nat Prod. 1984; 47: 1-43.
9. http://www.entheology.org/edoto/anmviewer.asp?a=155
10. BHATTACHARJEE I, CHATTERJEE SK, CHATTERJEE S, CHANDRA G. Antibacterial potentiality of Argemone mexicana solvent extracts against some pathogenic bacteria. Mem Inst Oswaldo Cruz. 2006; 101: 645-648.
11. WILLCOX ML, GRAZ B, FALQUET J, SIDIBÉ O, FORSTER M, DIALLO D. Argemone mexicana decoction for the treatment of uncomplicated falciparum malaria. Trans R Soc Trop Med Hyg. 2007; 101: 1190-1198.
12. The list of vegetal extracts not admitted in dietary supplements in Italy can be found at http://www.salute.gov.it
13. BOSE BC, VIJAYVARGIYA R, SAIFI AQ, SHARMA SK. Chemical and pharmacological studies on Argemone mexicana. J Pharm Sci. 1963; 52: 1172-1175.
14. SCHMELLER T, LATZ-BRÜNING B, WINK M. biochemical activities of berberine, palmatine and sanguinarine mediating chemical defence against microorganisms and herbivores. Phytochem. 1997; 44: 257-266.
15. PENG WH, WU CR, CHEN CS, CHEN CF, LEU ZC, HSIEH MT. Anxiolytic effect of berberine on exploratory activity of the mouse in two experimental anxiety models: interaction with drugs acting at 5-HT receptors. Life Sci. 2004; 75: 2451-2462.
16. KARDOS J, BLASKÓ G, SIMONYI M. Enhancement of gamma-aminobutyric acid receptor binding by protopine-type alkaloids. Arzneimittelforschung. 1986; 36: 939-940.
17. ENRICA CAMPANINI. Dizionario di fitoterapia e piante medicinali. Ed. tecniche nuove 2004, p. 199.
18. CHMURA SJ, DOLAN EM, CHA A, MAUCERI KUFE DW, WEICHSELBAUM RR. In vitro and in vivo activity of protein kinase C inhibitor chelerythrine chloride induces tumor cell toxicity and growth delay in vivo. Clin Cancer Res. 2000; 6; 737-742.
19. CAPASSO A, PIACENTE S, PIZZA C, TOMMASI N, JATIVA C, SORRENTINO L. Isoquinoline alkaloids from Argemone mexicana reduce morphine withdrawal in guinea pig isolated ileum. Planta Med. 1997; 63: 326-328.
20. CAPASSO A, PIACENTE S, DE TOMMASI N, RASTRELLI L, PIZZA C. The effect of isoquinoline alkaloids on opiate withdrawal. Curr Med Chem. 2006; 13: 807-812.
21. VERMAA SK, DEVB G, TYAGIA AK, GOOMBERC S, JAIN GV. Case report: Argemone mexicana poisoning: autopsy findings of two cases. Forensic Sci Int. 2001; 115: 135-141.
22. GHOSH P, REDDY MMK, SASHIDHAR RB. Quantitative evaluation of sanguinarine as an index of argemone oil adulteration in edible mustard oil by high performance thin layer chromatography. Food Chem. 2005; 91: 757-764.
23. SHARMA BD, MALHOTRA S, BHATIA V, RATHEE M. Epidemic dropsy in India. Postgrad Med J. 1999; 75: 657-661.
24. http://toxnet.nlm.nih.gov/
25. WALTEROVA D, ULRICHOVA J, VALKA I, VICAR, J, VAVRECKOVA C, TABORSKA E, HARKRADER RJ, MEYER DL, CERNA H, SIMANEK V. Benzo[c]phenatridine alkaloids of sanguinarine and chelerythrine: biological activities and dental care applications. Acta Univ Palacki Olomuc Fac Med. 1995: 139; 7-16.
26. IBRAHIM HA, IBRAHIM H. Phytochemical screening and toxicity evaluation on the leaves of Argemone mexicana Linn (papaveraceae). Int Jor P App Scs. 2009; 3: 39-43.
27. RANIVAR P, CHATTERJEE VC. The toxicity of mexicana poppy (Argemone mexicana L.) seeds to rats. Vet Hum Toxicol. 1989; 31: 555-558.
28. KAUSAL KU, MUKUL D, ARVIND K, GIRIRAJ BS, SUBHASH KK. Biochemical toxicology of argemone oil. IV short-term and feeding response in rats. Toxicol. 1989; 58: 285-298.
29. El GAMAL AA. Phytochemistry, pharmacology and toxicology of Argemone mexicana L. PhD. Thesis, faculty of Pharmacy, university of Khartoum. 1995.
30. PRABHAKAR S, KHURANA D, GILL KD, CHOUDHARY S, LAL V, DAS CP. Neurologic complications of dropsy: from possibility to reality. Neurol India. 2000; 48: 144-148.
31. WAIZEL HAIAT S, WAIZEL BUCAY J. Algunas plantas utilizadas en México para el tratamiento del asma. An Orl Mex. 2009; 54: 145-171.
32. HUSAIN S, NARSIMHA R, RAO RN. Separation, identification and determination of sanguinarine in argemone and other adulterated edible oils by reversed-phase high-performance liquid chromatography. J Chromatogr A. 1999; 863: 123-126.

Argyreia nervosa
(Hawaiian baby woodrose)

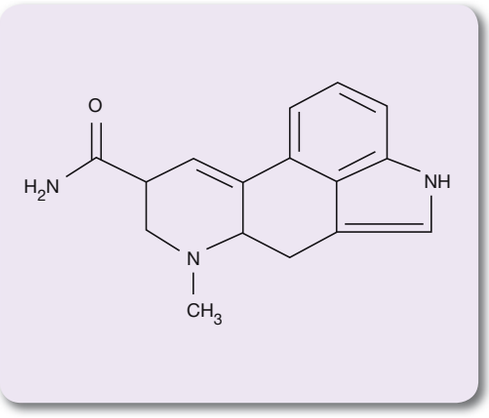
Name: *Argyreia nervosa, Argyreia speciosa*
Family: *Convolvulaceae*
Genus: *Argyreia Lour*
Species: *Argyreia nervosa* (Burm. F. Bojer)
Synonims: Hawaiian baby woodrose (HBWR), Elephant creeper, Woolly morning glory, Silver morning glory
Origin: Mountains of southern Mexico, Guatemala, Indian subcontinent, Subtropical America, Madagascar, Europa
Active compounds: ergine (lysergamide, lysergic acid amide), ergometrine, lysergic acid α-hydroxyethylamide, elymoclavine, chanoclavine

Ergine (Lysergamide, lysergic acid amide, LSA) and isoergine (ergine epimer with a lower pharmacological activity) are the principal psychoactive alkaloids (hallucinogens) contained in the seeds of the plant. Other alkaloids present are: ergometrine, lysergic acid α-hydroxyethylamide, elimoclavine and cianoclavine (Table 1). Ergine and isoergine are also present in the seeds of *Ipomea violacea* and *Rivea corymbosa*. The seeds of *Argyreia nervosa* contain from 0.5 to 0.9% ergolinic alkaloids, of which 0.14% (dry weight) is represented by ergine (or LSA) and 0.19% by isoergine [1,2]. Hylin and Watson, in a work published in 1965 in Science, noticed that the seeds of *Argyreia nervosa* contained 780 µg/g ergine and 555 µg/g isoergine per gram of wet weight [3]. A seed of *Argyreia nervosa* contains about 0.25 mg of LSA.
Although the active compounds are present in the seeds of the plant, the historical and traditional use refers to the entire plant. There are no reports on the identification of active principles in other plant parts.

Table 1. The principal alkaloids in the seeds of *Argyreia nervosa* [1]

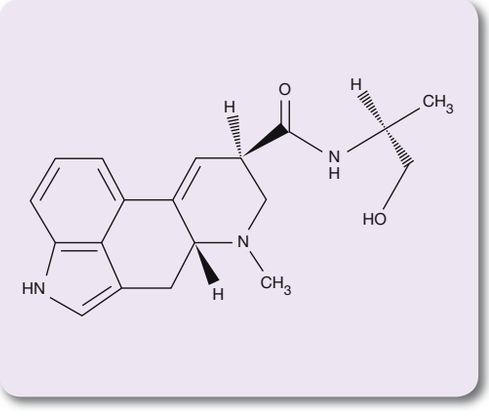
Alkaloids	Value espressed as % of total alkaloids	Value espressed as % of the seed dry weight
Chanoclavine-I	2.65	0.016
Elymoclavine	3.62	0.022
Ergine	22.68	0.136
Isoergine	31.36	0.188
Ergometrine	8.20	0.049
lysergic acid α-hydroxyethylamide	5.79	0.035
Minor unidentified alkaloids	18.82	0.113

Chemical formula and physico-chemical properties of the active compounds

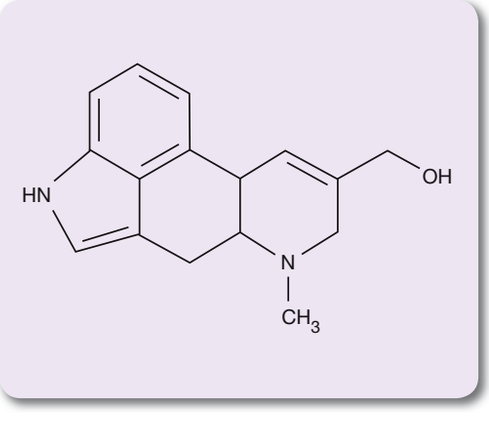


Name: Ergine (lysergamide, lysergic acid amide, LSA).
Molecular formula: $C_{16}H_{17}N_3O$ (molecular weight = 267.3).
Systematic name: 9,10,dihydro-6-methylergoline-8-β-carboxamide.
CAS registry number: 478-94-4.
Melting point: no data in the literature.
UVmax: no data in the literature.
Solubility: no data in the literature.

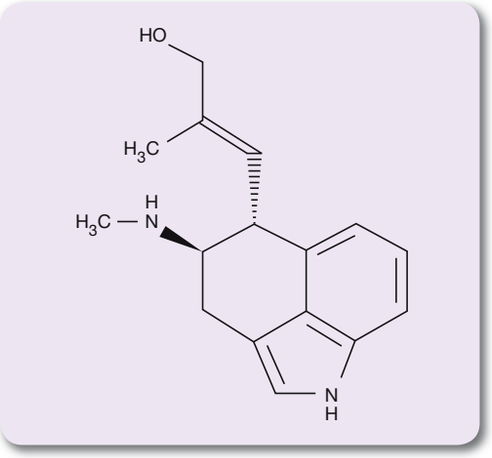
Name: Isoergine.
Molecular formula: $C_{16}H_{17}N_3O$ (molecular weight = 267,3). Since it is the epimer of ergine, it has the same molecular structure, but the spatial distribution is different.
Systematic name: 9,10,dihydro-6-methylergoline-8-α-carboxamide.
CAS registry number: 2889-26-1.
Melting point: no data in the literature.
UVmax: no data in the literature.
Solubility: no data in the literature.



Name: Ergometrine.
Molecular formula: $C_{19}H_{23}N_3O_2$ (molecular weight = 325.5).
Systematic name: 9,10,dihydro-N-(2-hydroxy-1-methylethyl)-6-methyl-8β-(S)-9-ergoline-8-carboxamide.
CAS registry number: 60-79-7.
Melting point: 162°C.
UVmax: no data in the literature.
Solubility: water.



Name: Elymoclavine.
Molecular formula: $C_{16}H_{18}N_2O$ (molecular weight = 254.3).
Systematic name: 8,9,dihydro-6-methylergoline-8-methyl alcohol.
CAS registry number: 548-43-6.
Melting point: no data in the literature.
UVmax: no data in the literature.
Solubility: no data in the literature.



Name: Chanoclavine.
Molecular formula: $C_{16}H_{20}N_2O$ (molecular weight = 256.3).
Systematic name: 2-Propen-1-ol, 2-methyl-3-(1,3,4,5-tetrahydro-4-(methylamino)benz(cd)indol-5-yl)-,(4R-(4α,5β(E).
CAS registry number: 2390-99-0.
Melting point: 221°C.
UVmax: no data in the literature.
Solubility: no data in the literature.

No data are present in the international literature concerning molecular formula, systematic name, CAS registry number, melting point, UVmax and solubility of lysergic acid α-hydroxyethylamide.

Historical use

Historically, the plant was prescribed in native medicine for the cure of gonorrhoea, urinary problems and for chronic ulcer. The roots are still used today by the Indians as a tonic, aphrodisiac, diuretic, anti-rheumatic and for the treatment of the nervous system ailments. The leaves have been used as rubefacient, vesicant and to stimulate circulation. The extracted oil of the seeds contains an unsaponifiable substance which, *in vitro* has shown antibacterial and antifungal activity.

Current use

The seeds of *Argyreia nervosa* (Hawaiian baby woodrose, HBWR), similar to *Ipomea violacea* and *Rivea corymbosa* seeds, are used for their ability to induce psychoactive effects like lysergic acid diethylammide (LSD), although of smaller intensity. The alkaloids of the plants in the *Argyreia* family are physiologically active nitrogenous bases. There is little scientific information regarding the necessary seeds amount for a "trip", but it seems that about four HBWR seeds are sufficient for a hallucinogenic effect, while a good "trip" is achieved using 5-10 seeds. The same effect is achieved swallowing 150-200 seeds of *Ipomea violacea*, whose content in LSA is 0.02% (dry weight) [4]. Commercially, the seeds of *Argyreia nervosa* are sold as "collectible seeds" although each pack contains just the necessary amount for an hallucinogenic " trip".

Legislation

In the USA, the ergine is a controlled substance (Schedule III drug in the Controlled Substances Act) as a depressant, and it is present in the list of U.S. Code of Federal Regulations as a possible forerunner of the LSD, but the plant and the seeds are freely sold. From April 2009, *Argyreia nervosa* has been declared illegal in Russia. In Italy, the lysergic acid amide (ergine) is included in Table I containing narcotic and psychotropic substances subject to the supervision and control under Article 14 of Republic Presidential Decree 309/90 and subsequent updates. With a 2007 Ministerial Decree (n. 27 of September 25th 2007, subsequently published in the Official Bulletin n. 237 of October 11th 2007) also the *Argyreia nervosa* seeds have been included In the Table I of article14 of 309/90 RPD.

Pharmaco-toxicological properties

The hallucinogenic activity of the ergine (LSA) becomes evident when a minimum of 2-5 mg is used [5]. There are few pharmacodynamic studies published on ergine. Similar to the ergot alkaloids (such as ergometrine), ergine seems to bind to the dopamine receptors D2, whose stimulation causes inhibition of adenylate cyclase and reduction of cyclic adenosine monophosphate (AMPc) formation [6]. The discovery of the ergot alkaloids in the seeds of *Rivea corymbosa*, *Ipomea violacea* and *Argyreia nervosa* in the early sixties has been rather unexpected and of particular interest from a phytochemical

point of view, since until than the lysergic acid alkaloids had been isolated only from the mushrooms of the *Claviceps*, *Penicillium* or *Rhizopus* family, rather than the Convolvulaceae [3,7-8]. LSA has psychotomimetic effects (mind and state of awareness alterations, perceptions, hallucinations) similar to those caused by LSD (lysergic acid diethylamide), although the LSD effects are 50 to 100 times more powerful than LSA. The effects of the LSA can last approximately 4-8 hours and they are associated with a sensation of tranquillity, dysphoria, psychedelic visual effects (vision of bright colours). Effects comparable with those of the *Argyreia nervosa* , similar to those caused by LSD use, are produced by the seeds of *Ipomea Violacea* (Tlitlitzin) and *Rivea corymbosa* (ololiuhqui).
Pharmacokinetic studies on ergine conducted in animals (beef) demonstrate that the pharmacokinetics in the serum after a single intravenous administration of 14 µg/Kg presents three different phases. The first phase (0-10 minutes), characterised by a steady state volume of distribution, is followed by a second phase (that starts immediately after the injection and lasts for about an hour) with similar ergine concentrations in serum and tissues. In the third, metabolism phase begins [9].
Elymoclavine and chanoclavine, although present in the seeds in lower percentage, seem to contribute to the hallucinogenic activity . The contribution of ergometrine (present in the seeds in trace amounts) to the pharmacotoxicological properties of the plant has not been sufficiently studied.

Data regarding the acute toxicity of ergine
In man - TDLo following oral administration: 14 µg/kg [1]
In rat and in rabbit – LDLo following intravenous administration: 2500 µg/kg

There is no data of acute toxicity with respect to other active compounds of the plant.

Adverse Effects

The greatest psychotic adverse effects are dissociative reactions and schizophrenic relapses that can occur following the ingestion of seeds [10].
There is a case report in the literature of toxic psychosis induced by the ingestion of *Argyreia nervosa* seeds characterised by hallucinations, orientation problems, anxiety and psychomotor agitation [11]. In another case, an 18-year-old boy has been admitted to emergency wards with psychotic manifestations following the ingestion of the plant seeds [12]. Another 18-year-old boy was admitted after ingestion of 12 *Argyreia nervosa* seeds, complaining vomiting, nausea, dizziness, auditory hallucinations, blurred vision and diaphoresis [13]. A month later, the patient complained of auditory hallucinations flashbacks every time he smoked cigarettes.
The above-reported clinical cases indicate that it is important to pay a strict attention to a differential diagnosis between the episodes of adolescent acute psychosis and those that can be caused by the ingestion of this or another hallucinogenic drug particularly in the young persons.

Pharmacological interactions

There are no well-known pharmacological interactions of the ingested *Argyreia nervosa* with other medications. However, it has been demonstrated that the metabolism of the LSD, an analogue of LSA present in the plant, is inhibited by medications used for the treatment of HIV [14]. This suggests the possibility that in patients treated with antiretroviral medication who use LSD or *Argyreia nervosa* there is an increased risk of toxicity induced by these hallucinogens.

Effects in pregnancy

The ingestion of the seeds of *Argyreia nervosa* during the pregnancy is risky. The ergine, similar to LSD from a structural point of view, is a potential inducer of uterine contractions [15,16]. Therefore, the drug can increase the risk of spontaneous abortions.

Analytical determinations

Scientific literature reports a series of analytical methodologies to measure active principles of *Argyreia nervosa* both in urine [17,18], in blood [18] and in plant seeds [19]. The method used for the analysis of seeds is quite dated and involved the use of thin layer chromatography (TLC) coupled to ultraviolet spectrophotometric detection.
The assay applied for the determination of lysergic acid amide in blood and urine samples from two intoxication cases used an ultra-performance liquid chromatograph (UPLC) coupled to a time of flight mass spectrometer [17].
Finally, the methodology developed for urine samples only is based on liquid chromatography coupled to tandem mass spectrometer [18].

The method below reported is a brief outline useful to the investigator to set up the analysis. For specific details, it is advisable to refer to the original text.

Determination of lysergic acid amide (LSA) in urine

(From: BJÖRNSTAD K, BECK O, HELANDER A. A multi-component LC-MS/MS method for detection of ten plant-derived psychoactive substances in urine. J Chromatogr B Analyt Technol Biomed Life Sci. 2009; 877: 1162-1168) [18].

The analysis is carried out on urine samples by liquid chromatography coupled to tandem mass spectrometry.

Extraction of the compounds

A 50 µl volume of urine sample is diluted with 150 µl distilled water containing 200 µg/L psylocine-D4 as internal standard. A 10 µl volume of this latter solution is injected into the chromatograph.

Analytical conditions

Chromatographic column: Hypersil GOLD (100 x 2.1mm x 5µm)
Mobile phase A: 10 mM formic acid- acetonitrile (99:1 v/v)
Mobile phase B: 10 mM formic acid- acetonitrile (40:60 v/v)
Separation: linear gradient (mobile phase B, from 0 to 100% in 10 minutes, then to 100 to 0% in 4 minutes for a total of 14 minutes)
Flow rate: 0.2 ml/min
Detector: mass spectrometer with positive mode electrospray (ESI) interface
Source temperature: 350°C
Nebulization gas pressure: 20 psi
Capillary voltage: 5000 V
Collision energy: 30 eV

Retention times of the tested compounds

Ergine (LSA): 5.86 minutes
Psylocine-D4 (internal standard): 5.39 minutes

Characteristic fragments for the tested compounds

Ergine (LSA): m/z 268 ⟶ 223, 208
Psylocine-D4 (internal standard): m/z 209 ⟶ 164

Standards

The LSA standard used in this assay has been gently donated by Dr. Fleger (Microbiology Institute, Science Academy, Prague). The internal standard, Psylocine-D4 can be purchased from THC PHARM (Frankfurt, Germania).

Calibration curve

The calibration curve in urine covered the LSA concentration range: 10-5000 µg/L.

Results

Analyzed urine samples showed LSA concentrations between 31 and 49 µg/L.

References

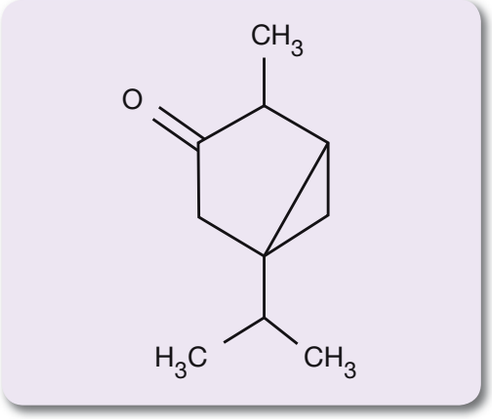
1. CHAO JM., DER MAERDEROSIAN AH. Ergoline alkaloidal constituents of Hawaiian Baby Wood Rose, *Argyreia nervosa* (Burm.f.) Bojer. J Pharm Sci. 1973; 62: 588-591.
2. SRIVASTAVA A, SHUKLA YN, JAIN SP, KUMAR S. Chemistry and pharmacology of the elephant creeper *Argyreia speciosa* - a review. J Med Arom Plant Sci. 1998; 20: 774-778.
3. HYLIN JW, WATSON DP. Ergoline alkaloids in tropical wood roses. Science. 1965; 148: 499-500.
4. HALPERN JH. Hallucinogens and dissociative agents naturally growing in United States. Pharmacol Ther. 2004; 102: 131-138
5. USDIN E, EFRON DH. Psychotropic drugs and related compounds. 2nd ed. Washington, DC, 1972: 72.
6. LARSON BT, HARMON DL, PIPER EL, GRIFFIS LM, BUSH LP. Alkaloid binding and of D2 dopamine receptors in cell culture. J Anim Sci. 1999; 77: 942-947.
7. TABER WA, HEACOCK RA, MAHON ME. Ergot-type alkaloids in vegetative tissue of *Rivea corymbosa* (L.) Hall.f. Phytochemistry. 1963; 2: 99-101.
8. TABER WA, HEACOCK RA. Location of ergot alkaloid and fungi in the seed of *Rivea corymbosa* (L.) Hall. f., "ololiuqui". Can J Microbiol. 1962; 8: 137-143.
9. MOUBARAK AS, PIPER EL, JHONSON ZB, FLIEGER M. HPLC method for detection of ergotamine, ergosine, and ergine after intravenous injection of a single dose. J Agric Food Chem. 1996; 44: 146-148.
10. MILLER MD. Isolation and identification of lysergic acid amide and isolysergic acid amide as the principal ergoline alkaloids in *Argyreia nervosa*, a tropical Wood rose. J AOAC. 1970; 53: 123-127.
11. GOPEL C, MARAS A, SCHMIDT MH. [Hawaiian baby rose wood: case report of an argyreia nervosa induced toxic psychosis] Psychiatr Prax. 2003; 30: 223-224.
12. GERTSCH JH, WOOD C. Case report: an ingestion of Hawaiian Baby Woodrose seeds associated with acute psychosis. Hawaii Med J. 2003; 62: 127-129.
13. AL-ASSMAR SE. The seeds of the Hawaiian baby woodrose are a powerful hallucinogen. Arch Intern Med. 1999; 159: 2090.
14. ANTONIOU T, TSENG AL, VAN HEESWIJK RP, WALKER SE, GIGUERE P, PHILLIPS EJ. Steady-state pharmacokinetics and tolerability of indinavir-lopinavir/ricombination therapy in antiretroviral-experienced patients. Ther Drug Monit. 2005; 27: 779-781.
15. MCGLOTHLIN WH, SPARKERS RS, ARNOLD DO. Effect of LSD on human pregnancy. JAMA. 1970; 212: 1483-1487.
16. JACOBSEN CB, BERLIN CM. Possible reproductive detriment in LSD users. JAMA. 1972; 222: 1367-1373.
17. KLINKE HB, MÜLLER IB, STEFFENRUD S, DAHL-SØRENSEN R. Two cases of lysergamide intoxication by ingestion of seeds from Hawaiian Baby Woodrose. Forensic Sci Int. 2009 (in press).
18. BJÖRNSTAD K, BECK O, HELANDER A. A multi-component LC-MS/MS method for detection of ten plant-derived psychoactive substances in urine. J Chromatogr B Analyt Technol Biomed Life Sci. 2009; 877: 1162-8.
19. KIM W, CRAWFORD MS. The Identification of Lysergic Acid Amide in Baby Hawaiian Woodrose By Mass Spectrometry. J Forensic Sci. 1970; 15: 588-594.

Artemisia absinthium
(Wormwood, Absinthe)

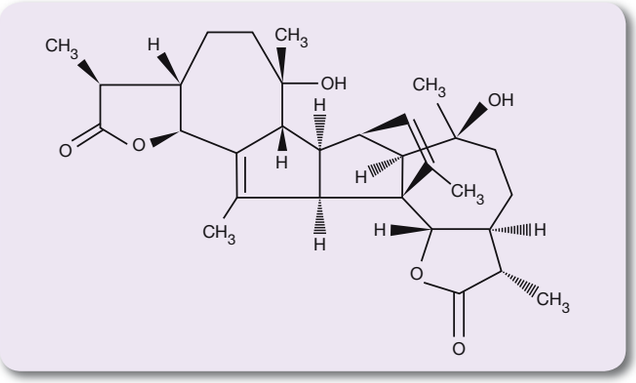
Name: *Artemisia absinthium*
Family: *Compositae*
Genus: *Artemisia* L.
Specie: *Artemisia absinthium* L.
Synonims: absinthe wormwood, grand wormwood
Origin: Europe (grows spontaneously, particularly in Italy)
Active compounds: α- and β-thujone, absynthin, anabsynthin, artabsine, anabsine and anabsinin. Thujone is an active ingredient found in *Salvia Officinalis* as well.

The active compounds mentioned above can be found in the leaves, in the stalks and in the flowering tops of the plant. α- and il β-thujone, monoterpenes, are molecules present both in the essential oils and in certain parts of *Artemisia absinthium*, *Salvia Sclarea*, *Tanacetum Vulgaris* and in several kinds of juniper and cedar. The ratio between the α- and β-thujone varies depending on the plant from which they are extracted. In *Artemisia absinthium* the concentrations of α- and β-thujone are equal to 0.53 – 1.22%, and 17.5-42.3%, respectively [(1)]. Based on data from the XIX century, it seems that the liqueur absinthe was prepared from *Artemisia absinthium* and it contained about 260 ppm of thujone (260 mg/l). Today, some authors believe that the absinthe, as it was prepared in the XIX century, contained a high concentration of thujone [(2)]. However, recent data did not confirm this hypothesis, highlighting that in older drinks (dated 1930), prepared according to the original recipe, the thujone concentration was extremely low (~1,8 mg/l) [(3)].
The (+)-absynthin, has been isolated in 1953 when it has been also classified as the principal dimeric guaianolide extracted from *Artemisia absinthium* L. The complete molecular structure of this triterpene has been completed only in 1980, using techniques such as nuclear magnetic resonance and X– ray crystallography [(4)]. The absynthin is responsible for the extremely bitter taste of the plant.

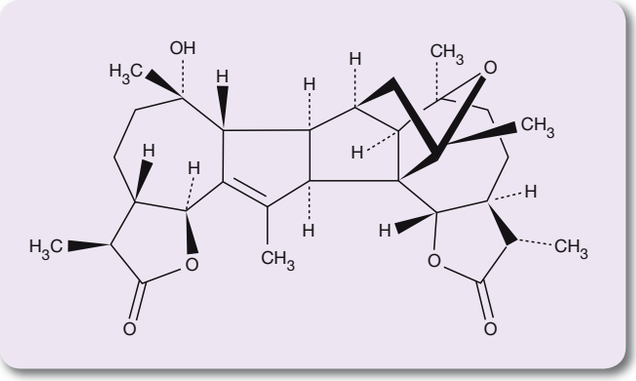
Chemical formula and physico-chemical properties of the active compounds



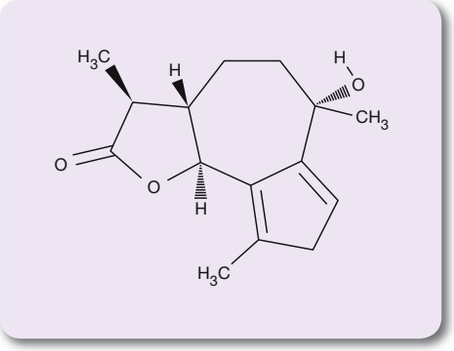
Name: Thujone (absinthol).
Molecular formula: $C_{10}H_{16}O$ (molecular weight = 152.2).
Systematic name: 4-methyl-1-(1-methylethyl)bicyclo[3.1.0]-esan-3-one;3-thujanone.
CAS registry number: 546-80-5.
Melting point: data in the literature.
UVmax: in isooctane, 300nm.
Solubility: practically insoluble in water, soluble in alcohol and other organic solvents [(5)].



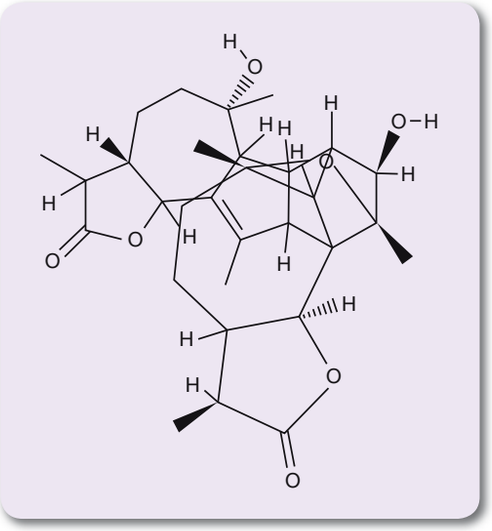
Name: Absynthin.
Molecular formula: $C_{30}H_{40}O_6$ (molecular weight = 496.6).
Systematic name: (1R,2R,5S,8S,9S,12S,13R,14S,15S,16R,17S,20S,21S,24S)-12,17-dihydroxy-3,8,12,17,21,25-hexamethyl-6,23-dioxaheptacyclo[13.9.2.0(1,16).0(2,14).0(4,13).0(5,9).0(20,24)]hexacosa-3,25-diene-7,22-dione.
CAS registry number: 1362-42-1.
Melting point: 179-180°C.
UVmax: no data in the literature.
Solubility: no data in the literature.



Name: Anabsynthin.
Molecular formula: $C_{30}H_{40}O_6$ (molecular weight = 496.6).
Systematic name: 3,3a,4,5,6,6a,6b,7,7a,8,9,10,10a,13a,13c,14b- hexadecahydro-3,6,8,11,14,15- hexamethyl- 2H-8,15-epoxy- 13b ethanepentalen(1",2":6,7;5",4":6',7')dicycloepta(1,2-b:1',2'-b')difuran-2,12(11H)-dione.
CAS registry number: 6903-12-4.
Melting point: 267°C (anhydrous), 210°C (monohydrate).
UVmax: no data in the literature.
Solubility: no data in the literature.



Name: Artabsin.
Molecular formula: $C_{15}H_{20}O_3$ (molecular weight = 248.3).
Systematic name: (3S,3aS,6S,9bS)-6-hydroxy-3,6,9-trimethyl-3a,4,5,6,8,9b-hexahydroazuleno[4,5-b]furan-2(3H)-one.
CAS registry number: 24399-20-0.
Melting point: no data in the literature.
UVmax: no data in the literature.
Solubility: no data in the literature.



Name: Anabsine.
Molecular formula: $C_{30}H_{40}O_7$ (molecular weight = 512.6).
Systematic name: no data in the literature.
CAS registry number: 72542-39-3.
Melting point: 276°C.
UVmax: no data in the literature.
Solubility: it dissolves in warm alkaline solutions.

No data are present in the international literature concerning molecular formula, systematic name, CAS registry number, melting point, UVmax and solubility of anabsinin.

Historical use

The therapeutic properties of *Artemisia absinthium* were known and used from the antiquity: in fact, the plant it is cited in an Egyptian papyrus of 1600 B.C.

In 150 B.C. Plinio and Plutarco, reported that the wormwood was used in the fields for its insecticide quality.

Above all, the leaves and the flowers of *Artemisia absinthium* are known foremost as ingredients used for the preparation of a particular liqueur known as absinthe (wormwood) [6]. Historically, the inventor of the liqueur was a French doctor, Pierre Ordinaire, who in 1792, after having escaped the French Revolution, settled in Couvet, Switzerland. Like many country doctors, he was preparing himself the remedies against the most common sicknesses using medicinal plants. In Switzerland he found the grand wormwood (*Artemisia absinthium*) and knowing the use of this plant since ancient times, tried to use it himself. Doctor Ordinaire distilled a strong liqueur (about 60° alcohol volume) which contained in addition to wormwood also anise, hyssop, dictamus, calamus, lemon-balm (a type of mint) and various quantities of other common plants. His absinthe became extremely famous as a miracle cure in Couvet and was named *Fée Verte*, the Green Fairy. It is believed that Ordinaire left his secret recipe to the Henriod sisters in Couvet, but it is also possible that the sisters were producing absinthe already much before Pierre Ordinaire. In the XIX century many distilleries appeared in France and in Switzerland producing different types of absinthe which became well-known in France, popular with the writers and Parisian artists at the end of XIX century and at the beginning of the XX [7].

Absinthe was the inspiration for the *bohemiènne* lifestyle and it was the favorite drink of famous artists, such as Van Gogh and Toulouse Lautrec. The liqueur was not usually drunk “in one gulp”, but used following a quite elaborate ritual in which a specific teaspoon with a sugar cube was put over the glass, and ice water was poured over it until it reached a volume five times that of the liqueur. The accessories used were extremely odd: the glasses and the teaspoons with the strange shapes were giving to the drinking ritual a fascinating and mysterious atmosphere.

The success of the absinthe in Europe was very quick, but its decline was also swift: it disappeared in all the markets of Europe and abroad after not more than a decade. The reasons for this were threefold: first of all the strong movement that was fighting against alcoholism in whole Europe in the early 1900s; then the scientific studies that singled out the thujone as a neurotoxin able to cause convulsions and death in the laboratory animals; and at last the pressure exercised by the French wine producers that were afraid of the growing popularity of the absinthe.

Current use

The liqueur is known in Europe under different names: in France, its natural country, it is known by the name *absinthe*, in England as *wormwood*, in Germany under the *wermuth* name, in Italy as *assenzio*. The vermut, produced in Piedmont owes its name to the wormwood (the German *wermuth*) that is used in its preparation, and that confers to the wine a particular aroma and a special bitter taste.

The absinthe usually has a pale green color (hence the name "Green fairy") and has a taste similar to a liqueur based on anise, but with a harsher aroma due to the many plants which are used in the preparation, and a lightly bitter aftertaste. The alcoholic content of the drink is extremely high (between 45 and 90%).

"Green fairy", the liqueur tied to alchemy, whose fame has been fed by the passion of a whole generation of artists, is stirring up again the interest of a new generation of consumers, attracted above all by the myth tied to the use of the liqueur by famous and popular artists like Van Gogh, Toulouse Lautrec, Hemingway, Oscar Wilde and Picasso.

Legislation

At European level, the Annex II of the Directive 88/388/EEC [9] limited the maximum allowed thujone level (α and β) at 0,5 mg/Kg in food and beverages in which aromatizing agents have been used. There are exceptions, when higher levels of thujone are allowed: 1) the alcoholic beverages with no more than 25% in alcohol volume (5 mg/Kg); 2) the alcoholic beverages with more than 25% of alcohol in volume (10 mg/Kg); food preparations based on sage (25 mg/Kg), beers (35 mg/Kg). Thujone cannot be added as such to food products.

France and the Great Britain have limited the daily maximum quantity of thujone that can be consumed by an individual. In France, it is 15.6-44.3 µg/kg body weight/day. In Great Britain, the levels are lower: 3.9-14.2 µg/kg body weight/day. These limits are in line with the maximum amounts proposed by the European Council in the year 2000. Most of the thujone found in comestibles is derived from the plant *Salvia officinalis* both, in food products as well as in alcoholic beverages.

In France, the Decree 88-1.024 of November 2nd, 1988 has confirmed the Law of March 16th 1915 restoring the prohibition of the sale of wormwood and of similar liqueurs and listing the chemicals whose presence puts any liqueur into the same category of prohibition as the wormwood.

In the United States of America the wormwood as traditional drink is forbidden because of its thujone content. The thujone as additive to food is forbidden in agreement with the section 801st of Federal Food, Drug, and Cosmetic Act (August 1972) [11].

In Italy, the monarchy forbade the wormwood after a referendum in 1931, but the legislation of January 25th 1992 No 107[8], in spite of a never abrogated law, seems to allow the sale of the wormwood in Italy (in *e-commerce*), similar to the free circulation of goods in the European Union. The Italian Ministry of Health included the oil of'*Artemisia absinthium L.* to the list of the vegetable extracts not admitted in dietary supplements [10].

Pharmaco-toxicological properties

The toxicity of the wormwood is attributable to the monoterpene thujone and its metabolites. The wormwood contains essential oils and sesquiterpenelactones such as absynthin, anabsynthin, artabsine, anabsine and anabsinin to which the pharmacological properties of the plant can be ascribed.

Two neutral urinary metabolites have been identified: 3-α-hydroxy-β-thujone and 3-β-hydroxy-β-thujone. These metabolites indicate that the reduction reaction is stereospecific, depending on the configuration of the two methyl groups. In addition, experiments performed on mouse microsomal preparations have shown how that the metabolism of α-thujone generates one major metabolite, 7-hydroxy-α-thujone and five secondary metabolites, 7-hydroxy-α-thujone and five secondary metabolites (4-hydroxy-α-thujone, 4-hydroxy-β-thujone, 8-hydroxy-α-thujone, 10-hydroxy-α-thujone and 7,8-dihydro-α-thujone) [12].

The stereospecific reactions and the species-specific differences in the metabolism of the thujone diastereoisomers have been studied *in vitro* in the hepatic microsomes of rats, mice and man, while *in vivo* only in rats and mice. The 2-hydroxylation has been studied only in mice, where the conjugated metabolite represents the major urinary product. The

4-hydroxylation of the α- and β-thujone represents another metabolic route with the 4-hydroxy-thujone being the major urinary metabolite in the rat. The 7-hydroxylation generates a secondary conjugated urinary metabolite in the mouse. The position-specific glucuronidation favors the conjugation of (2R)-9-hydroxy and 4-hydroxy-thujone glucuronide compared to other hydroxythujones [1].

In the popular medicine the wormwood has been used for the cure of dyspeptic symptoms, moreover as eupeptic (substance of intense bitter taste that favors the digestion) and carminative (substance that favors the elimination of gas from the gastrointestinal tract) [13].

The wormwood possesses also antimicrobial properties. In a recent study, the ability of the essential oil to inhibit the growth of *Candida albicans* and *Saccharomyces cerevisiae* has been observed [14].

In addition, the wormwood exercises a protective effect towards toxic insults to the liver that seems to be partially associated with the inhibition of the hepatic microsomal enzymes. A study in rats has highlighted that the plant extract given as an oral dose of 500 mg/kg is able to exert a preventive and prophylactic action towards the hepatic damage induced by paracetamol and carbon tetrachloride (CCl_4), two widely used experimental models for the study of hepatotoxicity[15].

Other biological effects of thujone stem from studies made on cultures of embryonic cells of chicken liver. These studies show that the thujone is porphyrogenic (it causes a copro- and protoporphyrine accumulation) and can be the cause of acute porphyria. The accumulation of porphyrin induced by terpene is further boosted by concomitant administration of desferoxamine, a chelating agent for iron, which inhibits the biosynthesis of heme and mimics the block that occurs in cases of acute porphyria. From these studies is therefore clear that the administration of thujone can be dangerous in patients with a deficit in the heme biosynthesis at the hepatic level [16].

Knowing that *in vitro* wormwood extracts exert suppressive action on tumor necrosis factor (TNF-α) in a recent study, the possible effects of wormwood extracts on the course of Crohn's disease were investigated. The selected patients were treated for six weeks with wormwood powder, in addition to their usual therapy. In comparison with the control group, in the treated subjects, a decrease in serum levels of TNF-α, an improved mood and the remission of symptoms were noted [17].

Toxicity

In accordance with European legislation (88/388/EEC 1988), the highest concentration of thujone in alcoholic beverages can not be greater than 5 mg/kg and in any case the percentage should not exceed 25% of the alcohol volume of and 35 mg/kg in the bitters.

The component responsible for the psychoactive and toxic effects of the wormwood is α-thujone, in spite of being present in smaller proportion compared to β-thujone.

The neurotoxicity of α-thujone has been associated with its ability of blocking the γ-amino butyric acid receptors at cerebral level (GABA). Particularly a study led by Hold et al., [12] has demonstrated that thujone acts as an antagonist of the $GABA_A$ receptors. α-thujone is about 2-3 times more active and subsequently more toxic than β-thujone. Less potent than α-thujone, the metabolites 7-hydroxy-α-thujone and dihydro-α-thujone show also these neurotoxic effects. The reduction of the gabaergic activity of the α-thujone in the wormwood seems to favor the start of anomalous neuronal electric discharges responsible for the clinical manifestations of epileptic seizures [12,18]. It has been hypothesised that the proconvulsive activity of α-thujone can be correlated with a reduction in the response to the 5-HT3 receptor for serotonin[19].

The thujone, besides having a structural similarity to delta-9-tetrahydrocannabinol, the psychotropic component of cannabis, possesses a slight affinity for the cannabinoid receptors without inducing cannabis-mimetic effects [18].

The appearance of toxic effects is concentration dependent. Extracts of wormwood given for 13 weeks to rats in the drinking water at a concentration less than 2% (equivalent to 1.27 g/kg/day in males and 2.06 g/kg/day in females) did not produce toxic effects [19].

The European Union in 2002 has drafted a document regarding the safety of alcoholic drinks or foods containing thujone based aromatizing agents. In this context, it has been established that the consumption of 1 liter of an alcoholic drink (25% of alcohol) containing 5 mg/L thujone it is equivalent in a 60 Kg adult with a dose of thujone of 4.8 mg (0.08 mg/kg). These values are 100 times less than those established in the NOEL studies (No Obseved Effect Level) in rats (NOEL for the convulsions in male rats: 12 mg/Kg) [1].

Data regarding the acute toxicity of thujone

In rabbit - LD 50 following intravenous administration: 0.031 mg/Kg.

In rat - LD50 following oral administration: 500 mg/Kg.

Adverse Effects

The symptoms associated with acute poisoning are represented by convulsions (cortical neuronal discharges), hypotension due to generalized vasodilatation, decrease of the cardiac rhythm and respiratory difficulties.

In the past (XIX and in XX century), the chronic abuse of absinthe (the liqueur based on wormwood) has been the principal cause of a syndrome defined as "absinthism", characterized by an initial sensation of wellbeing which was followed by hallucinations and depression. The prolonged wormwood use, besides causing convulsions, could also cause blindness, hallucinations and mental deterioration. A typical case is that of Vincent van Gogh, who in the last years of his life experienced hallucinations, which have been attributed to the psychosis he was suffering of. In fact it has been verified that the artist was a strong absinthe drinker and had probably developed the syndrome of the absinthism, presumably responsible for the anomalous behavior [20].

Recent studies indicate that the actual thujone contained in the liqueur prepared according to the original prescription is not sufficient to cause the toxic effects that are shown as a consequence of chronic use. The authors of this finding suggest that the "undesirable effects" observed might be in fact caused by the chronic alcohol abuse contained in the liqueur and the mixture of some toxic plants (*Acorus calamus*, *Tanacetum vulgare*) that were used as adulterants of the liqueur, or other adulterants such as zinc or antimony chloride [3].

Although the results of these studies seem to suggest that the thujone content in the wormwood liqueur is rather low, it is worthwhile mentioning that there are still clinical cases in which adverse effects (epileptic attacks) are described in individuals who have used essential oils containing thujone [1].

A clinical case of a patient hospitalized for convulsive episodes associated with rabdomiolysis, renal insufficiency and congestive cardiac failure following erroneous use of 10 ml of essential wormwood oil is described. The symptomatology disappeared with a normalization of the laboratory test results after a period of 17 days in bed [21].

Pharmacological interactions

In the laboratory animals, the wormwood extracts are able to exert an inhibitory effect on the hepatic microsomal enzymes and they can prolong the sleep induced by the pentobarbital and increase the toxicity of the strychnine [14].

Since the thujone contained in the wormwood can reduce the clinical efficacy of phenobarbital, although the mechanism of this interaction is still unclear, the concomitant use with such drugs should be avoided [22].

Effects in pregnancy

The American Herbal Products Association has assigned the wormwood to the class 2b (not to be used in pregnancy), 2c (not to be used during breastfeeding) and 2d (not to be used for long periods and not exceeding the advisable dose) [23].

Analytical determinations

No methodologies have been reported in the international literature concerning the analysis of *Artemisia Absinthium* active principles in biological fluids of eventual consumers. Neither, assays for the identification of the active principles in plant portions have been published. A gas-chromatography-mass spectrometry method for thujone determination in beverages containg *Artemisia absinthium* developed in the "Drug abuse and Doping" Unit laboratories of Italian National Institute of Health is here reported.

Determination of thujone in beverages based on *Artemisia absinthium*

(methodology developed in the Laboratories of the "Drug abuse and Doping Unit" laboratories of Italian National Institute of Health).

The analysis is carried out on beverages of *Artemisia absinthium* using gas chromatography coupled with mass spectrometry.

Extraction of the compounds

50 µl of 1N sodium hydroxide pH 9 is added to 1 ml of sample. After vortexing for 1 minute, a liquid - liquid extraction with 2 ml of diethyl ether is performed and the mixture is centrifuged at 3000 rpm for 15 minutes. The organic phase is transferred to a clean tube. The ethyl ether extraction is repeated 2 more times and the pooled organic phase is evaporated under nitrogen. The dry residue is reconstituted with 200 µl of hexane and 2 µl are injected into a gas chromatograph.

Analytical conditions

Chromatographic column: 5MS (0.25 mm x 30 m x 0.25 µm)
Injector temperature: 250°C
Carrier gas: Helium at 11.60 psi
Injection mode: splitless
Temperature program: 60°C for one minute, 60°C-240°C at 4 C°/min, 240°C for five minutes
Detector: mass spectrometer with electron impact interface.

Retention times of the tested compounds

Thujone: 5.8 minutes

Characteristic fragments for the tested compounds

Thujone: m/z 110, 68, 55

Standard

The thujone standard used for the analysis was purchased from Sigma (St. Luis, MI, USA).

Calibration curve

Stock solution of the analyte (1 mg/ml) was prepared in methyl alcohol. The working standards (concentration range: 1-100 µg/ml) were prepared by serial dilution of the stock standard and were kept at -20°C until the day of the analysis. The calibration standards (concentration range: 50 - 1000 µg/ml for thujone) were prepared daily adding the methyl alcoholic solutions to previously tested drug-free samples.
The quality control samples were prepared in a similar fashion. These samples were included into each analytical batch to check the calibration, precision, accuracy and previously stored sample stability.

Results

The analysis of several samples with the above-mentioned methodology has highlighted a variable concentration of the active ingredient in the distilled product, from a minimum of 0.014 mg/lto a maximum of 28.5 ± 1.6 mg/l.

References

1. EUROPEAN COMMISSION- Health & Consumer Protection Directorate general- 2003. Opinion of the Scientific Committee on food on Thujone. Document SCF/CS/FLAV/FLAVOUR/23 ADD2 Final.
2. STRANG J, ARNOLD WN, PETERS T. Absinthe: what's your poison? BMJ. 1999; 319: 1590-1592.
3. LACHENMEIER DW, EMMERT J, KUBALLA T, SARTOR G. Thujone – cause of absinthism? Forensic Sci Int. 2006; 158: 1-8.
4. ZHANG W, LUO S, FANG F, CHEN Q, HU H, JIA X, ZHAI H. Total synthesis of absinthin. J. Am. Chem. Soc. 2005; 127: 18-19.
5. THE MERCK INDEX An Enciclopedia of chemicals, drugs, and biologicals. 10th Ed. Merck & Co., Inc. 1983: 1346.
6. http://www.galenotech.org/assenzio.htm
7. http://www.lafeeabsinthe.com/it/faq.php
8. GAZZETTA UFFICIALE DELLA REPUBBLICA ITALIANA - Decreto Legislativo 25/0171992, N° 107 "Attuazione delle direttive 88/388/CEE e 91/71/CEE relative agli aromi destinati ad essere impiegati nei prodotti alimentari ed ai materiali di base per la loro preparazione" - G.U. n° 39 del 17.2.92.
9. EEC, 1988. Council Directive 88/388/EEC of 21 June 1988 on the approximation of the laws of the Member States relating to flavouring for use in foodstuffs and to source materials for their production. Official Journal of the European Communities, 15.07.1988, L184/61-67.
10. The list of vegetal extracts not admitted in dietary supplements in Italy can be found at http://www.salute.gov.it
11. http://erowid.org/chemicals/absinthe/absinthe_law.shtml
12. HÖLD KM, SIRISOMA NS, IKEDA T, NARAHASCHI T, CASIDA JE. A-thujone (the active component of absinthe): γ-aminobutyric acid type A receptor modulation and metabolic detoxification. Proc Natl Acad Sci. 2000; 97: 3826-3831.
13. BLUMENTHAL M, BUSSE WR, GOLDBERG A. (eds). The Complete German Commission E Monographs, 1st ed. American Botanical Council, Austin, TX; 1998.
14. JUTEAU F, JERKOVIC I, MASOTTI V, MILOS M, MASTELIC J, BESSIERE JM, VIANO J. Composition and antimicrobial activity of the essential oil of Artemisia absinthium from Croatia and France. Planta Med. 2003; 69: 158-161.
15. GILANI AH, JANBAZ KH. Preventive and curative effects of Artemisia absinthium on acetaminophen and CC_{l4}-induced hepatotoxicity. Gen Pharmacol. 1995; 26: 309-315.
16. BONKOVSKY HL, CABLE EE, CABLE JW, DONOHUE SE, WHITE EC, GREENE YJ, LAMBRECHT RW, SRIVASTAVA KK, ARNOLD WN. Porphyrogenic properties of the terpenes camphor, pinene, and thujone (with a note on historic implications for absinthe and the illness of Vincent van Gogh). Biochem Pharmacol. 1992; 43: 2359-2368.
17. KREBS S, OMER TN, OMER B. Wormwood (Artemisia absinthium) suppresses tumour necrosis factor alpha and accelerates healing in patients with Crohn's disease -A controlled clinical trial. Phytomedicine. 2009 in press, Epub ahead of print
18. RIETJENS I, MARTENA MJ, BOERSMA MG, SPIEGELENBERG W, ALINK GM. Molecular mechanisms of toxicity of important food-borne phytotoxins. Mol Nutr Food Res. 2005; 49: 131-158.
19. DEIML T, HASENEDER R, ZIEGLGANSBERGER W, RAMMES G, EISENSAMER B, RUPPRECHT R, HAPFELMEIER G. Alpha-thujone reduces 5-HT_3 receptor activity by an effect on the agonist-reduced desensitization. Neuropharmacology. 2004; 46: 192-201.
20. MESCHLER JP, HOWLETT AC. Thujone exhibits low affinity for cannabinoid receptors but fails to evoke cannabimimetic responses. Pharmacol Biochem Behav. 1999; 62: 473-480.
21. MUTO T, WATANABE T, OKAMURA M, MOTO M, KASHIDA Y, MITSUMORI K. Thirteen-week repeated dose toxicity study of wormwood (Artemisia absinthium) extract in rats. J Toxicol Sci. 2003; 28: 471-478.
22. ARNOLD WN. Vincent van Gogh and the thujone connection. JAMA. 1988; 260: 3042-3044.
23. WEISBORD SD, SOULE J, KIMMEL PL. Brief Report: Poison on line - acute renal failure caused by oil of wormwood purchased through the internet. N Engl J Med. 1997; 337: 825-827.
24. TYAGI A, DELANTY N. Herbal remedies, dietary supplements, and seizures. Epilepsia. 2003; 44: 228-235.
25. MCGUFFIN M, HOBBS C, UPTON R. American Herbal Products Association's Botanical Safety Handbook, CRC Press, Boca Raton, FL; 1997.

Ayahuasca
(Banisteriopsis caapi and Psychotria viridis)

Banisteriopsis caapi

Name: *Banisteriopsis caapi* (Spr. Ex Briesb)
Family: *Malpighiaceae*
Genus: *Banisteriopsis L*
Species: *Banisteriopsis caapi* (Spr. Ex Griesb)
Synonims: Daime, Yajé, Natema
Origin: tropical regions of South America

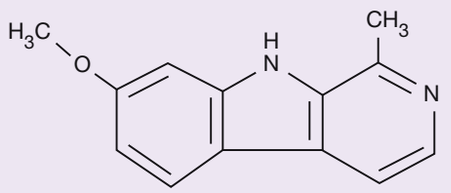
Psychotria viridis

Name: *Psychotria viridis*
Family: *Rubiaceae*
Genus: *Psychotria*
Species: *Psychotria viridis*
Synonims: chacruna
Origin: rain forests of South America

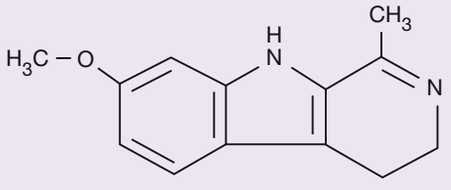
Active compounds: N,N-dimethyltryptamine, (*Psychotria viridis*); harmine, tetrahydroharmine, harmaline (*Banisteriopsis caapii*).

With the term "Ayahuasca" the native populations of the basin of the Amazon river used to indicate a drink with magic and curative powers, which was produced mixing different plants: classically the decoction is prepared with *Banisteriopsis caapi* and *Psychotria viridis*. The two plants are distinguishable by the content of active compounds; while the *Psychotria viridis* contains N,N-dimethyltryptamine (DMT), the *Banisteriopsis caapi* contains harmala alkaloids (*Peganum harmala* or Syrian Rue): harmine, tetrahydroharmine, harmaline. For the decoction preparation, the natives used the pulverized vines of *Banisteriopsis caapi* mixed with the *Psychotria viridis* leaves. The phytochemical analysis results state the average content of active compounds as: 1) *Psychotria viridis*: DMT: 7.50 mg/g dry weight of the plant; 2) *Banisteriopsis caapi*: harmine, 4.83 mg/g dry weight of the plant; harmaline 0.46 mg/g dry weight of the plant; tetrahydroharmine 1.00 mg/g dry weight of the plant [(1)]. Conversely, the analysis of 29 decoctions prepared according to traditional native prescriptions, produced the following average concentrations of active compounds: DMT, 2.09 ± 3.43 mg/ml; harmine, 4.95 ± 5.91 mg/ml; harmaline 0.23 ± 0.27 mg/ml; tetrahydroharmine 4.71 ± 6.01 mg/ml. The average tetrahydroharmine/harmine ratio in these preparations is close to unity, while in the pure extracts of the plants it is 1:5. It is not clear if such a discrepancy is to be attributed to the reduction of harmine and of harmaline to tetrahydroharmine during the preparation in an acid environment, or if simply, the tetrahydroarmine is more stable to the heat compared to the two other alkaloids [(2)]. DMT is a molecule with hallucinogenic properties, easily and quickly inactivated by the endogenous monoaminooxidases (MAO); the harmala alkaloids, such as harmine, tetrahydroarmine and harmaline, are inhibitors of MAO. For this reason, only the mixture of the two plants in the Ayahuasca will display the hallucinogenic effects.

Chemical formula and physico-chemical properties of the active compounds

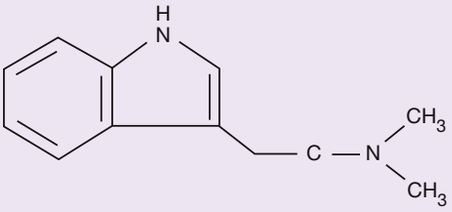


Name: Harmine.
Molecular formula: $C_{13}H_{12}N_2O$ (molecular weight = 212.2).
Systematic name: 7-methoxy-1-methyl-9H-pyrido(3,4-b)indole.
CAS registry number: 442-51-3.
Melting point: 273°C.
UVmax: 241, 301, 336 nm.
Solubility: hot water.



Name: Harmaline.
Molecular formula: $C_{13}H_{14}N_2O$ (molecular weight = 214.2).
Systematic name: 4,9-dihydro-7-methoxy-1-methyl-3H-pyrido(3,4-b)indole
CAS registry number: 304-21-2.
Melting point: 229-231°C.
UVmax: 218, 260, 376 nm.
Solubility: Mildly soluble in water, ethyl alcohol, ether. Soluble in hot ethyl alcohol.

Name: Tetrahydroharmine.
Molecular formula: $C_{13}H_{16}N_2O$ (molecular weight = 216.2)
Systematic name: 1,2,3,4-tetrahydro-7-methoxy-9H-pyrido(3,4-b) indole-1-methyl hydrochloride.
CAS registry number: 17019-01-1.
Melting point: 232-243°C.
UVmax: no data in the literature.
Solubility: no data in the literature.



Nome: N,N-Dimethyltryptamine (DMT).
Molecular formula: $C_{12}H_{16}N_2$ (molecular weight = 188.2).
Systematic name: N-dimethyl-1H-indole-3-ethylamine.
CAS registry number: 61-50-7.
Melting point: 44,6-46,8°C.
UVmax: 279-288 nm.
Solubility: acetic acid.

Historical use

The native people of Amazon river basin used long time ago the decoction produced from the mixture of the pulverized vines of *Banisteriopsis caapi* and the leaves of *Psychotria viridis* for religious ceremonies and for magic-therapeutic reasons. The oldest known object tied to the ceremonial use of the *Ayahuasca* is a cup extracted from a stone carved and decorated with engravings, found in the Ecuadorian forests and tied to the Pastaza culture (500 b.C.-50 a.C). This demonstrates that the *Ayahuasca* has been known and used for at least 2500 years.

Current use

The religious syncretism that has led to the development of movements such as Santo Daime, União do Vegetal and Barquinia, is based on the use of the *Ayahuasca* in the religious ceremonies to induce hallucinations in the master of ceremonies and in the participants.

Some of these movements have followers also in Europe, and because of the use of the *Ayahuasca* in religious ceremonies there were quite a few incidents with legal implications.

In the 90's, in North America and in Europe the preparation and use of the so called "analogues of *Ayahuasca*"or "*anahuasca*" became widespread. These were psychoactive drinks obtained from plants sometimes different from those used in the traditional Ayahuasca, but containing the same active compounds as the original plants. One of the plants most used in Europe and in Italy for the preparation of analogues of the *Ayahuasca* it is the *Peganum harmala* or Syrian Rue, specifically its seeds are used [3].

Legislation

In Europe different judicial measures have been implemented regarding the initiates of the Santo Daime sect (one of the religious communities that base their ceremonies on the consumption of the *Ayahuasca* beverage). In Holland, in the October 1999, two managers of the sect have been arrested. A criminal investigation into the charging of the two managers, ended with a sentence of "not guilty" from the Amsterdam Court in the May 2000. In France as well, in November 1999, managers of the Churches of Santo Daime have been arrested. In this case, the court of Appeal of Paris, with the decision of January 13, 2005 freed the defendants and legalized the ritual use of *Ayahuasca* in France.

However, from May 2005 France included *Banisteriopsis caapi* e la *Psychotria viridis* in the list of controlled substances.

In Italy, DMT is included in Table I containing narcotic or psychotropic substances subject to the supervision and control under Article 14 of Republic Presidential Decree 309/90 and subsequent updates, but nor *Psychotria viridis* as whole plant nor parts of it are included in that table. In Italy, between 2004 and 2005 in Perugia province (Umbria) a series of *Ayahuasca* seizures and related arrests have been reported.

The DMT is illegal in the United States of America (USA) and is included in the Schedule I drugs of the Controlled Substances Act. It is also included in the Schedule I of the Convention of the Psychotropic Substances of 1971 under the control of the International Narcotics Control Board.

Between 2006 and 2009, some USA courts decided in favor of religious use of *Ayahuasca*. Nevertheless, the use of *Ayahuasca* is still prohibited in USA unless one is not a member of União do Vegetal (UDV) and Santo Daime churches.

The legality of the consumption of *Ayahuasca* on account of religious ceremonies has been debated for a long time in Brazil (the country of Santo Daime) and at last, in the second half of the eighties, the *Ayahuasca* has been legalized, for use in the context of religious rituals but not for selling it.

Pharmaco-toxicological properties

The medical and toxicological properties of the *Ayahuasca* are the result of the combined actions of the different active compounds. The main psychotropic agent present in the extracts of the *Psychotria viridis* is dimethyltryptamine (DMT), structurally correlated to serotonine, able to bind to the central serotoninergic receptors 5HT2A$_{A/2C}$, acting as an agonist [4,5].

The synthetic DMT is usually smoked as free base or administered parenterally. Taken by these routes, the substance causes intense psychedelic episodes, with immediate onset (the effects appear within 30 sec), manifesting with perception altera-

tions (of the own identity and of the surrounding reality), hallucinations (the indios believed that the *Ayahuasca* is a door that opens to let to the body receive the arrival of spirits that produce visions, related to the jungle and to the spirits of the animals) and with a state of alertness. The visions caused by *Ayahuasca*, as for other hallucinogens, depend in part on the individual's emotional state. In any case, such episodes are usually of short duration (5-10 minutes).

The DMT acts also on the sympathetic nervous system causing stimulation of the adrenergic system with increase in the cardiac frequency, blood pressure and mydriasis.

The DMT, taken orally, is degraded quickly at peripheral level by monoamino oxidases (MAO), particularly MAO-B.

The *Ayahuasca* is generally consumed orally but thanks to the simultaneous presence of beta-carbolines, harmine, harmaline and tetrahydroharmine (powerful inhibitors of the MAO), the peripheral degradation of the DMT is avoided and the active component can get to the central level justifying its pharmacological action [4,6].

For the DMT, the dose (following intravenuous administration) at which hallucinogenic effects are shown is 0.2 mg/kg. Other effects caused by DMT include an increase in the plasma levels of: β-endorphin, corticotropine, cortisol, prolactin and growth hormone (GH); on the contrary the levels of melatonin remain unchanged [7].

In a study on human model , the effects of a single administration of two different oral doses (0.6 mg of DMT/kg and 0.85 mg of DMT/kg) of a drink based on *Ayahuasca* were observed. The drink produced significant perceptive alterations and it generated a positive state of mind in the examined subjects. The effects reached maximum intensity between 1.5 and 2 hours after the administration of the drink. The pharmacokinetic analysis highlighted an average maximum serum concentration (Cmax) of DMT of 12.14 ng/ml after the administration of the smaller dose, and 17.44 ng/ml after the administration of the elevated dose. The average time for the achievement of Cmax (Tmax) was 1.5 hours for both doses. The Tmax for the DMT coincided with the peak of the reported subjective effects [8].

Recently, the *Ayahuasca* has been also used for therapeutic purposes. The extracts of the plant, appeared effective in the treatment of alcoholism and drug abuse dependence [9]. Besides, it has been suggested that the *Ayahuasca* could be useful for the treatment of mental disorders with a suspected serotoninergic deficiency. Alterations of this type are considered to be at the base of a series of disorders such as depression, autism, schizophrenia, attention deficit disorder and hyperactive syndrome. Therefore, the long time treatment with the extracts of *Ayahuasca* might be effective for the treatment of these pathological conditions [10]. The regular use of the *Ayahuasca* has been associated with potential immunomodulatory effects with antineoplastic effects [11]. Nonetheless, at the moment there is still not enough scientific evidence to support this theory.

The harmine possesses in vitro antiparasitic properties to fight the *Tripanosoma lewsii* [12]. This effect allowed the rationalization of *Ayahuasca* use in the prophylaxis of malaria and of several other parasitosis [13].

At last, recently it has been observed that the *Ayahuasca* is able to alleviate the symptoms of Parkinson disease. This effect has been attributed to the double ability of the extracts of the plant of inhibiting the MAOs-A responsible for the degradation of dopamine, and of stimulating at the same time the release of the same neurotransmitter from the nigrostriatal cells [14].

Toxicity

Data regarding the acute toxicity of the dimethyltriptamine [15]

In human - TDLo: 1 mg/kg

In mouse - LD50 following intraperitoneal administration: 47 mg/kg.

In rat - LD50 following intravenous administration: 32 mg/kg.

Data regarding the acute toxicity of harmine

In mouse - LD50 following subcutaneous administration: 243 mg/kg.

In rabbit - LDLo following intravenous administration: 60 mg/kg.

In rat: LD50: following intravenous administration 200 mg/kg.

Data regarding the acute toxicity of harmaline

In mouse - LD50 following subcutaneous administration: 120 mg/kg.

In rabbit - LDLo following intravenous administration 20 mg/kg.

In rat: LD50: following intravenous administration: 120 mg/kg.

There is no toxicity data for tetrahydroharmine.

Adverse Effects

The β-carbolines, being a potent MAO inhibitors, can block the deamination of serotonin increasing its cerebral levels. This effect seems to be the origin of the sedation observed in the users of *Ayahuasca* [9].

In addition, because of an excessive increase of the serotonin levels, the beginning of a serotoninergic syndrome can be observed. This syndrome, which exhibits with behavioural problems (state of confusion, hypomania, agitation), dysfunction of the autonomous nervous system (diarrhoea, shivers, fever, perspiration, changes in the blood pressure, nausea, vomiting) and neuromuscular alterations (myoclonus, hyperreflexia, tremor and difficulty in the coordination of the movements), can be potentially fatal [16].

Occasionally, the *Ayahuasca* users can show gastrointestinal disturbances (nausea, vomiting and diarrhoea) [17].

The case of a 25-year-old man who died as a consequence of ingesting a preparation based on *Ayahuasca* is described. The toxicological analysis has shown high blood levels of DMT, harmine, harmaline and tetrahydroharmine associated to this fatal poisoning [18].

Pharmacological interactions

The β-carbolines harmine, harmaline and to a smaller degree tetrahydroharmine, are potent inhibitors of MAO. The concomitant administration of *Ayahuasca* and of selective serotonin reuptake inhibitors (SSRI) can potentially induce a severe serotoninergic syndrome. The patients treated with these medicines should not therefore use the *Ayahuasca* products [19].

Regarding the MAO inhibitors, the concomitant ingestion of *Ayahuasca* and of foods rich in tyramine should be absolutely avoided, since upon accumulation it causes hypertensive episodes due to noradrenaline release [17].

Effects in pregnancy

There is no data regarding use in pregnancy or during lactation.

Analytical determinations

Scientific literature reports analytical methodologies to measure active principles of *Ayahuasca* (both DMT and harmine and harmaline) in biological fluids and tissues [18,20,21], in commercial products [22], and in the beverage [23,24]. The method for the determinations of active principles in commercial products involves both a gas and a liquid chromatograph coupled with a mass spectrometer [22]. The active principles present in the beverages are identified by gas chromatography coupled to mass spectrometry [23] or nitrogen-phosphorous detector [24].

The method below reported is a brief outline useful to the investigator to set up the analysis. For specific details, it is advisable to refer to the original text.

Determination of dimethyltriptamine, harmine, and harmaline in blood and urine

(From: SKLEROV J, LEVINE B, MOORE KA, KING T, FOWLER D. A fatal intoxication following the ingestion of 5-methoxy-N,N-dimethyltryptamine in an ayahuasca preparation. J Anal Toxicol. 2005; 29: 838-841) [18].

The analysis is carried out in various biological fluids (central and periferic blood, urine, gastric juice, bile) and biological tissues (kidney, brain and liver) by liquid chromatograph coupled to a mass spectrometer.

Extraction of the compounds

An amount of 2 ml sodium borate, 50 µl of internal standard (5-fluorotriptamine; 0.01mg/ml) and 2 ml n-chlorobutane are added to 1 ml sample (blood or urine). The solution is vortex-mixed for 10 minutes and then centrifuged at 3500 rpm for 10 minutes. The organic layer is evaporated under a stream of nitrogen at 40°C and the dry residue reconstituted with 100 µl of mobile phase and 2 µl are injected into the liquid chromatograph.

Analytical conditions

Chromatographic column: Xterra MS C18 (100 mm x 3mm x 3.5µm)
Mobile phase: 75% ammonium formiate 0.02M and 25% acetonitrile
Separation: isocratic
Flow rate: 0.4 ml/min
Column temperature: 35°C
Detector: mass spectrometer with positive mode electrospray (ESI) interface
Evaporation gas temperature: 350°C
Evaporation gas flow: 12ml/min
Nebulization gas pressure: 30 psi
Capillary voltage: 100, 150 and 200 V
Fragmentor voltage: 110V

Retention times of the tested compounds

DMT: 1.96 minutes
Harmaline: 2.66 minutes
Harmine: 4.70 minutes
5-fluorotryptamine (internal standard): 2.00 minutes

Characteristic fragments for the tested compounds

DMT: m/z 189
Harmaline: m/z 215
Harmine: m/z 213
5-fluorotryptamine (internal standard): m/z 179

Standard

The DMT standard was obtained from the cerntral laboratory of Drug Enforcement Administration (Atalanta, GE, USA); harmine can be purchased from Sigma (St. Louis, MI, USA) and harmaline from Fisher Scientific (Pittsburgh, PA, USA).

Calibration curves

The calibration curves in both blood and urine was prepared covering the concentration range 0.01-2.5 mg/l.

Results

Samples analyzed with the applied methodology showed the following quantitative results:

DMT (peripheral blood)	0.01 mg/l
DMT (urine)	0.89 mg/l
harmine (peripheral blood)	0.08 mg/l
harmine (urine)	1.15 mg/l
harmaline (peripheral blood)	0.04 mg/l
harmaline (urine)	2.26 mg/l

References

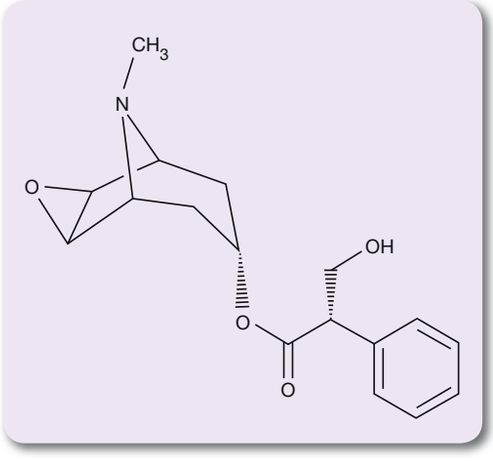
1. CALLAWAY JC, BRITO GS, NEVES ES. Phytochemical analyses of *Banisteriopsis Caapi* and *Psychotria viridis*. J Psychoactive Drugs. 2005; 37: 145-150.
2. CALLAWAY JC. Various alkaloid profiles in decoticons of *Banisteriopsis caapi*. J Psychoactive Drugs 2005; 37: 151-155.
3. http://www.samorini.net/doc/bib_it/bit_aya.htm
4. MCKENNA DJ, TOWERS GH, ABBOTT FS. Monoamine oxidase inhibitors in South American hallucinogenic plants Part 2: constituents of orally active Myristicaceous hallucinogens. J Ethnopharmacol. 1984; 12: 179-211.
5. PIERCE PA, PEROUTKA SJ. Hallucinogenic drug interactions with neurotransmitter receptor binding sites in human cortex. Psychopharmacology (Berlin). 1989; 97: 118-122.
6. SCHULTES R. Ethnotoxocological significance of additives to New World hallucinogens. Plant Sci Bull. 1972; 18, 34-41.
7. STRASSMAN RJ, QUALLS CR, UHLENHUTH EH, KELLNER R. Dose-response study of N,N-dimethyltryptamine in humans. II. Subjective effects and preliminary results of a new rating scale. Arch Gen Psychiatry. 1994; 51: 98-108.
8. RIBA J, VALLE M, URBANO G, YRITIA M, MORTE A, BARBANOJ MJ. Human pharmacology of ayahuasca: subjective and cardiovascular effects, monoamine metabolite excretion, and pharmacokinetics. J Pharmacol Exp Ther. 2003; 306: 73-83.
9. MCKENNA DJ. Clinical investigations of the therapeutic potential of ayahuasca: rationale and regulatory challenges. Pharmacol Ther. 2004; 102: 111-129.
10. CALLAWAY JC, AIRAKSINEN MM., MCKENNA DJ, BRITO GS, GROB CS. Platelet serotonin uptake sites increased in drinkers of ayahuasca. Psychopharmacology. 1994; 116: 385–387.
11. TOPPING DM. Ayahuasca and cancer: one man's experience. MAPS Newsletter. 1998; 8: 22-26 (on-line: http://www.maps.org/news-letters/v08n3/08322top.html).
12. HOPP KH, CUNNINGHAM LV, BROMEL MC, SCHERMEISTER LJ, KAHLIL SKW. In vitro antitrypanosomal activity of certain alkaloids against Trypanosoma lewisi. Lloydia. 1976; 39: 375-377.
13. RODRIGUEZ E, CAVIN JC, WEST JE. The possible role of Amazonian psychoactive plants in the chemotherapy of parasitic worms: a hypothesis. J Ethnopharmacol. 1982; 6, 303-309.
14. SCHWARZ MJ, HOUGHTON PJ, ROSE S, JENNER P, LEES AD. Activities of extract and constituents of Banisteriopsis caapi relevant to parkinsonism. Pharmacol Biochem Behav. 2003; 75: 627-633.
15. http://toxnet.nlm.nih.gov/
16. LEJOYEUX M ADES J, ROUILLON F. Serotonin syndrome: incidens, symptoms and treatment. CNS Drugs. 1994; 2: 132-143.
17. CALLAWAY JC, MCKENNA DJ, GROB CS, BRITO GS, RAYMON LP, POLAND RE, ANDRADE EN, ANDRADE EO, MASH DC. Pharmacokinetics of hoasca alkaloids in healthy humans. J Ethnopharmacol. 1999; 65, 243-256.
18. SKLEROV J, LEVINE B, MOORE KA, KING T, FOWLER D. A fatal intoxication following the ingestion of 5-methoxy-N,N-dimethyltryptamine in an ayahuasca preparation. J Anal Toxicol. 2005; 29: 838-841.
19. CALLAWAY JC, GROB CS. Ayahuasca preparations and serotonin reuptake inhibitors: a potential combination for severe adverse interactions. J Psychoactive Drugs. 1998; 30: 367-369.
20. BJÖRNSTAD K, BECK O, HELANDER A. A multi-component LC-MS/MS method for detection of ten plant-derived psychoactive substances in urine. J Chromatogr B Analyt Technol Biomed Life Sci. 2009; 877: 1162-1168.
21. CALLAWAY JC, RAYMON LP, HEARN WL, MCKENNA DJ, GROB CS, BRITO GS, MASH DC. Quantitation of N,N-Dimethyltryptaminea and Harmala Alkaloids in Human Plasma after Oral Dosing with Ayahuasca. J Anal Toxicol. 1996; 20: 492- 497.
22. KIKURA-HANAJIRI R, HAYASHI M, SAISHO K, GODA Y. Simultaneous determination of nineteen hallucinogenic tryptamines/β-carbolines and phenethylamines using gas chromatography-mass spectrometry and liquid chromatography- electrospray ionisation-mass spectrometry. J Chromatogr B. 2005; 825: 29-37.
23. GAMBELUNGHE C, ARONI K, ROSSI R, MORETTI L, BACCI M. Identification of N,N-dimethyltryptamine and beta-carbolines in psychotropic ayahuasca beverage. Biomed Chromatogr. 2008; 22: 1056-1059.
24. PIRES AP, DE OLIVEIRA CD, MOURA S, DÖRR FA, SILVA WA, YONAMINE M. Gas chromatographic analysis of dimethyltryptamine and beta-carboline alkaloids in ayahuasca, an Amazonian psychoactive plant beverage. Phytochem Anal. 2009; 20: 149-153.

Brugmansia arborea
(angel trumpet)

Name: *Brugmansia arborea*
Family: *Solanaceae*
Genus: *Brugmansia*
Species: *Arborea; candida; sanguinea*
Synonims: Tree Datura; Angel's Trumpet; Floripondio; Maikoa
Origin: Perù
Active compounds: tropane alkaloids (hyoscyamine, the racemic mixture atropine (*d* and *l*- hyoscyamine) which forms during plant drying and scopolamine.

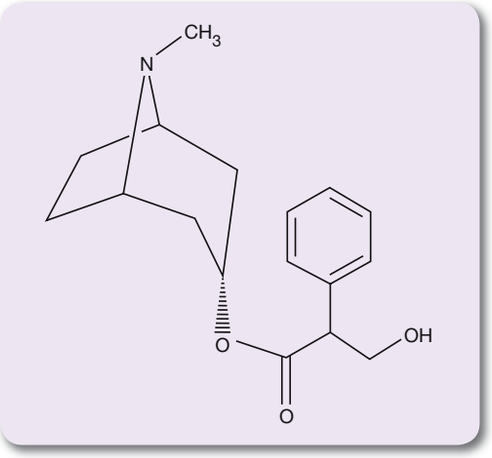
The plant belongs to the the solanacee group and its effects are entirely similar to those of *Datura stramonium* which originates from Colombia and Mexico. The *Brugmansia* genus includes different species, most of them from South America. The plant is commonly known as "angel's trumpet" but also "dead men's trumpet", because the native Peruvians considered its use dangerous, due to the presence of atropine and scopolamine.

Chemical formula and physico-chemical properties of the active compounds[1]



Name: Scopolamine.
Molecular formula: $C_{17}H_{21}NO_4$ (molecular weight = 303.4).
Systematic name: [7(S)-(1α,2β,4β,5α,7β)]-α-(hydroxymethyl) benzoylacetic acid 9-methyl-3-oxa-9-aza-triciclo-[3.3.1.$0^{2,4}$]non-7-yl-ester.
CAS registry number: 51-34-3.
Melting point: 59°C.
UVmax: 246, 252, 258, 300 nm.
Solubility: warm water, alcohol, ether, chloroform, acetone.

Name: Hyoscyamine.
Molecular formula: $C_{17}H_{23}NO_3$ (molecular weight = 289.4).
Systematic name: α-(hydroxymethyl)-(3-endo)-8-methyl-8-azabicyclo (3.2.1)oct-3-yl-ester(α-S)-benzoylacetic acid.
CAS registry number: 101-31-5.
Melting point: 108,5°C.
UVmax: 252, 258, 264 nm.
Solubility: alcohol and dilute acids.



Name: Atropine.
Molecular formula: $C_{17}H_{23}NO_3$ (molecular weight = 289.4).
Systematic name: (1R,5S)-8-methyl-8-azabicyclo[3.2.1]octan-3-yl] 3-hydroxy-2-phenilpropanoate).
CAS registry number: 51-55-8.
Melting point: 108,5°C.
UVmax: 258 nm (methyl alcohol).
Solubility: alcohol and dilute acids.

Historical use

The plants from the *Brugmansia* genus were well-known to the inhabitants of Central America and were used only for the preparation of intoxicating and narcotic potions, and not for therapeutic reasons. The plant is not mentioned in the texts of the ancient European medicine. Only towards the end of 1700 it started to be used for medical reasons [(2)]. Its seeds were used by the magicians for their narcotic properties, for the fantastic visual effects they were causing and for their presumed aphrodisiacal power.

Current use

At present, *Brugmansia arborea* is widely used since it causes an initial hallucinatory phase, followed by strong sedation, apathy and retrograde amnesia. It is used as a psychedelic mixture called “zombies haitian mixture” with effects that swing between visual hallucinations and a trance-like state [(3)].

Legislation

In Europe and in the USA *Brugmansia arborea* is not subjected to any legal control. In Italy, neither scopolamine, hyoscyamine, atropine, nor the whole plant or parts of it are included in the tables containing narcotic or psychotropic substances subject to the supervision and control under Article 14 of Republic Presidential Decree 309/90 and subsequent updates.

Pharmaco-toxicological properties

Hyoscyamine is an anticholinergic, antimuscarinic compound, which works by blocking acethylcholine action at the peripheral level of the parasympathetic nervous system (smooth musculature) and at the central nervous system level. This affects mainly the antagonism of muscarinic receptors, less than of nicotinic ones, and at the ganglia and neuromuscolar ends level.

Parasympaticolytic effects :1) spasmolytic effect (smooth musculature); 2) midriasis and paralysis of the visual accommodation; 3) decrease of the secretory activity of the exocrine glands; 4) tachicardia; 5) suppression of nausea and vomiting. Atropine, the racemic mixture of hyoscyamine, is the ester of tropic acid with tropane: the alkaloid found naturally is the (-)-hyoscyamine. Atropine is formed by the racemisation at the chiral carbon atom of the tropic acid during the isolation process. While the dextrogire isomer of the atropine is inactive, the levogire (-)-hyoscyamine is about twice as potent as atropine. Its action and/or uses are similar to those of antimuscarinic compounds, with exception that hyoscyamine, differently from atropine, is not used in ophtalmology but almost exclusively like an antispasmodic medicine [(4,5)]. A rabbit liver homogenate containing (-)-hyoscyamine, hydrolized the molecule of atropine and (-)-tropic acid, but has no effect on (+)-hyoscyamine [(5)]. About 80-90 % of the atropine is excreted in the 24 hours urine: 50 % unchanged, 2 % as tropic acid and tropine, and about 30 % as unidentified metabolites [(6)]. Atropine is the generic treatment used against the poisoning with nervine gases; in fact, atropine inhibits the effect of the acetylcholine at the cholinergic receptor level limiting the poisonous effect of the nervine gas. For this reason the soldiers who operate in environments potentially at risk of contami-

nation with nervine gases receive atropine syringes for immediate autoadministration.
Scopolamine is the common name given to (-)-hyoscine, the natural alkaloid of *Brugmansia arborea*. Similar to hyoscyamine, scopolamine is an anticholinergic compound and its action is acethylcholine- competitive at the muscarinic receptor level. For this reason, it seems that scopolamine is able to correct the acethylcholine and noradrenaline imbalances which may occur in certain motor disorders [7].
The oral administration of 0.90 mg of scopolamine in a healthy individual result in a peak plasma level of about 2 ng/ml within an hour. It reversibly binds to plasma proteins and is able to cross the blood-brain barrier. Similarly, it crosses the placenta and concentrates in the maternal milk. Although the metabolic pathway and excretion pattern of scopolamine is not entirely clear, it is believed that the molecule should be almost completely metabolized (by conjugation) in the liver and excreted in the urine [7].
Recently, using aqueous extracts of *Brugmansia arborea*, it has been demonstrated that its compounds can interact also with the serotoninergic receptors [8].

Toxicity

An uncontrolled ingestion of *Brugmansia arborea* can have major consequences, especially if the ingestion occurs together with alcohol or psychotropic agents. It is possible to go from hallucinations to delirium, convulsions, major visual disturbances, up to coma by cerebral anoxia (lack of oxygen to the brain) and to death.
The toxic dose of atropine is variable and it depends on the individual sensitivity. There are cases of death for doses of 50-100 mg and cases of recovery with doses of 1 g. Doses equal to 10 mg can be fatal for children and for the sensitive individuals [9]. The toxicity induced by the scopolamine, characterised by a classic anticholinergic syndrome, usually derives from accidental ingestion of adulterated products or of plants containing the substance in question (in this case *Brugmansia arborea*). The classic manifestations occuring as a result of scopolamine ingestion include hallucinations and urinary incontinence [10]. A deficit in the cognitive and motor processes induced by scopolamine has been widely demonstrated in the animal and human models, although it is not entirely clear what role acethylcholine has in the mnemonic processes [11].
There are poisoning cases described in the literature, induced by scopolamine alone or in association with other drugs of abuse (for example heroin). The manifestations of an overdose with such drugs (apparently “cut” with scopolamine) include lethargy, agitation, hallucinations, paranoia, tachicardia, moderate hypertension, dry skin, urinary retention [7]. In the Spring 1995 a great number of intoxications presented in the Emergency Departments of several New York hospitals following ingestion of heroin cut with scopolamine, showing symptoms of a severe anticholinergic poisoning. A 90 % of the treated subjects needed hospital admission with half of them being treated in the respective intensive care unit [12].

Data related to acute toxicity of hyoscyamine [13]
In mouse - LD50 following intravenous administration: 95 mg/kg
In human - TDLo: 1.471 mg/kg
In human probable LD following oral dose is 5 mg/kg [14]

Data related to acute toxicity of scopolamine [13]
In mouse - LD50 following oral administration: 1275 mg/kg
In mouse - LD50 following subcutaneous administration: 1700 mg/kg
In human – TDLo following intramuscular administration: 0.004 mg/kg
In human - TDLo following subcutaneous administration: 0.002 mg/kg

Data related to acute toxicity of atropine [13]
In mouse - LD50 following oral administration: 75mg/kg
In mouse - LD50 following subcutaneous administration: 428 mg/kg
In human - TDLo following intramuscular administration: 0.001 mg/Kg
In human - TDLo following subcutaneous administration: 0.033 mg/Kg

Adverse Effects

Atropine and scopolamine are anticholinergic agents which can cause symptoms such as dry mouth, dilated and not reactive pupils, increase of the blood pressure and tachycardia.
Elevated doses may cause excessive thirst, hallucinations, loss of conscience and in some cases even death. Atropine is the typical anticholinergic drug, it competes with the acetylcholine at the muscarinic receptor level; it is well absorbed by the intestine and distributed throughout the body [15].

Pharmacological interactions

Atropine increases the anticholinergic activity of the tricyclic antidepressants. Scopolamine should be used carefully in the patients who are prescribed other drugs that act on the central nervous system (sedatives, tranquilizers, alcohol) and in association with the drugs with anticholinergic activity, such for example antihistamines, triciclic antidepressants, muscle relaxants [16].

Analytical determinations

No methodologies have been reported in the international literature concerning the analysis of *Brugmansia arborea* active principles in biological fluids of eventual consumers. However, analytical methods have been published regarding the determination of these active principles in urine of consumers of *Datura stramonium*, a plant which, similarly to *Brugmansia arborea*, contains scopolamine and hyoscyamine [17].
Furthermore, international literature reports the determination of active principles in the root of *Brugmansia arborea* by liquid chromatography coupled to photodiode array detection [18].

The method below reported is a brief outline useful to the investigator to set up the analysis. For specific details, it is advisable to refer to the original text.

Determination of scopolamine and hyoscyamine in serum and urine of an individual intoxicated by *Datura stramonium*

(From: NAMERA A, YASHIKI M, HIROSE Y, YAMAJI S, TANI T, KOJIMA T. Quantitative analysis of tropane alkaloids in biological materials by gas chromatography–mass spectrometry. Forensic Sci Int. 2002: 130; 34-43) [17].

The analysis is carried out on serum and urine samples by gas chromatography coupled to mass spectrometry.

Extraction of the compound

An amount of 0.5 ml sample (serum or urine) is diluted in 100 mM borate buffer, pH 9. The mixture is applied to an Extrelut extraction column and alkaloids are eluted with 10 ml methylene chloride. The organic layer is evaporated under a stream of nitrogen at 40°C and the residue derivatized with 20 µl N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) at 80°C for 15 minutes. After derivatization, the sample is diluted with 100 µl methylene chloride and 1 µl solution is injected in the gas chromatograph.

Analytical conditions

Chromatographic column: HP-5MS (0.25 mm x 30 m x 0.25 µm)
Injector temperature: 250°C
Carrier gas: Helium at a flow rate of 0.8 ml/min
Injection mode: splitless
Temperature program: starting temperature at 50°C for 1 minute, then 50°C-300°C a 20°C/min, then 300°C for 5 minutes.
Detector: mass spectrometer with electron impact interface

Retention times of the tested compounds

Hyoscyamine: 13.1 minutes

Scopolamine: 13.7 minutes

Characteristic fragments for the tested compounds

Hyoscyamine: m/z 361, 140, 124

Scopolamine: m/z 375, 154, 138

Standard

The standard of hyoscyamine and scopolamine used in the analyses were purchased from Sigma (St. Louis, MI, USA).

Calibration curves

The calibration curves of the compounds, both in serum and urine, ranged from 10 to 5000 ng/ml.

Results

Both hyoscyamine and scopolamine were identified in serum after acute intoxication with *Datura Stramonium* and quantified with a concentration of 12 ng/ml hyoscyamine and traces of scopolamine (< 10 ng/ml). No values for urine samples were reported.

References

1. THE MERCK INDEX An Enciclopedia of chemicals, drugs, and biologicals. 10th Ed. Merck & Co., Inc. 1983.
2. FASSINA G. Lezioni di farmacognosia. Droghe vegetali. Antonio Milani (Ed.), 1974: 265-266.
3. ULRIKE PREISSEL, HANS-GEORG PREISSEL Brugmansia and Datura Angel's Trumpets and Thorn Apple Buffalo, New York: Firefly Books. 2002: 106-129.
4. OSOL A. (ed.) Remington's Pharmaceutical sciences. 16th ed. Easton, Pennsylvania: Mack Publishing Co., 1980, p. 856.
5. WERNER G. Metabolism of tropane alkaloids. V. Enzymatic preparation of (+-)-hyosciamine sulfate H_2O. Arzneim-forsch 1967; 17: 1467.
6. Clarke's isolation and identification of drugs. The pharmaceutical press (ed.) 1986: 364.
7. American Society of Health System Pharmacists. AHFS Drug Information 2008. Bethesda, Maryland 2008, p. 1298-1323.
8. CAPASSO A, DE FEO V. In vitro binding receptors study by Valeriana adscendens, Iresine herbstii and Brugmansia arborea extracts. Med Chem. 2007; 3: 599-604.
9. GOODMAN AND GILMAN'S, in: J.G. Hardman, L.E. Limbird (Eds.), The Pharmacological Basis of Therapeutics, 9th ed., McGraw-Hill, New York, 1995, pp. 141-160.
10. DART RC. (ed). Medical toxicology. Third Edition, Lippincott Williams & Wilkins. Philadelphia, PA. 2004, p. 564.
11. THOMAS E, SNYDER PJ, PIETRZAK RH, JACKSON CE, BEDNAR M, MARUFF P. Specific impairments in visuospatial working and short-term memory following low-dose scopolamine challenge in healthy older adults. Neuropsychologia 2008; 46 (10): 2476-84.
12. HAMILTON RJ, PERRONE J, HOFFMAN R, HENRETIG FM, KARKEVANDIAN EH, MARCUS S, SHIH RD, BLOK B, NORDENHOLZ K. A descriptive study of an epidemic of poisoning caused by heroin adulterated with scopolamine. J Toxicol Clin Toxicol. 2000; 38(6): 597-608.
13. http://toxnet.nlm.nih.gov
14. GOSSELIN RE, HODGE HC, SMITH RP, GLEASON MN. Clinical toxicology of commercial products. 4th ed. Baltimore: Williams and Wilkins, 1976, p. 2-157.
15. DEWITT MS, SWAIN R, GIBSON LB JR. The dangers of jimson weed and its abuse by teenagers in the Kanawha Valley of West Virginia. WV Med J. 1997; 93: 182-5.
16. THOMSON HEALTH CARE INC.; Physicians' Desk Reference 62 ed., Montvale, NJ 2008, p. 2192-2193.
17. NAMERA A, YASHIKI M, HIROSE Y, YAMAJI S, TANI T, KOJIMA T. Quantitative analysis of tropane alkaloids in biological materials by gas chromatography–mass spectrometry. Forensic Sci Int. 2002: 130; 34-43.
18. NINO J, GALLEGO C M, CORREA Y M, MOSQUERA O M Production of scopolamine by normal root cultures of Brugmansia Plant Cell, Tissue and Organ Culture 2003; 74 : 289-291.

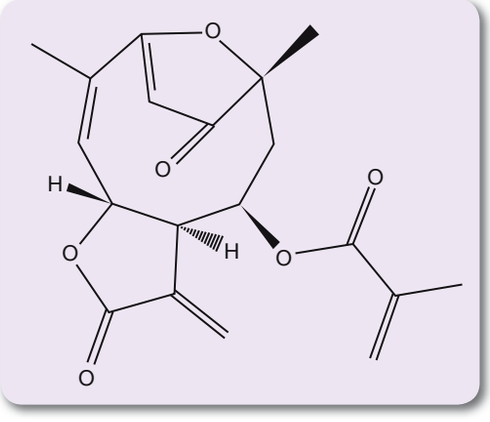
Calea zacatechichi Schl.

(Leaf of God)

Name: *Calea zacatechichi*
Family: *Compositae*
Genus: *Calea*
Species: *Calea zacatechichi* Schl.
Synonims: dream herb, cheech, bitter grass, leaf of the god, herb of the dog, mother leaf
Origin: Mexico and Costa Rica
Active compounds: sesquiterpenic lactones (calaxin, ciliarine); germacranolids (1 β-acetoxy-zacatechinolide, 1-oxo-zacatechinolide) [1]

The plant contains a large number of active compounds. However, some of these molecules do not seem to have the oneirogenic effect/hallucinogenic effect reported by the users of the *Calea zacatechichi*.

Chemical formula and physico-chemical properties of the active compounds



Name: Calaxin.
Molecular formula: $C_{19}H_{20}O_6$ (molecular weight = 344.4).
Systematic name: 2-Propenoic acid, 2-methyl-, 2,3,3a,4,5,6,7,11a-octahydro-6,10-dimethyl-3-methylene-2,7-dioxo-6,9-epoxycyclodeca(b)furan-4-yl ester, (3aR-(3aR*,4R*,6R*,10Z,11aR*)).
CAS registry number: 30412-86-3.
Melting point: no data in the literature.
UVmax: no data in the literature.
Solubility: no data in the literature.

Regarding the other active compounds: ciliarine, 1 β-acetoxy-zacatechinolide, 1-oxo-zacatechinolide, there are no data in the literature describing their molecular formula, systemate name, CAS registry number, melting point, UV max and solubility.

Historical use

Calea zacatechichi is a plant used in Mexico (used by the indigenous Chontal of the Mexican state of Oaxaca) in the traditional and shamanistic medicine in the pre-Columbian period. *Zacatechichi* is a Nahuatl (aztec) word which means "bitter grass". Most probably *Calea zacatechichi* corresponds to "chichixihuitl", a plant used by the ancient Aztecs to induce dreams [2]. The plant is extensively used in the Mexican popular medicine: an infusion of roots, leaves and stem was used in the treatment of gastrointestinal disorders, as a digestive, cathartic and fever reducing agent. Together with other *Compositae* the dried plant is used as insecticide [1]. In the shamanistic medicine it was used for the induction of particularly vivid dreams with real vision of profound knowledge and imagination.

Current use

In treating certain pathologies, Mexicans from the Oaxaca region use the plant even today. Some authors refer to the experience of the Chontal Indians who used the leaves of the plant (smoked or drunk in an infusion form) to get divine messages through dreams. The websites, that advertise the smart drugs, include the plant in the category of hallucinogenic grasses with "oneirogenic" effect (increase of the sensory perceptions of the dreams during sleep), even though the pharmacological properties of the psychoactive constituents have not yet been clarified in systematic clinical studies.

Legislation

With the exception of Poland where, since March 2009, all parts of *Calea zacatechichi* (e.g. seeds, leaves, extracts) are included in the same prohibition list of opiates, heroin and hydrocodone, in Europe there are no legal restrictions against *Calea zacatechichi* or its active ingredients. In Italy neither calaxin, nor ciliarine, 1β-acetoxy-zacatechinolide, 1-oxo-zacatechinolide, the whole plant or parts of the plant are included in the tables containing narcotic or psychotropic substances subject to the supervision and control under Article 14 of Republic Presidential Decree 309/90 and subsequent updates. In the USA, the possession, selling and cultivation of *Calea zacatechichi* are legal, although its use in food products has not been approved.

Pharmaco-toxicological properties

Calea zacatechichi is a plant used by the Chontal Indians of Mexico for oneiromancy (art of divination based on interpretation of dreams).

Since the active compounds responsible for the oneirogenic/hallucinogenic effect reported by the consumers of *Calea* are still unknown, it is impossible to establish which are the biochemical mechanisms at the base of the pharmacological effects, induced by the plant.

In the literature, the results of a double blind study using healthy volunteers who got either placebo or low doses of an extract of C*alea zacatechichi* (about 1 g/kg) are described. The volunteers who received the extract showed a significant increase of reaction time, an increase of the light sleep state and a greater number of spontaneous awakenings compared to the controls. Besides, in this cohort, an increase of the oneirogenic activity during the phases of light-sleep was observed. The ingestion of *Calea zacatechichi* infusion before sleeping produces the most realistic dreams and can also produce a sensation of wellbeing and relaxation that lasts for more days [(1)].

In rats, the aqueous *Calea zacatechichi* extract produces an anti-inflammatory action. Such an effect validated with the experimental model of carragenine-induced edema, has been attributed to the ability of the extract of inhibiting the prostaglandin and leucotriene synthesis. Nevertheless, at the present time, it is not clear which of the compounds is responsible for this pharmacological effect [(3)].

A study [(4)] reported how *in vitro*, the alcoholic *Calea zacatechichi* extract has an inhibitory effect of the NF kB transcription factor, involved in the inflammatory processes. The biological effect has been attributed to sesquiterpenic lactones present in the *Calea zacatechichi* extract as well as in other medicinal plants known for their anti-inflammatory activity such as *Arnica montana* and *Tanacetum parthenium* [(4)].

Another study conducted on experimental animals using several Mexican plants, has demonstrated a hypoglycemic effect of *Calea zacatechichi* [(5)].

Finally, some flavonoids extracted from *Calea zacatechichi* resulted active against the Dd2 strain of Plasmodium falciparum resistant to chloroquine [(6)].

Toxicity

At moment, there are no animal studies to establish the toxicity of *Calea zacatechichi*.

The organic *Calea zacatechichi* extracts cause alterations to the electroencephalogram and drowsiness in cats. Elevated doses induce increased salivation, ataxia and, occasionaly, vomiting [(1)].

Adverse Effects

On the internet web sites, commercializing the plant, it has been reported that *Calea zacatechichi* used in elevated, but not quite definite doses, causes tachicardia, hypertension, anxiety, irritability and insomnia [7].

Pharmacological interactions

There are no pharmacological interactions reported in the literature.

Effects in pregnancy

There is no data regarding the use of *Calea zacatechichi* during pregnancy or lactation.

Analytical determinations

There are no described analytical methodologies for the determination of active compounds of the *Calea zacatechichi* neither in biological fluids of eventual consumers nor in plant parts.

References

1. MAYAGOITIA L, DIAZ JL, CONTRERAS CM. Psychopharmacologic analysis of an alleged oneirogenic plant: Calea zacatechichi. J Ethnopharmacol.1986; 18: 229-243.
2. http://amazing-nature.com/info/1124.htm
3. VENEGAS-FLORES H, SEGURA-COBOS D, VAZQUEZ-CRUZ B. Antiinflammatory activity of the aqueous extract of Calea zacatechichi. Proc West Pharmacol Soc. 2002; 45: 110-111.
4. BORK PM, SCHMITZ ML, KUHNT M, ESCHER C, HEINRICH M. Sesquiterpene lactone containing Mexican Indian medicinal plants and pure sesquiterpene lactones as potent inhibitors of transcription factor NF-kappaB. FEBS Lett. 1997; 402: 85-90.
5. ROMAN RAMOS R, ALARCON-AGUILAR F, LARA-LEMUS A, FLORES-SAENZ JL. Hypoglycemic effect of plants used in Mexico as antidiabetics. Arch Med Res. 1992; 23: 59-64.
6. KOHLER I, JENETT-SIEMS K, SIEMS K, HERNANDEZ MA, IBARRA RA, BERENDSOHN WG, BIENZLE U, EICH E. In vitro antiplasmodial investigation of medicinal plants from El Salvador. Z Naturforsch [C]. 2002; 57: 277-281.
7. http://www.albanesi.it/Mente/ecstasy.htm

Citrus aurantium
(Bitter Orange)

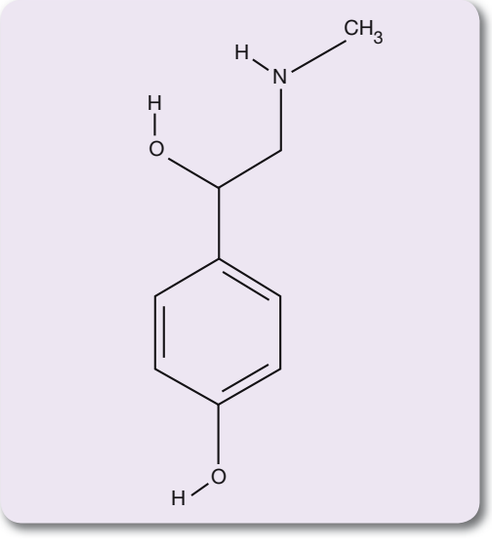
Name: *Citrus aurantium*
Family: *Rutaceae*
Genus: *Citrus*
Species: *Citrus aurantium* L.
Synonims: Sour orange, Bitter orange, Seville orange.
Origin: South East Asia, but by now it already got introduced to the temperate climate countries. In Europe it is grown mostly in Spain and Italy, particularly in Sicily.
Active compounds: (±)-*p*-synephrin; (±)-*p*-octopamine

The *Citrus aurantium* contains a wide range of constituents, such as flavonic glucosides (e.g. hesperidin), cumarine, polymethoxyflavones, aldehydes, amines and monoterpenes. The principal compounds are two: octopamine and synephrine, found mostly in the peel (from which an essential oil is extracted) and in the pulp of the fruit.
The principal pharmacologically active compound of the fruits (oranges) of *Citrus aurantium* is the synephrine (called also p-synephrine or oxedrine). Chemically there are six possible isomers of the synephrine (ortho-, meta- and para- and, for each one of them, the form *d* (+) o *l* (-)). The isomer found in the *Citrus aurantium* seems to be the para-synephrine, although some authors have noticed also the presence of the meta-synephrine (known also as phenylephrine or neosynephrine). Phenylephrine, an α adrenergic receptor agonist, is used in the clinical practice as nasal decongestant and to induce midriasis. Synephrine seems to act directly on the α-1 adrenergic receptors; its effects are in fact blocked by the administration of the prazosin, an antagonist of the above-mentioned receptors. Structurally, synephrine is closely related to the endogenous neurotransmitters (adrenaline) and to ephedrine, the principal alkaloid of *Ephedra sinica* (Ma-huang) [1,2]. From a chemical point of view, there are two differences between synephrine and ephedrine: in the first substance, indeed, one of the carbon atoms in the benzenic ring is hydroxylated, and a methylic group of the lateral chain is substituted with a hydrogen.
Octopamine is a biological amine derived by the β-hydroxylation of tyramine by dopamine β-hydroxylase. The natural *d* (-) form is three times more powerful than the *l* (+) form in producing adrenergic type cardiovascular response. In the nervous system of the invertebrates, octopamine can act as a neurotransmitter [3]. The octopamine is used as a cardiotonic in the treatment of hypotension.
Synephrine seems to be present in slightly greater amounts in the immature dried fruit of *Citrus aurantium* compared to the dried fruit left to achieve complete maturation (0.26% vs 0.22%). In any case, the fresh fruit contains a lesser amount of active component (*d*,*l*-synephrine) compared to the dried fruit (0.02% vs about 0. 3%), while the dry extract of *Citrus aurantium* contains a rather high percentage of *d*,*l*-synephrine (3%). Some of the commercially available erboristic products based on *Citrus aurantium*, analysed in order to quantify their synephrine and octopamine content, showed variable concentrations of the compounds , as shown in Table 1[4].

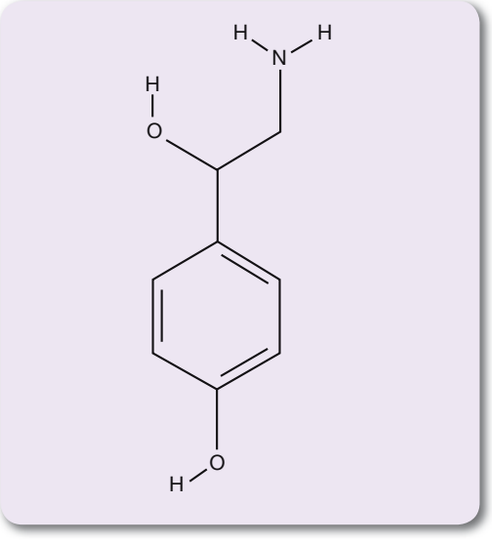
Table 1. *dl*-octopamine and *dl*-synephrine content in *Citrus aurantium* L. bitter variety [4]

Compound	Octopamine (%)	Synephrine (%)
Fresh fruit	<LOQ	0.020
Esiccated fruit	<LOQ	0.352
Dry extract 1	0.028	3.003
Dry extract 2	0.023	3.079
Erboristic product 1	0.013	0.989
Erboristic product 2	0.147	0.664
Erboristic product 3	0.015	0.250

Chemical formula and physico-chemical properties of the active compounds



Name: Synephrine (oxedrine).
Molecular formula: $C_9H_{13}NO_2$ (molecular weight = 167.2).
Systematic name: 1-(4-hydroxyphenyl)-2-methylaminoethyl alcohol
CAS registry number: 94-07-5.
Melting point: 184-185°C; 151-152°C as hydrochloride [5].
UVmax: 224 nm. 231, 272 nm in acetate buffer pH 4.9:acetonitrile (99:1 v/v).
Solubility: water.



Name: Octopamine.
Molecular formula: $C_8H_{11}NO_2$ (molecular weight = 153.1).
Systematic name: 1-(4-hydroxyphenyl)-2-aminoethyl alcohol.
CAS registry number: 104-14-3.
Melting point: 160°C.
UVmax: 224 nm. 231, 272 nm in acetate buffer pH 4.9:acetonitrile (99:1 v/v).
Solubility: water. The *d*(-) form crystallizes from hot water; at 160°C it changes in a compound with boiling point >250°C.

Historical use

Historically "*Citrus aurantium*", the bitter orange, has been rarely used in the culinary field, due to the strong sour taste of the fruit. The ripe fruits however, are still consumed in Iran, while the fresh fruits in Mexico are eaten flavoured with salt and chili. The fresh peel of the fruit (orange) has been often used in the preparation of jams, while the dried peel is used to aromatize some beers (like the Belgian Orange Muscat), and liqueurs (Curaçao, Cointreau, etc.). The flowers have been used to prepare infusions, and the essential oil extracted from the flowers is used in preparation of perfumes. The most frequent and historically most important use of the plant is in traditional medicine. The erboristic Asiatic medicine recognizes the useful properties of the young dried fruit (Zhi-schi in Chinese, Kijitsu in Japanese) in the treatment of digestive tract problems. In the traditional western medicine *Citrus aurantium* is used for the appetite and gastric secretion stimulation [3].

Current use

In addition to all these uses, the *Citrus aurantium* is also used as dietary supplement promoting weight loss. A recent communication of the American Food and Drug Administration (FDA) about the banning of the ephedra based products, has increased the interest among the United States mass media in the so called "ephedra-free" food additives. The products advertised as "ephedra-free" contain a wide range of erboristic mixtures mostly based on coffee (for example, green tea extract [*Camellia sinensis*], guaranà [*Paullinia cupana*], cola nut [*Cola nitida*] yerba mate [*Ilex paraguariensis*]), but the ones based on bitter orange (containing synephrine) have attracted the FDA's attention, putting the products based on *Citrus aurantium* under surveillance [6]. It is well-known that the majority of the dietary supplements contain mixtures or extracts of different plants: hence, it is not uncommon to find additives based, for example, on coffee and synephrine, with a synergistic effect difficult to assess. The dietary supplements used for weight loss contain on average 100-200 mg of bitter orange extract (*Citrus aurantium*), which supply around 10-40 mg of synephrine per dose. A pure extract can contain up to 95% of synephrine. Today, the extracts used in the majority of the dietary supplements for weight loss contain a larger amount of synephrine compared to the dried fruit or peel extracts used in the traditional medicine [7].

Legislation

The sale of weight loss products containing *Citrus aurantium* or synephrine is prohibited in Canada [10]. In the USA the *Citrus aurantium* (bitter orange) was placed in the Poisonous Plants Database (PPD), but, although suggested by the FDA, the sale of products containing plant extracts or synephrine is not prohibited. The oil extracted from the peel, the flowers and the leaves have been included in the American database of food additives (EAFUS, or "Everything Added to Food in the United States). In the orange juice concentrate, the volume of bitter orange cannot exceed 5%.

No news on particular European Legislation on Citrus aurantium has been reported to date.

In Italy, in the Decree No. 3 of July 18th 2002 published in the Official Bulletin N. 188 dated August 12th 2002, specific considerations for products containing certain vegetal ingredients are mentioned. Regarding the *Citrus aurantium* the Bulletin says: "the daily intake of synephrine must not exceed 30 mg, contained in about 800 mg of *Citrus aurantium* (considering a 4% synephrine content); Caution: do not exceed the advisable daily dose. In case of cardiovascular diseases and/or hypertension, consult a physician before ingesting the product. The use in pregnancy, during breastfeeding and under 12 years of age is not advisable [8].

The Italian Ministry of Health included *Citrus aurantium* in the list of vegetal extracts admitted in dietary supplements[9].

Pharmaco-toxicological properties

The most important compounds of the *Citrus aurantium* extracts are *p*-synephrine and *p*-octopamine, two substances present also in vertebrates. Synephrine is a sympatomimetic, α-1 adrenergic receptor agonist [11]. It can stimulate the sympathetic nervous system in a specific "fight or flight" fashion. Both synephrine and octopamine seem to be involved in the pathogenesis of headache [12].

At peripheral level, synephrine, through the stimulation of the α-1 adrenergic receptors, causes vasoconstriction and an

increase in blood pressure [13].

In the United States of America, the substance is used as nasal decongestant and as vasoconstrictor, while in Europe it is used for the treatment of asthma and of hypotension. Clinical tests have demonstrated that, in healthy individuals, the intravenous administration of synephrine at a rate of 4 mg/min, increases the systolic pressure and the average arterial pressure, decreases peripheral vascular resistance, but it does not affect the diastolic pressure and the cardiac frequency [7].

The extracts of bitter orange and synephrine are found in numerous slimming preparations due to the β-3 adrenergic receptor agonist effect of synephrine which may correlate to a lipolytic activity.

The pharmacological effects of synephrine are strictly correlated to the dosage, isomeric form and to the route of administration. Once absorbed at systemic level, the half life of this compound is approximately 2 hours and its elimination is mostly through urine; a part of the compound is eliminated unchanged, and the rest as p-hydroxymandelic acid, the principal urinary metabolite [5].

The octopamine is the second most important component of the *Citrus aurantium*. It is considered, among the catecholamine analogues, the substance with the greatest affinity for the β-3 adrenergic receptors. *In vitro*, octopamine stimulates the lipolysis and the oxygen use in the rat and dog adipocytes while it is much less active in the human cells [14].

It is believed that the octopamine, naturally present in small concentrations in the vertebrate central nervous system, could be a metabolic product of the catecholamines. In the rat, *m*- and *p*-octopamine are present in equal concentration in the heart, the liver and the spleen. In the kidneys, intestine, bladder and lungs the *m*-octopamine is more abundant compared to the *p*-octopamine. The principal urinary metabolites of octopamine are the *o*-, *m*- and *p*-hydroxymandelic acid.

Studies on rat model showed a possible protective action of hesperidine, related to its antioxidant properties, on toxic action from bonzopyrene at testicular level1 [5].

Toxicity

Acute toxicity data for octopamine and synephrine on animal model are reported in Table 2.

In rats, the administration of standardized extracts of *Citrus aurantium* containing different concentrations of synephrine (4 and 6%) can cause death due to the onset of ventricular arrhythmias associated with enlargement of QRS complex on the ECG [16].

In mice, the acute toxicity after oral administration of an extract of *Citrus aurantium* (2.5% *p*-synephrine) at a dose of 300-5000 mg / kg is characterized by reduced locomotor activity. Also in mouse model, the acute oral administration of *p*-synephrine dosage of 150-2000 mg/kg produced reduced locomotor activity, salivation, piloerection, exophthalmos. These effects have been linked to stimulation of the adrenergic system [17].

Table 2: Acute toxicity data for octopamine and synephrine in an animal model [7].

Administration	Species	LDLo/LD50/TDLo
(±)-p-octopamine		
s.c.	Mouse	LD50 = 2070 mg/kg (13.51 mmol/kg)
i.p.		LD50 = 600 mg/kg (3.92 mmol/kg)
i.v.		LD50 = 75 mg/kg (0.49 mmol/kg)
i.c.		LD50 = 2100 mg/kg (13.71 mmol/kg)
Oral		LD50 = 4200 mg/kg (27.42 mmol/kg)
s.c.	Rat	LD50 = 350 mg/kg (2.28 mmol/kg)
i.p.		LD50 = 1350 mg/kg (8.813 mmol/kg)
Oral		LD50 = 1240 mg/kg (1.31 mmol/kg)
i.v.	guinea pig	LDLo = 200 mg/kg (1.31 mmol/kg)
(±)-p-synephrine		
s.c.	Mouse	LDLo = 1500 mg/kg (8.971 mmol/kg)
	Rat	LDLo = 1500 mg/kg (8.971 mmol/kg)
i.p.	Mouse	LD50 =1000 mg/kg (5.981 mmol/kg)
i.v.	Mouse	LD50 = 270 mg/kg (1.61 mmol/kg)
	Rabbit	LDLo = 150 mg/kg (0.897 mmol/kg)
(S)-(+)-p-synephrine		
s.c.	Mouse	LDLo = 700 mg/kg (4.19 mmol/kg)
Oral	Rat	TDLo = 1 mg/kg (6 µmol/kg)
(±)-p-synephrine		
s.c.	Mouse	LDLo = 400 mg/kg (1.96 mmol/kg)
	Rat	LDLo = 320 mg/kg (1.57 mmol/kg)
	guinea pig	LDLo = 500 mg/kg (2.45 mmol/kg)

i.c. = intracerebral; i.p. = intraperitoneal; i.v. = intravenous; LD50 = lethal dose for 50% of the test animals; LDLo = minimum letal dose; s.c. = subcutaneous; TDLo = minimum toxic dose for previous modes of administrations which demonstrated carcinogenic or teratogenic effect in animals or humans , or or any toxic effect in humans..

Adverse Effects

In the United States of America, due to synephrine vasoconstricting effect, the FDA has established that in the sprays used as nasal decongestants the warnings should be appended: “Use the product at the prescribed dose for not more than three days”. Adverse effects associated with such preparations can include: tachicardia, increased blood pressure, insomnia, irritation, tremors, migraines and urinary problems. The FDA advises that in the case of cardiac problems, hypertension and/or urinary problems a physician should be consulted prior to use of synephrine containing nasal decongestants.

The slimming products based on *Citrus aurantium* or on synephrine can cause heavy cardiovascular adverse effects which manifest with tachicardia, cardiac arrest, fibrillation and collapse.

Between January 1st, 1998 and February 24th, 2004, the Health Canada (Canadian Ministry of Public Health) has received 16 reports of cardiovascular adverse effects shown in subjects that had ingested products based on bitter orange or on

synephrine. All cases were of remarkable gravity. In seven cases, the supplements were containing also caffeine, while in eight cases ephedrine was also present. One case involved a patient who had ingested products based on bitter orange without caffeine or ephedrine. Two of the indicated patients have died.
Based on these cases it is difficult to evaluate the danger of synephrine or of the *Citrus aurantium* since there was insufficient information on the quantity and on the composition of the ingested mixture [18].
There are many case reports of adverse effects associated with ingestion of supplements containing bitter orange or synephrine described in the scientific literature.
One of the described cases refers to a 55-year-old woman hospitalized because of an acute infarct following the ingestion of a slimming product based on bitter orange and caffeine [19]. An other described case is of a 38-year old patient hospitalized because of an acute ischemic attack following the ingestion of dietetic products which contained synephrine [20].
Finally, there is the case of a 22-year-old girl who, as a consequence of the ingestion of a product based on *Citrus aurantium*, has presented with a major syncope with the a prolongation of the QT interval in the electrocardiogram [21].
Nonetheless, it is important to underline that in neither of these cases was the association between the ingestion of *Citrus aurantium* extracts and adverse effects, established with certitude [22].
In the United States of America, following the banning of ephedra in the food supplements, the FDA has focused on the preparations based on *Citrus aurantium*, which replaced ephedra in the weight loss products. Correlating adverse reactions to the use of *Citrus aurantium* has been contested in a counter-analysis done by American Herbal Products Association (AHPA), the company that in the United States of America represents the herbal industries [23].

Pharmacological interactions

In vitro, the bitter orange, because of the presence of furanocumarine, is able to inhibit the CYP3A4 isoform of the cytochrome P-450 and it can therefore interact with numerous drugs metabolized by this system [24].
Pharmacological interactions have been observed particularly with midazolam and sesquinavir [25]. In addition, studies in animals have demonstrated that orange juice can inhibit the metabolism of cyclosporine [26].
In human, the bitter orange can increase the bioavailability of the calcium channel blocker felodipine [25].
The association between synephrine and the MAO inhibitors can cause acute, serious hypertension.
At last, associating synephrine with phenylephrine, phenylpropanolamine, ephedrine or pseudoephedrine itself can produce an increase of sympatomimetic effects induced by such drugs.

Effects in pregnancy

There is no data regarding the use of *Citrus aurantium* during pregnancy or lactation.

Analytical determinations

Scientific literature reports analytical methodologies to measure active principles of *Citrus aurantium* in blood [27], in urine [28], in dietary supplements [29], in fresh and dried plant fruits [4]. The method for the active principles determination in dietary supplements involves a gas chromatograph coupled with a mass spectrometer [29], while active principles in fresh and dried fruits are determined by liquid-chromatography coupled to ultraviolet spectrophotometric detection [4].

The method below reported is a brief outline useful to the investigator to set up the analysis. For specific details, it is advisable to refer to the original text.

Determination of synephrine in urine after *Citrus aurantium* consumption

(From: KUSU F, MATZUMOTO K, ARAI K, TAKAMURA K. Determination of synephrine enantiomers in food and conjugated synephrine in urine by high-performance liquid chromatography with electrochemical detection. Anal Biochem. 1996; 235: 191-194)[28].

The analysis is carried out on urine samples using liquid chromatography coupled with electrochemical detection.

Extraction of the compounds

1 ml urine acidified to pH 1-2 with 6 N hydrochloric acid is hydrolyzed at 100 ° C for 15 minutes. After hydrolysis, the pH is lowered to 9, and 1 ml hydrolyzed urine is loaded into the Extrelut column and synephrine is eluted with 5 ml ethyl acetate containing 2% n-butanol. This procedure is repeated twice. The organic phase is evaporated to dryness and the residue redissolved in 225 µl water and 25 µl di *l*-phenylephedrine, used as internal standard. A 5 µl volume is injected into the chromatograph.

Analytical conditions

Chromatografic column: Sumichiral OA-6000 (150 x 4,6mm x 5 µm)
Mobile phase: aqueous solution containing 1 mM copper (II) and 20 mM ammonium acetate pH 6.4
Separation: isocratic
Flow rate: 1.5 ml/min
Detector: electrochemical detector
Electrochemical cell potential: 1.0 V

Retention times of the tested compounds

***l*-synephrine:** 9.0 minutes
***d*-synephrine:** 13.5 minutes
***l*-phenylephedrine (internal standard):** 17.0 minutes

Standards

The standard of *dl*-synephrine used in the analysis can be purchased from the Sigma-Aldrich (St. Louis, MO). The internal standard can be obtained by Koyo Tokyo Kasei (Tokyo, Japan).

Calibration curve

A calibration curve in urine was not reported. The method was linear in the range of 1.0 to 500 pmoles of synephrine injected into the instrument.

Results

The authors report the times of excretion of synephrine in urine after administration of 240 to 275 grams of pulp of *Citrus aurantium*. The maximum excretion of synephrine is observed 2-3 hours after administration. At 16 hours after administration, synephrine is no longer detectable in urine.

References

1. FOUGH-BERMAN A, MYERS A. *Citrus aurantium*, an ingredient of dietary supplements marketed for weight loss: current status of clinical and basic research. Exp Biol Med. 2004; 229: 698-704.
2. ALLISON DB, CUTTER G, POEHLMAN ET, MOORE DR, BARNES S. Exactly which synephrine alkaloids does *Citrus aurantium* (bitter orange) contain? Int J Obes. 2005; 29: 443-446
3. THE MERCK INDEX An Enciclopedia of chemicals, drugs, and biologicals. 10th Ed. Merck & Co., Inc. 1983: 971.

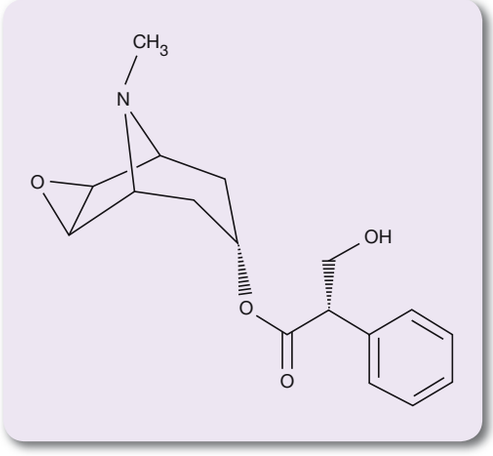
4. PELLATI F, BEVENUTI S, MELEAGRI M, FIRENZUOLI F. Determination of adrenergic agonists from extracts and herbal products of *Citrus aurantium* L. var. amara by LC. J Pharmacol Anal. 2002; 29: 1113-1119.
5. THE MERCK INDEX An Enciclopedia of chemicals, drugs, and biologicals. 10th Ed. Merck & Co., Inc. 1983: 1295.
6. BLUMENTHAL M. Bitter orange peel and synephrine. Part 1 WholeFoods 2004 and Part 2. WholeFoods 2005. ®American Botanical Council.
7. NTP. Bitter orange (*Citrus aurantium* var. amara). Extracts and constituents (±)-p-synephrine [CAS No.94-07-5] and (±)-p-octopamine [CAS No. 104-14-3]. Review of toxicological literature. National Toxicology Program. Jun 2004.
8. ITALIA.Circolare n.3, 18 luglio 2002. Applicazione della procedura di notifica di etichetta di cui all'art.7 del Decreto legislativo n.111/1992, ai prodotti a base di piante e derivati aventi finalità salutistiche. Gazzetta Ufficiale n. 188, 12 agosto 2002.
9. The list of vegetal extracts admitted in dietary supplements in Italy can be found at http://www.salute.gov.it
10. HEALTH CANADA Products containing bitter orange or synephrine: suspected cardiovascular adverse reactions. Canadian Adverse reaction newsletter 2004; 14: 3-4.
11. HOFFMAN BB, TAYLOR P. Neurotransmission. In: Hardman JG, Limbird LE, eds. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 10th ed. New York, NY: McGraw-Hill, 2001: 115-153.
12. D'ANDREA G, TERRAZZINO S, FORTIN D, COCCO P, BALBI T, LEON A. Elusive amines and primary headaches: historical background and prospectives. Neurol Sci. 2003; 24: S65-S67.
13. PENZAK SR, JANN MW, COLD JA, HON YY, DESAI HD, GURLEY BJ. Seville (sour) orange juice: synephrine content and cardiovascular effects in normotensive adults. J Clin Pharmacol. 2001; 41: 1059-1063.
14. CARPENE C, GALITZKY J, FONTANA E, ATGIE C, LAFONTAN M, BERLAN M. Selective activation of beta3-adrenoceptors by octopamine: comparative studies in mammalian fat cells. Naunyn Schmiedebergs Arch Pharmacol. 1999; 359: 310-321.
15. ARAFA HM, ALY HA, ABD-ELLAH MF, EL-REFAEY HM. Hesperidin attenuates benzo[alpha] pyrene-induced testicular toxicity in rats via regulation of oxidant/antioxidant balance. Toxicol Ind Health. 2009; 25: 417-427.
16. CALAPAI G, FIRENZUOLI F, SAITTA A. Antiobesity and cardiovascular toxic effects of Citrus aurantium extracts in the rat: a preliminary report. Fitoterapia. 1999; 70: 586-592.
17. ARBO MD, LARENTIS ER, LINCK VM, ABOY AL, PIMENTEL AL, HENRIQUES AT, DALLEGRAVE E, GARCIA SC, LEAL MB, LIMBERGER RP. Concentrations of p-synephrine in fruits and leaves of Citrus species (Rutaceae) and the acute toxicity testing of Citrus aurantium extract and p-synephrine. Food Chem Toxicol. 2008 Aug; 46: 2770-2775.
18. JORDAN S., MURTY M., PILON K. Products containing bitter orange or synephrine: suspected cardiovascular adverse reactions. CMAJ. 2004 Oct 12; 171: 993-4.
19. NYKAMP DL, FACKIH MN, COMPTON AL. Possible association of acute lateral-wall myocardial infarction and bitter orange supplement. Ann Pharmacother. 2004; 38: 812-816.
20. BOUCHARD NC, HOWLAND MA, GRELLER HA, HOFFMAN RS, NELSON LS. Ischemic stroke associated with use of an ephedra-free dietary supplement containing synephrine. Mayo Clin Proc. 2005; 80: 541-545.
21. NASIR JM, DURNING SJ, FERGUSON M, BAROLD HS, HAIGNEY MC. Exercise-induced syncope associated with QT prolongation and ephedra free Xenadrine. Mayo Clinic Proc. 2004; 79: 1059–62.
22. HALLER CA, BENOWITZ NL, JACOB P 3rd. Hemodynamic effects of ephedra-free weight-loss supplements in humans. Am J Med. 2005; 118: 998-1003.
23. MCGUFFIN M. FDA spins numbers on bitter orange AERs: AHPA analysis finds only 1 actual report associated with bitter orange. AHPA Report 2004; 19: 2-4.
24. GUO L, TANIGUCHI M, CHEN Q et al: Inhibitory potential of herbal medicines on human cytochrome P450-mediated oxidation: Properties of Umbelliferous or Citrus crude drugs and their relative prescriptions. Jpn J Pharmacol 2001; 85: 399-408.
25. MALHOTRA S, BAILEY D, PAINE M et al: Seville orange juice-felodipine interaction: comparison with dilute grapefruit juice and involvement of furocoumarins. Clin Pharmacol Ther 2001; 69: 14-23.
26. HOU YC, HSIU SL, TSAO CW et al: Acute intoxication of cyclosporin caused by coadministration of decoctions of the fruits of Citrus aurantium and the Pericarps of Citrus grandis. Planta Med 2000; 66: 653-655.
27. BEYER J, PETERS FT, KRAEMER T, MAURER HH. Detection and validated quantification of nine herbal phenalkylamines and methcathinone in human blood plasma by LC-MS/MS with electrospray ionization. J Mass Spectrom. 2007; 42: 150-160.
28. KUSU F, MATZUMOTO K, ARAI K, TAKAMURA K. Determination of synephrine enantiomers in food and conjugated synephrine in urine by high-performance liquid chromatography with electrochemical detection. Anal Biochem. 1996; 235: 191-194.
29. MARCHEI E, PICHINI S, PACIFICI R, PELLEGRINI M, ZUCCARO P. A rapid and simple procedure for the determination of synephrine in dietary supplements by gas chromatography-mass spectrometry. J Pharm Biomed Anal. 2006; 41: 1468-1472.

Datura stramonium
(devil's weed)

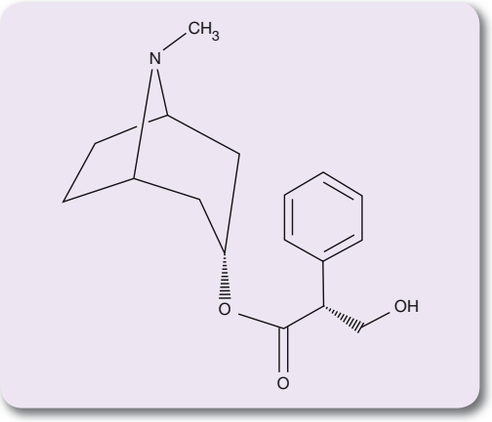
Name: *Datura stramonium*
Family: *Solanaceae*
Genus: *Datura L.*
Species: *Datura stramonium L.*
Synonims: devil's weed, stinkweed
Origin: The plant grows in the subtropical regions and in the temperate climates and is common in America, Asia and Europe. Its origin is unclear. In Italy, this species is found in all the regions, where it grows sporadically in uncultivated grounds, close to damps and at the edge of the roads.
Active compounds: tropane alkaloids (mainly *l*-hyoscyamine, atropine as the racemic mixture of *d* and *l*-hyoscyamine, which is formed by drying the plant and scopolamine).

The tropane alkaloids are found mostly in the plants belonging to the *Atropa*, *Datura* e *Duboisia genera* [1]. The international literature states that hyoscyamine is the principal *Datura stramonium* alkaloid [1,3]. The leaves contain 0.2 to 0.45% of total alkaloids (dry weight), while the seeds approximately 0.2-1.2 % [1,4]. *L*-hyoscyamine and its racemic mixture atropine (*d* and *l*-hyoscyamine) and scopolamine are among the principal natural alkaloids used in the medical field [3]. Atropine is not present in the fresh drug, but it forms by racemization during the process of drying, especially at high temperatures [4]. Classified as anticholinergic, such alkaloids are widely used in ophthalmology for inducing dilation of the pupil and like antispastic and preanaesthetic agents. The alkaloids of natural origin are used as model for the synthesis of artificial alkaloids which are more efficient and with lower toxicity [5]. Homatropine, for example, [6] is prepared synthetically by the esterification of mandelic acid with 3α-tropine. Its effects are superimposable to those of the atropine although they are ten times less pronounced. Hyoscyamine and scopolamine, whose anticholinergic peripheral effects are qualitatively identical, are different at the central nervous system level. At this level, atropine and *l*-hyoscyamine can induce changes only at toxic doses, with strong cortical excitement. In contrast, scopolamine has a very powerful sedative and narcotic effect in humans [4].

Chemical formula and physico-chemical properties of the active compounds [7]



Name: Scopolamine.
Molecular formula: $C_{17}H_{21}NO_4$ (molecular weight = 303.4).
Systematic name: acido [7(S)-(1α,2β,4β,5α,7β)]-α-(hydroxymethyl) benzoylacetic acid 9-methyl-3-oxa-9-aza-triciclo-[3.3.1.02,4]non-7-l-estere.
CAS registry number: 51-34-3.
Melting point: 59°C.
UVmax: 246, 252, 258 nm.
Solubility: warm water, alcohol, ether, chloroform, acetone.



Name: Hyoscyamine.
Molecular formula: $C_{17}H_{23}NO_3$ (molecular weight = 289.4).
Systematic name: α-(hydroxymethyl)-,(3-endo)-8-methyl-8-azabicyclo (3.2.1)oct-3-yl,ester,(αS)-benzoylacetic acid.
CAS registry number: 101-31-5.
Melting point: 108,5°C.
UVmax: 252, 258, 264 nm.
Solubility: alcohol and dilute acids.

Name: Atropine.
Molecular formula: $C_{17}H_{23}NO_3$ (molecular weight = 289.4).
Systematic name: (1R,5S)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl) 3-hydroxy-2-phenylpropanoate.
CAS registry number: 51-55-8.
Melting point: 108,5°C.
UVmax: 258 nm (methyl alcohol).
Solubility: alcohol and dilute acids

Historical use

The name *Datura* derives from the name of a poison, the *dhât*, prepared in India from the native daturas. The plants of the genus *Datura* were well-known to the Arabs, Greeks, Indians and the populations of Central America, but they were used only to prepare intoxicating and narcotic potions, and not for therapeutic reasons. The plant is not mentioned in the ancient European medical textbooks. It started to be used for therapeutic purposes only towards the end of 1700 [4]. The seeds of the plant were used by magicians for their narcotic properties, for their visual hallucination causing properties and for their presumed aphrodisiac power. Together with the *belladonna* and the *henbane* (*Hyoscyamus niger*), *stramonium* was contributing to the aberrant effect of poisoning that was described in the meetings of the witches during sabbats reported in *Malleus Maleficarum* (the hammer of witches) drafted in 1486 by the Dominican friars Jacob Sprenger and Heinrich Institor Kramer and in the *Compendium maleficarum* of Francesco Mario Guazzo of 1608.

Current use

Stramonium is called also “the grass of the witches” because in the Middle Ages it was thought that its hallucinogenic vision causing properties were used in the potions prepared by the witches during sabbats. *Stramonium* picks the curiosity of people looking for strong emotions similar to those produced by hallucinogenic mushrooms widespread in Central and South America and among the initiates of religious and satanic cults. It is used more and more frequently by youngsters in search of strong sensations, who smoke the *Stramonium* leaves or eat the flowers or the seeds in order to experience their hallucinogenic effects. Such a practice is very dangerous with sometimes negative consequences throughout the whole life.

Legislation

Among the European Community countries, in Norway and in England, *Datura stramonium* can be bought, sold and possessed legally. In Canada and in USA the plant is not controlled, meaning that it is legal to cultivate, possess, buy and sell parts of the plant or the plant extracts. In Italy neither scopolamine, hyoscyamine, atropine, nor the whole plant or parts of it are included in the tables containing narcotic and psychotropic substances subject to the supervision and control under Article 14 of Republic Presidential Decree 309/90 and subsequent updates.

Pharmaco-toxicological properties

Hyoscyamine is an anticholinergic, antimuscarinic compound, which works by blocking acethylcholine action at the peripheral level of the parasympathetic nervous system (smooth musculature) and at the central nervous system level. This affects mainly the antagonism of muscarinic receptors, less than of nicotinic ones, and at the ganglia and neuromuscolar ends level. Parasympaticolytic effects:

1) spasmolytic effect (smooth musculature);
2) midriasis and paralysis of the visual accommodation;
3) decrease of the secretory activity of the exocrine glands;
4) tachicardia;
5) suppression of nausea and vomiting.

Atropine, the racemic mixture of hyoscyamine, is the ester of tropic acid with tropane: the alkaloid found naturally is the (-)-hyoscyamine. Atropine is formed by the racemisation at the chiral carbon atom of the tropic acid during the isolation process. While the dextrogire isomer of the atropine is inactive, the levogire (-)-hyoscyamine is about twice as potent as atropine. Its action and/or uses are similar to those of antimuscarinic compounds, with exception that hyoscyamine, differently from atropine, is not used in ophtalmology but almost exclusively like an antispasmodic medicine [9,10].

About 80-90% of the atropine is excreted in the urine in 24 hours: 50% unchanged, 2% as tropic acid and tropine, and about 30% as unidentified metabolites [11].

Atropine is the generic treatment used against the poisoning with nervine gases; in fact, atropine inhibits the effect of the acetylcholine at the cholinergic receptor level limiting the poisonous effect of the nervine gas. For this reason the soldiers who operate in environments potentially at risk of contamination with nervine gases receive atropine syringes for immediate autoadministration.

Scopolamine is the common name given to (-)-hyoscine, the natural alkaloid of *Datura stramonium*. Similar to hyoscyamine, scopolamine is an anticholinergic compound and its action is acethylcholine- competitive at the muscarinic receptor level. For this reason, it seems that scopolamine is able to correct the acethylcholine and noradrenaline imbalances which may occur in certain motor disorders [12].

The oral administration of 0.90 mg of scopolamine in a healthy individual result in a peak plasma level of about 2 ng/ml within an hour. It reversibly binds to plasma proteins and is able to cross the blood-brain barrier. Similarly, it crosses the placenta and concentrates in the maternal milk. Although the metabolic pathway and excretion pattern of scopolamine is not entirely clear, it is believed that the molecule should be almost completely metabolized (by conjugation) in the liver and excreted in the urine [12].

Toxicity

An uncontrolled ingestion of *Datura stramonium* can have major consequences, especially if the ingestion occurs together with alcohol or psychotropic agents. It is possible to go from hallucinations to delirium, convulsions, major visual disturbances, up to coma by cerebral anoxia (lack of oxygen to the brain) and to death.

The toxic dose of atropine is variable and it depends on the individual sensitivity. There are cases of death for doses of 50-100 mg and cases of recovery with doses of 1 g. Doses equal to 10 mg can be fatal for children and for the sensitive individuals [6].

The toxicity induced by the scopolamine, characterised by a classic anticholinergic syndrome, usually derives from ac-

cidental ingestion of adulterated products or of plants containing the substance in question (in this case the *Datura stramonium*). The classic manifestations occuring as a result of scopolamine ingestion include hallucinations and urinary incontinence [13]. A deficit in the cognitive and motor processes induced by scopolamine has been widely demonstrated in the animal and human models, although it is not entirely clear what role acethylcholine has in the mnemonic processes [14]. There are poisoning cases described in the literature, induced by scopolamine alone or in association with other drugs of abuse (for example heroin). The manifestations of an overdose with such drugs (apparently "cut" with scopolamine) include lethargy, agitation, hallucinations, paranoia, tachicardia, moderate hypertension, dry skin, urinary retention, etc [12]. In the Spring 1995 a great number of intoxications presented in the Emergency Departments of several New York hospitals following ingestion of heroin cut with scopolamine, showing symptoms of a severe anticholinergic poisoning. A 90 % of the treated subjects needed hospital admission with half of them being treated in the respective intensive care unit [15]. There is also the case of two old women treated in the hospital for symptoms characteristic to an anticholinergic syndrome (agitation, confusion, urinary retention, dry mouth, midriasis) which appeared approximately 3 hours after the ingestion of dried *Datura stramonium* seeds. The two women, following 5 days of hospitalization with a conservative pharmacological therapy, have been discharged with the complete resolution of the symptomatology [16].
A case of anisocoria (unilateral midriasis) has been described in an 11-year old child following simple contact with Angel's Trumpet. The midriasis resolved without further complications 48 hours after the exposure [17].

Data related to acute toxicity of hyoscyamine [18]
In mouse- LD50 following intravenous administration: 95 mg/kg
In human - TDLo: 1.471 mg/kg
In human probable LD following oral dose is of 5 mg/Kg [19]

Data related to acute toxicity of scopolamine [18]
In mouse- LD50 following oral administration: 1275 mg/kg
In mouse - LD50 following subcutaneous administration: 1700 mg/kg
In human - TDLo following intramuscular administration: 0.004 mg/Kg
In human - TDLo following subcutaneous administration: 0.002 mg/Kg

Data related to acute toxicity of atropine [18]
In mouse - LD50 following oral administration: 75 mg/kg
In mouse - LD50 following subcutaneous administration: 428 mg/kg
In human - TDLo following intramuscular administration: 0.001 mg/Kg
In human - TDLo following subcutaneous administration: 0.033 mg/Kg

Adverse Effects

Adverse effects ensuing from the ingestion of the plant include tachicardia, dry mouth, dilated pupils, obscured vision, hallucinations, confusion, aggressive behaviour, difficulty to urinate. A higher degree of toxicity has been associated with coma and tremors, but death is rare as reported in a case involving nine teenagers intoxicated by the ingestion of *Datura stramonium* [20].

Pharmacological interactions

In mouse, hyoscyamine (0.3 mg/Kg) inhibits salivation with cholinergic and adrenergic drugs without influencing the diurnal variation of body temperature. When given 30 minutes before the administration of an adrenergic sialagogue, it does not inhibit the salivation induced by d-anphetamine, but reduces the salivation induced by isopreterenol bitartrate [21]. Scopolamine should be used carefully in patients who take other drugs that act on the central nervous system (sedatives, tranquillizers, alcohol). Particular attention should be paid to the drugs that possess anticholinergic activity, such as antihistamines, triciclic antidepressants, muscle relaxants [22].

Effects in pregnancy

A study done on chicken embrios has evaluated the effects of a mixture of scopolamine and hyoscyamine on the development of these embryos. The same dose of the mixture was administered to the embryos in two different development phases, by an injection in the yolk sack. This treatment has caused the death of the embryos at different stages of growth , as well as the development of several malformations. The results prove that the injection of scopolamine/hyoscyamine mixture in the yolk sack has teratogenic effects in this animal model [23].

For the American Food and Drug Administration (FDA) the risk of taking scopolamine and hyoscyamine in pregnancy is C, meaning that the fetal health could be affected if taken during the gestational period even though the studies have been done only in animal models not in humans [23].

Analytical determinations

Scientific literature reports analytical methodologies to measure active principles of *Datura stramonium* both in serum and in urine [24] and in the root, leaves, seeds and tree branch [1,3]. In case of plant parts determination, gas chromatography [1] or liquid chromatography [3] coupled to mass spectrometry are used.

Please refer to the monograph of *Brugmansia arborea* for analytical details of the determination of hyoscyamine and scopolamine in serum and urine samples of plant consumers [24].

References

1. MIRALDI E, MASTI A, FERRI S, BARNI COMPARINI I. Distribution of hyoscyamine and scopolamine in Datura stramonium. Fitoterapia. 2001; 72: 644-648.
2. LOUNASMAA M, TAMMINEN T. IN: G.A. CORDELL (Ed.), The Alkaloids, vol. 44, Academic Press, New York, 1993, pp. 1–114.
3. STEENKAMP PA, HARDING NM, VAN HEERDEN FR, VAN WYK BE. Fatal Datura poisoning: identification of atropine and scopolamine by high performance liquid chromatography/photodiode array/mass spectrometry. Forensic Sci Int. 2004; 145: 31-39

4. FASSINA G. Lezioni di farmacognosia. Droghe vegetali. Antonio Milani (Ed.), 1974: 265-266.
5. MATEUS L, CHERKAOUI S, CHRISTEN P, VEUTHEY JL. Capillary electrophoresis for the analysis of tropane alkaloids: pharmaceutical and phytochemical applications. J Pharm Biomed Anal. 1998; 18: 815-25.
6. Goodman and Gilman's, in: J.G. Hardman, L.E. Limbird (Eds.), The Pharmacological Basis of Therapeutics, 9th ed., McGraw-Hill, New York, 1995, pp 141–160.
7. THE MERCK INDEX An Enciclopedia of chemicals, drugs, and biologicals. 10th Ed. Merck & Co., Inc. 1983
8. FOYE WO, LEMKE TL, WILLIAMS DA. Principi di chimica farmaceutica. Piccin (ed) III Edizione.
9. OSOL A (ed.). Remington's Pharmaceutical sciences. 16th ed. Easton, Pennsylvania: Mack Publishing Co., 1980, p. 856.
10. WERNER G. Metabolism of tropane alkalods. V. Enzymic preparation of (+-)-hyosciamine sulfate hydrate. Arzneim-forsch (1967); 17: 1467.
11. Clarke's isolation and identification of drugs. The pharmaceutical press (ed.) 1986: 364.
12. American Society of Health System Pharmacists. AHFS Drug Information 2008. Bethesda, Maryland 2008, pp.1298-1323.
13. DART RC. (ed). Medical Toxicology. Third Edition, Lippincott Williams & Wilkins. Philadelphia, PA. 2004, p. 564.
14. THOMAS E, SNYDER PJ, PIETRZAK RH, JACKSON CE, BEDNAR M, MARUFF P. Specific impairments in visuospatial working and short-term memory following low-dose scopolamine challenge in healthy older adults. Neuropsychologia 2008; 46: 2476-2484.
15. HAMILTON RJ, PERRONE J, HOFFMAN R, HENRETIG FM, KARKEVANDIAN EH, MARCUS S, SHIH RD, BLOK B, NORDENHOLZ K. A descriptive study of an epidemic of poisoning caused by heroin adulterated with scopolamine. J Toxicol Clin Toxicol. 2000; 38(6): 597-608.
16. SUK SH, KWAK YT. Toxic encephalopathy after taking dried seeds of Datura stramonium in two elderly subjects. Geriatr Gerontol Int. 2009;9:326-328.
17. ANDREOLA B, PIOVAN A, DA DALT L, FILIPPINI R, CAPPELLETTI E. Unilateral mydriasis due to Angel's trumpet. Clin Toxicol (Phila). 2008 ;46:329-31.
18. http://toxnet.nlm.nih.gov/
19. GOSSELIN RE, HODGE HC, SMITH RP, GLEASON MN. Clinical toxicology of commercial products. 4th ed. Baltimore: Williams and Wilkins, 1976., p. 2-157.
20. DEWITT MS, SWAIN R, GIBSON LB JR. The dangers of jimson weed and its abuse by teenagers in the Kanawha Valley of West Virginia. W V Med J. 1997; 93: 182-185.
21. KOPPANYI T, MALING HM. Salivation in mice as an index of adrenergic activity. II. The effects of atropines and ganglionic blocking agents on adrenergic salivation and temperature responses in mice. Arch Int Pharmacodyn Ther. 1972; 199: 333-43.
22. Thomson Health care Inc.; Physicians' Desk Reference 62 ed., Montvale, NJ 2008, pp. 2192-2193.
23. MAGRAS IN, KOTSAKI-KOVATSI VP, KOVATSIS A, ADAMIDOU L. Teratogenic effects of a mixture of scopolamine and hyoscyamine in chick embryos. Vet Hum Toxicol. 1993; 35: 434-5.
24. NAMERAA A, YASHIKIA M, HIROSEB Y, YAMAJIC S, TANIC T, KOJIMA T. Quantitative analysis of tropane alkaloids in biological materials by gas chromatography–mass spectrometry. Forensic Sci Int. 2002: 130; 34-43.

Ephedra sinica
(Ma huang)

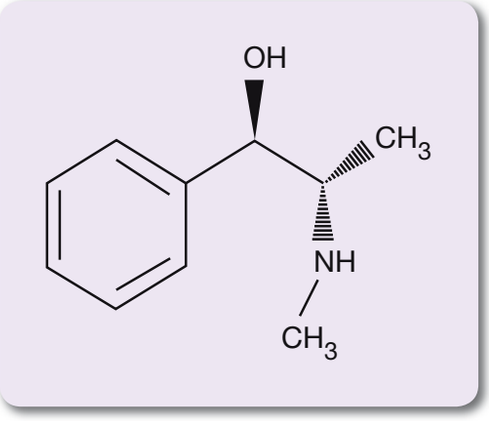
Name: *Ephedra sinica*
Family: *Ephedraceae*
Genus: *Ephedra L.*
Species: *Ephedra sinica Stapf.*
Synonyms: mao, ma-huang
Origin: Asia (China, Korea, Japan).
Active compounds: ephedrine, *d*-pseudoephedrine, N-methylephedrine, N-methylpseudo-ephedrine, norpseudoephedrine and the norephedrine (phenylpropanolamine)

Ephedrine, the principal alkaloid of the plant, is a white, crystalline solid, with bitter taste and slightly aromatic smell. Ephedrine and its optic isomer, pseudoephedrine, are structurally quite similar to methamphetamine and to dobutamine. The clandestine laboratories, in which amphetamine and amphetamine derivatives are illicitly synthesized use a simple dehydrogenation to obtain methamphetamine from ephedrine. Ephedrine contains two asymmetrical carbon atoms; only *l*-ephedrine and the racemic ephedrine are used in clinical practice.

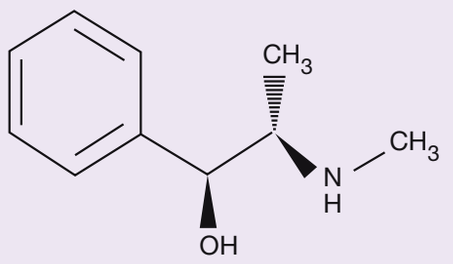
Different kinds of *Ephedra* contain variable percentages (between 0.02% and 3.4%) of the above-reported six alkaloids concentrated essentially at the level of the internodes of the stems of the trees. Ephedrine is the most abundant alkaloid (50-85% of the alkaloids contained in the dried grass), followed by *d*-pseudoephedrine (~25%) and a smaller percentage of norephedrine, norpseudoephedrine, N-methylephedrine, N-methylpseudoephedrine. Glycans (ephedran A-E), volatile oils (limonene, caryophyllene, phellandrene and others) are also present, as well as small quantities of saponins, catechines and tannins [(1)].

Norephedrine is structurally identical to the phenylpropanolamine, a synthetic molecule used in the past for weight loss and as decongestant of the nasal mucous membrane, until several studies have demonstrated the increased risk of stroke after treatment with this substance. Subsequently the pharmaceutical companies were forced into voluntary withdrawal from the market of all the products based on phenylpropanolamine. This substance is a racemic mixture of norephedrine: in fact the *Ephedra sinica* contains only the (-) isomer. Phenylpropanolamine seems to have important pharmacodynamic interactions with caffeine, so much so that since 1983 the Food and Drug Administration in USA has banned the combination between the two molecules [(2)].

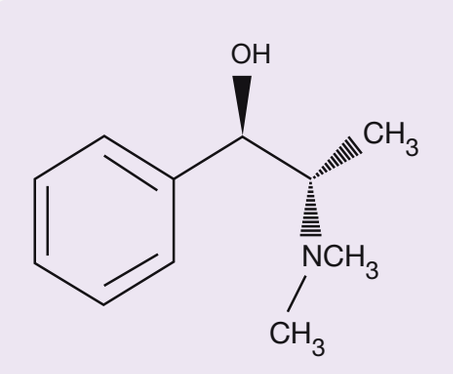
Chemical formula and physico-chemical properties of the active compounds



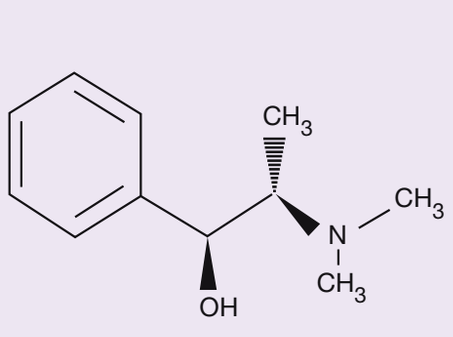
Name: Ephedrine.
Molecular Formula: $C_{10}H_{15}NO$ (molecular weight = 165.2).
Systematic name: (1R,2S)-2-methylamino-1-phenylpropanol.
CAS registry number: 299-42-3.
Melting point: 38°C.
UVmax: 251 nm.
Solubility: water, alcohol, chloroform, ether, glycerol and liquid paraffin.



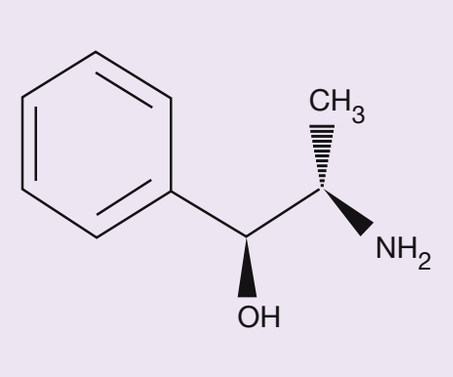
Name: Pseudoephedrine.
Molecular Formula: $C_{10}H_{15}NO$ (molecular weight = 165.2).
Systematic name: (1S,2S)-2-methylamino-1-phenylpropanol.
CAS registry number: 90-82-4.
Melting point: 116-119°C.
UVmax: 251 nm.
Solubility: ethyl alcohol, ether, partially soluble in water.



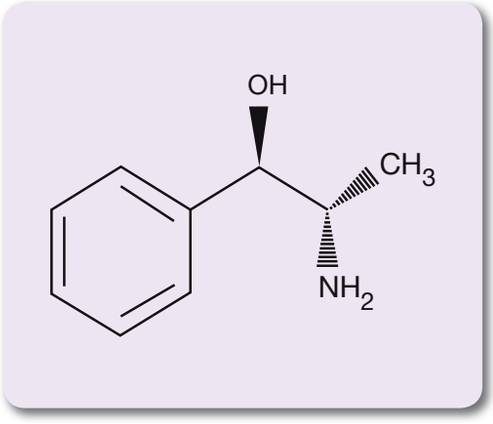
Name: Methylephedrine
Molecular Formula: $C_{11}H_{17}NO$ (molecular weight = 179.3).
Systematic name: (1R,2S)-2-dimethylamino-1-phenylpropanol.
CAS registry number: 17605-71-9.
Melting point: 190°C.
UVmax: 251 nm.
Solubility: chloroform and ether.



Name: Methylpseudoephedrine.
Molecular Formula: $C_{11}H_{17}NO$ (molecular weight = 179.3).
Systematic name: (1S,2S)-2-dimethylamino-1-phenylpropanol.
CAS registry number: 51018-28-1.
Melting point: 190°C.
UVmax: 251 nm.
Solubility: chloroform and ether.



Name: Norpseudoephedrine (cathine).
Molecular Formula: $C_9H_{13}NO$ (molecular weight = 151.2).
Systematic name: (1S,2S)-2-amino-1-phenylpropanol.
CAS registry number: 36393-56-3.
Melting point: 75-78°C.
UVmax: 251 nm.
Solubility: alcohol, chloroform and ether.



Name: Norephedrine (phenylpropanolamine).
Molecular Formula: $C_9H_{13}NO$ (molecular weight = 151.2).
Systematic name: (1R,2S)-2-amino-1-phenylpropanol.
CAS registry number: 14838-15-4.
Melting point: 101°C.
UVmax: 251 nm.
Solubility: alcohol, chloroform and ether.

Historical use

The Chinese term *Ma-huang* might be roughly translated into English as "yellow astringent", "yellow equisetum", or, as, "yellow hemp" (the term *huang* means yellow) and it indicates specifically the aerial parts of the *Ephedra sinica*. The Chinese traditional medicine recognises the medicinal properties of the green stalks of the plant, which are dried, boiled in hot water and used as a tea. The recommended dose corresponds to 1.5-9 g of boiled grass per day. Another three *Ephedra* species seem to contain alkaloids, although they are not recognised by the Chinese pharmacopoeia (*Ephedra minuta* Florin, *Ephedra distachya L.*, *Ephedra gerardian*a Wall). *Ephedra gerardiana* has been used for a long time in the Indian traditional medicine. Although in the past China has been the greatest producer of *Ma-huang* in the world, at present India and the Pakistan are recognized as principal producers of the plant [(1)]. In the past ephedrine, the principal alkaloid of the *Ephedra sinica*, has been used in the treatment of the Stokes-Adams syndrome and as stimulant of the central nervous system in narcolepsy and in depression. Ephedrine and its salts have been used in the treatment of mild bronchial asthma and of bronchospasm; nevertheless, at present the most selective (β_2-stimulants) bronchodilators have superseded its use, due to the fact that ephedrine acts also as a stimulator of the α and β_1 adrenergic receptors. Ephedrine has been also used to counteract urinary incontinence, although its efficacy has not been clearly demonstrated. In fact, ephedrine causes urinary retention, especially in males with benign prostate hyperplasia. It has been also used in the treatment of the arterial hypotension following spinal anaesthesia [(3,4)].

Current use

The majority of the dietary supplements containing *Ephedra sinica*, are marketed with the indication of helping weight loss or to improve athletic performances of the users. Often such supplements are sold together with natural sources of caffeine (*Paullinia cupana* or guaranà and *Cola nitida* or kolanut), to the purpose of increasing the effects of the ephedrine and to obtain a combination of "exciting drugs" used for example in the disco scene. A typical quantity of caffeine found in the "mix" of grasses varies between 40 and the 200 mg [(2)]. It is estimated that, only in 1999, 12 million individuals, in the United States of America, have used 3 milliard doses of Ephedra alkaloids [(5)].

In the Italian smart-shops the products based on *Ephedra* are extremely heterogeneous with respect to the content of active compounds and for the association with other herbal extracts containing pharmacologically active molecules (caffeine, theobromine, theophylline). A great number of the commercial products contain Ephedra together with kola nut (caffeine), *Sida cordifolia* (ephedrine), guaranà (caffeine), ginseng (ginsenosides), damiana (damianine), yohimbe (yohimbine). Such associations can potentiate the effects of the individual compounds of the mixture. Indeed, the herbal products based on ephedrine are often called by the general name of "*herbal ecstasy*". This substance is in fact largely used as a sympathomimetic for stimulant effects, and for its lipolitic properties in diets in association with other substances such as caffeine and acetylsalicylic acid. The consumption of uncontrolled quantities of these preparations can result in the ingestion of dangerous amounts of the active compounds, particularly ephedrine.

Legislation

In Italy neither ephedrine, nor pseudoephedrine, or methylephedrine, methylpseudoephedrine or norephedrine, as the whole plant or parts of it are included in the tables containing narcotic or psychotropic substances subject to the supervision and control under Article 14 of Republic Presidential Decree 309/90 and subsequent updates. However, norpseudoephedrine (or cathine) is included in the above-mentioned tables. The Italian Ministry of Health included *Ephedra sinica* in the list of vegetal extracts non admitted in dietary supplements [6].

Ephedrine, pseudoephedrine and norephedrine are still included into the category 1 of the Annex I for the classified substances in the decree No 258 of April 12th, 1996 (Official Bulletin n.112 of May 15th, 1996) regarding the directive 92/109/EEC relative to the illicit manufacturing and marketing of certain drugs and psychotropic substances. Such an annex is present in the updated text of the Presidential Decree 309/90 (Official Bulletin n. 62 of March 15th, 2006). Ephedrine, pseudoephedrine and methylephedrine are included in the list of biologically or pharmacologically active substances which, according to the article 1 of the law No 376/00: "Discipline of the medical supervision of sporting activities and of the fight against doping" published in the Official Bulletin n.294 of December 18th, 2000. Such a list has been approved by the Ministerial Decree in October 15th, 2002 and published in Official Bulletin n. 278 in November 27th, 2002.

In addition, this substance is added to the list of prohibited substances published by the World Anti-Doping Agency (WADA). An urine sample is considered positive in a drug test when the concentration of ephedrine is equal or higher than 10 μg/ml [7].

Due to the scientific evidence showing heavy side effects on the cardiovascular system and central nervous system caused by the herbal preparations based on ephedrine and related alkaloids [8], recently, the Food and Drug Administration (FDA) has concluded that the above-mentioned dietary supplements constitute short and long term health hazards. Because of this, the FDA has decided that the dietary supplements based on ephedrine must be considered adulterated and decided to ban all products that contain derivatives of ephedrine [9,10].

Pharmaco-toxicological properties

The pharmacological properties of the Ephedra are due to the presence of ephedrine, pseudoephedrine and other structurally related alkaloids. Ephedrine and pseudoephedrine are simpathomimetic agents with direct or indirect agonist activity towards the α and β-adrenergic receptors and stimulating the central nervous system [11,12].

Ephedrine can be inhaled (ephedrine salts are used as nasal decongestants) and is well absorbed also through the skin when is used as an ointment. Ophtalmic ephedrine drops at 0.1% are effective for the treatment of the allergic conjunctivitis. Ephedrine drops at 0.1% applied to the eyes are effective for the treatment of allergic conjunctivitis. When the ephedrine is orally administered for its bronchodilatatory and decongestant effects, the average dose is 25-50 mg/kg/day to be repeated if necessary every 3-4 hours. The daily total dose should not to exceed, in any case, 150 mg. The pediatric dose is of 2-3 mg/kg/day of body weight or 100 mg/m^2/day dof body surface, subdivided in 4-6 doses [8]. In case of parenteral administration, the minimum effective dose (12.5-25 mg) must be injected.

After oral administration ephedrine is quickly and completely absorbed in the intestine. Once absorbed, it reaches peak plasma concentration an hour after administration and has a high volume of distribution. The plasma half life of the compound varies between 3 to 6 hours depending on the urinary pH. The pharmacological effects last for about 1 hour. Ephedrine and the related compounds are lipophilic and can cross the blood-brain barrier interacting with the central nervous system. Only a small proportion of ephedrine is metabolized by the liver; the main reactions in the metabolism are N-demethylation (8-20%) and deamination (4-13%). A greater proportion of ephedrine (about 53-74%) is instead excreted unchanged in the urine. Because of the presence of an ionizable amino group, the urinary excretion is favoured by the acidic pH of urine [4,5,13].

Ephedrine is a powerful stimulant of the central nervous system; it is used as a central anorectic in slimming products and for the treatment of narcolepsy and depression [4].

At cardiovascular level it increases the strength of of the heart contractions, increases the cardiac output and peripheral vasoconstriction. This is translated in an increase of the systolic blood pressure rather than the diastolic blood pressure [14].

The alkaloid, stimulating the adrenergic receptors induces relaxation of the bronchial smooth muscle, reduces the intestinal motility, produces bladder relaxation and reduces uterine activity.
In mice, ephedrine and pseudoephedrine have an anti-inflammatory effect in the case of carragenine induced edema [15]. In addition, *Ephedra sinica* extract *in vitro* has the ability of inhibiting the classic activation pathway of the complement [16] as well as an antibacterial effect towards the *Staphylococcus aureus* [17], a cytotoxic effect towards the hepatoblastoma HepG2 and the neuroblastoma Neuro-2a [18], and a hepatoprotective effect against the citotoxicity induced by carbon tetrachloride [19].

Toxicity

Some authors suggest that the neurotoxic potential of the ephedra extracts is greater compared to that of the synthetic ephedrine, probably because of the synergistic effect exercised by the alkaloid fraction of the extract or for the presence in the plant of other still unidentified active compounds [18,20,21].
A study has demonstrated that the use of dietary supplements based on ephedra and caffeine increases the arterial pressure. Particularly it has been observed that the ingestion of a single oral dose containing both, ephedrine and caffeine (20 mg and 200 mg respectively) has caused an increase in the systolic blood pressure equal to 14 mm of Hg and a 6 mm of Hg in the diastolic blood pressure [2].
In another study on healthy subjects, using a placebo group, the blood pressure variations have been studied following the oral administration of ephedrine (0.1 mg/kg), caffeine (4 mg/kg) and the two substances together. For the caffeine, compared to placebo, an increase of the arterial blood pressure between 3 and 6 mm of Hg has been observed, for the ephedrine the increase in blood pressure was 12 mm of Hg. Finally, the association of the two substances has caused an increase of the pressure, compared to the placebo, of 15 mm of Hg [22,23].

Data regarding the acute toxicity of ephedrine [24]
In human - LDLo: 9 mg/kg
In mouse - LD50 following intraperitoneal administration: 350 mg/kg
In mouse - LD50 following intravenuous administration: 74 mg/kg

Data regarding the acute toxicity of pseudoephedrine [25]
In human - LDLo: 9 mg/kg
In human - TDLo: 64 mg/kg

Data regarding the acute toxicity of norephedrine
In children - TDLo: 0.938 mg/kg
In neonate - TDLo: 1.25 mg/kg
In human - TDLo: 9 mg/kg

Adverse Effects

The most commont central nervous system adverse effects associated with the use of ephedrine are: tremors, feelings of anxiety and of confusion, restlessness, insomnia and psychotic states; in cases of overdose paranoiac psychoses and hallucinations can be observed [11].
At cardiovascular level the ephedrine can induce arterial hypertension, vasoconstriction, tachycardia, palpitations, myocardial ischemia and cardiac arrest [26]. Moreover, the alkaloid can predispose to an ischaemic or hemorrhagic stroke [27].
The case of a 35-year-old woman having bronchospasms and cardiomiopathy as a result of chronic use of elevated doses of ephedrine is described [28].
As a consequence of repeated use, tachyphylaxis (reduction of the efficiency to the total loss of effect) may develop. Overdose of ephedrine manifests by nausea and vomiting followed by headache, agitation, anxiety, tremors, tachicardia and hypertension. The excessive increase in the blood pressure can lead to cerebral hemorrhage and to myocardial infarction. As a consequence of ventricular arrhythmias, it is possible to have cardiac arrest and death.

Ephedrine is contraindicated in case of hypertension, hyperthyroidism, pheochromocytoma and acute narrow-angle glaucoma. Patients suffering of prostatics hypertrophy or renal insufficiency, should take the substance with caution [2,4,29,30].
Recently, a meta-analysis of the clinical studies and data from clinical trials and from the system of reporting adverse reactions to the FDA, on the effects of preparations based on *Ephedra sinica* or ephedrine, used for slimming purpose or to improve athletic performance, has demonstrated that the use of *Ephedra sinica* or of ephedrine in association to caffeine increases the risk of cardiac arrhythmias and of gastrointestinal, psychiatric and autonomous nervous system disorders [31].
Regarding the manifestations of adverse events following the ingestion of ephedra based ditary supplements, it is worth mentioning that in 1998, in America more than 800 cases of collateral effects including psychosis, cardiac attacks and ictus have been reported [18]. The database of the Metabolife International, one of the biggest distributors of dietetic dietary supplements based on ephedra, contained about 2000 adverse effects after administration of these dietary supplements. Among other occurrences, there have been: 3 cases of death, 20 cases of cardiac attack, 24 cases of ictus, 465 episodes of thoracic pain and 966 cases of cardiac rhythm problems.
Also 46 cases of psychiatric syndromes requiring hospitalization and 82 cases in which emergency intervention has turned out to be necessary have been highlighted. The Metabolife report has also shown that 96% of the gross adverse reactions (stroke, cardiac attack, etc) occurred following therapeutic doses. In the cases where the age of the people who suffered adverse reactions was known, it emerged that 50% of the consumers were not older than 35 years and the majority of them were up to that moment in good health [31].
In literature it was reported a case of ischemic colitis with abdominal pain associated with bloody diarrhea in a man of 40 years likely due to *Ephedra sinica* [32].

Pharmacological interactions

Ephedrine can interact with the monoaminooxidase (MAO) inhibitors causing an increase in the noradrenaline levels with subsequent increase of the sympathetic tone. The interaction may cause headaches, fever, arrhythmias and hypertensive crisis. Therefore, ephedrine should not be taken by patients trated with with MAO inhibitors or patients who have stopped such treatment for less than 14 days [33,34].
Ephedrine can reduce the pharmacological efficacy of the antihypertensive medications [35]; associated with clonidine it can cause increase noradrenaline and arterial blood pressure [36].
Associated with non steroidal antiinflamatory drugs (NSAIDs), ephedrine may facilitate the occurence of lesions in the gastric mucosa [37]. Besides, it can increase the metabolism of the corticosteroids reducing their plasma levels. Asthmatic patients in treatment with such drugs should therefore avoid taking ephedrine based products [38].
The urinary excretion of ephedrine is pH-dependent. The medications listed below are able to alkalinize the urine and, as a result slow down the elimination of ephedrine [39]:

- Acetazolamide
- Antacids
- Ammonium chloride
- Sodium bicarbonate

A greater risk of adverse events of cardiovascular type (hypertension, tachicardia or cardiac arrhythmias) has been observed following the concomitant administration of ephedrine and the following drugs:

- Digoxin [34]
- Cyclopropanol [40]
- Phenylpropanolamine [41]
- Pseudoephedrine

Reserpine causing depletion of noradrenaline, can reduce the efficacy of ephedrine [42]. Theophyilline can cause a higher incidence of the central and gastrointestinal adverse effects (restlessness, insomnia and nausea) than the ones produced by ephedrine administration alone [43].

Finally, the association between ephedrine and caffeine can increase the sympatomimetic effects of the ephedrine and cause tachycardia, hypertension, ictus and cardiac arrhythmias. The concomitant use of this two substances should therefore be avoided [27].

Effects in pregnancy

Ephedrine can cross the placenta and also pass into the breast milk. The ingestion of the substance during pregnancy can cause in the foetus hyperactivity, irritability and tachycardia. For these reasons the FDA has assigned the products based on ephedra to the category 2c: not to use in pregnancy and/or during breastfeeding [44].

Analytical determinations

Scientific literature reports analytical methodologies to measure active principles of *Ephedra Sinica* both in plasma [45] and dietary supplements [46]. This latter method implies gas chromatography coupled to mass spectrometry [46].

The method below reported is a brief outline useful to the investigator to set up the analysis. For specific details, it is advisable to refer to the original text.

Determination of ephedrine, norephedrine, norpseudoephedrine, pseudoephedrine, methylephedrine, methylpseudoephedrine in plasma of consumers

(From: BEYER J, PETERS FT, KRAEMER T, MAURER HH. Detection and validated quantification of nine herbal phenalkylamines and methcathinone in human blood plasma by LC-MS/MS with electrospray ionization. J Mass Spectrom. 2007; 42: 150-60) [45].

The analysis is carried out in plasma by gas chromatography coupled with tandem mass spectrometry.

Extraction of the compounds

One ml plasma is diluted with 2 ml of an aqueous solution of 5 mM ammonium formate (adjusted to pH 3 with formic acid). After centrifugation at 1000 g for 3 minutes, the supernatant is separated and submitted to a solid phase extraction. The analytes are eluted with a solution of methyl alcohol-ammonia (98:2 v / v) then evaporated under nitrogen flow at 56 °C. The residue is reconstituted with 100 µl of 5 mM ammonium formate at pH 3 and 5 µl are injected into the instrument.

Analytical conditions

Chromatography column: Zorbax SCX (150 x 2,1mm x 2,1µm)
Mobile phase A: 5 mM ammonium formate (pH 3)
Mobile phase B: acetonitrile
Separation: gradient (mobile phase B: 5% 0-7 minutes; from 5 to 30% between 7.01-10.0 minutes; at 30% between 10.01 -11.0 minutes; from 30 to 5% between 11.01-17.0 minutes)
Flow: 1.5 ml/min
Detector: mass spectrometer with positive mode electrospray (ESI) interface
Source temperature: 630°C
Collision energy: 27 eV
Capillary voltage: 5500 V

Retention times of the tested compounds

Norephedrine (NE): 3.02 minutes
Norpseudoephedrine (NPE): 3.33 minutes
Ephedrine (EP): 4.55 minutes
Pseudoephedrine (PEP): 5.22 minutes
Methylephedrine (ME): 7.48 minutes
Methylpseudoephedrine (MPE): 8.90 minutes

Characteristic fragments for the tested compounds

Norephedrine (NE): m/z 152 ⟶ 117, 115
Norpseudoephedrine (NPE): m/z 152 ⟶ 117, 115
Ephedrine (EP): m/z 166 ⟶ 117, 91
Pseudoephedrine (PEP): m/z 166 ⟶ 133, 91
Methylephedrine (ME): m/z 180 ⟶ 147, 117
Methylpseudoephedrine (MPE): m/z 180 ⟶ 147, 91

Standards

NE, NPE, EP, PEP, ME and MPE used in the analysis can be purchased by Fluka (Neu-Ulm, Germany).

Calibration curves

Stock standard solutions of each compound were prepared at a concentration of 1 mg/ml using the mobile phase as solvent. The working solutions (0.001, 0.01 and 0.1 mg /ml) of each analyte were prepared by diluting the respective stock solution. Calibration standards (range of concentrations from 10 to 1000 ng/ml) were prepared daily by adding working solutions of known concentration in plasma samples of control.

Results

Table 1 shows plasma concentrations of NE, NPE, EP, PEP, ME and MPE after administration in healthy volunteers of aqueous extract of *Herba Ephedra*-based products and anti cold drugs containing PEP and EP.

Table 1. Plasma concentrations (ng/ml) of norephedrine (NE), norpseudoephedrine (NPE), ephedrine (EP), pseudoephedrine (PEP), methylephedrine (ME) and methylpseudoephedrine(MPE) after the administration of Herba Ephedra-based products and anti cold drugs [45]

Analyzed sample	NE	NPE	EP	PEP	ME	MPE
Herba Ephedra	< LLOQ	14.9	20.0	16.0	< LLOQ	n.d.
Herba Ephedra	< LLOQ	20.3	25.5	15.8	< LLOQ	< LLOQ
Pseudoephedrine (30 mg)	n.d.	n.d.	n.d.	106.7	n.d.	n.d.
Pseudoephedrine (30 mg)	n.d.	n.d.	n.d.	114.1	n.d.	n.d.
Ephedrine (6.2 mg)	n.d.	n.d.	21.5	n.d.	n.d.	n.d.
Ephedrine (6.2 mg)	n.d.	n.d.	21.6	n.d.	n.d.	n.d.

< LLOQ = lower limit of quantification (10 ng/ml); n.d.: not detected

References

1. ABOURASHED EA, EL-ALFY A, KHAN IA, WALKER L. Ephedra in perspective - a current review. Phytother Res. 2003; 17: 703-712.
2. HALLER CA, JACOB P 3rd, BENOWITZ NL. Pharmacology of ephedra alkaloids and caffeine after single-dose dietary supplement use. Clin Pharmacol Ther. 2002; 71: 421-432.
3. GIOFIL–Banca Dati Sanitaria Farmaceutica.
4. http://ssn.giofil.it/protected/ite/LTUPHTHG.htm
5. GOODMAN AND GILMAN'S - The pharmacological basis of therapeutics. McGraw-Hill Medical Publishing Division. Tenth Edition 2001: pp. 237-238.
6. The list of vegetal extracts not admitted in dietary supplements in Italy can be found at http://www.salute.gov.it
7. http://www.wada-ama.org/
8. ANDRAWS R, CHAWLA P, BROWN D. Cardiovascular effects of Ephedra alkaloids: a comprensive review. Progress Car Dis. 2005; 47: 217-225.
9. DI CANDIA D, TIRELLI P. Valutazione di carattere tossicologico sull'utilizzo di efedrina negli integratori alimentari. Boll Farmacodip Alcolismo. 2003; 26: 13-15.
10. FOOD AND DRUG ADMINISTRATION - Final rule declaring dietary supplements containing ephedrine alkaloids adulterated because they present an unreasonable risk. Federal Register - February 11 - 2004; 69.
11. MARTINDALE. The Complete Drug Reference, 32nd edn. (Parfitt K, ed.). London: The Pharmaceutical Press, 1999.
12. WORLD HEALTH ORGANIZATION. WHO Monographs on Selected Medicinal Plants, vol 1. Geneva: World Health Organization, 1999.
13. SEVER PS, DRING LG, WILLIAMS RT. The metabolism of (-)-ephedrine in man. Eur J Clin Pharmacol. 1975; 9: 193-198.
14. MCEVOY GK (ed): AHFS Drug Information 1999. American Society of Health System Pharmacists, Bethesda, MD; 1999.
15. KASAHARA Y, HIKINO H, TSURUFUJI S, WATANABE M, OHUCHI K. Antiinflammatory actions of ephedrines in acute inflammations. Planta Med. 1985; 51: 325-331.
16. LING M, PIDDLESDEN SJ, MORGAN PB. A component of the medicinal herb ephedra blocks activation in the classical and alternative pathways of complement. Clin Exp Immunol. 1995; 102: 582-588.
17. CHANG HM, But PP-H, eds. Pharmacology and Applications of Chinese Materia Medica, vol 2. Singapore: World Scientific Publishing, 1987: 1119-1124.
18. LEE MK, CHENG BW, CHE CT, HSIEH DP. Cytotoxicity assessment of Ma-huang (Ephedra) under different conditions of preparation. Toxicol Sci. 2000; 56: 424-430.
19. LEE JW, CHOI JH, KANG SM. Screening of medicinal plants having hepatoprotective activity effect with primary cultured hepatocytes intoxicated using carbon tetrachloride cytotoxicity. Kor J Pharmacogn. 1992; 23: 268-275.
20. GURLEY BJ, GARDNER SF, WHITE LM, WANG PL. Ephedrine pharmacokinetics after the ingestion of nutritional supplements containing Ephedra sinica (ma huang). Ther Drug Monit. 1998; 20: 439-445.
21. WHITE LM, GARDNER SF, GURLEY BJ, MARX MA, WANG PL, ESTES M. Pharmacokinetics and cardiovascular effects of ma-huang (Ephedra sinica) in normotensive adults. J Clin Pharmacol. 1997; 37: 116-122.
22. JACOBS I, PASTERNAK H, BELL DG. Effects of ephedrine, caffeine, and their combination on muscular endurance. Med Sci Sports Exerc. 2003; 35: 987-994.
23. BERLIN I, WAROT D, AYMARD G, ACQUAVIVA E, LEGRAND M, LABARTHE B, PEYRON I, DIQUET B, LECHAT P. Pharmacodynamics and pharmacokinetics of single nasal (5 mg and 10 mg) and oral (50 mg) doses of ephedrine in healthy subjects. Eur J Clin Pharmacol. 2001; 57: 447-455.
24. ARENA JM, SPRIGFIELD IL, THOMAS CC. Poisoning: toxicology, symptoms, treatments. 2nd ed. 1970; 2: p. 73.
25. BURKHART KK. Intravenous propranolol reverses hypertension after sympathomimetic overdose: two case reports. J Toxicol Clin Toxicol. 1992; 30: 109-114.
26. PENTEL P. Toxicity of over-the-counter stimulants. JAMA. 1984; 252: 1898-1903.
27. HALLER CA, BENOWITZ NL. Adverse cardiovascular and central nervous system events associated with dietary supplements containing ephedra alkaloids. N Engl J Med. 2000; 343: 1833-1838.
28. VAN MIEGHEM W, STEVENS E, COSEMANS J. Ephedrine-induced cardiopathy. Br Med J. 1978; 1: 816.
29. HALLER CA, JACOB P, BENOWITZ NL. Short-term metabolic and hemodynamic effects of ephedra and guarana combinations. Clin Pharmacol Ther. 2005; 77: 560-571.
30. http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?./temp/~mYqZaZ:1
31. SHEKELLE PG, HARDY ML, MORTON SC, MAGLIONE M, MOJICA WA, SUTTORP MJ, RHODES SL, JUNGVIG L, GAGNE J. Efficacy and safety of ephedra and ephedrine for weight loss and athletic performance: a meta-analysis. JAMA. 2003; 289: 1537-1545.
32. SONG HJ, SHIM KN, RYU KH, KIM TH, JUNG SA, YOO K. A case of ischemic colitis associated with the herbal food supplement ma huang. Yonsei Med J. 2008 Jun 30;49(3):496-9. PubMed PMID: 18581601; PubMed Central PMCID: PMC2615353.
33. U.S. House of Representatives, Committee on Government Reform, Minority Staff Report, "Adverse Event Reports from Metabolife", 2002.
34. BLUMENTHAL M, BUSSE WR, GOLDBERG A, HALL T, RIGGINS CW, RISTER RS, EDS. KLEIN S, RISTER RS. The Complete German Commission E Monographs – Therapeutic Guide to Herbal Medicines. Boston: Integrative Medicine Communications; Austin, TX: American Botanical Council, 1998.
35. ZAHN KA, LI RL, PURSSELL RA. Cardiovascular toxicity after ingestion of "herbal ecstacy." J Emerg Med. 1999; 17: 289-291.

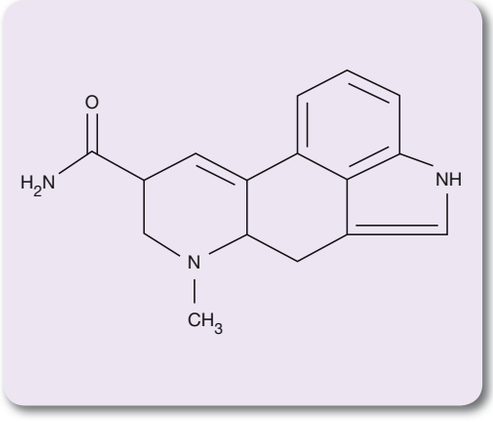
36. NISHIKAWA T, KIMURA T, TAGUCHI N, DOHI S. Oral clonidine preanesthetic medication augments the pressor responses to intravenous ephedrine in awake or anesthetized patients. Anesthesiology. 1991; 74: 705-710.
37. CHO S, HONG T, JIN GB, YOSHINO G, MIURA M, AIKAWA Y, YASUNO F, CYONG JC. The combination therapy of ephedra herb and loxoprofen caused gastric lesions in mice. Am J Chin Med. 2002; 30: 571-577.
38. BROOKS SM, SHOLITON LJ, WERK EE, ALTENAU P. The effects of ephedrine and theophylline on dexamethasone metabolism in bronchial asthma. J Clin Pharmacol. 1977; 17: 308-318.
39. BRATER DC, KAOJARERN S, BENET LZ, LIN ET, LOCKWOOD T, MORRIS RC, MCSHERRY EJ, MELMON KL. Renal excretion of pseudoephedrine. Clin Pharmacol Ther. 1980; 28: 690-694.
40. Product Information: Ephedrine sulfate injection USP. Abbott Hospital Products, North Chicago, IL; 1997.
41. ONUIGBO M, ALIKHAN M: Over-the-counter sympathomimetics: a risk factor for cardiac arrhythmias in pregnancy. South Med J. 1998; 91: 1153-1155.
42. HANSTEN PD, HORN JR. Drug Interaction and Updates. Malvern, Pa: Lea & Febiger; 1990.
43. BIERMAN CW, PIERSON WE, SHAPIRO GG. Exercise-induced asthma: pharmacological assessment of single drugs and drug combinations. JAMA 1975; 234: 295-298.
44. BERKOWITZ RL, COUSTAN DR, NOCHIZUKI TK. Handbook for Prescribing Medications During Pregnancy. Little, Brown and Co, Boston, MA; 1981.
45. BEYER J, PETERS FT, KRAEMER T, MAURER HH. Detection and validated quantification of nine herbal phenalkylamines and methcathinone in human blood plasma by LC-MS/MS with electrospray ionization. J Mass Spectrom. 2007; 42: 150-160.
46. MARCHEI E, PELLEGRINI M, PACIFICI R, ZUCCARO P, PICHINI S. A rapid and simple procedure for the determination of ephedrine alkaloids in dietary supplements by gas chromatography-mass spectrometry. J Pharm Biomed Anal. 2006; 41: 1633-1641.

Ipomoea violacea
(morning glory)

Name: *Ipomoea violacea*
Famly: *Convolvulaceae*
Genus: *Ipomoea* L.
Species: *Ipomoea violacea* L.
Synonyms: Morning glory, Heavenly blue, Pearly Gates, Flying Saucers, Blue Star, Wedding Bells, Summer Skies, Badoh negro. The seeds are called Tlilitzin or ololiuhqui.
Origin: Mexico
Active compounds: Ergine (Lysergamide or lysergic acid amide LSA), isoergine, ergometrine, chanoclavine, lysergol

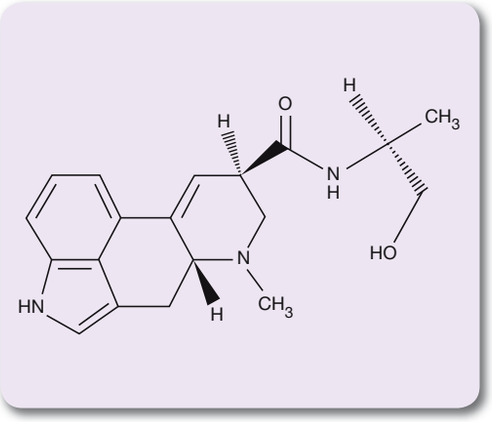
Ergine (Lysergamide or lysergic acid amide LSA) is the principal psychoactive (hallucinogen) alkaloid contained in the seeds of the plant. Other alkaloids present are: isoergine with much lower activity than its epimer ergine, ergometrine, chanoclavine and lysergol. Ergine and isoergine are also present in the seeds of *Argyreia nervosa* and *Rivea corymbosa*. These active compounds are present in the seeds of the plant, nevertheless the historical and traditional use refers to the entire plant. There are no studies reporting on the active compounds in parts of the plant. Based on the data in the literature, the alkaloids percentage measured in the seeds can vary between 0.005%-0.079% fresh weight [(1)]. Ergine and isoergine can be also found in the seeds of *Argyreia nervosa* and *Rivea corymbosa*.

Chemical formula and physico-chemical properties of the active compounds

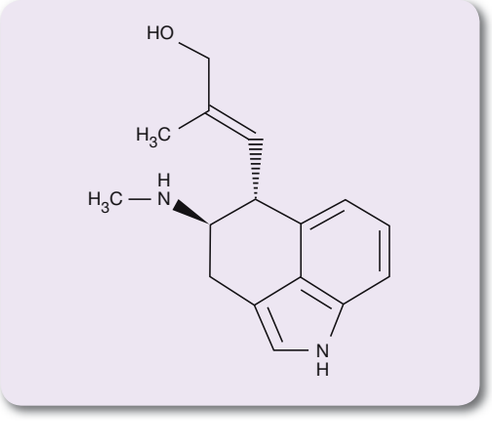


Name: Ergine (Lysergamide or lysergic acid amide LSA) .
Molecular Formula: $C_{16}H_{17}N_3O$ (molecular weight = 267.3).
Systematic name: 9,10-didehydro-6-methylergoline-8-β-carboxamide.
CAS registry number: 478-94-4.
Melting point: no data in the literature.
UVmax: no data in the literature.
Solubility: no data in the literature.

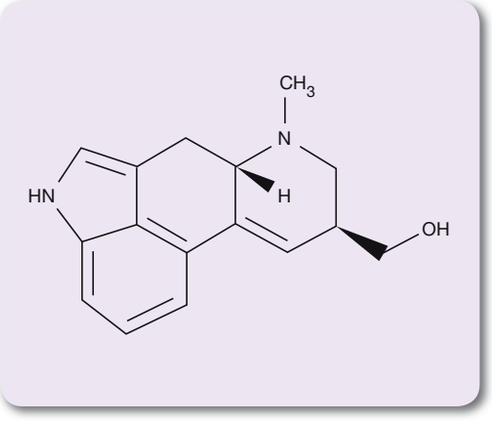
Name: Isoergine.
Systematic name: $C_{16}H_{17}N_3O$ (molecular weight = 267.3). It is the epimer of ergine, it has the same molecular structure, but the spatial distribution is different.
Systematic name: 9,10-didehydro-6-methylergoline-8-α-carboxamide.
CAS registry number: 2889-26-1.
Melting point: no data in the literature.
UVmax: no data in the literature.
Solubility: no data in the literature.



Name: Ergometrine.
Molecular Formula: $C_{19}H_{23}N_3O_2$ (molecular weight = 325.5).
Systematic name: 9,10-didehydro-N-(2-hydroxy-1-methylethyl)-6-methyl-8β-(S)-9-ergoline-8-carboxamide
CAS registry number: 60-79-7.
Melting point: 162°C.
UVmax: no data in the literature.
Solubility: water.



Name: Chanoclavine.
Molecular Formula: $C_{16}H_{20}N_2O$ (molecular weight = 256.3).
Systematic name: 2-Propen-1-ol, 2-methyl-3-(1,3,4,5-tetrahydro-4-(methylamino)benz(cd)indol-5-yl)-,(4R-(4-α,5β(E).
CAS registry number: 2390-99-0.
Melting point: 221°C.
UVmax: nno data in the literature.
Solubility: no data in the literature.



Name: Lysergol.
Molecular Formula: $C_{16}H_{18}N_2O$ (molecular weight = 254,.3).
Systematic name: 9,10-didehydro-8-hydroxymethyl-6-methylergoline.
CAS registry number: 602-85-7.
Melting point: no data in the literature.
UVmax: no data in the literature.
Solubility: no data in the literature.

Historical use

Similar to *Rivea corymbosa* and A*rgyreia nervosa, Ipomoea violacea* was traditionally used by the native Americans for religious ceremonies. After the conquest of Mexico, the Spanish chroniclers wrote that the ololiuqui and tlitliltzin (the name of the plant seeds) were important divinatory hallucinogens in the Aztec religion, with magical and medicinal properties.
Ololiuqui is the little round and brownish seed of a climbing plant, coatl-xoxouhqui ("plant of the snake"), with heart shaped leaves and white flowers; tlitliltzin is a black seed with sharp ends. They were identified as seeds of both, *Rivea corymbosa* and *Ipomoea violacea*. Until the nomenclature of this family was not clarified, these two kinds were called sometimes *Turbina corymbosa* and *Ipomoea tricolor*, respectively. While much was written about the ololiuqui, the tlitliltzin was only mentioned in ancient writings. The ololiuhqui is still used by the tribes of zapotec, chinantec, mazatec and mixtec indians which till recently were still living in isolation in the remote mountains of southern Mexico. The first reports about this drug were written by the Spaniards in the sixteenth century, when they also mentioned peyote and teonanacatl (divine mushrooms).

Current use

The seeds of *Ipomoea violacea*, as those of *Argyreia nervosa* and *Rivea corymbosa*, are sought today for their ability to induce effects psychoactive effects similar to those of lysergic acid diethylamide (LSD), although of lower intensity. There are numerous varieties of *Ipomoea violacea*, cultivated for ornamental reasons, because of the beautiful flowers. The varieties containing ergolinic alkaloids are: Heavenly Blue, Pearly Gates, Flying Saucers, Blue Star, Wedding Bells and Summer Skies.

Legislation

The ergine or lysergic acid amide is subject to control in the United States of America (Schedule III drug in the Controlled Substances Act) as depressant, and is on the list of the U.S. code of Federal Regulations as a possible precursor of LSD.
In Italy, the lysergic acid amide is included in Table I containing narcotic and psychotropic substances subject to the supervision and control under Article 14 of Republic Presidential Decree 309/90 and subsequent updates. According to the Ministerial Decree in September 25th, 2007 published in the Official Bulletin n. 237 in October 11th, 2007, also the seeds of *Ipomea violacea* have been placed on the list of illicit and psychotropic substances of the Table I of article 14 of the RPD n.309/90.

Pharmaco-toxicological properties

The hallucinogenic activity of ergine (lysergic acid amide, LSA) is observed after ingesting 2-5 mg. Similar to the ergot alkaloids (for example ergometrine) it seems that ergine can attach to the D_2 dopaminergic receptors whose stimulation causes adenylate cyclase inhibition and reduction of the formation of adenosine cyclic monophosphate (AMP_c) [(2)].
The discovery of the ergot alkaloids in the seeds of *Rivea corymbosa*, *Ipomoea violacea* and *Argyreia nervosa* in the early ’60 has been rather unexpected and of particular interest from a phytochemical point of view, since, until then, the lysergic acid alkaloids had been isolated only in the mushrooms of the family *Claviceps*, *Penicillium* or *Rhizopus*, and for the first time they were isolated in superior plants (Fanerogame), of the Convolvulaceae family [(3-5)]. LSA has psychotomimetic effects (mind and perception alterations [hallucinations] and of consciousness conscience) similar to those caused by LSD, although LSD is 50 to 100 times more potent than LSA. The effects of the LSA, which last about 4-8 hours, are associated with a sensation of quiet, dysphoria, psychedelic visual effects, colour vision. The ingestion of *Ipomoea violacea* seeds (Tlitlitzin) produces effects comparable with those produced by the seeds of *Argyreia nervosa*. Studies on ergine made in bovines (veal) demonstrate that on the average its pharmacokinetic profile after one single intravenous dose of 14 µg/Kg, has three distinct phases. The first phase (0-10 minutes), characterized by a steady state in the distribution volume, is followed by a second phase (that begins immediately after the injection and it persists for about an hour) with concentrations of ergine in steady state between blood and tissues. In the third phase the balance between tissues and blood is inverted and the molecule is eliminated through the liver [(6)]. Elymoclavine and chanoclavine, although present in a smaller percentage in the seeds of the plant, seem to contribute the to the hallucinogenic activity. The possible contribution of ergometrine (present in trace amounts in the seeds of the *Ipomoea violacea*) to the pharmacotoxicologic properties of the plant has not been sufficiently studied.

Toxicity

Data regarding the acute toxicity of ergine

In human - TDLo following oral administration: 14 µg/kg [(7)]
In rat and rabbit - LD following intravenous administration: 2500 µg/kg [(7)]

There is no data regarding the acute toxicity of the other active compounds of the plant.

Adverse Effects

Following the ingestion of seeds the most important psychotic advers effects are dissociative reactions and schizophrenic relapses [(8)].
There is a case of toxic psychosis induced by the ingestion of *Argyreia nervosa* seeds (the plant whose seeds contain LSA,

like *Ipomoea violacea*) described in the literature. The advers effects included hallucinations, orientation problems, anxiety and psychomotor agitation [9]. In another case, an 18-year-old male has been hospitalized because of an acute psychotic behaviour following the ingestion of the plant seeds [10].

These clinical cases emphasize the need to pay attention specifically to the differential diagnosis in adolescents between the episodes of acute psychosis and those caused by the ingestion of this one or of other hallucinogenic drugs.

Pharmacological interactions

There are no well-known interactions due to ingestion of *Argyreia nervosa*, *Ipomoea violacea* or *Rivea corymbosa* and other medications. Still, it has been demonstrated that the metabolism of LSD, an analogue of LSA present in the plant, is inhibited by medications used for the treatment of HIV [11]. This suggests the possibility that in patients treated with antiretroviral medications there is an increased risk of toxicity induced by hallucinogens such as LSD or seed of *Argyreia nervosa*, *Ipomoea violacea* or *Rivea corymbosa*.

Effects in pregnancy

The ingestion of the seeds of *Argyreia nervosa*, *Ipomoea violacea* or *Rivea corymbosa* during pregnancy is risky. In fact, ergine, structurally correlated to LSD, can induce uterine contractions [12,13] . The drug can, therefore increase the risk of spontaneous abortion.

Analytical determinations

No methodologies have been reported in the international literature concerning the analysis of *Ipomea violacea* active principles in biological fluids of eventual *Ipomea* consumers. However, analytical methods have been published regarding the determination of LSA in urine [14,15] , blood [15] and in the seeds of *Argyreia nervosa* [16].

Please refer to the monograph of *Argyreia nervosa* for analytical details of the determination of LSA in the urine samples [14].

References

1. DER MARDEROSIAN A, YOUGKEN HW. The distribution of indole alkaloids among certain species and varieties of Ipomoea, Rivea and Convolvolus (Convolvulaceae). Llodia. 1966; 29: 35-42.
2. LARSON BT, HARMON DL, PIPER EL, GRIFFIS LM, BUSH LP. Alkaloid binding and of D2 dopamine receptors in cell culture. J Anim Sci. 1999; 77: 942-947.
3. HYLIN JW, WATSON DP. Ergoline alkaloids in tropical wood roses. Science. 1965; 148: 499-500.
4. TABER WA, HEACOCK RA, MAHON ME. Ergot-type alkaloids in vegetative tissue of Rivea corymbosa (L.) Hall.f. Phytochemistry. 1963; 2: 99-101.
5. TABER WA, HEACOCK RA. Location of ergot alkaloid and fungi in the seed of Rivea corymbosa (L.) Hall. f., "ololiuqui". Can J Microbiol. 1962; 8: 137-143.
6. MOUBARAK AS, PIPER EL, JHONSON ZB, FLIEGER M. HPLC method for detection of ergotamine, ergosine, and ergine after intravenous injection of a single dose. J Agric Food Chem. 1996; 44: 146-148.
7. http://chem.sis.nlm.nih.gov/chemidplus/jsp/common/ChemFull.jsp?calledFrom=lite
8. USDIN E, EFRON DH. Psychotropic drugs and related compounds. 2nd ed. Washington, DC. 1972: 72.
9. MILLER MD. Isolation and identification of lysergic acid amide and isolysergic acid amide as the principal ergoline alkaloids in Argyreia nervosa, a tropical Wood rose. J AOAC. 1970; 53: 123-127.
10. DER MARDEROSIAN A. Psychotomimetic indoles in the Convolvulaceae. Am J Pharm Sci Support Public Health. 1967; 19-26.
11. FINK PG, GOLDMAN MJ, LYONS I. Morning glory seeds psychosis. Arch Gen Psychiat. 1966; 15: 209-213.
12. ISBELL H, GORODETZKY CW. Effect of alkaloids of Ololiuqui in man. Psychopharmacologia 1966; 8: 331-339.
13. INGRAM AL. Morning glory seed reaction. JAMA. 1964; 190: 107-108.
14. BJÖRNSTAD K, BECK O, HELANDER A. A multi-component LC-MS/MS method for detection of ten plant-derived psychoactive substances in urine. J Chromatogr B Analyt Technol Biomed Life Sci. 2009; 877: 1162-1168.
15. KLINKE HB, MÜLLER IB, STEFFENRUD S, DAHL-SØRENSEN R. Two cases of lysergamide intoxication by ingestion of seeds from Hawaiian Baby Woodrose. Forensic Sci Int. 2009 doi:10.1016/j.forsciint.2009.11.017 (in press).
16. KIM W, CRAWFORD MS. The Identification of Lysergic Acid Amide in Baby Hawaiian Woodrose By Mass Spectrometry. J Forensic Sci. 1970; 15: 588-594.

Lactuca virosa
(bitter lettuce)

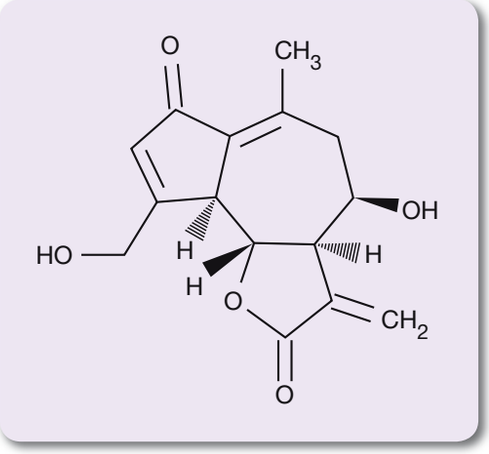
Name: *Lactuca virosa*
Family: *Compositae*
Genus: *Lactuca*
Species: *Lactuca virosa* L.
Synonims: bitter lettuce, poisonous lettuce, wild lettuce
Origin: Ubiquitous in the central-southern Europe, it grows along the roads and the canals on stony and basic grounds
Active compounds: lactucin, lactucopicrin (intybin), N-methyl-β-phenethylamine, hyoscyamine.

Lactucin is a neutral, white, crystalline substance, with bitter taste. The precise concentration of the active compounds in the green parts of the plant and in the latex extract is not known. Lactucopicrin (or intybin) is the parahydroxyacetic ester of lactucin.

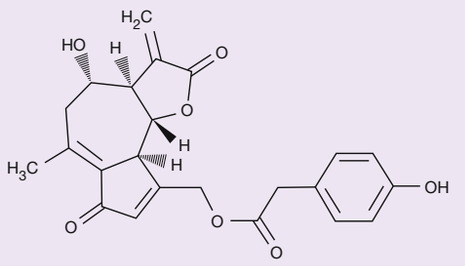
Usually, the leaves and the whitish latex that is obtained from the stem secretion are consumed. Once dried, the latex is also called "lactucarium" which has opioid like properties: more specifically, lactucarium can be smoked or used as a drink. The dried latex is also called "opium lettuce", although it does not contain any opioid substance [(1)].

According to internet sources, all the plants of the *Lactuca* genus contain the same active compounds as *Lactuca virosa* does, although in smaller quantity. Particularly *Lactuca sativa*, the common lettuce used as a food, has lost almost completely the original characteristics present in the wild varieties (*Lactuca virosa*) [(1)]. In a recent study, the content of sesquiterpene lactones extracted from different Lactuca varieties has been described: although no quantitative evaluation of the active compounds was reported, lactucin and lactucopicrin were both detected in *Lactuca sativa* as well [(2)]. There is no mention in the literature on the percentage of the active compounds in the whole plant but it has been established that the lactucarium contains about 0.2% of lactucin [(3)]. Some authors report the presence of hyoscyamine (the levorotatory isomer of atropine) in the green plant, a potent inhibitor of the parasympathetic nervous system [(3,4)], although its concentration in the plant is not mentioned.

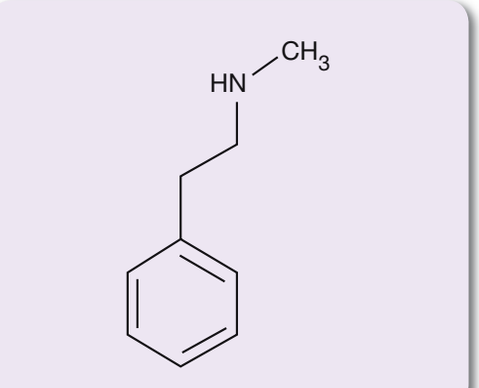
Chemical formula and physico-chemical properties of the active compounds



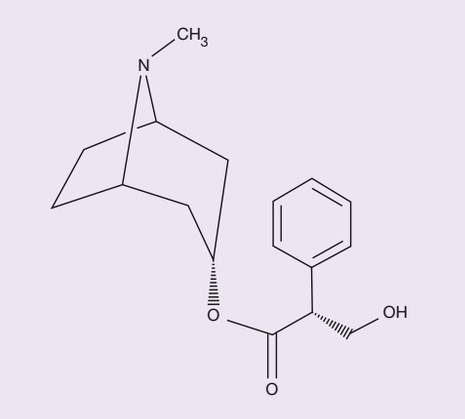
Name: Lactucin.
Molecular formula: $C_{15}H_{16}O_5$ (molecular weight = 276.3).
Systematic Name: (3aR,4R,9aS,9bR)-4-hydroxy-9-(hydroxymethyl)-6-methyl-3-methylene-4,5,9a,9b-tetrahydro-3aH-azuleno[5,4-d]furan-2,7-dione
CAS Registry Number: 1891-29-8.
Melting point: 228-233°C.
UVmax: 257 nm.
Solubility: water, ethyl alcohol, methyl alcohol, ethyl acetate, dioxane and anisole.



Name: Lactucopicrin (intybin).
Molecular formula: $C_{23}H_{22}O_7$ (molecular weight = 410.4).
Systematic Name: (4-Hydroxy-6-methyl-3-methylidene-2,7-dioxo-4,5,9a,9b-tetrahydro-3aH-azuleno[8,7-b]furan-9-yl)methyl 2-(4-hydroxyphenyl)acetate.
CAS Registry Number: 6466-74-6.
Melting point: no data in the literature.
UVmax: no data in the literature.
Solubilità: no data in the literature.



Name: N-methyl-β-phenethylamine.
Molecular formula: $C_9H_{13}N$ (molecular weight = 135.2).
Systematic Name: N-methyl-benzenethanamine.
CAS Registry Number: 589-08-2.
Melting point: 165-166°C.
UVmax: no data in the literature.
Solubility: very soluble in water.



Name: Hyoscyamine.
Molecular formula: $C_{17}H_{23}NO_3$ (molecular weight = 289.4).
Systematic Name: α-(hydroxymethyl)-,(3-endo)-8-methyl-8-azabicyclo (3.2.1)oct-3-yl,ester,(αS)-benzoylacetic acid.
CAS Registry Number: 101-31-5.
Melting point: 108,5°C.
UVmax: 252, 258, 264 nm.
Solubility: alcohol and dilute acids.

Historical use

Lactuca virosa was used in XIX century by physicians when opium was not available. It has been extensively studied by the Council of the Pharmaceutical Society of Great Britain in 1911. In that occasion, it was discovered that the two substances responsible for the sedative effects of *Lactuca virosa* were lactucopicrin and lactucin [5]. After having cut the plant, the Hopi Indians collected the sap, air-dried it and smoked it during ritual ceremonies [5].

Current use

The erboristic studies attribute sedative, narcotic, analgesic and antispasmodic properties to *Lactuca virosa*. The plant is known as antitussive and emollient as well. At present time it is used for recreational purpose for its ability to induce opium like sensations (although of smaller intensity).

Legislation

In Europe, there is no data of any restrictive measure for *Lactuca virosa* or its active compounds. In USA the *Lactuca virosa* is not a controlled plant either. This means that it is legal to cultivate, buy, possess and distribute all the parts of the plant and the respective plant extracts, without any authorization or prescription. If sold as a supplement, its marketing must conform to the USA laws with respect to food supplements. If sold as food or medicine, its marketing is regulated by the Food and Drug Administration (FDA).

In Italy, neither lactucin, intybin, N-methyl-β-phenethylamine and hyoscyamine nor the whole plant or parts of it are included in tables containing narcotic or psychotropic substances subject to the supervision and control under Article 14 of Republic Presidential Decree 309/90 and subsequent updates. However, *Lactuca virosa* is still on the list of vegetable extracts not admitted in dietary supplements by the Ministry of Health [6].

Pharmaco-toxicological properties

Lactuca virosa has been used as a opium substitute. In a work published in 1989 it is reported that: "The *Lactuca virosa* contains a milky and bitter juice called lactucarium, which has the smell and the effects similar to those of the opium. Among the narcotics that include the opium and its derivatives, there is the lactucarium, the smokable extract of the *Lactuca virosa*. The use of the lactucarium does not cause vision disturbances like opium, and the euphoria and the dreams due to the lactucarium intoxication are of longer duration" [7].

There is no pharmacokinetic and pharmacodynamic data available for lactucin and lactucopicrin up to now.

The analgesic and sedative properties of lactucin and lactucopicrin have been recently evaluated in the mouse model. In particular, it has been observed that both compounds, in doses of 15 -30 mg/kg, have analgesic effects comparable with those produced by ibuprofen administration. Lactucopicrin acts as a more powerful analgesic than lactucine [8].

The antimalarial activity of lactucin and lactucopicrin has been evaluated *in vitro* using a strain of *Plasmodium falciparum* sensitive to cloroquine and resistent to pyrimethamine. Both compounds resulted effective, with lactucine having a stronger effect compared to that of the lactucopicrine [9].

N-methyl-β-phenethylamine, is a monoaminergic alkaloid. In the brain, a neuromodulator or neurotransmitter function has been attributed to this substance. It has been found in different foods (for example, in the chocolate), and it is quickly inactivated by the monoamine oxydases, therefore avoiding that excessive quantities can reach the brain.

Hyoscyamine is a potent inhibitor of the parasympathetic nervous system [3,4]. Atropine used in the medical field is the racemic mixture of hyoscyamine. The toxicity data for atropine, shows that it is more toxic than hyoscyamine (LD 50 in the mouse following intravenous administration: 30 mg/Kg vs 95 mg/Kg for hyoscyamine). In any case, hyoscyamine can be considered as an antagonist of the cholinergic muscarinic receptors: data relative to atropine and to related compounds show that these molecules compete with acetylcholine and other muscarinic agonists for the ligands in the muscarinic receptors. There are no pharmacokinetic data for hyoscyamine itself, but those relative to the atropine demonstrate that the molecule is absorbed quickly in the gastrointestinal tract. Since hyoscyamine can not cross the blood-brain barrier, it has only minor effects on the central nervous system. Atropine has a half-life of about 4 hours; 50% of a given dose is eliminated through hepatic metabolism while the rest is eliminated unchanged into the urine [10].

Toxicity

All the parts of the plant can be toxic. Signs of toxicity include: nausea, vomiting, sedation, buzzing of the ears, drowsiness, numbing, respiratory depression that can lead to coma and to death.

The toxic doses for lactucopicrin and lactucin are not known.

Data regarding the acute toxicity of N-methyl-β-phenetylamine [11]

In mouse - LD50 following parenteral administration: 180 mg/kg

In mouse - LD50 following intraperitoneal administration: 190 mg/kg

In rat - LD50 following oral administration: 1400mg/kg

Data regarding the acute toxicity of hyoscyamine [11]

In human - LDLo mode of administration not reported: 1.471 mg/kg

In mouse - LD50 following intravenous administration: 95 mg/kg

Adverse Effects

In excessive doses the ingestion of lactucarium can produce headache, dizziness, nausea, vomiting, diarrhoea, excessive salivation, palpitations, midriasis, general excitement, low blood pressure and at last death by cardiac paralysis [12]. Normal doses can cause drowsiness, while elevated doses can cause irritability [13]. In an old case, published in 1876, a family who had consumed a mixed salad containing *Lactuca virosa* had been poisoned, exhibiting visual hallucinations associated with delirium [14].

Pharmacological interactions

There are no reported pharmacological interactions.

Effects in pregnancy

Do not use in pregnancy and breastfeeding [15].

Analytical determination

No methodologies have been reported in the international literature concerning the analysis of *Lactuca virosa* active principles in biological fluids of eventual consumers. However, analytical methods have been published regarding the determination of lactucin and lactupicrin in the plant latex [2].

The method below reported is a brief outline useful to the investigator to set up the analysis. For specific details, it is advisable to refer to the original text.

Determination of lactucin and lactucopicrin in *Lactuca virosa* latex

(From: SESSA RA, BENNETT MH, LEWIS MJ, MANSFIELD JW, BEALE MH, Metabolite profiling of sesquiterpene lactones from lactuca species. J Biol Chem. 2000; 275: 26877-26884) [2].

The analysis is carried out on the plant latex using liquid chromatography coupled with ultraviolet spectrophotometric detection.

Extraction of the compounds

The latex drops from the stalk are collected and 10 µl sample are immediately mixed with 1ml methyl alcohol containing 1% phosphoric acid. Without further extraction, the sample is centrifuged at 16000 g for 10 minutes, the supernatant filtered through a 0.45 µm membrane and 15 µl are injected into the liquid chromatograph.

Analytical conditions

Chromatografic column: RP-C18 (250 x 4.6 mm, 5 µm)

Mobile phase A: water containing 0.1% phosphoric acid

Mobile phase B: acetonitrile-water (90:10, v/v)

Separation : gradient (mobile phaseA: 99% at time zero, to 48% at 60 minutes)

Flow rate: 1 mL/min

Column temperature: 35°C

Detector: spectrophotometer with ultraviolet detection (200nm and 264nm)

Retention times of the tested compounds

Lactucin: 28 minutes

Lactucopicrin: 48 minutes

Standards

The source of the standards is not specified.

Calibration curve

The calibration curve is not described.

Results

The percentages of the active compounds in the entire plant are not described in the study.

References

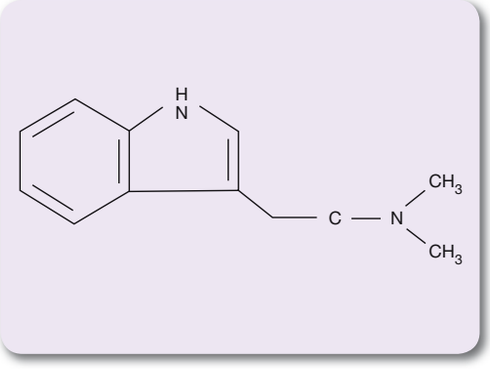
1. http://www.marijuanaalternatives.com/wild-lettuce.htm
2. SESSA RA, BENNETT MH, LEWIS MJ, MANSFIELD JW, BEALE MH. Metabolite profiling of sesquiterpene lactones from Lactuca species. J Biol Chem. 2000; 275: 26877-26884.
3. THE MERCK INDEX An Enciclopedia of chemicals, drugs, and biologicals. 11th Ed. Merck & Co., Inc. 1989: p. 843.
4. WEINER MA. Earth medicine, earth food. Ballatine books, 1980.
5. http://www.fungoceva.it/erbe_ceb/Lactuca_virosa.htm
6. The list of vegetal extracts not admitted in dietary supplements in Italy can be found at http://www.salute.gov.it
7. SIEGEL R. Intoxication: life in pursuit of artificial paradise. E.P. Dutton, New York, 1989.
8. WESOLOWSKA A, NIKIFORUK A, MICHALSKA K, KISIEL W, CHOJNACKA-WOJCIK E. Analgesic and sedative activities of lactucin and some lactucin-like guaianolides in mice. J Ethnopharmacol. 2006; 107: 254-258.
9. BISCHOFF TA, KELLEY CJ, KARCHESY Y, LAURANTOS M, NGUYEN-DINH P, AREFI AG. Antimalarial activity of lactucin and lactucopicrin: sesquiterpene lactones isolated from Cichorium intybus L. J Ethnopharmacol. 2004; 95: 455-457.
10. GOODMAN AND GILMAN'S. The pharmacological basis of therapeutics. 10th Edition. Hardman JG and Limbird Ed. 2001.
11. http://toxnet.nlm.nih.gov
12. NEGRI G. Nuovo erbario figurato. Hoepli Ed., Milano, 1979.
13. BROWN D. Encyclopaedia of herbs and their uses. Dorling Kinderseley, London. 1995.
14. BOE. Caso d'avvelenamento da Lactuca virosa. Gazzetta medica italiana, Province Venete 1876; 20: 99-100.
15. http://www.afisna.com/fitomedicina/plsvetope/lpls.html

Mimosa hostilis
(jurema)

Name: *Mimosa hostilis*
Family: *Leguminosae*
Genus: *Mimosa*
Species: *Mimosa hostilis*
Synonims: Mimosa tenuiflora, Poir, tepescohuite, jurema
Origin: Mexico, Central and South America (Honduras, Columbia, Guatemala, Brazil)
Active compounds: N,N-dimethyltryptamine (DMT)

Usually, the bark of the roots is dried and used for the infusions preparation. The dimethyltryptamine concentration in the roots is equal to about 0.57% of fresh weight [1,2]. The names *Mimosa hostilis* and *Mimosa tenuiflora* are used interchangeably, to indicate that the two plants belong in fact to the same species [3]. In particular, the term *Mimosa hostilis* is linked to the Brazilian ritual of jurema.

Chemical formula and physico-chemical properties of the active compounds



Name: N,N-dimethyltryptamine (DMT).
Molecular formula: $C_{12}H_{16}N_2$ (molecular weight = 188.2).
Systematic name: N-dimethyl-1H-indol-3-ethylamine.
CAS registry number: 61-50-7.
Melting point: 44,6-46,8°C.
UVmax: 279-288 nm.
Solubility: acetic acid.

Historical use

Since Middle Ages *Mimosa hostilis* has been used by the South American populations (particularly, Brazilian) in the so called "cult of the Jurema": religious and therapeutic rites of afro-Brazilian character which advocate the use of jurema or jurema wine based on *Mimosa hostilis*, producing visual hallucinations. The ritual of jurema enables the participants to get access to the world of the ancestors and of divine protectors. The participants recall the ancestors by singing and they, in turn, help and teach the participants [4]. In Central America, the local populations also used the bark of the plant to treat the burns or superficial lesions of the skin (an infusion of the bark was drunk or it was pulverised for use in ointments) [5].

Current use

At present, in the South American market there is a great variety of medicinal and cosmetic products based on *Mimosa hostilis* , although its use is entirely empirical [5]. The recreational use of the plant is based on Internet website offers; it is used often in conjunction with other herbal inhibitors of mononoamine oxidase, enzymes present in the postsynaptic neurons and in the synaptic wave that degrades the biogenic amines (for example, noradrenaline), but also DMT. This is the same mechanism as in case of *Ayahuasca*, where monoamine oxidase inhibitors are harmine and harmaline, the β-carbolines contained in the preparation. In the absence of the destruction and blocking of catecholamines reuptake, namely the reabsorption by the presinaptic neuron, an increase in the neurotransmitter action at the synaptic cleft and towards the

postsynaptic receptors is produced. Thus, because of its content in DMT, *Mimosa hostilis* can become part of the mixture of herbs that form *Ayahuasca* (see monograph) or can become part of blends alternative to those normally used to prepare *Ayahuasca* itself. In particular, the alternative blend is defined ANAHUASCA (ANAlogues of AYAHUASCA), i.e a beverage containing extracts from the seeds of *Peganum Armala* (Syrian rue, which contains β-carbolines, the powerful MAO inhibitors) and root bark of *Mimosa hostilis* [6]. The anahuasca is, with respect to ayahuasca, equally active and effective.

Legislation

DMT is illegal in the United States of America and is included in Schedule I drug in the "Controlled Substances Act". In addition, it is also on a list of the substances placed under the control of International Narcotics Control Board through Schedule I of the Convention on Psychotropic Substances of 1971. DMT is illegal in Europe. In May 2005, France has added *Mimosa hostilis* to the list of controlled substances. In Italy the DMT is included in Table I containing narcotic or psychotropic substances subject to the supervision and control under Article 14 of Republic Presidential Decree 309/90 and subsequent updates. Neither the whole plant nor parts of it are listed in the above-mentioned table.

Pharmaco-toxicological properties

DMT is a molecule related to serotonine, and, like other psychedelic drugs (LSD and mescaline), it binds to the serotoninergic receptors 5-HT_{2A} in the central nervous system, exerting its agonist behaviour. Studies performed on humans have shown that DMT given parenterally causes important perceptive modifications (of own identity and of the surrounding reality), which can be of remarkable intensity, but of relatively short duration. In addition, DMT is able to exert important effects over the autonomous nervous system, increasing blood pressure, cardiac frequency and causing midriasis.

In contrast to the majority of psychedelic drugs, DMT is inactive when given orally up to about 13 mg/Kg, because of the rapid degradation due to cellular monoamine oxidase (MAO) [7]. MAO are flavonoid enzymes (belonging to the class of the oxidoreductases) that catalyze the oxidation of primary amines, with formation of aldehyde and hydrogen peroxide. Molecules that inactivate monoamine oxidases would make DMT more efficacious: therefore *ayahuasca* (see monograph) is normally prepared by mixing plants that contain DMT with plants that contain β-carbolines, powerful MAO inhibitors. Some Internet sites claim that *Mimosa tenuifolia* can be effective even in the absence of MAO inhibitors, since it contains certain molecules ("kukulkanines") with inhibitory activity [3]. Up till now, there is no clear evidence that the plant extract is working in the absence of MAO inhibitors. For DMT, the minimum dose (intravenously administered) which produces clinical effects (hallucinations) compared to placebo, is 0.2 mg/Kg. These effects occur immediately, peak within 2 minutes and disappear within 20 or 30 minutes. An increase in the concentration of plasma β-endorphins, corticotropine, cortisol and prolactin occurs as well. In addition, the growth hormone (GH) plasma levels increase also [8].

In a study on human model, it has been established that the psychedelic effect is achieved when 120 mg of oral harmine (expressed as free base:1.5mg/kg) is administered together with 30 mg of DMT (concentration of 0.3-0.4 mg/kg) [9]. In the same study it is demonstrated that the MAO inhibitors render DMT and other tryptamines active when orally taken, but, in contrast reduce the activity of the above reported substances when administered by other routes. In other words, the MAO inhibitors are activators, not potentiators of the tryptamines effects [9].

The traditional use of the plant for wound care is supported by *in vitro* experiments showing that the *Mimosa tenuiflora* stimulates the activity and the proliferation of skin fibroblasts [10].

Toxicity

Data regarding the acute toxicity of dimethyltryptamine [11]

In human - TDLo: 1mg/Kg

In mouse - LD50 following intraperitoneal administration: 47 mg/kg

In rat - LD50 following intravenous administration: 32 mg/kg

Adverse Effects

When the plant is used as an infusion (eg. ayahuasca) there are no adverse effects described in the literature. Nevertheless, it is worth mentioning that there are reports found on the internet regarding the use of potentially dangerous mixtures of *Mimosa hostilis* and *Peganum harmala* with: cannabis, phencyclidine (PCP), scopolamine and cocaine [12].

Pharmacological interactions

There are no pharmacological interactions with DMT described in the literature.

Effects in pregnancy

In the rat the teratogenic action of the *Mimosa hostilis* seeds has been documented. In pregnant rats the administration of the seeds resulted in decreased body weight and development of bone malformations of the offsprings [13].

Analytical determinations

No methodologies have been reported in the international literature concerning the analysis of DMT in biological fluids of eventual *Mimosa hostilis* consumers, while assays exist to detect DMT in body fluids and tissues of *Ayahuasca* users [14-16].
An analytical method to determine DMT in bark powder, leaves, flowers and callus of *Mimosa tenuiflora* by liquid chromatography coupled to ultraviolet spectrophotometric detector as been reported [17].
Please refer to the monograph of *Ayahuasca* for analytical details of the determination of DMT in the urine and blood samples [14].

References

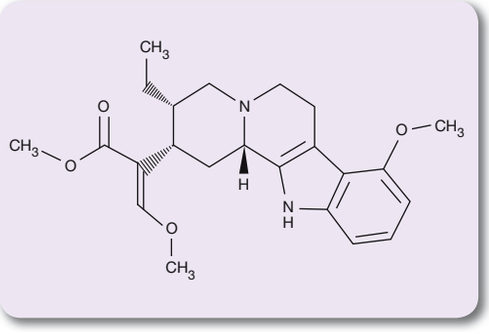
1. PACHTER, IJ, ZACHARIAS DE, RIBEIR O. Indole Alkaloids of Acer saccharinum (the Silever Maple), Dictyoloma incanescens, Piptadenia colubrina, and Mimosa hostilis. J Org Chem. 1959; 24: 1285-1287.
2. SCHULTES RE. The botanical and chemical distribution of hallucinogen. J Psychedelic drugs. 1977; 9: 247-263.
3. http://www.erowid.org/plants/mimosa/mimosa_info2.shtml
4. http://www.samorini.net/antrop/tx_ant/ant_jur.htm
5. CAMARGO-RICALDE SL. Description, distribution, anatomy, chemical composition and uses of Mimosa tenuiflora (Fabaceae-Mimosoideae) in Mexico. Rev Biol Trop. 2000; 48: 939-954.
6. http://leda.lycaeum.org/?ID=16774
7. SHULGIN AT. Profiles of psychedelic drugs.1. DMT. J Psychedelic drugs. 1976; 8: 167-168.
8. STRASSMAN RJ, QUALLS CR. Dose-response study of N,N-dimethyltryptamine in humans. Arch Gen Psychiatry. 1994; 51: 85-97.
9. OTT J. Pharmahuasca: human pharmacology of oral DMT plus harmine. J Psychoactive Drugs. 1999; 31: 171-177.
10. ZIPPEL J, DETERS A, HENSEL A. Arabinogalactans from Mimosa tenuiflora (Willd.) Poiret bark as active principles for wound-healing properties: specific enhancement of dermal fibroblast activity and minor influence on HaCaT keratinocytes. J Ethnopharmacol. 2009 ;124 391-396.
11. http://toxnet.nlm.nih.gov
12. http://erowid.org/experiences/subs/exp_Mimosa_hostilis.shtml
13. MEDEIROS RM, DE FIGUEIREDO AP, BENÍCIO TM, DANTAS FP, RIET-CORREA F. Teratogenicity of Mimosa tenuiflora seeds to pregnant rats. Toxicon. 2008 Feb; 51: 316-319.
14. SKLEROV J, LEVINE B, MOORE KA, KING T, FOWLER D. A fatal intoxication following the ingestion of 5-methoxy-N,N-dimethyltryptamine in an ayahuasca preparation. J Anal Toxicol. 2005; 29: 838-841.
15. BJÖRNSTAD K, BECK O, HELANDER A. A multi-component LC-MS/MS method for detection of ten plant-derived psychoactive substances in urine. J Chromatogr B Analyt Technol Biomed Life Sci. 2009; 877: 1162-1168.
16. CALLAWAY JC, RAYMON LP, HEARN WL, MCKENNA DJ, GROB CS, BRITO GS, MASH DC. Quantitation of N,N-Dimethyltryptaminea and Harmala Alkaloids in Human Plasma after Oral Dosing with Ayahuasca. J Anal Toxicol. 1996; 20: 492- 497.
17. DEL PILAR NICASIO M, VILLARREAL ML, GILLET F, BENSADDEK L, FLINIAUX MA. Variation in the accumulation levels of n,n-dimethyltryptamine in micro-propagated trees and in in vitro cultures of Mimonsa tenuiflora. Nat Prod Res. 2005; 19: 61-67.

Mitragina speciosa
(Kratom)

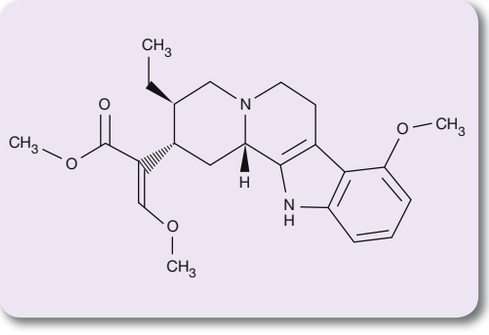
Name: *Mitragyna speciosa* (Kratom)
Family: *Rubiaceae*
Genus: *Mitragyna*
Species: *Mitragyna speciosa* Korth.
Synonims: Kratom, Ketum; kutum; Biak; Biak-biak
Origin: South East Asia (Thailand, Myanmar)
Active compounds: mitragynine (62.2%), speciogynine (6.6%), paynantheine (0.8%) speciociliatine (0.8%), 7-α-7-hydroxy-mitragynine (2%)

There have been over 25 different alkaloids isolated from Kratom, the most important ones being: mitragynine, paynantheine, speciogynine, speciociliatine and the 7-hydroxymitragynine (Takaiama) [1-2]. Chemically, mitragynine is the 9-methoxy-corynantheidine [3], a molecule structurally related to both yohimbine and voacangine. The molecular structure is somewhat similar to psilocibine type or LSD psychedelic drugs.
It seems that the kratom plants grown in different geographically distant places have a different alkaloids content .The alkaloids content seems to vary also during the year and according to several phases of the plant growth. *Mitragyna speciosa* leaves contain about 0.5% (weight-weight) alkaloids, half of this percentage being mitragynine. A leaf weighs on average 1.7 g if fresh and 0.43 g when dried. Twenty dried leaves contain about 17 mg mitragynine. The Thai Kratom has a 62.2% mitragynine (raw extract), 6.6% of speciogynine, 0.8% speciociliatine, 8.6 % paynantheine and 2% 7- hydroxy-mitragynine [2-4]. The same alkaloids have been extracted from *Mitragyna speciosa* originating in Malaysia, although mitragynine, which in this case is the principal alkaloid as well, represents 12% of the total alkaloids.

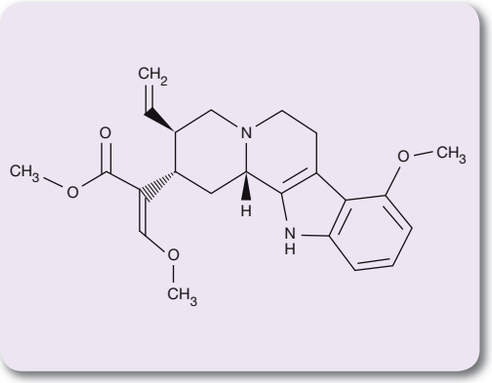
Chemical formula and physico-chemical properties of the active compounds:



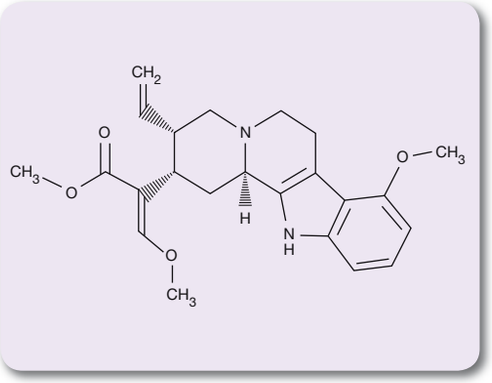
Name: Mitragynine.
Molecular formula: $C_{23}H_{30}N_2O_4$ (molecular weight = 398.5).
Systematic name: 16,17-didehydro-9,17-dimethoxy-17,18-seco-20-α-yohimban-16-carboxylic acid methyl ester.
CAS registry number: 4098-40-2.
Melting point: 104°C.
UVmax: 226, 292 nm.
Solubility: alcohol, chloroform and acetic acid.



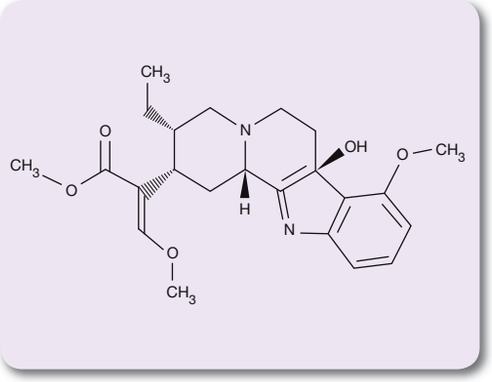
Name: Speciogynine.
Molecular formula: $C_{23}H_{30}N_2O_4$ (molecular weight = 398.5).
Systematic name: 16,17-didehydro-9,17-dimethoxy-17,18-seco-yohimban-16-carboxylic acid methyl ester.
CAS registry number: no data in the literature.
Melting point: 214°C.
UVmax: (in ethyl alcohol) 227, 274, 284, 293 nm.
Solubility: no data in the literature.



Name: Paynantheine.
Molecular formula: $C_{23}H_{28}N_2O_4$ (molecular weight = 396.5).
Systematic name: (α-E,2S,3R,12bS)-indolo(2,3-a)quinolizine-2-acetic acid-3-ethenyl-1,2,3,4,6,7,12,12b-octahydro-8methoxy-α-(methoxymethylene)-methyl ester.
CAS registry number: 1346-36-7.
Melting point: 98°C.
UVmax: (in ethyl alcohol): 227, 272, 283, 293 nm.
Solubility: no data in the literature.



Name: Speciociliatine.
Molecular formula: $C_{23}H_{28}N_2O_4$ (molecular weight = 396.5).
Systematic name: (3-β,16E,20-β)-17,18-seco-3-β,20-α-yohimban-16-, carboxylic acid 16,17-didehydro-9,17-dimethoxy- methyl ester.
CAS registry number: no data in the literature.
Melting point: no data in the literature.
UVmax: no data in the literature.
Solubility: no data in the literature.



Name: 7-α-7-hydroxy-mitragynine.
Molecular formula: $C_{23}H_{29}N_2O_5$ (molecular weight = 413.5).
Systematic name: 7 -α- 7-hydroxy-mitragynine.
CAS registry number: no data in the literature.
Melting point: no data in the literature.
UVmax: (in ethyl alcohol): 221, 245, 305 nm.
Solubility: no data in the literature.

Historical use

Kratom (*Mitragyna speciosa* Korth) is an original tree of South-East Asia, where the plant is used like a drug since ancient times. It belongs to the same botanic family of the coffee plant (*Rubiaceae*). In Thailand, the natives have always used the plant for its effects similar to those of opioids and cocaine. Traditionally, it is consumed by chewing the fresh leaves from the central rib. The dried leaves can be chewed as well, but, since they turn hard once they are dry, it is preferable to mince them or pulverize them before the use. The dried and minced leaves can be used also as an infusion which can be drunk as a tea. The Kratom can be smoked, but the effect turns out to be less intense than when it is chewed or drunk; the quantity of leaves necessary to obtain a typical dose is too high to be smoked. A paste like extract can be prepared if the fresh or dry leaves are boiled for a long time: this extract can be kept for a long time.
In low doses, Kratom it can be used as a stimulant; in elevated doses, like a sedative.

Current use

At present, the dried and pulverized Kratom leaves are used as legal stimulant or, otherwise for its analgesic, euphoric sedative effects.

Legislation

The Kratom is legal in whole Europe and in the United States of America while it is illegal in Australia by the February 2004 (Schedule 9 SUSPD: Standard for the Uniform Scheduling of Drugs and Poisons). In Thailand and Burma, the use of the plant has been forbidden because of its narcotic effects [2,5]. In Italy neither mitragynine, nor speciogynine, paynantheine, speciociliatine or 7-alpha-7 hydroxy-mitragynine, the whole plant or parts of the plant are included in the Tables containing narcotic and psychotropic substances subject to the supervision and control under Article 14 of Republic Presidential Decree 309/90 and subsequent updates.

Pharmaco-toxicological properties

The leaves of *Mitragyna speciosa* contain substances with psychoactive properties such as mitragynine and related alkaloids. They are used for recreational purpose as opium substitutes. Kratom has a lower effect than the pure mitragynine, due to the presence of other substances in the leaves which diminish its activity.

Mitragynine is an alkaloid with an indolic nucleus, structurally related to psilocybin and to ergine [6]. Its mechanism of action is based on the interaction with the opioid receptors acting as an agonist. Mitragynine has a depressing effect on the central nervous system similar to that induced by the opioids; the magnitude of mitragynine effect is about 26% the effect of morphine [7].

The analgesic activity of mitragynine seems to be due to the methoxyl group in position C9 of the indolic ring. Indeed, the corynantheidine (9-demethoxy mitragynine), an alkaloid with the indolic nucleus devoid of the methoxylic group, which is present in other genus of plants, does not present analgesic activities. It has been demonstrated that the replacement of the methoxyl group with an hydroxyl group (OH) or an hydrogen produces a variation in the molecular activity, changing from a pure agonist to a partial agonist or antagonist, respectively.

Mitragynine pseudoindoxyl, a synthetic oxydative derivative of mitragynine, has an *in vitro* opioid agonist activity which is 34-67% of the morphine activity [7].

The analgesic properties of mitragynine are comparable to those of codeine with some advantages. In contrast to codeine, mitragynine does not cause dyspnea or emesis, presents fewer withdrawal symptoms, less anticholinergic effect and causes less respiratory depression [8].

It has been demonstrated in animal model that the analgesic effects following intracerebroventricular administration of the receptor μ antagonists (ciprodime, 1-10 μg) and δ opioid receptor antagonist (natrindol 1-5 ng) and the preliminary treatment through the same route with the μ 1 receptor antagonist (naloxonazine 1-3 μg), interfere significantly with the antinociceptive activity of mitragynine. The study demonstrates that the analgesic properties of mytragynine are attributable to the stimulation of the μ and δ opioid receptors [9].

It has been demonstrated in the rat model that mitragynine has also the ability of reducing gastric secretion based on the stimulation of the opioid receptors [10].

Recently, it has been noted that the opioid-like effects of *Mitragyna speciosa* cannot be attributed exclusively to the weak opioid properties of mitragynine. Therefore, it has been hypothesized that the plant's properties are probably also due to the activity of 7-hydroxymitragynine, a molecule present (even if only in small quantities) in the leaves of *Mitragyna speciosa*, which is highly potent and has an affinity for the opioid receptors (particularly receptor μ) about 13 and 46 times higher than morphine and mitragynine, respectively [7].

It has been noticed that 7-hydroxymitragynine has an ED50 equal to 6.51 nmoles/mouse, morphine an ED50 equal to 3.20 nmoles/mouse and mitragynine an ED50 equal to 60.22 nmoles/mouse. The antinociceptive activity of the above-listed compounds is completely inhibited by naloxone (2 mg/kg) [7].

In the mouse, 7-hydroxymitragynine, given orally in doses of 5-10 mg/kg, has a higher antinociceptive activity than the equivalent dose of morphine [11].

Recently, it has been observed in an animal model that it is possible to develop a cross tolerance towards the analgesic effects of 7- hydroxymitragynine and morphine. Administration of naloxone to the animals treated chronically with 7-hydroxymitragynine can lead to heavy abstinence syndrome [12].

Other alkaloids present in the *Mitragyna speciosa* leaves are less potent compared to morphine (speciogynine: 3%, paynantheine: 1% and speciociliatine: 3% analgesic effect of morphine [13]. The speciociliatine (*cis*-quinolizidine) is the position C3 stereoisomer of mitragynine (*trans*-quinolizidine). Its lesser analgesic activity compared to mitragynine has been attributed to the bent *cis* configuration that seems to have a lower affinity for the opioid receptors [7]. A recent study investigating the effect of Kratom on the gastrointestinal tract of rats, showed antidiarrheal properties of methanolic extracts of the plant [14]. Among the activities of the extracts shown in the laboratory are also the anti-inflammatory and antinociceptive ones [15] and the ability to facilitate the entry of glucose in muscle cells, an activity which may explain its use as antidiabetic in popular medicine of Southeast Asia [16]. It has been described the use of Kratom by opiates addicts to improve pain in alleviating withdrawal symptoms. This effect has been attributed to the agonist action of the plant alkaloids that exert to the supraspinal μ and δ receptors for opioids [17].

Toxicity

In adults, the ingestion of 50 mg of pure mitragynine produces motor agitation, positive Romberg sign and tremors in the face, in the extremities and in the tongue.

Data regarding the acute toxicity: There is no data of acute toxicity for any of the alkaloids present in *Mitragyna speciosa*.

Adverse Effects

There are no systematic scientific studies on the adverse effects associated with the consumption of kratom in humans. There is only one study presenting human data by Jansen et al [6]. In this study, addiction cases in people from Thailand are described. The study participants presented with excessive thinness, dilated stomach, dark lips and cutaneous dryness. On the other hand, the administration of mitragynine to five healthy volunteers produced effects similar to those caused by cocaine. A case of kratom dependence has also been described; the subject in question chronically used the drug presenting with abstinence syndrome at discontinuation of use. At the same time, he never tried to increase the dose, remaining in good health without losing weight and maintaining a “rather normal” physical and mental state.

An early study, carried out in 1975 on 30 kratom consumers in Thailand, highlighted that the subjects (habitual consumers for more than five years) maintained on the whole a state of good health. A 90% subjects had chewed the fresh *Mitragyna speciosa* leaves or had ingested the powder. The leaves were chewed 3-10 times per day. The adverse effects shown in the study group included dryness of the jaws, frequent urination, constipation, loss of the appetite, cardiac irregularities and weight loss. The abstinence syndrome included manifestations of aggressiveness, skeletal-muscle pains, absence of tears and spasmodic movements [18].

Conversely, when kratom is used as a “Smart Drug“ with the purpose of recreational effects, it is suggested to use the substance occasionally (not more than once in week, better if once each 15 days) in order not to generate drug dependence. It is noteworthy that in Thailand some cases of *Mitragyna speciosa* dependence have been described with manifestations of muscular pains, irritability, weeping, rhinorrhea, diarrhoea and cramps.

Pharmacological interactions

The kratom has a depressant effect on the central nervous system, therefore it is advised not to use it in combination with other substances with central inhibitory activity such as yohimbine, cocaine, alcohol, benzodiazepines and narcotics.

Effects in pregnancy

There are no data on use in pregnancy or during lactation.

Analytical determinations

Scientific literature reports analytical methodologies to measure mitragynine, the principal active compound of *Mitragyna speciosa*, in urine of consumers [19]. While no assay exists to determine active principles in different plant parts.

The method below reported is a brief outline useful to the investigator to set up the analysis. For specific details, it is advisable to refer to the original text.

Determination of mitragynine in urine of consumers

(From: LU S, TRAN BN, NELSEN J L, ALDOUS KM. Quantitative analysis of mitragynine in human urine by high performance liquid chromatography-tandem mass spectrometry. J Chromatogr B 2009; 877: 2499-2505) [19].

The analysis was performed in urine, using liquid chromatography coupled with tandem mass spectrometry.

Extraction of the compound

2 ml of urine is mixed with 500 µl of phosphate buffer pH 11 for 30 seconds. After the addition of 3 ml methyl tert-butyl ether, the organic phase is separated and evaporated at 45°C under a nitrogen flow. The dry extract is resuspended in 1 ml methyl alcohol and a 10 µl volume is injected in the liquid chromatograph.

Analytical conditions

Chromatografic column: Atlantis HILIC (50mm x 3.0 mm x 3µm)
Mobile phase A: 5 mM ammonium acetate
Mobile phase B: methyl alcohol
Separation : gradient (mobile phase B: 90% to 100% in 3 minutes. From 100% to 90% in 3.9 minutes)
Flow rate: 0.25 ml/min
Column temperature: 40°C
Collision energy: 45 eV
Spray voltage: 4500 V
Source temperature: 550°C
Detector: mass spectrometer with positive mode electrospray (ESI) interface

Retention times of the tested compound

Mitragynine: 2.6 minutes

Characteristic fragments of the tested compound

Mitragynine: m/z 399 ⟶ 238, 226 ,174

Standard

As mytragynine standard, pulverized Kratom leaves purchased by web sites were used.

Calibration curve

The calibration curve samples (range 0.01-5.0 ng/ml) were prepared by adding stock standard solutions of known concentration to blank urine samples.

Results

The performed analysis showed that the urine contained mitragynine at a concentration well above the range of the calibration curve. The sample nedded to be diluted 20 times with methyl alcohol and reanalysed. The concentration of mitragynine was found to be 167 ± 15 ng/ml of urine.

References

1. SHELLARD EJ, The alkaloids of Mitragyna with special reference to those of *Mitragyna speciosa*, Kort Bull Narc. 1974; 26: 41-55.
2. TAKAYAMA H. Chemistry and pharmacology of analgesic indole alkaloids from the Rubiaceous plant, *Mitragyna speciosa*. Chem Pharm Bull. 2004; 52: 916-928.
3. THE MERCK INDEX An Enciclopedia of chemicals, drugs, and biologicals. 10th Ed. Merck & Co., Inc. 1983: p. 891.
4. PONGLUX D, WONGSERIPIPATANA S, TAKAYAMA H, KIKUCHI M, KURIHARA M, KITAJIMA M, AIMI N, SAKAI SI. A new indole alkaloid, 7α-hydroxy-7H-mitragynine, from *Mitragyna speciosa* in Thailand. Planta Med. 1994; 60: 580-581.
5. JANSEN KL, PRAST CJ. Ethnopharmacology of kratom and the Mitragyna alkaloids. J Ethnopharmacol. 1988; 23: 115-119.
6. JANSEN KL, PRAST CJ. Psychoactive properties of mitragynine (kratom). J Psychoactive Drugs 1988; 20: 455-457.
7. TAKAYAMA H, ISHIKAWA H, KURIHARA M, KITAJIMA M, AIMI N, PONGLUX D, KOYAMA F, MATSUMOTO K, MORIYAMA T, YAMAMOTO LT, WATANABE K, MURAYAMA T, HORIE S. Studies on the synthesis and opioid agonistic activities of mitragynine-related indole alkaloids: discovery of opioid agonists structurally different from other opioid ligands. J Med Chem. 2002; 45: 1949-1956.
8. TAKAYAMA H, AIMI N, SAKAI S. Chemical studies on the analgesic indole alkaloids from the traditional medicine (Mitragyna speciosa) used for opium substitute. Yakugaku Zasshi 2000; 120: 959-967.
9. THONGPRADICHOTE S, MATSUMOTO K, TOHDA M, TAKAYAMA H, AIMI N, SAKAI S, WATANABE H. Identifcation of opioid receptor subtypes in antinociceptive actions of supraspinally-administrated mitragynine in mice. Life Sci. 1998; 62: 1371-1378.
10. TSUCHIYA S, MIYASHITA S, YAMAMOTO M, HORIE S, SAKAI S, AIMI N, TAKAYAMA H, WATANABE K. Effect of mitragynine, derived from Thai folk medicine, on gastric acid secretion through opioid receptor in anesthetized rats. Eur J Pharmacol. 2002; 443: 185-188.
11. MATSUMOTO K, HORIE S, ISHIKAWA H, TAKAYAMA H, AIMI N, PONGLUX D, WATANABE K. Antinociceptive effect of 7-hydroxymitragynine in mice: Discovery of an orally active opioid analgesic from the Thai medicinal herb *Mitragyna speciosa*. Life Sci. 2004; 74: 2143-2155.
12. MATSUMOTO K, HORIE S, TAKAYAMA H, ISHIKAWA H, AIMI N, PONGLUX D, MURAYAMA T,WATANABE K. Antinociception, tolerance and withdrawal symptoms induced by 7-hydroxymitragynine, an alkaloid from the Thai medicinal herb *Mitragyna speciosa*. Life Sci. 2005; 78: 2-7.
13. HORIE S, KOYAMA F, TAKAYAMA H, ISHIKAWA H, AIMI N, PONGLUX D, MATSUMOTO K, MURAYAMA T. Indole alkaloids of a Thai medicinal herb, *Mitragyna speciosa*, that has opioid agonistic effect in guinea-pig ileum. Planta Med. 2005; 71: 231-236.
14. CHITTRAKARN S, SAWANGJAROEN K, PRASETTHO S, JANCHAWEE B, KEAWPRADUB N. Inhibitory effects of kratom leaf extract (Mitragyna speciosa Korth.) on the rat gastrointestinal tract. J Ethnopharmacol. 2008; 116: 173-178.
15. SHAIK MOSSADEQ WM, SULAIMAN MR, TENGKU MOHAMAD TA, CHIONG HS, ZAKARIA ZA, JABIT ML, BAHARULDIN MT, ISRAF DA. Anti-inflammatory and antinociceptive effects of Mitragyna speciosa Korth methanolic extract. Med Princ Pract. 2009; 18: 378-384.
16. PURINTRAPIBAN J, KEAWPRADUB N, KANSENALAK S, CHITTRAKARN S, JANCHAWEE B, SAWANGJAROEN K. Study on glucose transport in muscle cells by extracts from Mitragyna speciosa (Korth) and mitragynine. Nat Prod Res. 2008 10:1-9.
17. BABU KM, MCCURDY CR, BOYER EW. Opioid receptors and legal highs: Salvia divinorum and Kratom. Clin Toxicol (Phila). 2008; 46: 146-152.
18. SUWANLERT S. A study of kratom eaters in Thailand. Bull Narc. 1975; 27: 21-27.
19. LU S, TRAN BN, NELSEN JL, ALDOUS KM. Quantitative analysis of mitragyne in human urine by high performance liquid chromatography-tande, mass spectrometry. J Chromatogr B 2009; 877: 2499-2505.

Muira puama
(potency wood)

Name: *Muira puama*
Family: *Olacaceae*
Genus: *Ptychopetalum ovata*
Species: *Ptychopetalum olacoides*
Synonims: acanthea marapama, muiratã, muiratam, marapuama, potency wood
Origin: Amazon rainforest
Active compounds: muirapuamine

The properties of the root and the bark of *Muira puama* have been attributed to certain components, such as numerous free long chain fatty acids, essential oils, phytosterols, cumarin and to a specific alkaloid "muirapuamine". These compounds are present in the plant at the following percentages: muirapuamine (0.5 %), fats (4 %), alkaloids (5 %), phlobaphene (6 %), α resinic acid (6%), β resinic acid (7%), as well as tannins and volatile oils [1-4]. Although the mechanism of action of the active components in the roots and bark is not clear, *Muira puama* is best known for its tonic effect at the nervous, muscular and circulatory systems level.

Chemical formula and physico-chemical properties of the active compound

The chemical structure, molecular formula, systematic name, CAS registry number, melting point, UVmax and solubility of muirapuamine have not been found in the current scientific literature.

Historical use

The numerous beneficial properties attributed to *Muira puama* by the Brazilian herbalistic tradition have contributed to its dissemination during colonial times also in western countries, particularly in France and in England.
The first European explorers of the 1920-1930s noticed that the natives of the Amazon rainforest were using *Muira puama* as aphrodisiac and tonic. Once it spread to Europe, it became part of the English herbalistic medicine.
As early as in 1925, there was a pharmacological study which showed the efficacy of *Muira puama* in the treatment of nervous system problems and decreased libido [5]. The British Herbal Medicine Association recognizes its long history of use in England. *Muira puama* can be found in "British Herbal Pharmacopoeia" - an authoritative source of herbalistic medicine which recommends it for the treatment of dysentery and impotence. It is part of the Brazilian Pharmacopoeia since 1950.

Current use

At present, *Muira puama* is used as a psychophysical tonic; a sexual stimulant action is reported and for this reason the plant is indicated in some forms of impotence, both, in males and females. Its aphrodisiacal properties are owed essentially to the presence of the muirapuamine, an alkaloid with alleged pharmacological action similar to that of yohimbine (alkaloid of the *Pausinystalia yohimbe*), with excellent sexual stimulant properties. In addition, *Muira puama* is also used as digestive, neurotonic, antireumatic and antineuralgic. It was reported as useful in cases of gastrointestinal and circulatory asteny, in ovarian atony and for alleviating menstrual pains. Because of its neurotonic properties, in addition to cases of impotence, it can be useful for the treatment of exhaustion and light nervous depression.

Legislation

There are no legal restrictions either in Europe, or in the United States of America for the use of *Muira puama* or of its active ingredients. In Italy, neither muirapuamine, nor the whole plant of *Muira puama* or parts of it are are included in the tables containing narcotic and psychotropic substances subject to the supervision and control under Article 14 of Republic Presidential Decree 309/90 and subsequent updates.

Pharmaco-toxicological properties

The ethanolic extracts of *Muira puama* have nootropic, antioxidant and neuroprotective properties [6-8]. Studies in mice on both short and long term memory highlighted the effect of this plant also on the beta-adrenergic receptors and D1 dopamine receptor. These above-mentioned effects on the central nervous system attributed to the alcoholic extracts of *Muira puama* are increased by pharmacologically active compounds such as spiperone and pindol - serotonin $5HT_{2A}$ antagonists [9].

The best known effects are due to the plant ability of acting as a tonic at the nervous, muscular and circulatory systems level. Most probably *Muira puama* acts on the "catecholaminergic" systems of the central nervous system and its active ingredients would act as precursors of cerebral neurotrasmitors. Clinical studies have demonstrated that the use of *Muira puama* improves the erectile state and the sexual functions in men, the vigour and the libido both in men and in women [10].

Toxicity

A study using extracts of *Muira puama* did not present any evidence of toxicity [11].

Adverse effects

There is no data on any adverse effects due to *Muira puama* use.

Pharmacological interactions

Possible pharmacological interactions have not been reported.

Effects in pregnancy

There is no data on *Muira puama* use during pregnancy and/or breast feeding.

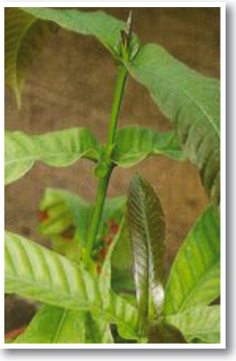
Analytical determinations

No methodologies have been reported in the international literature concerning the analysis of *Muira puama* active principles in biological fluids of eventual plant consumers, nor in the plant or parts of it.

References

1. IWASA, J., KIMURA Y. Constituents of Muira puama. Yakugaku Zasshi (Japan) 1969; 89: 1172–74.
2. AUTERHOFF, H. "Components of Muira puama." Arch. Pharm. Ber. Dtsch. Pharm. Ges. 1968; 301: 481–89.
3. AUTERHOFF, H. "Components of Muira puama II." Arch. Pharm. Ber. Dtsch. Pharm. Ges. 1969; 302: 209–12.
4. AUTERHOFF, H. "Lipophilic constituents of Muira puama." Arch. Pharm. Ber. Dtsch. Pharm. Ges. 1971; 304: 223–28.
5. DIAS DA SILVA, R. "Medicinal plants of Brazil. Botanical and pharmacognostic studies. Muira puama." Rev. Bras. Med. Pharm. 1925; 1(1): 37–41.
6. DA SILVA AL, PIATO ALS, BARDINI S, NETTO CA, NUNES DS, ELISABETSKY E. Memory retrieval improvement by *Ptychopetalum olacoides* in young and aging mice, J Ethnopharmacol 2004; 95 : 199–203.
7. SIQUEIRA IR, CORDOVA CS, CRECZYNSKI-PASA T, ELISABETSKY E, D.S. NUNES DS, NETTO CA. Antioxidant action of an ethanolic extract of *Ptychopetalum olacoides* Bentham (Olacaceae), Pharm Biol 2002; 40: 374–379.
8. SIQUEIRA IR, CIMAROSTI H, FOCHESATTO C, NUNES DS, SALBEGO C,. ELISABETSKY E.. Neuroprotective effects of *Ptychopetalum olacoides* Bentham (Olacaceae) on oxygen and glucose deprivation induced damage in rat hippocampal slices. Life Sci 2004;75:1897–1906.
9. DA SILVA A L , FERRIERA JG, DA SILVA MARTINS B, OLIVEIRA S, MAI N, NUNES DS, ELISABETSKY E. Serotonin receptors contribute to the promnesic effects of P. olacoides (Muirapuama). Physiol & Bahavior 2008;95: 88-92
10. ROWLAND DL, TAI W. A review of plant-derived and herbal dysfunctions approaches to the treatment of sexual dysfunctions. J. Sex Marital Ther. 2003;29:185–205.
11. OLIVEIRA CH, MORAES MEA, MORAESFERNANDO MO, BEZERRA AF, ABIB E, DE NUCCI G. Clinical toxicology study of an herbal medicinal extract of *Paullinia cupana, Trichilia catigua, Ptychopetalum olacoides* and *Zingiber officinale* (Catuama®) in healthy volunteers. Phytother. Res. 2005; 19: 54–57.

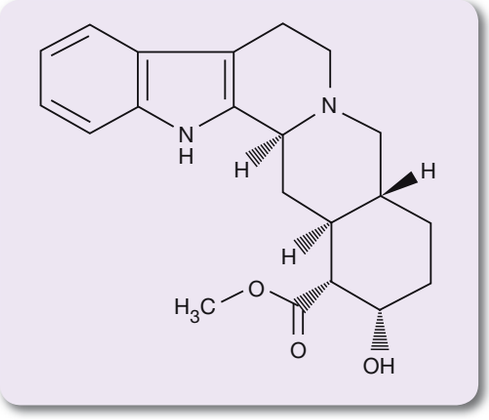
Pausinystalia yohimbe
(Yohimbe)



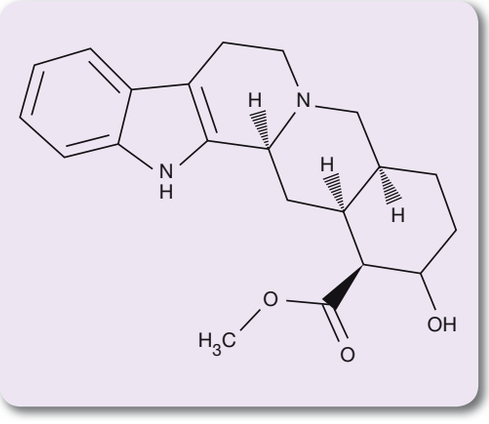
Name: *Pausinystalia yohimbe*
Family: *Rubiaceae*
Genus: *Pausinystalia (Corynanthe)*
Species: *Pausinystalia yohimbe* [K.Schumann]
Synonims: Corynanthe yohimbe; yohimbe
Origin: West Africa: Nigeria, Camerun, Congo
Active compounds: yohimbine, α-yohimbine, δ-yohimbine, allo-yohimbine, corynanthine [1]

The plant *Pausinystalia yohimbe* contains up to 6% of total alkaloids of which 10-15% is yohimbine [1]. Yohimbine is the principal alkaloid (as well as the most studied one) extracted from the bark of *Pausinystalia yohimbe* of West Africa. The quality and the quantity of yohimbine in the bark is highly variable, the optimum quantity-quality ratio being reached in the bark of the principal trunk. The concentration of the active compounds is subject also to seasonal changes, being the highest during the rainy season and lowest during the dry season. Normally, the minced bark is used after dissolving it in water or alcohol.

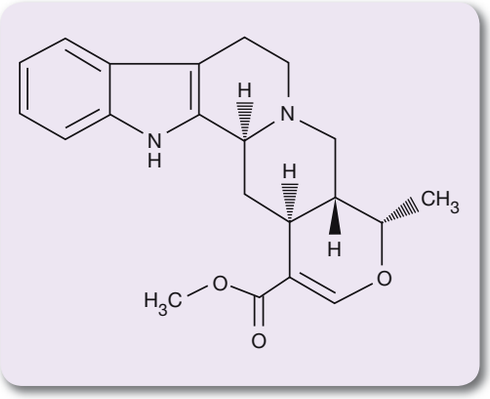
Chemical formula and physico-chemical properties of the active compounds



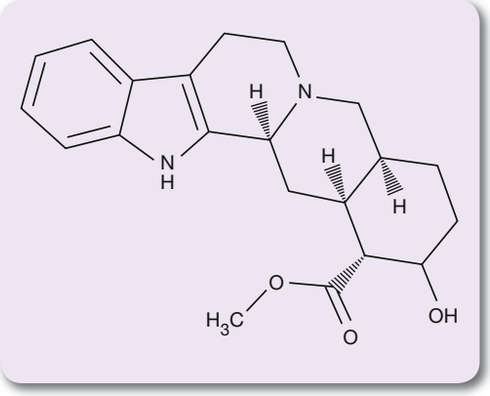
Name: Yohimbine.
Molecular formula: $C_{21}H_{26}N_2O_3$ (molecular weight = 354.4).
Systematic name: yohimban-16-α-carboxylic acid, 17-α-hydroxy-, methyl ester.
CAS registry number: 146-48-5.
Melting point: 241°C.
UVmax: (methyl alcohol) 226, 280, 291 nm.
Solubility: Limited solubility in water, moderately soluble in ether, soluble in alcohol, cloroform, warm benzene.



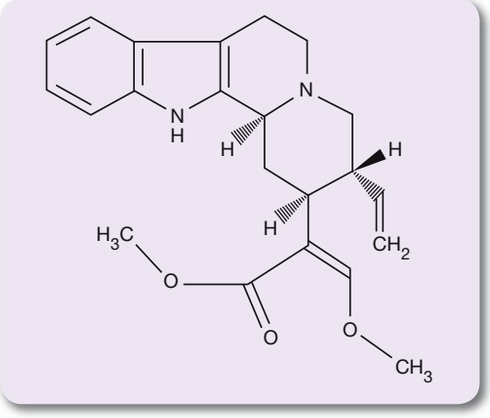
Name: α-Yohimbine.
Molecular formula: $C_{21}H_{26}N_2O_3$ (molecular weight = 354.4).
Systematic name: 20-α-yohimban-16-β-carboxylic acid, 17-α-hydroxy-methyl ester.
CAS registry number: 131-03-3.
Melting point: 243.5°C.
UVmax: (methyl alcohol) 227, 281 nm.
Solubility: Insoluble in water, moderately soluble in ether and benzene, methyl alcohol and warm ethyl alcohol.



Name: δ-Yohimbine.
Molecular formula: $C_{21}H_{24}N_2O_3$ (molecular weight = 352.4).
Systematic name: Oxayohimban-16-carboxylic acid, 16,17-dide-hydro-19-α-methyl- methyl ester.
CAS registry number: 483-04-5.
Melting point: 258°C.
UVmax: no data in the literature.
Solubility: no data in the literature.



Name: allo-Yohimbine.
Molecular formula: $C_{21}H_{26}N_2O_3$ (molecular weight = 354.4).
Systematic name: yohimban-20-α-16-α-arboxylic acid, 16, 17-de-hydro, 19-α-methyl-methyl ester.
CAS registry number: 522-94-1.
Melting point: 135-140°C.
UVmax: (methyl alcohol) 225, 280, 290 nm.
Solubility: insoluble in water, soluble in methyl alcohol, ethyl alcohol, pyridine..



Name: Corynanthine.
Molecular formula: $C_{22}H_{26}N_2O_3$ (molecular weight = 366.4).
Systematic name: 17-18 secoyohimban-16–carboxylic acid,16, 17, 18,19- tetradehydro, 17-methoxy-methyl ester.
CAS registry number: 18904-54-6.
Melting point: the α isomer melts at 103-107°C, the β isomer melts at 165-166°C.
UVmax: (methyl alcohol) 227, 280, 291 nm.
Solubility: corynanthine chlorhydrate is soluble in alcohol and slightly in water.

Historical use

Traditionally *Pausinystalia yohimbe* (or yohimbe) has been used in the treatment of the male impotence and in the treatment of a wide range of vascular problems. The historical use, is in any case related to the presumed "aphrodisiac" effect [(2)]. The plant is pulverized by grinding the bark, and after boiling in water with other herbal products the infusion can be drunk.

Current use

The yohimbe is used sometimes by the athletes as a performance enhancer, as well as by the singers to obtain a higher clarity of the tone of voice during prolonged touring. The western consumers search mostly for its aphrodisiac properties . It is possible to find and purchase yohimbe in capsules, often mixed with other herbs (damiana, ginseng, guaranà, *Muira puama*) on free Internet sites which sell "smart drugs". The consumer is attracted by the advertised aphrodisiac properties: "…increases the erotic and sexual feelings, it is an aphrodisiac and heightens the sexual vigor...".

Legislation

In Finland, Norway, Australia and Canada the selling and trading of *yohimbe* is illegal, since it is considered a health hazard. In the USA the *yohimbe* is not controlled: it is possible in fact to buy, sell, cultivate and possess without license or prescription the whole plant or its extracts. If the plant or its derivatives are sold like food additives, they have to follow the United States laws relative to the dietary supplements. In Italy, neither yohimbine, nor α-yohimbine, delta-yohimbine, allo-yohimbine, or corynanthine, as the whole plant or part of it are found in the tables containing narcotic and psychotropic substances subject to the supervision and control under Article 14 of Republic Presidential Decree 309/90 and subsequent updates. Nonetheless, the Italian Ministry of Health included *yohimbe* in the list of vegetal extract not admitted in dietary supplements [3].

Pharmaco-toxicological properties

Most of the research related to *Pausinystalia yohimbe* has been carried out on yohimbine, the principal alkaloid present in the extracts of the plant. The yohimbine is a powerful antagonist of the α-1 adrenergic receptors [4]; increasing doses block serotoninergic and dopaminergic receptors, while at elevated doses yohimbine can act as a local anesthetic [5].

At central level, yohimbine is able to block the pre-synaptic α-2 adrenergic receptors with the inhibition of noradrenaline release. As a consequence of this effect, the metabolism of noradrenaline is increased and its cerebral and spinal levels are reduced [6]. This effect is higher effect on the pre-synaptic, rather than in the post-synptic α-adrenergic receptors [7-8].

In vivo animal studies have demonstrated that the intraperitoneal yohimbine given in 0.5 – 1 mg/kg doses, by affecting the adrenergic receptors, significantly inhibit sthe effect of LSD (lysergic acid diethyl amide) on the animal's behavior [9].

At the sympathetic level, yohimbine increases the cholinergic activity and reduces the adrenergic activity. It acts similarly to reserpine on the peripheral blood vessels, although the effect is faster but shorter [10].

In the human, yohimbine can affect the sexual behaviour [11]. The alkaloid, in fact, by blocking the α-2 adrenergic receptors, increases the blood flow to the *corpus cavernosus* constantly refilling it and maintaining the erection [12]. It is worth mentioning that, according to the American Food and Drug Administration (FDA), although up to the middle of the 90's, yohimbine has been widely used for the treatment of erectile dysfunctions, its effectiveness in this sense has never been proven in an unequivocal way [13]. This lack of proof of efficaciousness has been confirmed also by the American Urological Association, which has recently published the guidelines for the treatment of erectile dysfunctions [14].

Yohimbine is a stimulant of the central nervous system and at high doses it can have an anxiogenic effect. The reason for this effect might be the blocking of the α-2 adrenergic receptors and an increase in the cortical release of colecistochinine-like substances [15]. The anxiogenic effect can be prevented by diazepam administration [15].

In a study on healthy volunteers, it has been demonstrated that oral yohimbine inhibits *ex-vivo* the platelet aggregation induced by adrenaline. The substance has been administered in single doses of 4, 8 and 12 mg. The antiplatelet effect has been observed at the 8 mg dose, while at the 12 mg dose the duration of the effect was at least 10 hours. None of the administered doses resulted in changes of the blood pressure, heart rate or plasma levels of catecholamines and glucose [16].

Yohimbine easily crosses the blood-brain barrier and, once in the central nervous system, inhibits the peripheral serotoninergic receptors and causes excitement, increased motor activity, tremors and release of the antidiuretic hormone (ADH). In turn, the release of ADH causes water retention, increased blood pressure and heart rate [17].

Toxicity

Data regarding the acute toxicity of yohimbine

In human - TDLo following oral administration: 0.643 mg/Kg

In mouse - LD50 following intraperitoneal administration: 16 mg/kg.

In mouse - LD50 following oral administration: 43 mg/kg.

In mouse - LD50 following subcutaneous administration: 37 mg/kg.

In rabbit - LDLo following intravenous administration: 11 mg/kg.

In rabbit - LDLo following subcutaneous administration: 50 mg/kg.

Data regarding the acute toxicity of corynanthine

In mouse - LD50 following intravenous administration: 35 mg/kg

Data regarding the acute toxicity of α-yohimbine

In mouse - LD50 following intraperitoneal administration: 80 mg/kg

In mouse - LDLo following oral administration: 2500 mg/kg

In rat - LD50 following intraperitoneal administration: 50mg/kg

Data regarding the acute toxicity of allo-yohimbine

In mouse - LDLo following oral administration: 2500 mg/kg

Data regarding the acute toxicity of δ-yohimbine

In child - TDLo following oral administration: 12.5 mg/Kg

In mouse - LD50 following intraperitoneal administration: 165 mg/kg

In mouse - LD50 following oral administration: 400 mg/kg

In mouse - LD50 following intravenous administration: 20 mg/kg

In rat - LD50 following intraperitoneal administration: 2000 mg/kg

In rat - LD50 following intravenous administration: 24 mg/kg

In rat - LD50 following oral administration: 750 mg/kg

Adverse Effects

The adverse effects associated with yohimbine use include increase of the blood pressure and heart rate [18], anxiety, drowsiness and maniac symptoms [19-21]. Seldom it causes piloerection, runny nose, decreased urine output and a state of generalized central excitation that manifests by increase of the motor activity, agitation, irritability and tremors. In some cases, there have been reports of numbness, movement coordination difficulties and dissociative states.

In literature it is reported the case of a 42-year-old man who, after taking yohimbine for the treatment of impotence, has developed erythrodermic rash accompanied by progressive renal failure and a lupoid-like syndrome [22].

In another patient suffering from impotence, an adverse reaction characterised by bronchospasm has been attributed to yohimbine administration. The authors hypothesized that the basis of the bronchospasm has been an increase of the cholinergic tone with subsequent increase of contractions and of bronchial secretion [23]. It was also described a severe case of intractable priapism associated with the ingestion of yohimbe extract. Management required insertion of a proximal cavernosal spongiosum shunt (Quackles shunt) in the operating room [24].

In an article in the “Consumer Report“ (official scientific review of the Consumers Trade Union of the United States of America) yohimbine has been reported as “potentially dangerous” and it has been placed on a list of twelve plant products associated with the onset of major adverse effects [25].

In November 2001 the Food and Drug Administration (FDA) warned consumers from using dietary supplements for weight loss containing yohimbine and other farmacologically active compounds (norephedrine, caffeine, diiodothyronine and sodium usniate) because of suspect of hepatotoxicity [26].

Yohimbine overdose manifests with increased salivation, midriasis, diarrhea, hypotension and with a negative inotropic effect. Death can occur from heart failure [19].The treatment involves the emptying of the intestinal tract (induction of vomiting and/or gastric lavage), administration of activated charcoal, treatment of cardiac rhythm disturbances, administration of physostigmine, administration of electrolytes and infusion of sodium bicarbonate to treat the possible metabolic acidosis [19].

In recent years, the use of yohimbine among body-builders has spread for its alleged lipolytic and sympathomimetic effects. The case of a 37 year- old body builder who developed symptoms such as illness, vomiting, high blood pressure, unconsciousness and convulsions due to ingestion of large amounts of yohimbine (5 g) as been reported [27].

Pharmacological interactions

Yohimbine can interact with numerous drugs causing adverse reactions characterized by hypertension, tremors, insomnia, palpitations and anxiety [28].

In particular, interactions can be with:

- Monoamine oxydase inhibitors: it is possible to have an increase of the hypertensive effects [29].
- Sibutramine: used for appetite control in obese subjects. The drug inhibits serotonine and noradrenaline uptake in the peripheral tissues; yohimbine increases this effect and as a result it can increase the risk of hypertension [30].
- Clonidine: yohimbine can compete with clonidine at the α2 adrenergic receptor level causing inhibition of the antihypertensive activity.
- Morphine: yohimbine can increase the analgesia and the adverse effects associated with the use of morphine [31].
- Naloxone: together with yohimbine it causes irritation, anxiety, tremors, palpitations, nausea, changes in temperature and alterations in plasma cortisol levels [32].

Yohimbine can cause a reduction in the antihypertensive efficiency of the following drugs [31]:

- ACE-inhibitors
- Angiotensin receptor antagonists
- β blockers
- Calcium channel blockers
- Diuretics
- Guanabenz
- Guanadrel
- Guanethydine
- Guanfacine
- Hydrazaline
- Minoxidil
- Reserpine
- Methyldopa

Yohimbine can cause an increase in the risk of manic episodes in bipolar patients and others, treated with one of the following [34]:

- Carbamazepinae
- Lithium
- Valproic acid
- Desipramine

Yohimbine can cause hypertension if associated with the following drugs [28]:

- Tiroxine
- Clomipramine
- Synephrine and ephedrine

Effects in pregnancy

Yohimbe should not be used in pregnancy [35].

Analytical determinations

Scientific literature reports analytical methodologies to measure yohimbine, the principal active compound of *Pausinystalia yohimbe*, in urine [36] and to determine different active principles in the bark of the tree [37,38]. The first assay to analyze active substances in the plant bark implies the use of gas chromatography coupled to mass spectrometry [37], while the second one employs capillary electrophoresis coupled to diode array spectrophotometric detector and also gas chromatography coupled to mass spectrometry [38].

The method below reported is a brief outline useful to the investigator to set up the analysis. For specific details, it is advisable to refer to the original text.

Determination of yohimbine in urine of consumers

(From: BJÖRNSTAD K, BECK O, HELANDER A. A multi-component LC-MS/MS method for detection of ten plant-derived psychoactive substances in urine. J Chromatogr B Analyt Technol Biomed Life Sci. 2009; 877: 1162-1168)[36].

The analysis is carried out on urine samples by liquid chromatography coupled to tandem mass spectrometry.

Extraction of the compounds

A 50 µl volume of urine sample is diluted with 150 µl distilled water containing 200 µg/l dimethyltriptamine-D4 as internal standard. A 10 µl volume of this latter solution is injected into the chromatograph.

Analytical conditions

Chromatografic column: Hypersil GOLD (100 x 2.1mm x 5µm)
Mobile phase A: 10 mM formic acid- acetonitrile (99:1 v/v)
Mobile phase B: 10 mM formic acid- acetonitrile (40:60 v/v)
Separation: linear gradient (mobile phase B, from 0 to 100% in 10 minutes, then to 100 to 0% in 4 minutesfor a total of 14 minutes)
Flow rate: 0.2 ml/min
Detector: mass spectrometer with positive mode electrospray (ESI) interface
Source temperature: 350°C
Nebulization gas pressure: 20 psi
Capillary voltage: 5000 V
Collision energy: 41 eV

Retention times of the tested compounds

Yohimbine: 7.67 minutes
Dimethyltriptamine-D4 (internal standard): 6.37 minutes

Characteristic fragments for the tested compounds

Yohimbine: m/z 355 ⟶ 144, 212
Dimethyltriptamine-D4 (internal standard): m/z 193 ⟶ 148

Standard

Yohimbine standard has been obtained by Chromadex (Santa Ana, CA, USA). The internal standard has been synthesized in the analytical laboratory.

Calibration curves

Calibration curves have been prepared with blank urine covering a yohimbine concentration range from 5 to 5000 µg/L.

Results

Analyzed urine samples came from individuals attending the emergency ward with a suspected intoxication by *Pausinystalia yohimbe* herbal preparations. However, no positive result has been obtained in the examined samples.

References

1. http://www.pureworld.com/redirect_pw2n.html
2. SUNDERLAND T, TCHOUNDJEU Z, NGO-MPECK. The exploitation of Pausinystalia yohimbe. Med Plant Cons. 2000; 6: 21-22.
3. yohimbe. Med Plant Cons. 2000; 6: 21-22.
3. The list of vegetal extracts not admitted in dietary supplements in Italy can be found at http://www.salute.gov.it
4. LANGER SZ. Presynaptic regulation of the release of catecholamines. Pharmacol Rev. 1980; 32: 337-362.
5. GOLDBERG MR, ROBERTSON D. Yohimbine: a pharmacological probe for study of the alpha-2-adrenoreceptor. Pharmacol Rev. 1983; 35: 143-180.
6. ANDEN N, GRABOWSKA M. Pharmacological evidence for a stimulation of dopamine neurons by noradrenaline neurons in the brain. Eur J Pharmacol. 1976; 39: 275-282.
7. BROWN J. Effects of alpha adrenoceptor agonists and antagonists and of antidepressant drugs on pre and postsynaptic alpha adrenoceptors. Eur J Pharmacol. 1980; 67: 33-40.
8. DREW GM. Effects of alpha adrenoceptor agonists and antagonists on pre- and post synaptically located alpha adrenoceptors. Eur J Pharmacol. 1976; 36: 313-320.
9. MUSTAFA SM., BAVADEKAR SA., MA G., MOORE BM., FELLER DR., MILLER DD. Synthesis and biological studies of yohimbine derivatives on human α_{2c}-adrenergic receptors. Bioorg Med Chem Lett. 2005; 15: 2758-2760.
10. ANON. Yohimbine hydrochloride. Mosby, Inc 1998. Available at: http://www.Rxlist.com (cited 1/6/00).
11. RILEY AJF. Yohimbine in the treatment of erectile disorder. Br J Clin Pract Suppl. 1994; 48:133-136.
12. BAUM NY. Treatment of impotence. 1. Nonsurgical methods. Postgrad Med. 1987; 81: 133-6.
13. BRINDLEY GS. Pilot experiments on the actions of drugs injected into the human corpus cavernosum penis. Br J Pharmacol. 1986; 87: 495-500.
14. http://www.fda.gov/ohrms/dockets/ac/00/transcripts/3602b1c.pdf
15. http://www.auanet.org/guidelines/
16. BECKER C, HAMON M & BENOLIEL JJ. Prevention by 5-HT1A receptor agonists of restraint stress- and yohimbine-induced release of cholecystokinin in the frontal cortex of the freely moving rat. Neuropharmacology. 1999; 38: 525-532.
17. BERLIN I, CRESPO-LAUMONNIER B, COURNOT A et al. The alpha-2-adrenergic receptor antagonist yohimbine inhibits epinephrine-induced platelet aggregation in healthy subjects. Clin Pharmacol Ther. 1991; 49: 362-369.
18. HARDMAN JG, LIMBIRD LE, MOLINOFF PB et al. Goodman and Gilman's The Pharmacological Basis of Therapeutics, 9th ed. McGraw-Hill, New York, NY, 1996.
19. LACOMBLEZ L, BENSIMON G, ISNARD F et al. Effect of yohimbine on blood pressure in patients with depression and orthostatic hypotension induced by clomipramine. Clin Pharmacol Ther. 1989; 45: 241-251.
20. FLEMING T. PDR for Herbal Medicines, Medical Economics company, Montvale, NJ. 1998.
21. TYLER VE. Herbs of Choice: The Therapeutic use of phytomedicinals. Pharmaceutical Products Press, New York, NY. 1991.
22. SANDLER B, ARONSON P. Yohimbine-induced cutaneous drug eruption, progressive renal failure, and lupus-like syndrome. Urology. 1993; 41: 343-345.
23. LANDIS E, SHORE E. Yohimbine-induced bronchospasm. Chest. 1989; 96: 1424.
24. MYERS A, BARRUETO F JR. Refractory priapism associated with ingestion of yohimbe extract. J Med Toxicol. 2009; 5: 223-225.
25. [AUTORI NON ELENCATI]. Dangerous supplements: still at large. Consum Rep. 2004; 69: 12-17.
26. [AUTORI NON ELENCATI]. Dietary supplement warning. FDA Consum. 2002; 36: 4
27. GIAMPRETI A, LONATI D, LOCATELLI C, ROCCHI L, CAMPAILLA MT. Acute neurotoxicity after yohimbine ingestion by a body builder. Clin Toxicol (Phila). 2009; 47: 827-829.
28. FIRENZUOLI F. Interazioni tra erbe, alimenti e farmaci. Ed. Tecniche Nuove. 2001
29. FUGH-BERMAN A. Herb-drug interactions. Lancet. 2000; 355: 134-138.
30. JORDAN J, SHARMA AM. Potential for sibutramine-yohimbine interaction? Lancet. 2003; 361: 1826.
31. GEAR RW, GORDON NC, HELLER PH et al. Enhancement of morphine analgesia by the alpha2-adrenergic antagonist yohimbine. Neuroscience. 1995; 66: 5-8.
32. CHARNEY DS, HENINGER GR. Alpha-2-adrenergic and opiate receptor blockade. Synergistic effects on anxiety in healthy subjects. Arch Gen Psychiatry. 1986; 43: 1037-1041.

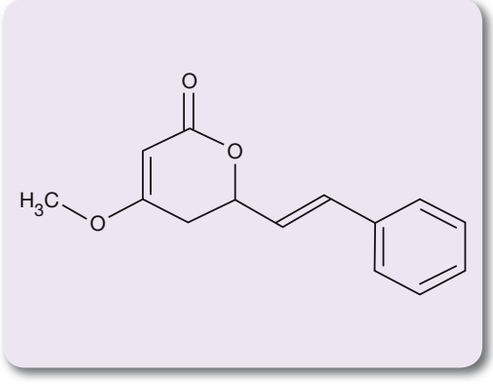
33. MUSSO NR, VERGASSAOL C, PJENDE A. Yohimbine effects on blood pressure and plasma catecholamines in human hypertension. Am J Hypertens. 1995; 8: 565-571.
34. PRICE LH, CHARNEY DS, HENINGER GR. Three cases of manic symptoms following yohimbine administration. Am J Psychiatry. 1984; 141:1267-1268.
35. FETROW CW, AVILA JR. Professional's Handbook of Complementary and Alternative Medicines. Springhouse Co, Springhouse, PA, 1999.
36. BJÖRNSTAD K, BECK O, HELANDER A. A multi-component LC-MS/MS method for detection of ten plant-derived psychoactive substances in urine. J Chromatogr B Analyt Technol Biomed Life Sci. 2009; 877: 1162-1168.
37. BETZ JM, WHITE KD, DER MARDEROSIAN AH. Gas chromatographic determination of yohimbine in commercial yohimbe products. J AOAC Int. 1995; 78: 1189-1194.
38. QINHUA C, PENG L, ZHUI Z, KAIJUN L, JIA L, QIANG L. Analysis of yohimbe alkaloid from Pausinystalia yohimbe non-aqueous capillary electrophoresis and gas chromatography-mass spectrometry J Sep Sci 2008; 31: 2211-2218.

Piper methysticum
(Kava-kava)

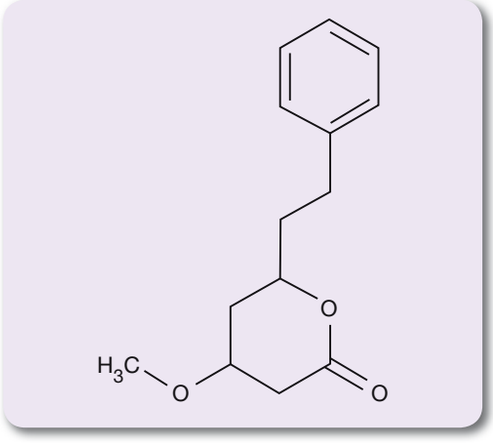
Name: *Piper methysticum, kava-kava*
Family: *Piperacee*
Genus: *Piper*
Species: *Piper methysticum*
Synonims: Ava, ava pepper, ava pepper shrub, ava root, awa, cavain, gea, gi, intoxicating long pepper, intoxicating pepper, kao
Origin: Oceania
Active compounds: kavalactons: such as kavain, dihydrokavain, methysticin, dehydromethysticin, yangonin

Kava-kava (or *Sakau* or *Kawa Kawa*) is an old drink, based on an herb (*Piper methysticum*), used by the populations of the South Pacific. It was thought that the plant originates from Melanesia, even if it has long been rooted in the Polinesian islands [(1)]. The plant is part of the black pepper family, whose principal active ingredient called kavain is concentrated in the roots. The dried roots are pounded and then pulverized. The powder is sold in bags in the local supermarkets and shipped around the world. If taken in little doses, *kava-kava* produces a sensation of wellbeing, sharpens the intellectual faculties and makes difficulties easier to take. When it is used at medium doses it acts as a muscular relaxant and as spasmolitic producing a quiet and pacifying sleep, rich in pleasant dreams. High doses lead a deep sleep.

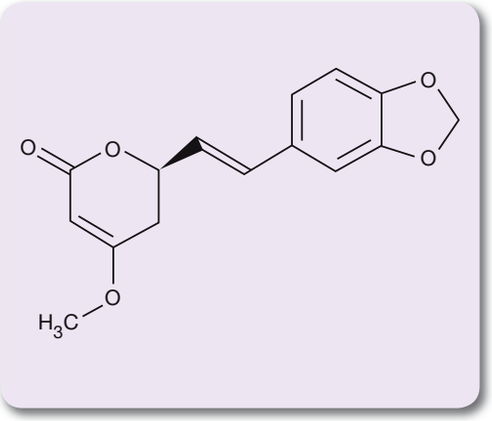
Chemical formula and physico-chemical properties of the active compounds



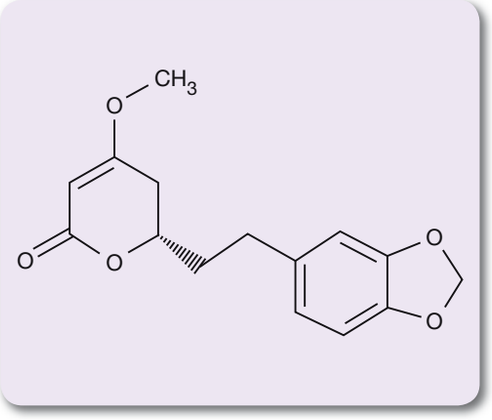
Name: Kavain
Molecular formula: $C_{14}H_{14}O_3$ (molecular weight = 230.3).
Systematic name: 2,6-heptadienoic acid, 5-hydroxy-3-methoxy-7-phenyl-, δ-lactone.
CAS registry number: 1635-33-2.
Melting point: 146°C.
UVmax: 210, 245, 282 nm.
Solubility: acetone, ether, methyl alcohol, slightly soluble in hexane



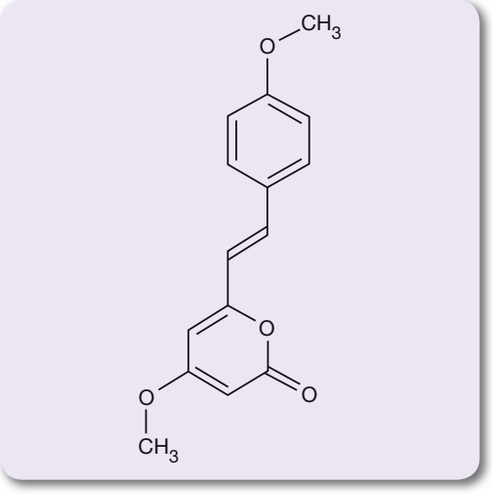
Name: Dihydrokavain.
Molecular formula: $C_{14}H_{16}O_3$ (molecular weight = 232.2).
Systematic name: 2H-pyran-2-one, 5,6-dihydro-4-methoxy-6-phenethyl-.
CAS registry number: 587-63-3.
Melting point: no data in the literature.
UVmax: no data in the literature.
Solubility: no data in the literature.



Name: Methysticin.
Molecular formula: $C_{15}H_{14}O_5$ (molecular weight = 274.2).
Systematic name: (R)-5,6-Dihydro-4-methoxy-6-(3,4-(methylenedioxy)styryl)-2H-pyran-2-one.
CAS registry number: 495-85-2.
Melting point: 137°C.
UVmax: 226, 267, 306 mn.
Solubility: alcohol, ether, acetone



Name: Dehydromethysticin.
Molecular formula: $C_{15}H_{16}O_5$ (molecular weight = 276.2).
Systematic name:(2S)-2-(1,3-benzodioxy-5-yl)ethyl)-4-methoxy-2,3-dihydropyran-6-one.
CAS registry number: 19902-91-1.
Melting point: no data in the literature.
UVmax: no data in the literature.
Solubility: no data in the literature.



Nome: Yangonin.
Molecular formula: $C_{15}H_{14}O_4$ (molecular weight = 258.2).
Systematic name: 4-methoxy-6-2-(4-methoxyphenyl)vinyl]-2H-pyran-2-one.
CAS registry number: 500-62-9.
Melting point: 156°C.
UVmax: 360 nm.
Solubility: alcohol, glacial acetic acid, acetone, partially soluble in benzene, ether.

Historical use

For the past 3000 years at least, *kava-kava* has been the «national drink» of Polynesia and Melanesia, where it has got an important cultural role. The royalty and nobility used the drink for religious and political ceremonies and almost each tribe had an own ritual for the use of *kava-kava*. This traditional drink plays a key role as well in Fiji, Samoa and Tonga, where it is drunk during the ceremonies to honor the hosts, to join the participants and to reinforce social identities. In the western societies, *kava-kava* has been widely used for the selfmedicating treatment of depression and anxious states and of the premenstrual syndrome, due to its sedative, anxiolitic, antidepressant and muscle relaxant properties [(2)].

Current use

Today it is still believed that *kava-kava* restores the physical resistance, acts as an aphrodisiac, alleviates stomach pain and many other indispositions. As well as drinking the root extract, the *kava-kava* is also used to purify the environment in order to drive out diseases. Its anxiolitic and sedative effect is particularly useful in subjects being in a state of anxiety and emotional distress, with obvious difficulty to fall asleep, having nervous ticks, tremors, hyperexcitability, muscular tension, etc [3]. Another effect of *kava-kava*, at the level of the central nervous system, is that of a muscle relaxant [4]. It can cause skin problems such as cutaneous allergies, which nevertheless disappear quickly after discontinuing the use. In addition, it can increase the effect of almost all the psychotropic agents and the alcohol effect on the body.

Legislation

Kava-kava has always proved to be a safe remedy [5]. However, in recent times it has been associated with some cases of hepatic toxicity, which prompted its withdrawal from the commerce in numerous countries starting from 2001. In Italy, for the health protection of the citizens, the Ministry of Health according to the May 29th, 2002 Decree published in the Official Bulletin n. 141 in June 18th, 2002 has forbidden the sale of *kava-kava* homoeopathic products. In addition, the Ministry of Public Health has added *Piper methysticum* to the list of vegetable extracts non admitted in dietary supplements [6]. In Italy, neither the kavain nor the *Piper methysticum* whole plant or parts of the plant are included in the tables containing narcotic or psychotropic substances subject to the supervision and control under Article 14 of Republic Presidential Decree 309/90 and subsequent updates.

Pharmaco-toxicological properties

The acqueous *kava-kava* extracts are used in animal model to counteract the hyper motility induced by amphetamines; this activity has turned out to be comparable to the one produced by antipsychotic drugs such as haloperidol and chlorpromazine [7]. In 1998, Baum and colleagues observed that the *kava-kava* relaxing, slightly euphoric effects might be caused by the activation of the dopaminergic neurons of the mesolimbic system, while the hypnosis inducing effects would be due to a reduction in the serotonine concentration [10]. It has been highlighted that the limbic structure, and particularly the amygdala is the preferential action site of the D,L-kavain compared to the whole plant extract [3] and more recently, that the kavapyrons influence the GABA receptors of the hyppocampus and of the amygdala [8]. Based on experiments carried out on rodent brain, it has been demonstrated that not only the GABA-A receptors, but also D2 for dopamine, for opioids (μ and δ) and for histamine (H1 and H2) might be involved in the pharmacological activity of *kava-kava*. Scientific experiments proved that the *kava-kava* leaves are more active than the root (which is traditionally used) in inhibiting the connection of several neurotransmitters with their own receptors; the IC50 is respectively 3 μg/ml for the leaves and between 5-87 μg/ml for the root. When different types of *kava-kava* containing the same amount of kavalactones were tested the results indicated different pharmacological activities; therefore the authors hypothesized that other constituents could also play an important role. The same research has highlighted also that the bindings for serotonine (5-HT6 and 5-HT7) and benzodiazepine receptors are weakly inhibited by the *kava-kava* leaves and by the roots of different types of cultivation [9]. The methysticine and the kavain inactivate the sodium channels at the neurons level of the hyppocampus [11]. The synthetic kavapyrone reduces the current of the Na^+ and Ca^{2+} channels [12]. The kavain and the dihydromethysticine act on the calcium channels in additive terms and together they increase the effects of ipsapyrone, a serotonine antagonist with anxiolytic activity [13]. It has been demonstrated that the two natural kavapyrons: (+)-methysticine and (+)-kavain, and the synthetic one, (+/-)-kavain are powerful as *in vitro* inhibitors (the last two more than methysticine) of the [3H]-noradrenaline reuptake [14]. The anxiolytic activity of the *kava-kava* extracts might be mediated in part also by dihydrokavain [15]. The *kava-kava* has a neuroprotective effect in animals who suffered an ischemic damage. This effect is probably mediated by methysticine and dihydromethysticine [16]. Lastly, it has been noticed that in an animal experimental model of Parkinson disease, 200 mg/kg (+/-)-kavain stops completely the inhibition of the immunoreactivity of T cells and the loss of neurons of *Substancia nigra*. Because of this effect, it has been proposed that (+/-)-kavain can be potentially considered a new candidate for future studies on Parkinson disease and other diseases with glutamatergic hyperactivity [17]. Summarizing, *kava-kava* inhibits the calcium and sodium channels, influences the GABA neurotransmitters, glutamate, dopamine and

serotonin. The most important active compounds and mechanism of action are well-known, but there might be also other substances and mechanisms which can increase the therapeutic effects.

Toxicity

In 2001 some cases of poisoning with *kava-kava* have been reported. They concerned individuals who attended the hospital emergency wards with symptomatology such as jaundice, tiredness, indisposition, lack of appetite and who had severe liver function alterations with transaminases increased up to 60-70 times the normal values as well as alterations of total bilirubin and alkaline phosphatase levels [18].

Data regarding the acute toxicity of the kavalactons [19]

In the animals in general - LD50 following intraperitoneal administration: 300-400 mg/kg

Data regarding the acute toxicity of the dihydrokavain [19]

In the mouse - LD50 following oral administration: 950 mg/kg

Data regarding the acute toxicity of the dihydromethysticin [19]

In the mouse - LD50 following oral administration: 1050 mg/kg

Adverse Effects

In the chronic consumers of high doses of *Piper methysticum*, facial turgidity, hematuria, macrocitic anaemia, ataxia, increased patellar reflexes, weight and hair loss, cutaneous eruptions, dyspnea, vision problems, hepatotoxicity, gastrointestinal troubles, allergic cutaneous reactions, headache, photosensitivity, asthenia, agitation, drowsiness and tremors have been observed [20].

Pharmacological interactions

Alcohol and certain medicaments with effect on the central nervous system can potentiate the effects of *kava-kava*, leading to a temporary reduction of the cognitive performance or to a partial loss of conscience [21]. *Piper methysticum* can potentiate the effects of anticoagulants with the subsequent risk of hemorrhagic complications [22]. Possible pharmacological interactions can be attributed to the inhibition of certain isoformes of cytochrome P450 responsible for the metabolism of numerous drugs [7]. At the present time interactions of this plant with foods are not known [21].

Effects in pregnancy

Kava-kava is contraindicated in pregnancy and during lactation [21].

Analytical determinations

Scientific literature reports a series of analytical methodologies to measure *Piper methysticum* active principles in different biological matrices [23,26], and in dietary supplements [27]. In this latter case, analysis is carried out by liquid chromatography coupled to a to ultraviolet spectrophotometric detection [27].

The method below reported is a brief outline useful to the investigator to set up the analysis. For specific details, it is advisable to refer to the original text.

Determination of some active principles and human urinary metabolites of *Piper methysticum*

(From: DUFFIELD AM, JAMIESON DD, LIDGARD RO, DUFFIELD PH, BOURNE DJ. Identification of some human urinary metabolites of the intoxicating beverage kava. J Chromatogr. 1989; 475: 273-281) [24].

The analysis is carried out by both gas chromatography coupled with mass spectrometry and liquid chromatography coupled to diode array spectrophotometric detection.
Details of the gas chromatographic-mass spectrometric assay are reported.

Extraction of the compounds

An amount of 100 ml urine, acidified with 2 M hydrochloric acid are extracted three times with 30 ml chloroform. The organic layer is dried after washing two times with 20 ml 5% sodium carbonate and 20 ml water. The dried extract is derivatized with 50 µl N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) at 80 °C for 30 minutes. A 2 µl amount is injected into the gas chromatograph.

Analytical conditions

Chromatographic column : Quartz BP-10 (25 m x 0,3 mm)
Carrier gas: Helium at 1 ml/min
Injection mode: Splitless
Temperature program: 100°C for 1 minute, 10-300°C at 6°C/minute
Detector: mass spectrometer with chemical ionization interface

Retention times of the tested compounds

Retention times of tested compounds are not reported.

Characteristic fragments of the tested compounds

Dihydrokavain: m/z 233
Kavain: m/z 231
Methysticin: m/z 275
Dihydromethysticin: m/z 277
Dehydromethysticin: m/z 273
Yangonin: m/z 259
Desmethoxyyangonin: m/z 229
Tetrahydroyangonin: m/z 263
11-methoxytetrahydroyangonin: m/z 293

Standards

The company from where standard compounds were obtained is not reported.

Calibration curve

Since a qualitative assay is presented, the preparation of the calibration curve is not described.

Results

With the presented assay, all the major kava lactones excreted in urine after the consumption of a drink prepared from *Piper methysticum* could be identified. The kavain could not be determined in urine samples and a previous study showed that the amount detectable in blood is very low [25].

References

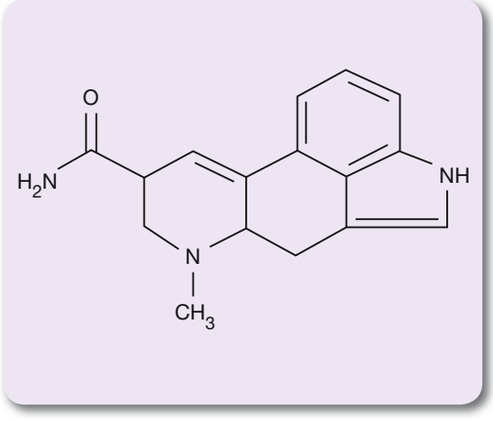
1. SINGH Y N. Kava: an overview J. Ethnopharmacol. 1992; 37: 13-45.
2. NOWAKOWSKA E, OSTROWICZ A, CHODERA A.. Kava-Kava preparations: alternative anxiolytics Polski Merkuriusz lekarski 1998; 4: 179-180.
3. HOLM E, STAEDT U, HEEP J, KORTSIK C, BEHNE F, KASKE A, MENNICKE I. The action profile of D,L kavain. Cerebral sites and sleep-wakefullness-rhythm in animals. Arzn Forsch Drug res 1991; 41: 469-474.
4. SINGH Y N, Effects of kava on neuromuscular transmission and muscle contractility J. Ethnopharmacol 1983; 7: 267-276.
5. STEVINSON C, HUNTLEY A, ERNSI E. A systematic review of the safety of kava extract in the treatment of anxiety. Drug Saf 2002; 25: 251-261.
6. The list of vegetal extracts not admitted in dietary supplements in Italy can be found at http://www.salute.gov.it
7. DUFFIELD PH. Effect of aqueous and lipid soluble extracts of kava on the conditioned avoidance response in rat.Arch Int Pharmacodyn Ther 1989; 301: 81-90.
8. JUSOFFIE A, SCHMIZ A, HIEMKE C. Kavapyrone enriched extract from Piper Methysticum As Modulator Of The GABA Binding Site In Different Regions Of Rat Brain. Psychopharmacology (Berl) 1994;116:469-474.
9. DINH LD, SIMMEN U, BUETER KB, BUETER B, LUNDSTROM K, SCHAFFNER W. Interaction of various Piper methysticum cultivars with CNS receptors in vitro. Planta Med. 2001; 67: 306-311.
10. BAUM SS, HILL R, ROMMELSPACHER H. Effect of kava extract and individual kavapyrones on neurotransmitter levels in the nucleus accumbens of rats. Prog Neuropsychopharmacol Biol Psychiatry. 1998; 22: 1105-1120.
11. MAGURA EI. Kava extract ingredients, (+)-methysticin and (+/-)-kavain inhibit voltage-operated Na(+)-channels in rat CA1 hippocampal neurons. Neuroscience 1997;81:345-351.
12. SCHIRRMACHER K. Effects of (+/-)-kavain on voltage-activated inward currents of dorsal root ganglion cells from neonatal rats. Eur Neuropsychopharmacol 1999; 9: 171-176.
13. WALDEN J Effects of kawain and dihydromethysticin on field potential changes in the hippocampus. Prog Neuropsychopharmacol Biol Psychiatry 1997; 21:697-706.
14. SEITZ U [3H]-monoamine uptake inhibition properties of kava pyrones. Planta Med 1997; 63: 548-549.
15. SMITH KK. Anxiolytic effects of kava extract and kavalactones in the chick social separation-stress paradigm. Psychopharmacology 2001; 155: 86-90.
16. BACKHAUSS Extract of kava (Piper methysticum) and its methysticin constituents protect brain tissue against ischemic damage in rodents. Eur J Pharmacol 1992; 215: 265-269.
17. SCHMIDT N Neuroprotective effects of (+/-)-kavain in the MPTP mouse model of Parkinson's disease. Synapse 2001; 40: 47-54.
18. RUSSMAN S Kava hepatotoxicity Ann Intern Med 2001; 135: 68-69.
19. SPILLANE P K Neurological manifestations of Kava intoxication Med J Aust 1997 4; 167: 172-173.
20. EDZARD E. The risk-benefit profile of commonly used herbal therapies: Ginkgo St. John's Wort, Ginseng, Echinacea, Saw Palmetto and Kava. Ann Intern Med 2002; 136: 42-53.
21. ERNST E. Kava update: a European perspective. NZ Med J 2004; 117: 1143-1146.
22. WOOLTORTON E. Herbal kava: reports of liver toxicity. CMAJ 2002; 166: 777.
23. KÖPPEL C, TENCZER J. Mass spectral characterization of urinary metabolites of D,L-kawain. J Chromatogr. 1991; 562: 207-211.
24. DUFFIELD AM, JAMIESON DD, LIDGARD RO, DUFFIELD PH, BOURNE DJ. Identification of some human urinary metabolites of the intoxicating beverage kava. J Chromatogr. 1989; 475: 273-281.
25. TARBAHA F, MAHLERA H, KARDELA B, WEINMANNB W, HAFNERC D, DALDRUP T. Kinetics of kavain and its metabolites after oral application. J Chromtogr B. 2003; 789: 115-130.
26. VILLAIN M, CIRIMELE V, TRACQUI A, RICAUT FX, LUDES B, KINTZ P. Testing for kavain in human hair using gas chromatography–tandem mass spectrometry. J Chromatogr B. 2003; 798: 351–354.
27. HU L, JHOO JW, ANG C TW, DINOVI M, MATTIA A. Determination of six kavalctones in dietary supplements and selected functional food containig piper methysicum by isocratic liquid chromatography with internal standard J AOAC International 2005; 88: 17-22.

Rivea corymbosa
(Turbina corymbosa)

Name: *Rivea corymbosa*
Family: *Convolvulaceae*
Genus: *Turbina Raf.*
Species: *Rivea corymbosa*
Synonims: Christmas vine, ololiuqui (seeds)
Origin: Mexico
Active compounds: ergine (lysergamide or lysergic acid amide), isoergine, ergometrine, lysergic acid α-hydroxyethylamide, elymoclavine, chanoclavine.

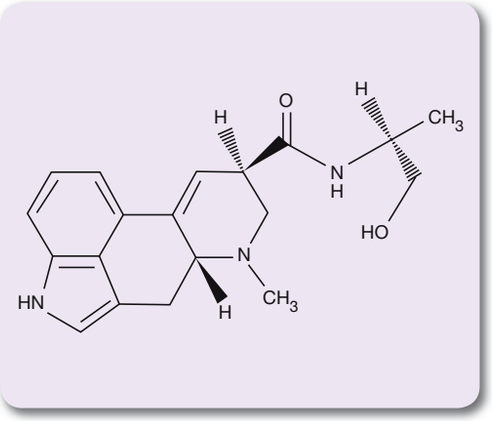
Ergine (Lysergamide or lysergic acid amide LSA) is the principal psychoactive (hallucinogen) alkaloid contained in the seeds of the plant. Other alkaloids present are: isoergine with much lower activity than its epimer ergine, ergometrine, lysergic acid alpha-hydroxyethylamide, isolysergic acid alpha-hydroxyethylamide, elymoclavine, chanoclavine. Ergine and isoergine are also present in the seeds of *Ipomea violacea* and *Argyreia nervosa*.
These active compounds are present in the seeds of the plant, nevertheless the historical and traditional use refers to the entire plant. There are no studies regarding research on active compounds in parts of the plant. The percentage of alkaloids found in the seeds of *Rivea corymbosa* varies between 0.02 and 0.06% [(1)].

Chemical formula and physico-chemical properties of the active compounds

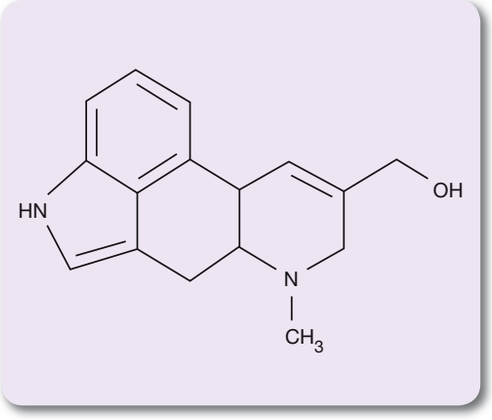


Name: Ergine (Lysergamide or lysergic acid amide LSA).
Molecular Formula: $C_{16}H_{17}N_3O$ (molecular weight = 267.3).
Systematic name: 9,10-dihydro-6-methylergoline-8-β-carboxamide.
CAS registry number: 478-94-4.
Melting point: no data in the literature.
UVmax: no data in the literature.
Solubility: no data in the literature.

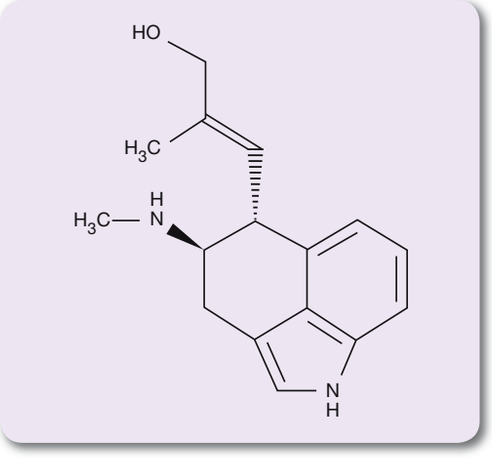
Name: Isoergine.
Molecular Formula: $C_{16}H_{17}N_3O$ (molecular weight = 267.3). Since it is the epimer of ergine, it has the same molecular structure, but the spatial distribution is different.
Systematic name: 9,10-dihydro-6-methylergoline-8-α-carboxamide.
CAS registry number: 2889-26-1.
Melting point: no data in the literature.
UVmax: no data in the literature.
Solubility: no data in the literature.



Name: Ergometrine.
Molecular formula: $C_{19}H_{23}N_3O_2$ (molecular weight = 325.5).
Systematic name: 9,10-dihydro-N-(2-hydroxy-1-methylethyl)-6-methyl-8-β-(S)-9-ergoline-8-carboxamide.
CAS registry number: 60-79-7.
Melting point: 162°C.
UVmax: no data in the literature.
Solubility: water.



Name: Elymoclavine.
Molecular formula: $C_{16}H_{18}N_2O$ (molecular weight = 254.3).
Systematic name: 8,9-dihydro-6-methylergoline-8-methyl alcohol.
CAS registry number: 548-43-6.
Melting point: no data in the literature.
UVmax: no data in the literature.
Solubility: no data in the literature.



Name: Chanoclavine.
Molecular formula: $C_{16}H_{20}N_2O$ (molecular weight = 256.3).
Systematic name: Propen-1-ol,2-methyl-3-(1,3,4,5-tetrahydro4(methylamino)benz(cd)indol-5-yl-(4R-(4-α,5-β(E).
CAS registry number: 2390-99-0.
Melting point: 221°C.
UVmax: no data in the literature.
Solubility: no data in the literature.

There is no literature data on the molecular formula, the systematic name, CAS registry number, melting point, solubility and UVmax of lysergic acid α-hydroxyethylamide.

Historical use

Coming from the tropical America, it has been relatively recent that the plant has been identified as belonging to the family of "morning glory", although its use, by the native Americans, has a long tradition. Ololiuqui is the aztec name for *Rivea corymbosa* seeds: they contain LSA and have a long history of use in central Mexico. According to some historical sources the plant seeds would seem to have had greater importance in religious rituals compared to the hallucinogenic mushrooms like the peyote. Besides, the plant was used also in the traditional medicine to treat flatulence, pain and as a remedy for tumors. Today the seeds are still used in some tribes (Zapotechi, Mazatechi, etc.) who live in complete isola-

tion in the remotest mountains of southern Mexico. An excellent revision of the historical, botanic, ethnologic aspects of the Ololiuqui has been provided by Schultes in 1941 in his monograph "A Contribution of our knowledge of *Rivea corymbosa*: the narcotic ololiuqui of the Aztechs" [2]. In 1959, Richard Schultes sent seeds of the morning glory plant cultivated in Mexico (*Rivea corymbosa*, specifically) to doctor Albert Hofmann, a discoverer of the LSD (lysergic acid diethylamide). Schultes declared that these seeds were used by the shamans. In 1960, Hofmann analyzed the seeds and concluded that they contained ergot-like alkaloids. It was difficult for the scientists of that period to trust in Hofmann's results. Up to that moment, in fact, such alkaloids had been found only in some mushrooms. But Hofmann was right: the seeds contained the amide of lysergic acid, the LSA.

Current use

The *Rivea corymbosa* seeds, similar to those from *Ipomea violacea* and *Argyreia nervosa* (Hawaiian baby woodrose), are used today for their ability of inducing psychoactive and hallucinogenic effects just like LSD, although of smaller intensity.

Legislation

The ergine or lysergic acid amide is subject to control in the United States of America (Schedule III drug in the Controlled Substances Act) as depressant, and is on the list of the U.S. code of Federal Regulations as a possible precursor of LSD.
In Italy, the lysergic acid amide is included in Table I containing the narcotic and psychotropic substances subject to the supervision and control under Article 14 of Republic Presidential Decree 309/90 and subsequent updates. According to the Ministerial Decree in September 25th, 2007 published in the Official Bulletin n. 237 in October 11th, 2007, also the seeds of *Rivea corymbosa* have been placed on the list of illicit and psychotropic substances of the Table I of article 14 of the DPR n.309/90.

Pharmaco-toxicological properties

The hallucinogenic effect of the ergine (LSA) starts from the ingestion of 2-5 mg of the active ingredient. There are few published studies on the pharmacodynamics of ergine. Similar to the ergot alkaloids (for example ergometrine), it seems to bind to the D2 dopaminergic receptors, causing inhibition of adenilate cyclase and reduction of cyclic adenosine monophosphate (cAMP) production [3]. The discovery of the ergot alkaloids in the seeds of *Rivea corymbosa*, *Ipomea violacea* and *Argyreia inervosa* in the beginning of the 60s has been rather unexpected and of particular interest from a phytochemical point of view, since the alkaloids of the lysergic acid had been isolated until then only in the mushrooms of the *Claviceps*, *Penicillium* or *Rhizopus* family and it was the first time that they were isolated in the superior plants (Phanerogams), in the Convolvulaceae family [4-6]. LSA has psychotomimetic type effects (alterations of the mind, of perceptions [hallucinations] and of the level of conscience) similar to those caused at the LSD (Lysergamide), although this later one is 50 to 100 times more powerful than the LSA. The effects of LSA, which last about 4-8 hours, are characterized by a general sensation of quiet, which can be accompanied by dysphoria, and psychedelic visual effects (vision of bright colors). Effects comparable with those of *Rivea corymbosa* are produced by the seeds of *Ipomea violacea* (Tlitlitzin) and of *Argyreia nervosa*. These effects, although of smaller intensity, are similar to the LSD effects.
Studies on cows (veal) demonstrate that the average serum pharmacokinetics of ergine in after a single intravenous dose of 14 µg/kg presents three different phases. The first phase (0-10 minutes), characterized by a balance in the distribution volume, followed by a second phase (which begins immediately after the injection and it lasts for about an hour) with blood and tissue levels of ergine in equilibrium. In the third phase, the ratio between tissue and blood is inverted because of the epatic elimination of ergine [7]. Elymoclavine and chanoclavine, even though are present in a lower percentage in the seeds of the plant, seem to contribute to the hallucinogenic activity. The possible contribution of ergometrine (present in trace amounts in the seeds of *Rivea corymbosa*) on the pharmacotoxic effects of the plant has not been sufficiently studied.

Toxicity

Data regarding the acute toxicity of ergine

In humans - TD Lo following oral administration: 14 µg/kg [8]

In rats and rabbits - LD following intravenous administration: 2500 µg/kg [8]

There are no toxicity data regarding the other active compounds of the plant.

Adverse effects

The biggest psychotic adverse effects that can occur following the ingestion of the seeds are dissociative reactions and schizophrenic relapses [9].

There is a case of toxic psychosis induced by the seeds of *Argyreia nervosa* (plant in which the seeds have the same active compound as in *Rivea corymbosa*) described in the literature. The effect manifested in hallucinations, orientation problems, anxiety and psychomotor agitation [10]. In another case, an 18-year-old youngster has been hospitalized because of increasing psychotic behavior following the ingestion of plant seeds [11]. Another 18-year-old boy has been admitted after the ingestion of 12 seeds of *Argyreia nervosa*, complaining of vomiting, nausea, dizziness, auditory hallucinations, obscured vision and perspiration [12]. One month later, the patient was complaining of auditory hallucination fashbacks each time he was smoking cigarettes.

The above mentioned clinical cases indicate that it is necessary to pay a particular attention to the differential diagnosis between adolescent acute psychotic episodes and those which can occur in youngsters following the ingestion of this or of other hallucinogenic drugs.

Pharmacological interactions

There are no known interactions due to the ingestion of *Rivea corymbosa* and pharmaceuticals. However, it has been reported that the metabolism of LSD, similar to LSA present in the plant, is inhibited by drugs used for the treatment of HIV [13]. This suggests the possibility that in patients in therapy with antiretroviral drugs taking also LSD or *Rivea corymbosa* there will be an increased toxicity induced by such hallucinogens.

Effects in pregnancy

The ingestion of *Rivea corymbosa* seeds during pregnancy is risky. The ergine in fact, is related from the structural point of view to LSD which can induce uterine contractions [14,15]. The drug can therefore, increase the risk of spontaneous abortions.

Analytical determinations

No methodologies have been reported in the international literature concerning the analysis of *Rivea corymbosa* active principles in biological fluids of eventual *Rivea* consumers. However, analytical methods have been published regarding the determination of LSA in urine [16,17] , blood [17] and in the seeds of *Argyreia nervosa* [18].

Please refer to the monograph of *Argyreia nervosa* for analytical details of the determination of LSA in the urine samples [16].

References

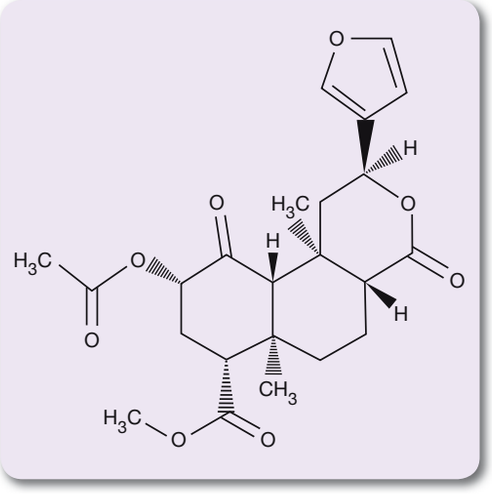
1. DER MARDEROSIAN A, YOUGKEN HW. The distribution of indole alkaloids among certain species and varieties of *Ipomoea*, *Rivea* and *Convolvolus* (Convolvulaceae). Llodia 1966; 29: 35-42.
2. SCHULTES RE. "A Contribution to our Knowledge of Rivea Corymbosa: The Narcotic Ololiuqui of the Aztecs ", Botanical Museum, Harvard Univ., Cambridge, Mass., 1941.
3. LARSON BT, HARMON DL, PIPER EL, GRIFFIS LM, BUSH LP. Alkaloid binding and of D2 dopamine receptors in cell culture. J.Anim. Sci. 1999; 77: 942-947.
4. HYLIN JW, WATSON DP. Ergoline alkaloids in tropical wood roses. Science. 1965; 148: 499-500.
5. TABER WA, HEACOCK RA, MAHON ME. Ergot-type alkaloids in vegetative tissue of rivea corymbosa (L.) Hall.f. Phytochemistry. 1963; 2: 99-101.
6. TABER WA, HEACOCK RA. Location of ergot alkaloid and fungi in the seed of *Rivea corymbosa* (L.) Hall. f., "ololiuqui". Can J Microbiol. 1962; 8: 137-143.
7. MOUBARAK AS, PIPER EL, JOHNSON ZB, FLIEGER M. HPLC method for detection of ergotamine, ergosine, and ergine after intravenous injection of a single dose. J Agric Food Chem. 1996; 44: 146-148.
8. http://toxnet.nlm.nih.gov/
9. MILLER MD. Isolation and identification of lysergic acid amide and isolysergic acid amide as the principal ergoline alkaloids in *Argyreia nervosa*, a tropical Wood rose. J AOAC. 1970; 53: 123-127.
10. GOPEL C, MARAS A, SCHMIDT MH. Hawaiian baby rose wood: case report of an argyreia nervosa induced toxic psychosis. Psychiatr Prax. 2003; 30: 223-224.
11. GERTSCH JH, WOOD C. Case report: an ingestion of Hawaiian Baby Woodrose seeds associated with acute psychosis. Hawaii Med J. 2003; 62: 127-129.
12. AL-ASSMAR SE. The seeds of the Hawaiian baby woodrose are a powerful hallucinogen. Arch Intern Med. 1999; 159: 2090.
13. ANTONIOU T, TSENG AL, VAN HEESWIJK RP, WALKER SE, GIGUERE P, PHILLIPS EJ. Steady-state pharmacokinetics and tolerability of indinavir-lopinavir/ricombination therapy in antiretroviral-experienced patients. Ther Drug Monit. 2005; 27: 779-781
14. MC GLOTHLIN WH, SPARKERS RS, ARNOLD DO. Effect of LSD on human pregnancy. JAMA. 1970; 212: 1483-1487.
15. JACOBSEN CB, BERLIN CM. Possible reproductive detriment in LSD users. JAMA 1972; 222: 1367-1373.
16. BJÖRNSTAD K, BECK O, HELANDER A. A multi-component LC-MS/MS method for detection of ten plant-derived psychoactive substances in urine. J Chromatogr B Analyt Technol Biomed Life Sci. 2009; 877: 1162-1168.
17. KLINKE HB, MÜLLER IB, STEFFENRUD S, DAHL-SØRENSEN R. Two cases of lysergamide intoxication by ingestion of seeds from Hawaiian Baby Woodrose. Forensic Sci Int. 2009 doi:10.1016/j.forsciint.2009.11.017 (in press).
18. KIM W, CRAWFORD MS. The Identification of Lysergic Acid Amide in Baby Hawaiian Woodrose By Mass Spectrometry. J Forensic Sci. 1970; 15: 588-594.

Salvia divinorum
(magic mint)

Name: *Salvia divinorum*
Family: *Labiatae*
Genus: *Salvia*
Specie: *Salvia divinorum* Epling & Jativa
Synonims: Hojas de Maria, Yerba Maria, Hierba de la pastora, Ska Maria pastora, Magic Mint, Diviner's sage
Origin: Mexico
Active compounds: Salvinorin A

Salvinorin A is the principal farmacologiclly active molecule of *Salvia divinorum*. The other compounds, like Salvinorin B, C, D, E and F are pharmacologically inactive [1-4].
The concentration of the active component can vary between 0.89 and the 3.7 mg/g of leaves (dry weight). Salvinorin A is a neoclerodane diterpene (probably the unique known psychoactive terpenoide), representing the only non nitrogenous selective agonist of the κ opioid receptors known today. The chemical structure of salvinorin A is completely different from that of other natural hallucinogens (N, N-dimethyltriptamine, psilocybin, mescaline) [5,6].

Chemical formula and physico-chemical properties of the active compound



Name: Salvinorin A.
Molecular formula: $C_{23}H_{28}O_8$ (molecular weight = 432.5).
Nome sistematico: (2-α,4-α,6-β,7-β,9-β,10-α,10-β)-2H-naphto(2,1-c)pyran-7-carboxylic acid,9-(acetoxy)-2-(3-furanil) dodecahydo-6a,10b-dimethyl-4,10-dioxo-methylester.
CAS registry number: 83729-01-5.
Melting point: 242-244°C.
UVmax: 238 nm.
Solubility: methyl alcohol, chloroform and acetonitrile

Historical use

Salvia divinorum has been known for many centuries and used by the mazatec shamans from the Oaxaca region. The mazatecs knew its hallucinogenic properties and the shamans used it in the spiritual healing ceremonies. Those who were being initiated in the shamanistic practices had to follow a path which brought them closer to the gods first through the consumption of sage, then the consumption of the seeds of *Rivea corymbosa* and finally of hallucinogenic mushrooms. The mazatec Indians attributed to *Salvia* names to remember its connection with the Virgin Mary (in Spanish: Hojas de Maria-Mary leaves, Yerba Maria- Mary herb, Hierba de la pastora- shepherdess herb, Ska Maria pastora). The plant is

believed to be the incarnation of the Virgin Mary [7].

Traditionally, in Mexico the fresh leaves are chewed in a dark and silent place until a vision appears: then the shaman - healer is able to discover, by getting in touch with the supernatural, the causes of diseases, to predict the future, to answer important questions (to discover the perpetrators of crimes or simply to find lost items). *Salvia divinorum* has been also used for the treatment of numerous pathologies, like headache, rheumatism, bloating, diarrhoea [8-10].

Current use

Sold up to 2005 (the year in which it was place into the Table I containing narcotic or psychotropic substances subject to the supervision and control under Article 14 of Republic Presidential Decree 309/90 and subsequent updates) in "smart shops", *Salvia divinorum* is used in different ways: the fresh leaves can be chewed or used to make a tea; the dried leaves can be chewed or smoked [11]. Some studies suggest a liquid extraction of the active compound (in isopropanol) to obtain a very potent and efficient stock tincture, in which case Salvinorin A can be vaporized and inhaled [12].

Legislation

At present, in many states of the Europe, America and Asia the use of *Salvia divinorum* is not forbidden since neither the whole plant nor any of its parts or constituents are placed on the list of controlled substances. In the United States of America the posession and the marketing of *Salvia divinorum* (with exception of Louisiana and St. Peter in Missouri) is permitted; in Europe, the use of *Salvia divinorum* is forbidden in Denmark, Finland, Spain and Belgium. Since 2006 it is also forbidden in Sweden and in 2008, in Germany, *Salvia divinorum* has been added to the list of substances with narcotic effect. In 2009, its use was forbidden also in Russia. In Australia and in South Korea is forbidden as well, while in Japan it is one of the controlled substances [11]. According to the January 2005 Ministerial Decree n. 11, published in the Official Bulletin n. 54 in March 7th, 2005 in Italy *Salvia divinorum* and Salvinorin A were included in the Table I containing narcotic or psychotropic substances subject to the supervision and control under Article 14 of Republic Presidential Decree 309/90 and subsequent updates [13].

Pharmaco-toxicological properties

The hallucinogenic effect of *Salvia divinorum* is attributable to Salvinorin A, its principal constituent, identified by Ortega and isolated by Valdes in the early 80's [1,14].

The pharmacokinetics of salvinorin A has been studied in rats and primates. In these animal models, it is excreted relatively quickly, and its elimination half-life is about 75 minutes, the half-life in the brain varies between 8 and 36 minutes. The compound is metabolized by several oxidative enzymes and substrates for P-gp protein (membrane protein that has the ability to extrude substances from the cell) [15,16].

The pharmacological potency of Salvinorin A its comparable to the synthetic hallucinogens such as LSD (N,N-diethyllysergamide) and DOB (4-bromo-2,5-dimethoxy phenylisopropylamine), but the mechanism of action is different [5]. The compound is in fact a powerful κ oppioid receptor agonist, whose stimulation seems to be related to the psychotropic effects associated with the consumption of *Salvia divinorum* extracts. Studies performed *in vitro* and *in vivo* have demonstrated that Salvinorin A does not have any affinity for the serotonin 5-HT_{2A} receptor, which is the principal molecular target of the classic hallucinogens (LSD, N, N-dimethyltryptamine, psilocybin, mescaline). In addition, the total lack of affinity of Salvinorin A to other molecular targets such as G protein-coupled receptors, transporters and ion channels canals has been also demonstrated [17,18].

The inhalation of 200–500 μg of pure Salvinorin A causes the onset of hallucinations; the effects occur about 30 seconds after inhalation, reach a plateau phase in 5–10 minutes and disappear after 20–30 minutes.

The quantity of the active compounds present in the leaves varies between 0.89 and 3.7 mg/g of dry weight. In general, these concentrations contained in 1 gram of leaves, are sufficient to induce psychoactive effects [6].

In folk medicine, low doses of *Salvia divinorum* (4-5 fresh or dried leaves), has been used like tonic (to combat fatigue) and like a panacea, the only true medicine with magical properties. The infusions of larger amounts of the plant (20-60 fresh leaves) instead, act as hallucinogens [10]. The hallucinations are usually visual, auditory and tactile and involve

vision of two-dimensional surfaces, return to places of the past (particularly childhood), sensations of movement (to be pulled or twisted by an unknown force), sensations of loss of body or of identity, hysterical and uncontrollable laughter and distorted perception of reality (sensations of being in several places at the same time) [7].

The extracts of *Salvia divinorum* seem to possess also antidepressant properties. This effect seems to be in agreement with the observation that selective kappa-oppioid receptor agonists can have antidepressant effects. There is a case report of a 26-year-old woman suffering from depression who went into remission after taking *Salvia divinorum* [19].

Another potential therapeutic application of the Salvinorin A may relate to the treatment of diseases characterised by perception disturbances, such as schizophrenia, bipolar disorder and Alzheimer disease [5,17].

At last, a recent study has demonstrated that the administration of standardised extracts of *Salvia divinorum* to the guinea pig has an inhibitory effect on the enteric cholinergic transmission at the ilium level. The activation by Salvinorin A of prejunctional κ receptors for the opioids and CB receptors for the cannabinoids seems to be at the base of this pharmacological effect. The oppioid receptor agonists inhibit, in fact, the acethylcoline release from the myenteric plexus attenuating the contractions of the longitudinal smooth muscle. The results of this study provide the rational base to the traditional use of the plant in the treatment of the diarrhoea [20].

Toxicity

There is no data regarding the toxicity of *Salvia divinorum* in humans or laboratory animals.

Adverse Effects

There is an "Information Bulletin", by the Department of Justice of the United States of America [21], in which the following adverse effects are listed following the prolonged use of *Salvia divinorum* extracts: depression, schizophrenia and negative flashbacks (similar effects are reported for LSD). The same bulletin states that production, distribution and abuse of Salvia divinorum or Salvinorin A are not prosecuted in the United States of America, although there is a willingness from the Congress to include the plant and its active compounds in the "Controlled Substance Act".

There have been also reports, some of them subjective, of: nausea, motor uncoordination, dizziness, heart rate reduction and sensation of cold.

Under the influence of *Salvia divinorum* it is possible to run into a series of risks related to the alteration of the perception of the surrounding environment (it can be for example risky to be close to a window). Mixed with other substances, *Salvia divinorum* can have unforseen effects. A case of persistent psychosis characterized by echolalia (involuntary repetition of words or phrases spoken by others), paranoia, agitation and conflict of ideas was described in a young man of 21 years without past mental disorders who had smoked *Salvia divinorum* [22].

Pharmacological interactions

There are no reported pharmacological interactions in human. However, given that Salvinorin A is the substrate for various oxidative enzymes and for the P-glycoprotein [16] it can be supposed that drug interactions are possible.

Effects in pregnancy

There is no data regarding the effect in pregnancy or during lactation.

Analytical determinations

Scientific literature reports a series of analytical methodologies to measure Salvinorin A in different biological matrices [23,24] and in dry leaves of the plant [23,25]. The assays for determination of Salvinorin A in *Salvia divinorum* leaves are based on gas chromatography-mass spectrometry [23] and thin layer chromatography followed by gas chromatography and mass spectrometry [25].

The method below reported is a brief outline useful to the investigator to set up the analysis. For specific details, it is advisable to refer to the original text.

Determination of Salvinorin A in biological matrices of *Salvia divinorum* users

(From: PICHINI S, ABANADES S, FARRÈ M, PELLEGRINI M, MARCHEI E, PACIFICI R, DE LA TORRE R, ZUCCARO P. Quantification of the plant-derived hallucinogen Salvinorin A in conventional and non conventional biological fluids by gas chromatography/mass spectrometry after *Salvia divinorum* smoking. Rapid Commun Mass Spectrom. 2005; 19: 1649-1656) [23].

The analysis is carried out in biological matrices (urine, oral fluid, plasma and sweat) from *Salvia divinorum* users by gas chromatography coupled with mass spectrometry.

Extraction of the compounds

An amount of 1ml urine, saliva, plasma or sweat collected with a cotton ball from the forehead of a S*alvia divinorum* user following the addition of 0.5 µg internal standard, is extracted with 1.5 ml chloroform/isopropanol (90:10, v/v) mixture. After vortexing, the sample is centrifuged for 10 minutes in a table top centrifuge at 2500 rpm. The extraction is performed twice and the pooled organic phase is transferred to a clean tube and dried under a nitrogen flow. The residue is resuspended in 100 µl of ethyl acetate.

Analytical conditions

Chromatographic column : 5MS (0,25 mm x 30 m x 0,25 µm)
Injector temperature: 260°C
Carrier gas: Helium at 11.60 psi
Injection mode: Split: 15:1
Temperature program: 70°C for 3 minutes, 70-300°C at 30°C/minute
Detector: mass spectrometer with electron impact interface

Retention times of the tested compounds

Salvinorin A: 16.6 minutes
17α -methyltestosterone (internal standard): 12.9 minutes

Characteristic fragments of the tested compounds

Salvinorin A: m/z 91, 161, 229, 302
17α -methyltestosterone (internal standard): m/z 94, 273, 432

Standards

Salvinorin A and 17 α-methyltestosterone used in the analyses have been purchased from Sigma Aldrich (Milan - Italy). Since in March 7, 2005, according to the Ministerial Decree No 11 of January 2005, published in the Official Newspaper No 54 in March 7 2005, Salvinorin A has been included in the Table I of the list of narcotic or psychotropic substances subject to the supervision and control under Article 14 of Republic Presidential Decree 309/90 and subsequent updates, ministerial authorisation was needed for its purchase.

Calibration curves

The standard stock solutions of the analytes (1 mg/ml) were prepared in methyl alcohol. The working standard solutions at concentrations of 100, 10 and 1 µg/ml were prepared by diluting the stock solutions and keeping them at -20°C until the analysis. The internal standard was used at a concentration of 10 µg/ml. The calibration standards at 0.015-5 µg/ml were prepared daily by adding known concentrations of the methyl alcoholic solutions to previously tested, drug-free urine, plasma, oral fluid and sweat samples. Quality controls at 4.25 µg/ml (high concentration) 2 µg/ml (medium concentration) and 0.024 µg/ml (low concentration) were prepared by adding the methyl alcoholic solutions to biological matrices tested as drug-free. These quality controls were included into each analytical batch to check the calibration, precision, accuracy and the stability of the stored samples.

Results

In the saliva samples collected from two individuals approximately one hour after the smoking of *Salvia divinorum* leaves (containing on average 0.58 mg Salvinorin A), the Salvinorin A concentration was between 11.1 and 25 ng/ml, while in urine it was found to be between 2.4 and 10.9 ng/ml. Salvinorin A has not been found in the sweat samples collected from the consumers.

References

1. VALDES III LJ, BUTLER WM, HATFIELD GM, PAUL AG, KOREEDA M. Divinorin A, a psychotropic terpenoid, and divinorin B from allucinogenic Mexican mint, Salvia divinorum. J Org Chem. 1984; 49: 4716-4720.
2. MUNRO TA, RIZZACASA MA. Salvinorins D-F, new neoclerodane diterpenoids from Salvia divinorum, and an improved method for the isolation of salvinorin A. J Nat Prod. 2003; 66: 703-705.
3. BIGHAM AK, MUNRO TA, RIZZACASA MA, ROBINS-BROWNE RM. Divinatorins A-C, new clerodane diterpenoids from the controlled sage Salvia divinorum. J Nat Prod. 2003; 66: 1242-1244.
4. VALDES III LJ, CHANG HM, VISGER DC, KOREEDA M. Salvinorin C, a new neoclerodane diterpene from bioactive fraction of the hallucinogenic Mexican mint *Salvia divinorum*. Org Lett. 2001; 3: 3935-3937.
5. SHEFFLER DJ, ROTH BL Salvinorin A: the "magic mint" hallucinogen finds a molecular target in the kappa opioid receptor. Trends Pharmacol Sci. 2003; 24: 107-109.
6. GRUBER JW, SIEBERT DJ, DER MARDEROSIAN AH, HOCK RS. High performance liquid chromatographic quantification of Salvinorin A from tissues of Salvia divinorum Epling & Jativa-M. Phytochem Anal. 1999; 10: 22-25.
7. VALDES III LJ, DIAZ JL, PAUL AG. Ethnopharmacology of Ska Maria Pastora (Salvia divinorum, Epling & Jativa-M). J Ethnopharmacol. 1983; 7: 287-312.
8. EPLING C, JATIVA M. A new species of Salvia from Mexico. Bot Mus Leafl Harv Univ. 1962; 20: 75-76.
9. WASSON RG. A new Mexican psychotopic drug from the mint family. Bot Mus Leafl Harv Univ. 1962; 20: 77-84.
10. VALDES LJ, HATFIELD GM, KOREEDA M, PAUL AG. Studies of Salvia divinorum (Lamiaceae), an hallucinogenic mint from the Sierra Mazateca in Oaxaca, Central Mexico. Econ Bot. 1987; 41: 283-291.
11. http://www.sagewisdom.org
12. http://www.erowid.org/plants/salvia/salvia_extraction4.shtml
13. http://www.ministerosalute.it/imgs/C_17_normativa_484_allegato.pdf
14. ORTEGA A, BLOUNT JF, MANCHARD PS. Salvinorin, a new trans-neoclerodane diterpene from Salvia divinorum (Labiateae). J Chem Soc., Perkin Trans. I. 1982: 2505-2508.
15. HOOKER JM, XU Y, SCHIFFER W, SHEA C, CARTER P, FOWLER JS. Pharmacokinetics of the potent hallucinogen, salvinorin A in primates parallels the rapid onset and short duration of effects in humans. Neuroimage. 2008; 4: 1044-1050.
16. TEKSIN ZS, LEE IJ, NEMIEBOKA NN, OTHMAN AA, UPRETI VV, HASSAN HE, SYED SS,PRISINZANO TE, EDDINGTON ND. Evaluation of the transport, in vitro metabolism and pharmacokinetics of Salvinorin A, a potent hallucinogen. Eur J Pharm Biopharm. 2009; 72: 471-477.
17. ROTH BL, BANER K, WESTKAEMPER R, SIEBERT D, RICE KC, STEINBERG S, ERNSBERGER P, ROTHMAN RB. Salvinorin A: A potent naturally occurring nonnitrogenous kappa opioid selective agonist. Pro Natl Acad Sci. 2002; 99: 11934-11939.
18. GOODMAN AND GILMAN'S - The pharmacological basis of therapeutics. McGraw-Hill Medical Publishing Division. Tenth Edition 2001: p. 638.
19. HANES KR. Antidepressant effects of the herb Salvia divinorum: a case report. J Clin Psychopharmacol. 2001; 21: 634-635.
20. FICHNA J, SCHICHO R, JANECKA A, ZJAWIONY JK, STORR M. Selective natural kappa opioid and cannabinoid receptor agonists with a potential role in the treatment of gastrointestinal dysfunction. Drug News Perspect. 2009 Sep; 22(7): 383-92.
21. http://www.usdoj.gov
22. PRZEKOP P, LEE T. Persistent psychosis associated with salvia divinorum use. Am J Psychiatry. 2009; 166: 832.
23. PICHINI S, ABANADES S, FARRÈ M, PELLEGRINI M, MARCHEI E, PACIFICI R, DE LA TORRE R, ZUCCARO P. Quantification of the plant-derived hallucinogen Salivinorin A in conventional and non conventional biological fluids by gas chromatography/mass spectrometry after *Salvia divinorum* smoking. Rapid Commun Mass Spectrom. 2005; 19: 1649-1656.
24. MCDONOUGH PC, HOLLER JM, VORCE SP, BOSY TZ, MAGLUILO J JR, PAST MR. The detection and quantitative analysis of the psychoactive component of Salvia divinorum, salvinorin A, in human biological fluids using liquid chromatography-mass spectrometry. J Anal Toxicol. 2008; 32: 417-421.
25. JERMAIN J, EVANS H. Analyzing Salvia Divinorum and its active ingredient Savinorin A utilizing thin layer chromatography and gas Chromatography/mass spectrometry. J Forensic Sci 2009; 54: 612-616.

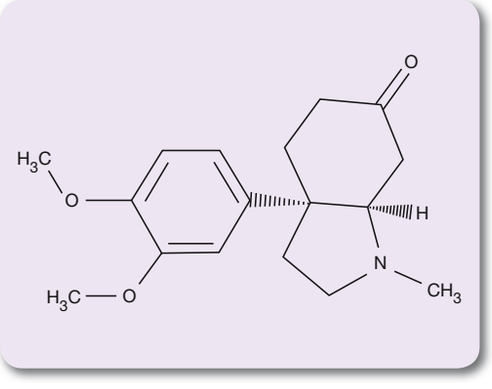
Sceletium tortuosum
(kanna)

Name: *Sceletium tortuosum*
Family: *Mesembryanthemaceae/Aizoaceae*
Genus: *Sceletium*
Species: *Sceletium tortuosum*
Synonims: kanna, channa
Origin: South Africa, in the provinces east and west of Cape Town
Active compounds: mesembrine, mesembrenone, 4'-O-demethylmesembrenol, tortuosamine [1]

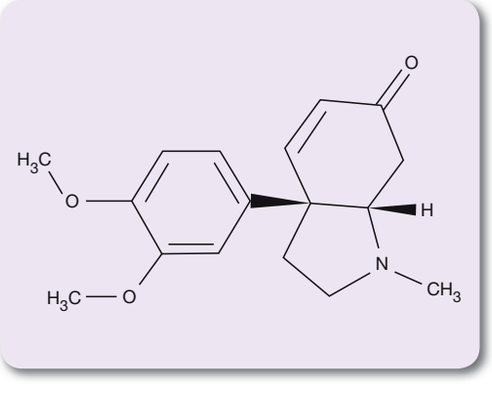
Sceletium tortuosum contains 1-1.5% alkaloids with mesembrine being the principal alkaloid at 0.3 and 0.86% in the leaves and in the trunk respectively [2]. Mesembrenone and 4'-O-demethylmesembrenol are present as well. Tortuosamine, isolated from *Sceletium* too, has a similar molecular structure as mesembrine: the principal difference between the two molecules is that the tortuosamine has the pyrrolic ring open. The active compounds can be found in the aerial parts of the plant.

The content of mesembrine and of 4'-O-demethylmesembrenol decreases during the phases of preparation of the vegetable mush of the plant according to the traditional method (see historical use); mesembrenone however, following the same treatment, undergoes an increase in concentration [3]. It is believed that this method of preparation could facilitate the microbial degradation or the sublimation of the oxalates contained in the plant in a high percentage (3.6-5%), resulting in a better taste [1].

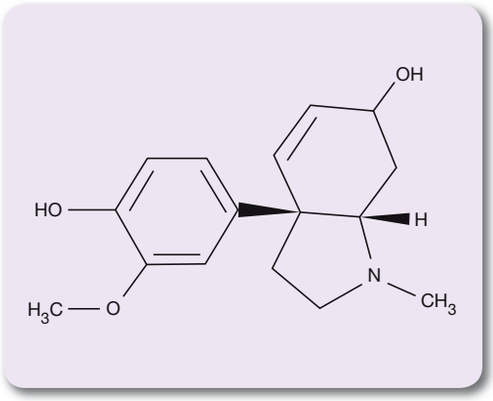
Chemical formula and physico-chemical properties of the active compounds



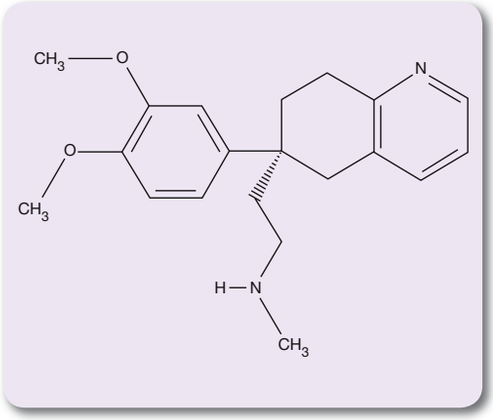
Name: Mesembrine.
Molecular formula: $C_{17}H_{23}NO_3$ (molecular weight = 289.4).
Systematic name: 3a-(3,4-dimethoxyphenyl)octahydro-1-methyl-6H-indol-6-one.
CAS registry number: 468-53-1.
Melting point: it boils without fusion at 186-190°C, its hydrochloride melts at 179-181°C.
UVmax: no data in the literature.
Solubility: alcohol, chloroform, acetone.



Name: Mesembrenone.
Molecular formula: $C_{17}H_{21}NO_3$ (molecular weight = 287.4).
Systematic name: 3a-(3,4-dimethoxyphenyl)-1-methyl-3,4,5,6,7,7a-hexahydro-2H-indol -6-one.
CAS registry number: no data in the literature.
Melting point: no data in the literature.
UVmax: no data in the literature.
Solubility: no data in the literature.



Name: 4'-O-demethylmesembrenol.
Molecular formula: $C_{16}H_{21}NO_3$ (molecular weight = 275.4).
Systematic name: 3a-(3,4-dimethoxy phenyl)- 1,2,3,3a,7,7a-hexahydro-1-methyl-6H-indol-6-one.
CAS registry number: no data in the literature.
Melting point: no data in the literature.
UVmax: no data in the literature.
Solubility: no data in the literature.



Name: Tortuosamine.
Molecular formula: $C_{20}H_{26}N_2O_2$ (molecular weight = 326.4).
Systematic name: 2-[6-(3,4-dimethoxyphenyl)-7,8-dihydro-5H-quinolin-6-il]-N-methyl- ethanamine.
CAS registry number: no data in the literature.
Melting point: no data in the literature.
UVmax: no data in the literature.
Solubility: no data in the literature.

Historical use

The traditional uses of the dry plant material (prepared to be chewed, smoked, or snuffed) have included hunger and the thirst suppression, sedation and mood elevation. The traditional use is traced back to the hottentots, a South African population initially living North of the Orange river, largely exterminated by the Boers in the XVIII century. The Hottentots were calling the plant "kanna" but with the arrival of the Dutch Boers the name was transformed in "kaugoed of kauwgoed" (chewing goods). The South African native groups were using the aerial parts of *Sceletium* to prepare the "kaugoed", a vegetable mush that was chewed repeatedly to extract from it the juice which was then swallowed. The kaugoed, prepared by collecting and choping the *Sceletium* with stones, was left to "ferment" in closed containers for several days before being consumed. Some authors report that kaugoed was used sometimes by itself or smoked with the *Cannabis sativa* leaves. There are stories about the Hottentots who, more than two centuries ago, were chewing *Sceletium*. It is reported that the effect of *Sceletium* was noticeable in these people: «… a revival of their wild spirits, their eyes sparkled and their faces showed laughter and good mood. They had thousands of delightful ideas, and a great time that allowed them to be amused in a simple way. Those using it in excess were losing their conscience and were falling in a terrible delirium…»[(2)]. In reality, in later times there were no reports of using this plant as a narcotic or as a hallucinogen.

Current use

Nowadays *Sceletium tortuosum* is sold on Internet sites as tablets or capsules, and is recommended for the treatment of anxiety and of depression, as an aid in smoking cessation, in attention deficit, and as an aid in intense study periods. The typical recommended dose is between 50 and 100 mg one or two times in the day, although there are reports on doses as high as 200 mg twice a day (if taken under medical supervision). If snorted, even 20 mg can produce substantial effects. The advertised effects are: mood elevator and a sense of closeness to others, although in "substantial" doses (albeit the amounts are not specified) it could cause delirium. The combination with alcohol and cannabis produces intense subjective hallucinatory effects.

Legislation

In Europe there are no legal restrictions for the use of *Sceletium tortuosum* or its active compounds. Posession, trade and cultivation of *Sceletium tortuosum* are legal in the United States of America, although its use in the food industry has not been approved . In Italy, neither mesembrine, mesembrenone 4'-O-demethylmesembrenol, or tortuosamine, nor the whole plant or parts of parts of it are included in the tables containing narcotic or psychotropic substances subject to the supervision and control under Article 14 of Republic Presidential Decree 309/90 and subsequent updates.

Pharmaco-toxicological properties

There are few studies regarding the pharmacological and toxicological effects of *Sceletium tortuosum* or of its active compounds. Mesembrine, well-known for its effects at the central nervous system level is a serotonin reuptake inhibitor and, in specific doses, it has antidepressant, mildly sedative and anxiolytic effects. Therefore, mesembrine might be used in the treatment of the mild to moderate depression, of psychological or psychiatric disorders characterised by anxiety, for alcohol or drug dependence, for bulimia nervosa or for obsessive compulsive disorders. The inhibitory activity has been noticed also at the level of the dopaminergic, adrenergic and nicotinic receptors.

Neither the "kougoed" nor the alkaloids of *Sceletium tortuosum* have any hallucinogenic activity but they act rather as narcotics-anxiolytics [(2)]. Mesembrenone has shown *in vitro* antitumoural activity towards the human Molt4 tumor cellular line [(4)].

Finally, there are no studies in animal models or humans regarding the pharmacological effects of tortuosamine.

Toxicity

There is no data regarding the acute toxicity of the active compounds of *Sceletium tortuosum*.

Adverse Effects

The adverse effects associated with the use of *Sceletium tortuosum* include strong headaches, lethargy, loss of appetite and depression [(2)].

Moreover, from a theoretical point of view it is possible that mesembrine, through inhibition of serotonin reuptake, which will lead to an increase of serotonin, could cause a potentially fatal serotonin syndrome . This syndrome manifests by behavioural problems (state of confusion with hypomania and agitation), dysfunctions of the autonomous nervous system (diarrhoea, chills, fever, sweating, changes in blood pressure, nausea, vomiting) and changes in the neuromuscular functions (myoclonus, hyperreflexia, tremor and difficulty in movement co-ordination) [(5)].

Pharmacological interactions

Mesembrine, being a serotonin reuptake inhibitor, can interact with selective serotonin reuptake or with monoamine oxidase (MAO) inhibitors. The use of *Sceletium tortuosum* should be therefore avoided in case of therapy with such drugs. In addition, *Sceletium tortuosum* can also interact with plants with similar pharmacological effects, such as: Syrian herb-of-grace (*Peganum harmala*), Ayahuasca (*Banisteriopsis caapi* and *Psychotria viridis*), Passionflower (*Passiflora incarnata*), Yohimbe (*Corynanthe yohimbe*) as well as with certain antidepressants. There is a report of the case of a polyuser who has experienced an episode of traumatic flashback as a consequence of the concomitant use of alcohol, cannabis and *Sceletium* [(2)].

Effects in pregnancy

There are no data regarding the use of Sceletium tortuosum in pregnancy or during lactation.

Analytical determinations

No methodologies have been reported in the international literature concerning the analysis of *Sceletium tortuosum* active principles in biological fluids of eventual consumers. However, analytical methods have been published regarding the determination of these active principles in the dry plant extract [6] and in herbal preparations [7]. In this latter case, capillary electrophoresis coupled to ultraviolet spectrophotometric detection is used [7], while in case of dry plant extract gas chromatography coupled to mass spectrometry is applied [6].

The method below reported is a brief outline useful to the investigator to set up the analysis. For specific details, it is advisable to refer to the original text.

Determination of mesembrine, mesembrenone and 4'-o-demethyl mesembrenol in the plant of *Sceletium tortuosum*

(From: SMITH MT, FIELD CR, CROUCH NR, HIRST M. The distribution of mesembrine alkaloids in selected taxa of the mesembryanthemaceae and their modification in the Sceletium derived "kougoed". Pharm Biol. 1998; 36: 173-179) [6].

The analysis is carried out on the dry extract of *Sceletium tortuosum* using gas chromatography coupled with mass spectrometry.

Extraction of the compounds

25 g dry material is mixed with 200 ml 95% ethyl alcohol and is extracted in a Soxhlet apparatus. The ethanolic extract is evaporated and resuspended in 20 ml 2M HCl. The acid solution is washed three times with a total of 150 ml ethyl ether to remove possible fats and pigments. The remaining acid solution is extracted using a 60 ml Extrelut column. The column is washed with 40 ml dichloromethane-isopropanol (85:15, v/v) mixture, alkalinised with ammonia and the elution is achieved with 40 ml dichloromethane-isopropanol (85:15 v/v) mixture. The eluate is reduced to 2 ml and then subjected to a further extraction using solid phase silica column, conditioned with dichloromethane. The alkaloids are eluted with 35 ml of 6 solvents with increasing polarity: dichloromethane, ethyl acetate, acetone, acetonitrile, methyl alcohol and acetic acid. The total eluate is concentrated to 2 ml and 1µl is injected into instrumentation.

Analytical conditions

Chromatographic column: DB5 (0.25 mm x 30 m x 0.25 µm)
Injector temperature: 350°C
Carrier gas: Helium at 11.6 psi
Injection mode: splitless
Temperature program: 230°C-260°C at 1 C°/min
Detector: mass spectrometer with electron impact interface.

Retention times of the tested compounds

Mesembrine: 12 minutes
Mesembrenone: 12.5 minutes
4'-O-mesembrenol: 11.5 minutes

Characteristic fragments for the tested compounds

Mesembrine m/z 289, 219, 204
Mesembrenone: m/z 287, 219, 70
4'-O-mesembrenol: m/z 275, 218, 204

Standards

The standards used for the analyses are extracted from the plant using thin layer chromatography .

Calibration curve

The preparation of the calibration curve is not described.

Results

Sceletium tortuosum examined dry extract contains 1-1.5%. alkaloids; mesembrine results to be the principal alkaloid (0.3 and 0.86% in the leaves and in the trunk, respectively).

References

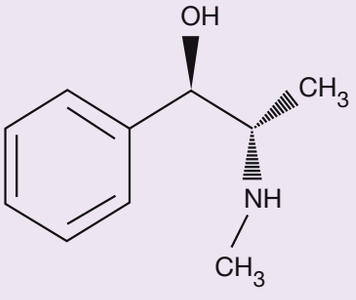
1. http://www.plantzafrica.com/medmonographs/scelettort.pdf
2. SMITH MT, CROUCH NR, GERICKE N, HIRST M. Psychoactive constituents of the genus Sceletium N.E.Br and other Mesembryanthemaceae: a review. J Ethnopharmacol. 1996; 50: 119-130.
3. PATNALA S, KANFER I. Investigation of phytochemical content of *Sceletium tortuosum* following the preparation of "Kougoed" by fermentation of plant material. J Ethnopharmacol. 2009; 121: 86-91
4. VENIGER B, ITALIANO L, BECK JP, BASTIDA J, BERGONON S, CODINA C, LOBSTEIN A, ANTON R. Cytotoxic activity of Amaryllidaceae alkaloids. Planta med. 1995; 61: 77-79.
5. LEJOYEUX M, ADES J, ROUILLON F. Serotonin syndrome: incidence, symptoms and treatment. CNS Drugs. 1994; 2: 132-143.
6. SMITH MT, FIELD CR, CROUCH NR, HIRST M. The distribution of mesembrine alkaloids in selected taxa of the mesembryanthemaceae and their modification in the Sceletium derived "kougoed". Pharm Biol. 1998; 36: 173-179.
7. PATNALA S, KANFER I. A capillary zone electrophoresis method for the assy and quality control of mesembrine in Sceletium tablets J Pharm Biomed Anal 2008; 48: 440-446.

Sida cordifolia
(malva branca)

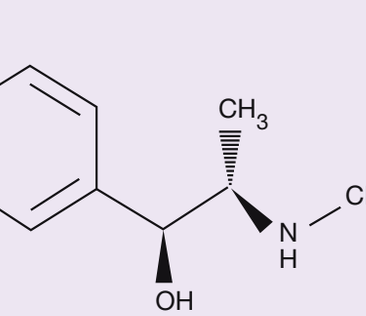
Name: *Sida cordifolia*
Family: *Malvaceae*
Genus: *Sida* L.
Species: *Sida cordifolia* L.
Synonims: llima;White mallow; Country mallow, bala, pinellia
Origin: India
Active compounds: ephedrine (0.8-1.2% in the plant extract), pseudoephedrine, vasicine and vasicinone [1]. These active compounds are present in the seeds, leaves and roots.

Ephedrine, the principal alkaloid of the plant, is a white, crystalline solid, with bitter taste and slightly aromatic smell. Ephedrine and its optic isomer, pseudoephedrine, are structurally quite similar to methamphetamine and to dobutamine. The clandestine laboratories, in which amphetamine and amphetamine derivatives are illicitly synthesized use a simple dehydrogenation to obtain methamphetamine from ephedrine. Ephedrine contains two asymmetrical carbon atoms; only *l*-ephedrine and the racemic ephedrine are used in clinical practice.

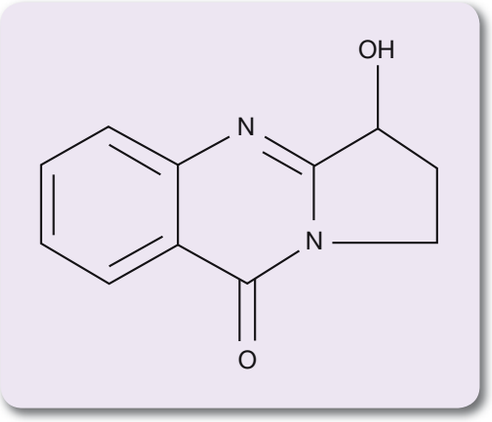
Chemical formula and physico-chemical properties of the active compounds



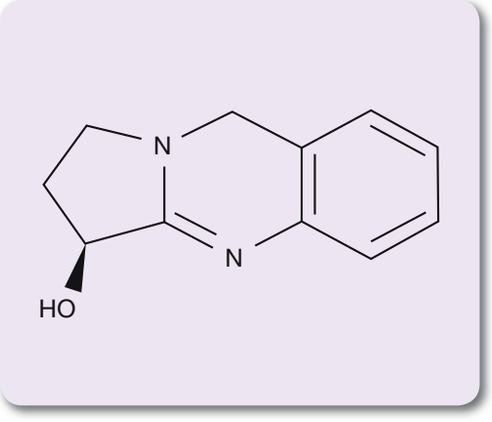
Name: Ephedrine.
Molecular formula: $C_{10}H_{15}NO$ (molecular weight = 165.2).
Systematic name: (1R,2S)-2-methylamino-1-phenylpropanol.
CAS registry number: 299-42-3.
Melting point: 38°C.
UVmax: 251nm.
Solubility: water, alcohol, chloroform, ether, glycerol and liquid paraffin.



Name: Pseudoephedrine.
Molecular formula: $C_{10}H_{15}NO$ (molecular weight = 165.2).
Systematic name: (1S,2S)-2-methylamino-1-phenylpropanol.
CAS registry number: 90-82-4.
Melting point: 116-119°C.
UVmax: 251 nm.
Solubility: ethyl alcohol, ether, partially soluble in water.



Name: Vasicinone.
Molecular formula: $C_{11}H_{10}N_2O_2$ (molecular weight = 202.2).
Systematic name:2,3-dihydro-3-hydroxypyrrol(2,1-b)quinazolin-9(1H)-one.
CAS registry number: 486-64-6.
Melting point: no data in the literature.
UVmax: no data in the literature.
Solubility: no data in the literature.



Name: Vasicine.
Molecular formula: $C_{11}H_{10}N_2O$ (molecular weight = 188.2).
Systematic name: 1,2,3,9-tetrahydropyrrol (2,1-b)quinazolin-3-ol.
CAS registry number: 6159-55-3.
Melting point: 210°C.
UVmax: no data in the literature.
Solubility: acetone, chloroform, alcohol, partially soluble in water, ether and benzene.

Historical use

Over the past two thousand years, the traditional Indian medicine has used the roots, the leaves, the seeds and the trunk of *Sida cordifolia*, each part with specific therapeutic properties, for the treatment of bronchial asthma, influenza and common cold, respiratory insufficiency, headaches, nasal congestion, osteoarticular pain, cough and swelling.

Current use

In the ayurvedic medicine *Sida cordifolia* is used as adjuvant in the treatment of of asthma: in the same context it is mixed also with other herbs known to increase "the vital energy" and the tone of the body.
Sida cordifolia extract, which can contain from 0.8 to 1.2% of ephedrine, is sold on specific Internet sites as a stimulant of the central nervous system producing an effect similar to amphetamine and is sought by users of herbal ecstasy (the generic name for ephedrine based herbal preparations) for its euphoric effects. It is found easily on the Internet, without any medical prescription or medical surveillance. The dry *Sida cordifolia* extract is present in numerous natural products, but with all the risks and contraindications of *Ephedra*. The greatest risks are for patients with heart problems, high blood pressure and those who are taking psychotropic drugs or other herbal stimulants.
For *Sida cordifolia*, like for *Ma-huang* there are alluring advertisements for the use of the plant. In fact, there are often advertisements such as: « … (omission) … *is the first natural ecstasy devoid of ephedra or Ma-huang. Definitely better than the original*... (omission)... *At present the strongest product among the ecstatic stimulants, has immediately found a strong popularity among the regular herbal ecstasy users. Perfect for Dance Floor, it develops an incredible sensuality, smiles, love, energy and passion*». The publicity, in this case, advertises a product that promises pleasant feelings without the need of taking ephedra extract or *Ma-huang*, but it does not advertise the fact that the active ingredient contained in *Sida cordifolia* is the same as in *Ma-huang* or in ephedra extract.

Legislation

The American Food and Drug Administration (FDA) recently banned the use of *Sida cordifolia*, having at the same time prohibited the sale of dietary supplements containing ephedra alkaloids, since they are considered as supplements that present an unreasonable risk to human health [2,3].

During 2004, in America some ayurvedic medicine societies have petitioned the FDA to legalize the sale and utilisation of the dietary supplements based on *Sida cordifolia*. To date, the outcome of the petitioning is not known [4].

In Switzerland the products containing ephedrine derivatives are labelled as pharmaceuticals which can be obtained only with medical prescription.

In Italy neither ephedrine, pseudoephedrine, vasicinone or vasicine, nor the whole plant or parts of the plant are included in the tables containing narcotic or psychotropic substances subject to the supervision and control under Article 14 of Republic Presidential Decree 309/90 and subsequent updates. Ephedrine and pseudoephedrine are however included in the category 1 of the Annex I for classified substances in the Decree n. 258 of April 12 1996 (G.U.112 of 5/15/1996) regarding the directive 92/109/EEC relative to the manufacturing and marketing of certain substances used in the illicit production of narcotic and psychotropic drugs. Such an annex is present in the updated text of the Decree 309/90 (Official Bulletin n. 62 of March 15th, 2006). Ephedrine and pseudoephedrine are also included in the list of biologically or pharmacologically active substances present in the article 1 of the law n. 376/00: "Discipline for the health protection of sports and for the fight against doping" published in the Official Bulletin n. 294 of December 18th, 2000. Such a list has been approved according to Ministerial Decree in October 15th, 2002 and published in the Official Bulletin n. 278 in November 27th, 2002. An urine sample is considered positive in a drug test when the concentration of ephedrine is equal or higher than 10 μg/ml [5,6].

Pharmaco-toxicological properties

The pharmacological properties of *Sida cordifolia* are determined by the presence of ephedrine, pseudoephedrine and other structurally related alkaloids. Ephedrine and pseudoephedrine are simpathomimetic agents with direct or indirect agonist activity towards the α- and β-adrenergic receptors and stimulating the central nervous system [7-8].

Ephedrine can be inhaled (ephedrine salts are used as nasal decongestants) and is well absorbed through the skin when applied as an ointment. Ephedrine drops at 0.1% applied to the eyes are effective for the treatment of allergic conjunctivitis. When the ephedrine is orally administered for its bronchodilatatory and decongestant effects, the average dose is 25-50 mg/kg/day to be repeated if necessary every 3-4 hours. The daily total dose should not to exceed, in any case, 150 mg. The pediatric dose is of 2-3 mg/kg/day of body weight or 100 mg/m^2/day of body surface, subdivided in 4-6 doses [3]. In case of parenteral administration, the minimum effective dose (12.5-25 mg) must be injected.

After oral administration ephedrine is quickly and completely absorbed in the intestine. Once absorbed, it reaches peak plasma concentration an hour after administration and has a high volume of distribution. The plasma half life of the compound varies between 3 to 6 hours depending on the urinary pH. The pharmacological effects last for about 1 hour. Ephedrine and the related compounds are lipophilic and can cross the blood-brain barrier interacting with the central nervous system.

Only a small proportion of ephedrine is metabolized by the liver; the main reactions in the metabolism are N-demethylation (8-20%) and deamination (4-13%). A greater proportion of ephedrine (about 53-74%) is instead excreted unchanged in the urine. Because of the presence of an ionizable amino group, the urinary excretion is favoured by the acidic pH of urine [9-11].

Ephedrine is a powerful stimulant of the central nervous system; it is used as a central anorectic in slimming products and for the treatment of narcolepsy and depression [9].

At cardiovascular level, it increases the strength of the heart contractions, the cardiac output and causes peripheral vasocostriction. This translates into an increase of both systolic and diastolic blood pressure [12].

The alkaloid, stimulating the adrenergic receptors, induces relaxation of the bronchial smooth muscle, decreased tone and intestinal motility, relaxation of the vescical walls and reduced uterine activity.

In mice, ephedrine and pseudoephedrine have an anti-inflammatory effect on the carragenine induced edema [13]. In addition, the *Ephedra sinica* extract has shown, in vitro, an ability to inhibit the classical activation pathway of the complement [14].

In vitro, the two substances show an antibacterial effect towards the *Staphylococcus aureus* [15], a cytotoxic effect towards hepatoblastoma HepG2 and neuroblastoma Neuro-2a [16], and a mild hepatoprotective effect in case of carbon tetrachloride induced cytotoxicity [17].

Sida cordifolia has been widely used by the tribes of the state of Gujarat in India for his alleged cardioprotective and vasodilatory effects. There are no clinical data to support such use. However, recently it has been shown that a hydroalcoholic extract of *Sida cordifolia* leaves administered orally in combination with propranolol produces cardioprotective effects in myocardial infarction induced experimentally in rats [18].

Also in rats, it has been shown that oral administration of a methanol extract obtained from aerial parts of *Sida cordifolia* has antipyretic effects and is able to protect against the ulcerogenic effects induced by aspirin and ethanol [19].

Toxicity

The majority of the studies on the toxicity on the natural ephedrine have been conducted on *Ma-huang* (*Ephedra sinica*) plant and not on *Sida cordifolia*. A recent study has demonstrated that taking food additives based on ephedra and caffeine increases the blood pressure. Particularly, it has been observed that taking a single oral dose of a mixture of ephedrine and caffeine (20 mg and 200 mg, respectively) has caused an increase of the systolic pressure by 14 mm of Hg and of diastolic pressure of 6 mm of Hg [20].

In another study on healthy subjects, using a placebo group as a comparison, the blood pressure variations following the oral administration of ephedrine (0.1 mg/kg), caffeine (4 mg/kg) and a combination of the two have been studied. In case of caffeine administration, an increase of the arterial pressure by 3 - 6 mm of Hg has been observed compared to the placebo group. For ephedrine the increase was 12 mm of Hg, and finally, the association of the two substances produced a 15 mm of Hg increase in the blood pressure of administered individuals, compared to the placebo group [21,22].

Data regarding the acute toxicity of ephedrine [23]

In human - LDLo: 9 mg/kg

In mouse - LD50 following intraperitoneal administration: 350 mg/kg

In mouse - LD50 following intravenous administration: 74 mg/kg

Data regarding the acute toxicity of pseudoephedrine [24]

In human - LDLo: 9 mg/kg.

In human - TDLo: 64 mg/kg

Adverse Effects

The most common adverse effects associated with the use of ephedrine are: tremors, states of anxiety and confusion, restlessness, insomnia and psychotic states; following an overdose, paranoic psychoses and hallucinations may occur [7].

At cardiovascular level, ephedrine can induce arterial hypertension, vasoconstriction, tachicardia, palpitations, myocardial ischaemia and cardiac arrest [25] . Moreover, the alkaloid can predispose to an ischaemic or hemorrhagic stroke [26].

The case of a 35-year-old woman having bronchospasms and cardiomiopathy as a result of chronic use of elevated doses of ephedrine is described [27].

As a consequence of repeated use, tachyphylaxis (reduction of the efficiency to the total loss of effect) may develop.

Overdose of ephedrine manifests by nausea and vomiting followed by headache, agitation, anxiety, tremors, tachicardia and hypertension. The excessive increase in the blood pressure can lead to cerebral hemorrhage and to myocardial infarction. As a consequence of ventricular arrhythmias, it is possible to have cardiac arrest and death.

Ephedrine is contraindicated in case of hypertension, hyperthyroidism, pheochromocytoma and acute narrow-angle glaucoma. Patients suffering of prostatics hypertrophy or renal insufficiency, should take the substance with caution [9,20,28,29].

Recently, a meta-analysis of the clinical studies and data from clinical trials and from the system of reporting adverse reactions to the FDA, on the effects of preparations based on *Ephedra sinica* or ephedrine, used for slimming purpose or to improve athletic performance, has demonstrated that the use of *Ephedra sinica* or of ephedrine in association to caffeine increases the risk of cardiac arrhythmias and of gastrointestinal, psychiatric and autonomous nervous system disorders [30].

Pharmacological interactions

Ephedrine can interact with monoamine oxidases (MAO) inhibitors causing an increase of the noradrenaline levels and subsequently an increase of the sympatheic tone. The interaction may cause headaches, fever, arrhythmias and hypertensive crisis. Therefore, ephedrine should not be taken by patients trated with with MAO inhibitors or patients who have stopped such treatment for less than 14 days [31].

Ephedrine can reduce the efficacy of the antihypertensive medications [32]; associated with clonidine it can cause increase in the noradrenaline levels and increased blood pressure [33].

Associated with non steroidal antiinflamatory drugs (NSAIDs), ephedrine may facilitate the occurence of lesions in the gastric mucosa [34]. Moreover, ephedrine can increase the metabolism of corticosteroids reducing their plasma levels. Asthmatic patients in treatment with such drugs should avoid taking ephedrine based products [35].

The urinary excretion of ephedrine is pH-dependent. The drugs listed below are able to alkalinize the urine and as a result slow down the elimination of ephedrine [36]:

- Acetazolamide
- Ammonium chloride
- Antacids
- Sodium bicarbonate

A greater risk of adverse events of cardiovascular type (hypertension, tachicardia or cardiac arrhythmias) has been observed following the concomitant administration of ephedrine and the following drugs [31,37,38].

- Digoxine
- Cyclopropanol
- Phenylpropanolamine
- Pseudoephedrine

Reserpine causing depletion of noradrenaline, can reduce the efficacy of ephedrine [39]. Teophylline can cause an increased incidence of central and gastrointestinal adverse effects (restlessness, insomnia and nausea) that occur following ephedrine administration [40].

Finally, the association between ephedrine and caffeine can in fact increase the sympatomimetic effects of the ephedrine and cause tachycardia, hypertension, ictus and cardiac arrhythmias. The concomitant use of this two substances should therefore be avoided [26].

Effects in pregnancy

Ephedrine is able to cross the placenta and pass into the breast milk. The ingestion of the substance during pregnancy can cause in the foetus hyperactivity, irritability and tachycardia. For these reasons the FDA has assigned the products based on ephedra to the category 2c: not to use in pregnancy and/or during breastfeeding [41].

Analytical determinations

No methodologies have been reported in the international literature concerning the analysis of *Sida cordifolia* active principles in biological fluids of eventual consumers. However, analytical methods have been published regarding the determination of these active principles in herbal preparations using gas chromatography coupled to mass spectrometry [42].

Furthermore, internation literature reports the determination of active principles of *Ephedra sinica*, some of which are the

same of those of *Sida cordifolia*.

Please refer to the monograph of *Ephedra sinica* for analytical details of the determination in the blood of ephedrine, and pseudoephedrine, active ingredients common to both plants [43].

References

1. FRANZOTTI EM, SANTOS CV, RODRIGUES HM, MOURAO RH, ANDRADE MR, ANTONIOLLI AR. Anti-inflammatory, analgesic activity and acute toxicity of *Sida cordifolia* L. (Malva-branca). J Ethnopharmacol. 2000; 72: 273-277.
2. CAPRINO L, BRAGANÒ MC, BOTRÈ F. Gli integratori fitoterapici nello sport: uso ed abuso. Ann Ist Super Sanità. 2005; 41: 35-38.
3. FDA - Final rule declaring dietary supplements containing ephedrine alkaloids adulterated because they present an unreasonable risk. Federal Register - February 11 - 2004; 69.
4. http://www.fda.gov/ohrms/dockets/dailys/04/aug04/082004/95n-0304-psa00001-vol398.pdf
5. http://www.wada-ama.org/
6. LEFEBVRE RA, SURMONT F, BOUCKAERT J, MOERMAN E. Urinary excretion of ephedrine after nasal application in healthy volunteers. J Pharm Pharmacol. 1992; 44: 672-675.
7. MARTINDALE. The Complete Drug Reference, 32nd edn. (Parfitt K, ed.). London: The Pharmaceutical Press, 1999.
8. WORLD HEALTH ORGANIZATION. WHO Monographs on Selected Medicinal Plants, vol 1. Geneva: World Health Organization, 1999.
9. GOODMAN AND GILMAN'S - The pharmacological basis of therapeutics. McGraw-Hill Medical Publishing Division. Tenth Edition 2001: pp. 237-238.
10. ANDRAWS R, CHAWLA P, BROWN D. Cardiovascular effects of Ephedra alkaloids: a comprensive review. Progress Car Dis. 2005; 47: 217-225.
11. SEVER PS, DRING LG, WILLIAMS RT. The metabolism of (-)-ephedrine in man. Eur J Clin Pharmacol. 1975; 9: 193-198.
12. MCEVOY GK (ed): AHFS Drug Information 1999. American Society of Health System Pharmacists, Bethesda, MD; 1999.
13. KASAHARA Y, HIKINO H, TSURUFUJI S, WATANABE M, OHUCHI K. Antiinflammatory actions of ephedrines in acute inflammations. Planta Med. 1985; 51: 325-331.
14. LING M, PIDDLESDEN SJ, MORGAN PB. A component of the medicinal herb ephedra blocks activation in the classical and alternative pathways of complement. Clin Exp Immunol 1995; 102: 582-588.
15. CHANG HM,, PAUL PH, YEUNG SCY, SHENG-YAO S. Pharmacology and Applications of Chinese Materia Medica, vol 2. Singapore: World Scientific Publishing, 1987: 1119-1124.
16. LEE MK, CHENG BW, CHE CT, HSIEH DP. Cytotoxicity assessment of Ma-huang (Ephedra) under different conditions of preparation. Toxicol Sci. 2000; 56: 424-430.
17. LEE JW, CHOI JH, KANG SM. Screening of medicinal plants having hepatoprotective activity effect with primary cultured hepatocytes intoxicated using carbon tetrachloride cytotoxicity. Kor J Pharmacogn. 1992; 23: 268-275.
18. KUBAVAT JB, ASDAQ SM. Role of Sida cordifolia L. leaves on biochemical and antioxidant profile during myocardial injury. J Ethnopharmacol. 2009 6; 124: 162-165.
19. PHILIP BK, MURALIDHARAN A, NATARAJAN B, VARADAMURTHY S, VENKATARAMAN S. Preliminary evaluation of anti-pyretic and anti-ulcerogenic activities of Sida cordifolia methanolic extract. Fitoterapia. 2008; 79: 229-231.
20. HALLER CA, JACOB 3rd P, BENOWITZ NL. Pharmacology of ephedra alkaloids and caffeine after single-dose dietary supplement use. Clin Pharm Ther. 2002; 71: 421-432.
21. JACOBS I, PASTERNAK H, BELL DG. Effects of ephedrine, caffeine, and their combination on muscular endurance. Med Sci Sports Exerc. 2003; 35: 987-994.
22. BERLIN I, WAROT D, AYMARD G, ACQUAVIVA E, LEGRAND M, LABARTHE B, PEYRON I, DIQUET B, LECHAT P. Pharmacodynamics and pharmacokinetics of single nasal (5 mg and 10 mg) and oral (50 mg) doses of ephedrine in healthy subjects. Eur J Clin Pharmacol. 2001; 57: 447-455.
23. ARENA JM, SPRIGFIELD IL, THOMAS CC. Poisoning: toxicology, symptoms, treatments. 2nd ed. 1970; 2: 73.
24. BURKHART KK. Intravenous propranolol reverses hypertension after sympathomimetic overdose: two case reports. J Toxicol Clin Toxicol. 1992; 30: 109-114.
25. PENTEL P. Toxicity of over-the-counter stimulants. JAMA. 1984; 252: 1898-1903.
26. HALLER CA, BENOWITZ NL: Adverse cardiovascular and central nervous system events associated with dietary supplements containing ephedra alkaloids. N Engl J Med. 2000; 343: 1833-1838.
27. VAN MIEGHEM W, STEVENS E, COSEMANS J. Ephedrine-induced cardiopathy. Br Med J. 1978; 1: 816.
28. HALLER CA, JACOB P, BENOWITZ NL. Short-term metabolic and hemodynamic effects of ephedra and guarana combinations. Clin Pharmacol Ther. 2005; 77: 560-571.
29. http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?./temp/~t63K7F:1
30. SHEKELLE PG, HARDY ML, MORTON SC, MAGLIONE M, MOJICA WA, SUTTORP MJ, RHODES SL, JUNGVIG L, GAGNE J. Efficacy and safety of ephedra and ephedrine for weight loss and athletic performance: a meta-analysis. JAMA. 2003; 289: 1537-1545.

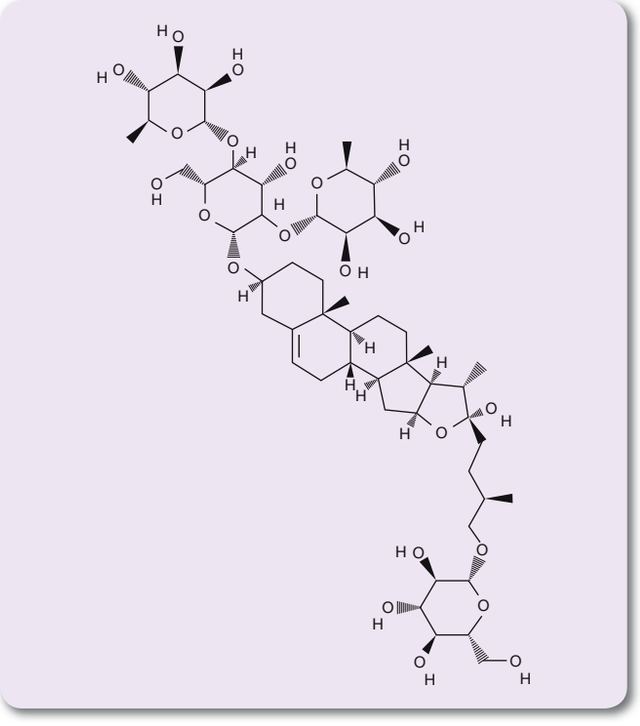
31. BLUMENTHAL M, BUSSE WR, GOLDBERG A, HALL T, RIGGINS CW, RISTER RS, EDS. KLEIN S, RISTER RS. The Complete German Commission E Monographs - Therapeutic Guide to Herbal Medicines. Boston: Integrative Medicine Communications; Austin, TX: American Botanical Council, 1998.
32. ZAHN KA, LI RL, PURSSEL RA. Cardiovascular toxicity after ingestion of "herbal ecstasy." J Emerg Med. 1999; 17: 289-291.
33. NISHIKAWA T, KIMURA T, TAGUCHI N. Oral clonidine preanesthetic medication augments the pressor responses to intravenous ephedrine in awake or anesthetized patients. Anesthesiology. 1991; 74: 705-710.
34. CHO S, HONG T, JIN GB, YOSHINO G, MIURA M, AIKAWA Y, YASUNO F, CYONG JC. The combination therapy of ephedra herb and loxoprofen caused gastric lesions in mice. Am J Chin Med. 2002; 30: 571-577.
35. BROOKS SM, SHOLITON LJ, WERK EE ALTENAU P. The effects of ephedrine and theophylline on dexamethasone metabolism in bronchial asthma. J Clin Pharmacol. 1977; 17: 308-318.
36. BRATER DC, KAOJARERN S, BENET LZ, LIN ET, LOCKWOOD T, MORRIS RC, MCSHERRY EJ, MELMON KL. Renal excretion of pseudoephedrine. Clin Pharmacol Ther. 1980; 28: 690-694.
37. Product Information: Ephedrine sulfate injection USP. Abbott Hospital Products, North Chicago, IL; 1997.
38. ONUIGBO M, ALIKHAN M. Over-the-counter sympathomimetics: a risk factor for cardiac arrhythmias in pregnancy. South Med J. 1998; 91: 1153-1155.
39. HANSTEN PD, HORN JR. Drug Interactions. Lea & Febiger, Philadelphia, PA; 1990.
40. BIERMAN CW, PIERSON WE, SHAPIRO GG. Exercise-induced asthma: pharmacological assessment of single drugs and drug combinations. JAMA. 1975; 234: 295-298.
41. BERKOWITZ RL, COUSTAN DR, NOCHIZUKI TK. Handbook for Prescribing Medications During Pregnancy. Little, Brown and Co, Boston, MA; 1981.
42. MARCHEI E, PELLEGRINI M, PACIFICI R, ZUCCARO P, PICHINI S. A rapid and simple procedure for determination of ephedrine alkaloids in dietary supplements by gas chromtography-mass spectrometry. J Pharm Biomed Anal 2006; 41: 1633-1641.
43. BEYER J, PETERS FT, KRAEMER T, MAURER HH. Detection and validated quantification of nine herbal phenalkylamines and methcathinone in human blood plasma by LC-MS/MS with electrospray ionization. J Mass Spectrom. 2007; 42: 150-160.

Tribulus terrestris
(Caltrop)

Name: *Tribulus terrestris* L
Family: *Zygophyllaceae*
Genus: *Tribulus* L.
Species: *Tribulus terrestris* L.
Synonims: caltrop, puncture vine, yellow vine, goathead, Bai Ji Li, Bindi, Dubblegee
Origin: the plant originates from India, but now it can be found as a weed in North America
Active compounds: protodioscin

The plant contains flavonoids, amides and alkaloids, although its properties seem to be attributed mostly to protodioscin. The parts used are the seeds and the fruit of the plant, and, generally, the aerial parts .

Chemical formula and physico-chemical properties of the active compound



Name: Protodioscin.
Molecular formula: $C_{51}H_{84}O_{22}$ (molecular weight = 1049.2).
Systematic name: no data in letterature.
CAS registry number: 18642-44-9.
Melting point: no data in letterature.
UVmax: no data in letterature.
Solubility: a mixture of water and acetonitrile.

Historical use

Tribulus terrestris has been used for a very long time in the asian herbal medicine, particularly in the ayurvedic medicine. In the Indian, Chinese, Bulgarian folk medicine and in other countries it is used for treating impotence, edema, abdominal swelling and cardiovascular diseases.

Current use

Tribulus terrestris is used as geriatric tonic and for the treatment of the generalised weakness. Preparations based on *Tribulus terrestris* extract, are sold in the United States of America like dietary supplements that claim a stimulating action

of the motor activity and of the muscle tone. Actually, the preparations based on *Tribulus terrestris* are used to improve sports performances and for the treatment of impotence. *Tribulus terrestris* is advertised on the Internet as "a powerful aphrodisiac with a positive influence on the sexual sphere, as a plant with the energizing and tonic properties, a stimulant of sexual activity and as a spermatogenesis". It is used also by some athletes as it is believed to increase the gonadal production of androgenic steroids according to a mechanism not yet clarified.

Legislation

There are no restrictive legal measures against the *Tribulus terrestris* or its active principles in European countries or outside Europe. In Italy, sales of *Tribulus terrestris* is not subject to any restrictions.

Pharmaco-toxicological properties

Protodioscin is a steroidal saponin that constitutes about 45% of the extract obtained from the aerial parts of *Tribulus terrestris*. The substance is able to increase the endogenous production of testosterone, dihydrotestosterone, luteinizing hormone, dehydroepiandrosterone and dehydroepiandrosterone sulphate. In experimental animals, these effects cause an increase in spermatogenesis and in the coupling frequency. In particular, it has been shown in the rabbit that the compound stimulates the release of nitric oxide (NO) from the vascular endothelium of corpus cavernosum having a pro-erectile effect. This effect is based on a mechanism which seems to involve a steroidal hormones pathway as well. In the human, protodioscin is used in the treatment of erectile dysfunctions, however, its efficacy has not yet been demonstrated [1,2].

In a study on a group of young volunteers versus placebo , following the administration of *Tribulus terrestris* at doses of 10 and 20 mg/kg, the plasma levels of testosterone, androstenedione and luteinizing hormone have been measured. After 4 weeks of treatment, the levels were similar to those in the untreated placebo group [3].

Another study on 15 athletes versus placebo, has shown that the intake for eight weeks of a product based on *Tribulus terrestris* (3.21 mg/kg/day) did not have significant differences between the treatment group and the control group with respect to muscle mass, and resistance to the physical exercise induced fatigue [4].

A Chinese study carried out in 406 patients suffering from *angina pectoris* has demonstrated that the protodioscin has coronary dilatation effect and can be useful in the treatment of this cardiac pathology [5]. The use of *Tribulus terrestris* extracts can significantly reduce blood lipids and counteract the damage from the vascular endothelial dysfunction caused by hyperlipidemia experimentally induced in rabbits [6].

In addition, the *Tribulus terrestris* extracts show antitumoural and antibacterial properties towards *Staphylococcus aureus* and *Escherichia coli* [7]. The saponins contained in *Tribulus terrestris* has antifungal properties against drug resistant strains of Candida albicans [8].

Finally, the aqueous extract of the plant can also affect oxalate metabolism by inhibiting both, glycol oxidase and dehydrogenase. This effect translates into reduction of hyperoxaluria, one of the principal causes of renal stone formation [9].

A study performed on rat model with the aim of assessing the effect of *Tribulus terrestris* on the endocrine system has shown a positive effect on sperm production in association with unchanged levels of circulating androgens. The same study did not show any significant effect in tissues of organs sensitive to hormones action such as prostate, seminal vesicles, uterus and vagina [10].

Toxicity

The aerial parts of *Tribulus terrestris* contain β-carbolines. In sheep, the continuous ingestion of the plant can cause the accumulation of such substances at the central nervous system level with progressive and irreversible locomotor disorders [11].

There is no acute toxicity data for protodioscin.

Adverse Effects

Recently, the clinical case of a 21-year-old male who had taken chronically *Tribulus* based products to improve his athletic performance, was described. The young man developed gynecomastia with altered hormonal profile (reduced levels

of follicle stimulating hormone and luteinizing hormone and testosterone with normal levels of prolactin, estradiol and progesterone); such an alteration was solved only after the boy discontinued the intake of *Tribulus terrestris* [12].

Pharmacological interactions

Pharmacological interactions are not reported

Effects in pregnancy

There is no data about the use of the plant during pregnancy and/or lactation.

Analytical determinations

No methodologies have been reported in the international literature concerning the analysis of *Tribulus terrestris* active principle in biological fluids of eventual consumer. There is, instead, an assay to determine protodioscin in pulverized plant [13], and another to measure this compound in rat plasma [14].

The method below reported is a brief outline useful to the investigator to set up the analysis. For specific details, it is advisable to refer to the original text.

Determination of protodioscine in rat plasma

(From: TIEJIE W, ZHOGBO L, JUN L, MIN Z, JUPENG L, XIAOHUI C, KAISHUN B. Determination of protodioscin in rat plasma by liquid chromatography-tandem mass spectrometry. J Chromatogr B 2007; 848: 363-368) [14].

The analysis is carried out on rat plasma by liquid chromatography coupled to tandem mass spectrometry.

Extraction of the compounds

An amount of 40 µl plasma is added with 100 µl acetonitrile and 20 µl methyl alcohol. The mixture is vortex mixed for 60 s and centrifuged at 4000 g for 10 minutes. A 20 µl volume of supernatant are then injected into the liquid chromatograph.

Analytical conditions

Chromatographic column: Carbosorb ODS-3 (50mm x 2.0 mm x 5 µm)
Mobile phase: acetonitrile-water-formic acid (80:20:0,1 v/v/v)
Separation: isocratic
Flow rate: 0.2 ml/min
Column temperature:30°C
Detector: mass spectrometer with positive mode electrospray (ESI) interface
Collision energy: 35 eV
Source temperature: 105°C

Retention times of the tested compounds

Protodioscin: 0.69 minutes

Characteristic fragments for the tested compounds

Protodioscin: m/z 1032, 869, 415

Standard

Protodioscin standard was obtained from the roots of *Discorsa Nipponica*.

Calibration curve

The calibration standards (range 20-125000 ng/ml) were prepared by diluting stock solution of protodioscin in blank plasma samples.

Results

The reported methodology allows only the identification of protodioscin in rat plasma after intravenous drug administration.

References

1. GAUTHMAN K, ADAIKAN PG, PRASAD RN. Aphrodisiac properties of Tribulus terrestris extract (protodioscin) in normal and castrated rats. Life Sci. 2002; 71: 1385-1396.
2. ADAIKAN PG, GAUTHAMAN K, PRASAD RNV, NG SC. Proerectile pharmacological effects of Tribulus terrestris extract on the rabbit corpus cavernosum. Ann Acad Med Singapore. 2000; 29: 22-26.
3. NEYCHEV VK, MITEV VI. The aphrodisiac herb Tribulus terrestris does not influence the androgen production in young men. J Ethnopharmacol. 2005; 101: 319-323.
4. ANTONIO J, UELMEN J, RODRIGUEZ R, EARNEST C. The effects of *Tribulus terrestris* on body comopsition and exercise performance in resistance-trained males. Int. J Sport Nutr Exerc Metab. 2000; 10: 208-215.
5. WANG B, MA L, LIU T. 406 cases of angina pectoris in coronary heart disease treated with saponin of Tribulus terrestris. Zhong Xi Yi Jie He Za Zhi. 1990; 10: 85-87.
6. TUNCER MA, YAYMACI B, SATI L, CAYLI S, ACAR G, ALTUG T, DEMIR R. Influence of Tribulus terrestris extract on lipid profile and endothelial structure in developing atherosclerotic lesions in the aorta of rabbits on a high-cholesterol diet. Acta Histochem. 2009; 111: 488-500.
7. ZAFAR R, LALWANI M. Tribulus terrestris Linn-a review of the current knowledge. Indian Drugs. 1989; 27: 148-153.
8. ZHANG JD, CAO YB, XU Z, SUN HH, AN MM, YAN L, CHEN HS, GAO PH, WANG Y, JIA XM, JIANG YY. In vitro and in vivo antifungal activities of the eight steroid saponins from Tribulus terrestris L. with potent activity against fluconazole-resistant fungal. Biol Pharm Bull. 2005; 28: 2211-2215.
9. SANGEETA D, SIDHU H, THIND SK NATH R. Therapeutic response of Tribulus terrestris (Gokhru) aqueous extract on hyperoxaluria in male adult rats. Phytother Res.1993; 7: 116-119.
10. MARTINO-ANDRADE AJ, MORAIS RN, SPERCOSKI KM, ROSSI SC, VECHI MF, GOLIN M, LOMBARDI NF, GRECA CS, DALSENTER PR. Effects of Tribulus terrestris on endocrine sensitive organs in male and female Wistar rats. J Ethnopharmacol. 2009 in press. Epub ahead of print.
11. BOURKE CA, STEVENS GR , CARRIGAN MJ. Locomotor effects in sheep of alkaloids identified in Australian Tribulus terrestris. Aust Vet J. 1992; 69: 163-165.
12. JAMEEL JKA, KNEESHAW PJ, RAO VSR, DREW PJ. Gynaecomastia and the plant product “Tribulus terrestris”. Breast. 2004; 13: 428-430.
13. TIEJIE W, ZHOGBO L, JUN L, MIN Z, JUPENG L, XIAOHUI C, KAISHUN B. Determination of protodioscin in rat plasma by liquid chromatography-tandem mass spectrometry . J Chromatogr B 2007; 848: 363-368.
14. GANZERA M, BEDIR E, KHAN IA. Determination of steroidal saponins in Tribulus Terrestris by Reversed-phase High- Performance liquid Chromatography and Evaporative Light scattering detection. J Pharm Sci. 2001; 90: 1752-1758.

The cacti from the Trichocereus family

Trichocereus macrogonus

Name: *Trichocereus macrogonus*
Family: *Cactaceae*
Genus: *Trichocereus*
Species: *Trichocereus macrogonus*
Synonims: unknown
Origin: South America
Active compounds: mescaline, 3-methoxytyramine, 3,4-dimethoxyphenethylamine, tyramine

Trichocereus pachanoi
(San Pedro cactus)

Name: *Trichocereus pachanoi*
Family: *Cactaceae*
Genus: *Trichocereus*
Species: *Trichocereus pachanoi*
Synonims: *Echinopsis pachanoi*, San Pedro cactus
Origin: Perù, Ecuador
Active compounds: mescaline, 3-methoxytyramine

Trichocereus peruvianus
(Peruvian Torch Cactus)

Name: *Trichocereus peruvianus*
Family: *Cactaceae*
Genus: *Trichocereus*
Species: *Trichocereus peruvianus*
Synonims: Peruvian Torch
Origin: Perù, on the west side of the Andes, at an altitude of approximately 2000m.
Active compounds: mescaline, 3-methoxytyramine, 3,4-dimethoxyphenethylamine, tyramine

Trichocereus validus

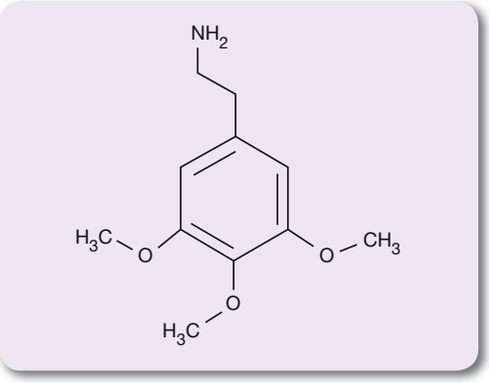
Name: *Trichocereus validus*
Family: *Cactaceae*
Genus: *Trichocereus*
Species: *Trichocereus validus*
Synonims: unknown
Origin: Bolivia
Active compounds: mescaline

Trichocereus werdermannianus

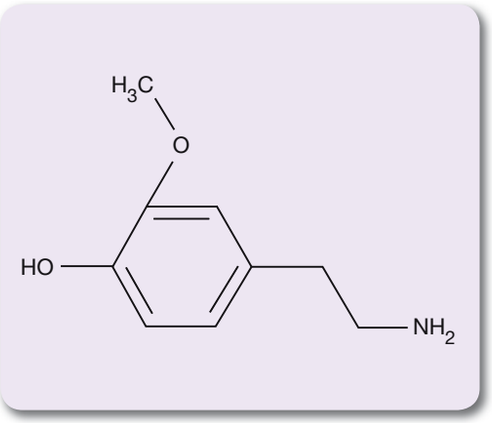
Name: *Trichocereus werdermannianus*
Family: *Cactaceae*
Genus: *Trichocereus*
Species: *Trichocereus werdermannianus*
Synonims: unknown
Origin: South America
Active compounds: mescaline, 3-methoxytyramine, 3,4-dimethoxyphenethylamine

There are about 40 different cactacee which belong to the *Trichocereus* plant genus. Mescaline is the plant alkaloid which produces the psychoactive effect. *Trichocereus macrogonus*, an other cactus belonging to the *Trichocereus* family, has a variable alkaloids content: 0.1 and 0.5 mg/g of fresh weight of the plant, with mescaline representing 50% of the entire alkaloid fraction that is, on average, 0.05% of fresh weight [(1)]. 3,4-dimethoxyphenethylamine, 3-methoxytiramine and tyramine represent 1-10% of the alkaloids fraction meaning, on the average, 0.0015% of the fresh weight of *Tricochereus macrogonus* [(1)]. Different publications report mescaline amount in *Trichocereus pachanoi* ranging from 0.1 to 2.3% dry weight [(2)], and similar percentages (0.8% plant dry weight) are referred for *Trichocereus peruvianus* [(3)]. Small amounts of 3-methoxytyramine (0.01%) have been found in *Trichocereus pachanoi* [(4)], while in *Trichocereus peruvianus* also traces of tyramine (0.0085%) and 3,4-dimethoxyphenethylamine have been detected. In *Trichocereus validus*, the only characterized active principle have been mescaline, with 0.25 mg/g plant fresh weight [(1)]. *Trichocereus werdermannianus* presents an alkaloids content ranging from 0.1 to 0.5 mg/g fresh weight, with mescaline being the 50% of the entire alkaloids content [(1)]. The 3,4-dimethoxyphenethylamine and 3 methoxytyramine have been found from 1 to 10% of the entire alkaloids content in *Tricochereus werdermannianus* [(1)].
According to some authors, based on the shape of the adult plant, it is possible to recognize the *Trichocereus* plants which contain great amounts of mescaline: generally those with the classic "candelabrum" shape contain high % mescaline, while those with columnar shape, do not [(1)].
Mescaline belongs to the family of compounds known as phenethylamines which are structurally different from other major psychedelic drugs (indolamines) such as LSD (diethylamide of lysergic acid), psilocybin, dimethyltryptamine (DMT), etc. [(5)].
Mescaline is abused orally. This active compound is generally extracted from the cactus plant and it is illegaly sold as powder, liquid or crystals [(6)].

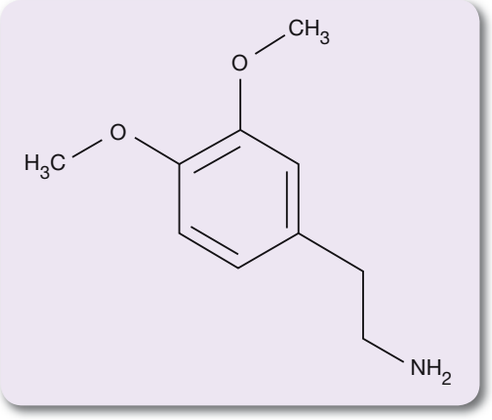
Chemical formula and physico-chemical properties of the active compounds



Name: Mescaline.
Molecular formula: $C_{11}H_{17}NO_3$ (molecular weight = 211.2).
Systematic name: 3,4,5-trimethoxyphenethylamine.
CAS registry number: 54-04-6.
Melting point: 35,5°C.
UVmax: no data in the literature.
Solubility: water, methyl alcohol.



Name: 3-methoxytyramine.
Molecular formula: $C_9H_{13}NO_2$ (molecular weight = 167.2).
Systematic name: 4-(2-aminoethyl)-2-methoxyphenol.
CAS registry number: 554-52-9.
Melting point: 213-215°C (as hydrochloride).
UVmax: no data in the literature.
Solubility: water.



Name: 3,4 dimethoxyphenylethylamine.
Molecular formula: $C_{10}H_{15}NO_2$ (molecular weight =181.2).
Systematic name: 3,4 dimethoxyphenylethylamine.
CAS registry number: 120-20-7.
Melting point: 12-15°C.
UVmax: no data in the literature.
Solubility: water.

Name: tyramine.
Molecular formula: $C_8H_{11}N_O$ (molecular weight = 137.1).
Systematic name: 4-(2-aminoethyl) phenol.
CAS registry number: 51-67-2.
Melting point: 165°C.
UVmax: no data in the literature.
Solubility: water.

Historical use

In South America there is an ancient cult tied to the big hallucinogenic cactus, the San Pedro (*Trichocereus pachanoi*) which grows in Perù and in Ecuador, particularly in the Andean regions. The archaeological data places the use of San Pedro to the preinca periods. The cults of peyote (*Lophophora williamsi*), the cactus most commonly known for its content of mescaline, and San Pedro (*Trichocereus pachanoi*) were developed in the geographic regions where they were growing naturally. So, while the Peyote is traditionally used by the shamans of Mexico, San Pedro is used by the shamans of the Andean regions. Even today the Andean curanderos use the cactus - cooked in a decoction called "*cimora*" in Ecuador - as a means of therapeutic or shamanic divination. In northern Peru the curanderos use the San Pedro during the therapeutic rituals (the mesadas).

There is no similar use reported for *Trichocereus macrogonus*, *peruvianus*, *validus* e *werdermannianus* by the native South American population.

Current use

At present time, the *Trichocereus* cacti are used primarily for recreational reasons by those who wish to try legal hallucinogens. They are sold on the Internet as the dry form or as seeds which, planted at home, will produce the adult plants.

Legislation

In the United States of America the San Pedro cactus and other cactuses of the *Trichocereus* genus containing mescaline are not subjected to restrictions, although mescaline is part of "Schedule I" of the Controlled Substances Act (CSA) . In Switzerland, mescaline is classified as an illicit drug of abuse. In Italy, together with its active component: mescaline, the most famous cactus "peyote" (*Lophophora williamsi*) is placed in Table 1 of in the list of narcotic or psychotropic substances subject to the supervision and control under Article 14 of Republic Presidential Decree 309/90 and subsequent updates. Conversely, the cacti of *Trichocereus* genus, are not placed in the above reported Table, hence it is possible to buy the plants as whole or parts of them.

Pharmaco-toxicological properties

The most important active principle of the hallucinogenic cacti belonging to the *Trichocereus* genus is mescaline.

Although its mechanism of action is not completely known, it seems that the hallucinogenic and behavioural effects are due to the stimulation of the serotoninergic and dopaminergic receptors at the central nervous system level [7].

Recently, it has been observed that mescaline (similar to other hallucinogens) behaves as partial agonist of the 5-HT_{2A} receptors for serotonine, which increases glutamate release at the level of the cerebral cortex which, in turn, seems to be responsible for the cognitive, perceptive and affective disorders caused by this compound [8].

At the dose of 350 mg, the first effects of the mescaline are observed at about 30 minutes after the ingestion such as nausea and vomiting, occasionaly accompanied by diarrhoea. During this phase, tachicardia, palpitations, increased blood pressure, breathing difficulties, mydriasis and obscured vision may occur. Subsequently, an hour post ingestion, the psychotomimetic effects, such as visual and audio hallucinations, anxiety, alterations of spatial, temporal, sensory, tactile perceptions become noticeable. Above all, visual alterations such as glittering, intensification of color perception, synesthesia, the ability of perceiving a stimulus using a different sensory pathway (for example, the ability of smelling the colors) and vision of images with wavy shapes may occur. At sensory level, the individual shows a strong empathy towards static objects or live organisms [9].

Suicidal tendencies, fear, violent behaviours and paranoia are rarely observed [10-13]. In some cases, flashback episodes have been reported [14].

Effects on the skin, such as flushing, diaphoresis and piloerection can be also noticed [13,14], Some of the effects of mescaline ingestion are muscular tremors, weakness, muscular hypertone and hyperreflexia [10-13].

The events and sensations that take place under the influence of mescaline are usually vividly remembered by the mescaline users.

In a study on healthy volunteers, it has been observed that the oral administration of a dose of 500 mg mescaline has produced about 3–4 hours later an acute psychotic state for about 12–15 hours [15]. The smallest dose of mescaline which produce a psychotic effect has been estimated at 200 mg for an average weight of 80 kg, given intramuscularly. A strong mescalinic intoxication is observed with approximately 3.75 mg/kg. In this case, the maximum toxic peak occurs within 2–4 hours post administration and it is resolved within the following 4–6 hours [16].

During the first two hours after administration, about 87% of the mescaline absorbed at intestinal level is excreted in the urine; of this about 55-60% is excreted unchanged while 27–30% becomes metabolized to 3,4,5-trimethoxyphenylacetic acid and 5% is transformed into N-acetil-(3,4-dimethoxy-5-hydroxy)-phenylethylamine [17].

Neurotoxic effects of 3,4-dimethoxyphenethylamine have been studied mainly in the nigrostriatal system in rats [18]. On the other hand, tyramine is a sympathomimetic amine with no effect on the organism when it is taken with food because it undergoes rapid biotransformation due to the intestinal and hepatic monoaminooxidases (MAO). Nevertheless, in therapies with MAO inhibitors, the foods containing more than 10 g tyramine (for example. seasoned cheese, fermented foods) can still provoke a strong hypertensive crisis [19].

Toxicity

Data regarding the acute toxicity of mescaline [20]

In human - TLD following intramuscular administration: 2.5 mg/kg
In mouse - LD50 following intravenus administration: 157 mg/kg
In mouse - LD50 following subcutaneous administration: 534 mg/kg
In mouse - LD50 following oral administration: 880 mg/kg
In rat - LD50 following intravenus administration: 157 mg/kg.
In rat - LD50 following subcutaneous administration: 534 mg/kg

Data regarding the acute toxicity of 3-methoxytyramine [21]

There are no data in the literature.

Data regarding the acute toxicity of 3,4-dimethoxyphenethylamine [21]

In mouse - LD50 following intravenus administration: 56 mg/kg
In mouse - LD50 following intraperitoneal administration: 181 mg/kg

Data regarding the acute toxicity of tyramine [21]

In mouse - LD50 following intravenus administration: 229 mg/kg
In mouse - LD50 following intraperitoneal administration: 800 mg/kg
In mouse - LD50 following subcutaneous administration: 225 mg/kg

Adverse Effects

The use of mescaline containing plants or preparation is rarely lethal. There are two clinical cases in the literature describing fatalities related to the use of mescaline. In the first case, the death has been caused by a trauma which occurred as a consequence of the drug induced delirium. The mescaline content in the blood and in the urine of patient was 9.7 µg/ml and 1163 µg/ml, respectively [22].

In the second case, in a 32-year-old man, the mescaline intoxication produced esophageal lacerations (Mallory-Weiss syndrome) with blood accumulation and marked pulmonary hemoaspiration (cause of the death). The mescaline plasma and urine concentration were 0.48 µg/ml and 61 µg/ml, respectively. The Mallory-Weiss syndrome has been determined probably by the profuse vomiting caused by mescaline [23].

The use of mescaline has been also associated with the induction of persistent psychoses, anxiety and depression [24].

Dependence and tolerance

Often mescaline can be used in association with other drugs; this can lead to dependence and tolerance [25]. Particularly, mescaline can induce crossed tolerance with other hallucinogens such as LSD and psilocybin [26]. In certain cases, the tolerance (defined as the reduction of the biological response to a constant dose of active component) tends to go down quickly, within a couple of days of stopping the drug.

Pharmacological interactions

Together with alcohol or methadone, mescaline can cause convulsions, coma, rabdomyolisis and renal insufficiency [27].

Physostygmine, administered together with mescaline increases the risk of mortality [27].

Lastly, the animal studies have demonstrated that, mescaline can increase the toxic effects induced by an overdose of insulin [28].

Effects in pregnancy

Mescaline can be considered a potential teratogen [29]. Given during the pregnancy, in rats it causes a greater incidence of spontaneous abortions; in addition, congenital malformations and reduced birth weight have been observed in the rat fetuses born to mothers treated with the alkaloid [30].

Analytical determinations

No methodologies have been reported in the international literature concerning the analysis of *Trichocereus* cacti active principles in biological fluids of eventual consumers, nor in any of the plant parts. Assays exist to detect mescaline, the principal alkaloid of *Trichocereus* cacti in biological fluids such as plasma [31] , urine [32] and cadaveric tissues [33]. Furthermore, the determination of mescaline in the Peyote cactus by liquid chromatography coupled to tandem mass spectrometry is reported [34].

The method below reported is a brief outline useful to the investigator to set up the analysis. For specific details, it is advisable to refer to the original text.

Determination of mescaline in human plasma

(From: HABRDOVA V, PETERS FT, THEOBALD DS, MAURER HH. Screening for and validated quantification of phenethylamine-type designer drugs and mescaline in human blood plasma by gas chromatography/mass spectrometry. J Mass Spectrom. 2005; 40: 785-795) [31].

The analysis is carried out on plasma samples from subjects taking controlled amounts of *Trichocereus pachanoi*, using gas chromatography coupled with mass spectrometry.

Extraction of the compounds

1 ml plasma is diluted with 2 ml of distilled water. After adding 0.1 ml of internal standard (1,0 µg/ml mescaline-d_9 in acetonitrile), the sample is vortexed and centrifuged for 3 minutes at 1000 g, then loaded on cartridges for solid phase extraction. The HCX cartridges are previously conditioned with 1 ml of methyl alcohol and 1 ml distilled water. After the sample is applied, the cartridges are washed with 1 ml distilled water, 1 ml of 0.01 M hydrochloric acid and 2 ml of methyl alcohol. The analytes are eluted with 1 ml methyl alcohol: ammonia (98:2 v/v) mixture. After organic layer is evaporated under a stream of nitrogen, derivatized with 20 µl of heptafluorobutyric anhydride and irradiated for 5 minutes at 450 W. After cooling, the derivatized extracts were briefly mixed (15 s) with 0.1 mL n-hexane, and the mixtures are centrifuged for 15 s at 10 000 g to avoid spilling when opening the reaction vials. Then, 0.2 mL an aqueous 0.5 M Na_3PO_4 solution was added and the vials were shaken on a rotary shaker for 3 min and centrifuged for 2 min at 10000 g. The organic layers were transferred to autosampler vials and 1 µl was injected into the gas chromatograph.

Analytical conditions

Chromatographic column: 5MS (0,25 mm x 30 m x 0,25 µm)
Injector temperature: 280°C
Carrier Gas: helium at 11.60 psi
Injection mode: splitless
Temperature program: 80°C for 30 seconds, followed by 80°C to 310°C at 30°C/min, and maintained at this temperature for 2 minutes
Detector: mass spectrometer with electronic impact interface

Retention times of the tested compounds

Mescaline: 6.40 minutes
Mescaline-d_9 (internal standard): 6.30 minutes

Frammenti caratteristici delle sostanze ricercate

Mescaline: m/z 193, 206, 419
Mescaline d_9 (internal standard): m/z 181, 194, 407

Standards

Pure mescaline and mescaline-d_9 used as internal standard, were obtained from Promochem. Since mescaline is included in the Table 1 of in the list of drugs and psychotropic substances subject to the supervision and control under Article 14 of Republic Presidential Decree 309/90 and subsequent updates, a special authorization for its purchase is needed.

Calibration curve

For the calibration curve points, to 1ml of blank plasma, stock standard solutions aliquots were added to obtain concentrations between 5-500 µg/l.

The quality control samples at 10 µg/L (low control), 250 µg/l (medium control) and 450 µg/l (high control) were prepared the adding a known amounts of stock standard to solution to blank plasma. These quality control samples were included into each analytical batch to check the calibration, precision, accuracy of the validated method and the stability of the stored samples.

Results

A dose of 500 mg mescaline, orally administered, produced a plasma concentration of 3.8 µg/ml and 1.5 µg/ml two and sever hours after the ingestion, respectively [30].

References

1. AGURELL S. Cactaceae alkaloids. I. Lloydia. 1969; 32: 206.
2. HELMLIN H, BRENNEISEN R. Determination of psychotropic phenylalkylamine derivatives in biological matrices by high-performance liquid chromatography with photodiode-array detection. J Chromatogr. 1992; 593: 87-94.
3. PARDANANI JH, McLAUGHLIN JL, KONDRAT RW, COOKS RG. Cactus alkaloids.XXXVI. Mescaline and related compounds from Trichocereus peruvianus. Lloydia. 1977; 40: 585-90.
4. CROSBY DM, McLAUGHLIN JL. Cactus alkaloids.XIX. Crystallization of mescaline HCl and 3-methoxytyramine HCl from Trichocereus pachanoi. Lloydia. 1973; 36: 416-418.
5. KOVAR KA. Chemistry and pharmacology of hallucinogens, entactogens and stimulants. Pharmacopsychiatry. 1998; 31: 69-72.
6. http://www.ildiogene.it/EncyPages/Ency=mescalina.html
7. TRULSON ME, CRISP T, HENDERSON LJ. Mescaline elicits behavioral effects in cats by an action at both serotonin and dopamine receptors. Eur J Pharmacol. 1983; 96: 151-154.
8. AGHAJANIAN GK, MAREK GJ. Serotonin and hallucinogens. Neuropharmacol. 1999; 21: 16S-23S.
9. SHULGIN AT. Mescaline: the chemistry and pharmacology of its analogs. Lloydia. 1973; 36: 46-58.
10. KAPADIA GJ, FAYEZ BN. Peyote constituents: chemistry, biogenesis, and biological effects. J Pharm Sci. 1970; 59: 1699-1727.
11. LUDWIG AM, LEVINE J. Patterns of hallucinogenic drug abuse. JAMA. 1965; 191: 104-108.
12. JACOBSEN E. The clinical pharmacology of hallucinogens. Clin Pharmacol Ther. 1963; 4: 480-503.
13. HOLLISTER LE, HARTMAN AM. Mescaline, lysergic acid diethylamide and psilocybin: comparison of clinical syndromes, effects on color perception, and biochemical measures. Compr Psychiatry. 1962; 3: 235-241.
14. TEITELBAUM DT, WINGELETH DC. Diagnosis and management of recreational mescaline self-poisoning. J Anal Toxicol. 1977; 1: 36-37.
15. FEHRENBACH RA, SPITZER M. Mescaline-induced psychopathological, neuropsychological, and neurometabolic effects in normal subjects: experimental psychosis as a tool for psychiatric research. Biol Psychiatry. 1992; 32: 976-991.
16. HALPERN JH. Hallucinogens and dissociative agents naturally growing in the United State. Pharmacol Ther. 2004; 102: 131-138.
17. DEMISCH L, KACZMARCZYK P, SEILER N. 3,4,5-Trimethoxybenzoic acid, a new mescaline metabolite in humans. Drug Metab Dispos. 1978; 6: 507-509.
18. KOSHIMURA I, IMAI H, HIDANO T, ENDO K, MOCHIZUKI H, KONDO T, MIZUNO Y. Dimethoxyphenylethylamine and tetrahydropapaverine are toxic to the nigrostriatal system. Brain Res. 1997; 773: 108-116.
19. HELLENHORN MJ, BARCELOUX DG. Medical toxicology - Diagnosis and treatment of human poisoning. New York: Elsevier Science Publishing Co., Inc. 1988.
20. http//www.chem.sis.nlm.nih.gov/chemidplus/jsp/common/ChemFull.jsp?calledFrom=lite
21. http//www.toxnet.nlm.nih.gov
22. REYNOLDS PC & JINDRICH EJ. A mescaline associated fatality. J Anal Toxicol. 1985; 9: 183-184.
23. NOLTE KB, ZUMWALT RE. Fatal peyote ingestion associated with Mallory-Weiss lacerations. West J Med. 1999; 170: 328.
24. KLEBER HD. Prolonged adverse reactions from unsupervised use of hallucinogenic drugs. J Nerv Ment Dis. 1967; 144: 308-319.
25. SCHWARTZ RH. Mescaline: a survey. Am Fam Physician. 1988; 37: 122-124.

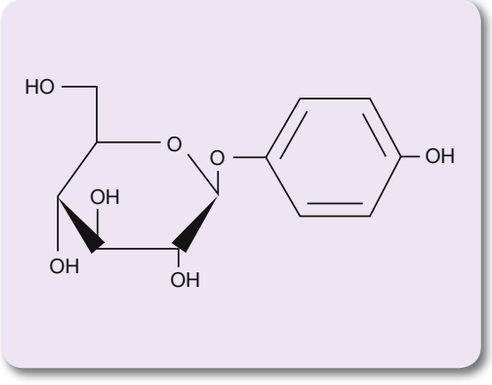
26. MARTIN WR, SLOAN JW. Pharmacology and classification of LSD-like hallucinogens. In Martin WR (ed) Drug Addiction II: Amphetamine, Psychotogen, and Marihuana Dependence, Handbuch der Experimentellen Pharmacologie, 42 Pt 2, Springer-Verlag, Berlin, Germany, 1977: 368.
27. JELLIN JM, GREGORY P, BATZ F. Pharmacist's Letter/Prescribers's Letter Natural Medicines Comprehensive Database. 3rd edition, Therapeutic Research Faculty, Stockton, CA, 2000: 825.
28. SPECK LB. Toxicity and effects of increasing doses of mescaline. J Pharmacol Exp Ther. 1957; 119: 78-84.
29. GILMORE HT. Peyote use during pregnancy. South Dakota J Med. 2001; 54: 27-29.
30. GEBER WF. Congenital malformations induced by mescaline, lysergic acid diethylamide, and bromolysergic acid in the hamster. Science. 1967; 158: 265-267.
31. HABRDOVA V, PETERS FT, THEOBALD DS, MAURER HH. Screening for and validated quantification of henethylamine-type designer drugs and mescaline in human blood plasma by gas chromatography/mass spectrometry. J Mass Spectrom. 2005; 40: 785-795.
32. BJORNSTAD K, HELANDER A, BECK O. Development and Clinical Application of an LC-MS-MS Method for Mescaline in Urine. J Anal Toxicol 2008; 32: 227-231.
33. HENRY JL, EPLEY J, ROHRIG TP. The analysis and distribution of mescaline in post-mortem tissues. J Anal. Toxicol. 2003; 27: 381.
34. CASADO R, URIARTE I, CAVERO RY, CALVO MI. LC_PAD Determination of Mescaline in Cactus "Peyote" (Lophophora williamsii). Chromatographia 2008; 67: 665-667.

Turnera aphrodisiaca
(Damiana)

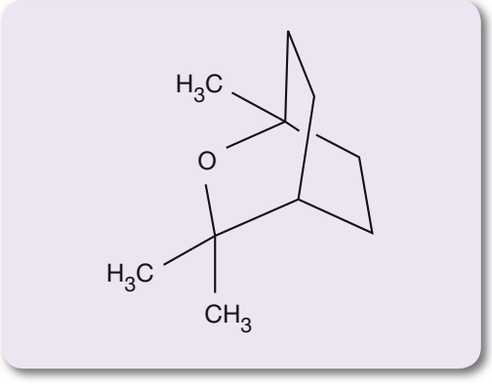
Name: *Turnera aphrodisiaca*
Family: *Turneraceae*
Genus: *Turnera*
Species: *Turnera diffusa var. Aphrodisiaca*
Synonims: Damiana, Mexican Damiana, Herba de la Pastora
Origin: Northern and Central America
Active compounds: arbutin, 1,8-cineole, α-pinene, β-pinene, p-cimene, gonzalitosin I, damianine, tetraphylline B, β-sitosterol, apigenin.

The family *Turnera* includes about 85 kinds of typical plants of the tropical and subtropical regions of America, Africa and Madagascar [1,2]. In the past the plant was known simply as "*Damiana*"; subsequently, different kinds and varieties have been individualised, therefore at present it is preferable to call Damiana as *Turnera diffusa var. Aphrodisiaca* [3]. The chemical composition is complex and not all the compounds have been identified. It is composed of 0.5-1 % of essential oil, 50 % of monoterpenes (1,8-cineole, α-pinene, β-pinene, p-cimene) and 50 % of sesquiterpenes [4,5], flavonoids (gonzalitosin I, apigenin) [6,7], cyanoglycosides (tetraphylline B) [8], arbutin [4], a bitter substance called damianine [9] of still not determinate structure, β-sitosterol [6].

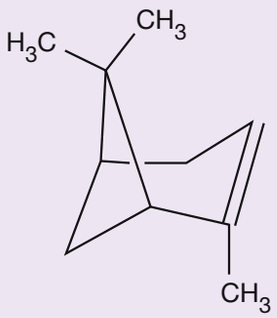
Chemical formula and physico-chemical properties of the active compounds [10,11]



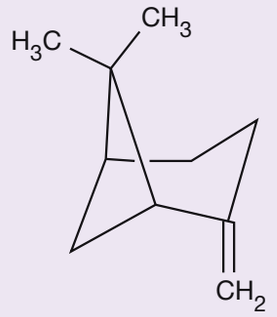
Name: arbutin.
Molecular formula: $C_{12}H_{16}O_7$ (molecular weight = 272.3).
Systematic name: 4-hydroxyphenyl-β-D-glucopyranoside.
CAS registry number: 497-76-7.
Melting point: 199, 5°C.
UVmax: 220, 283 nm.
Solubility: water and alcohol.



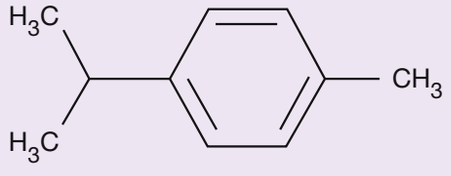
Name: 1,8-cineole or eucaliptol.
Molecular formula: $C_{10}H_{18}O$ (molecular weight = 154.2).
Systematic name: 1,3,3-trimethyl-2-oxabicyclo(2.2.2)octane.
CAS registry number: 470-82-6.
Melting point: 1,5°C.
UVmax: no data in the literature.
Solubility: alcohol, chloroform, ether, glacial acetic acid, oil.



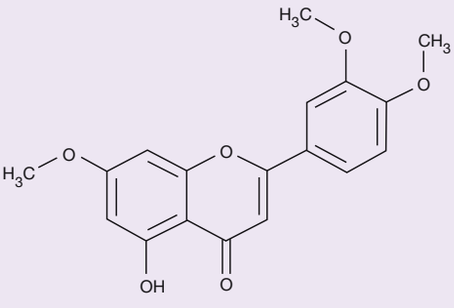
Name: α-pinene.
Molecular formula: $C_{10}H_{16}$ (molecular weight = 136.2).
Systematic name: 2,6,6-trimethylbicyclo(3.1.1)hept-2-ene.
CAS registry number: 80-56-8.
Melting point: -64°C (pure), 132°C (as hydrochloride).
UVmax: no data in the literature.
Solubility: alcohol, chloroform, ether, glacial acetic acid.



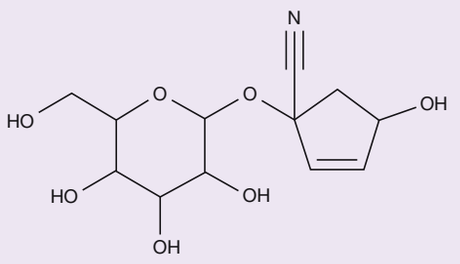
Name: β-pinene.
Molecular formula: $C_{10}H_{16}$ (molecular weight = 136.2).
Systematic name: 6,6-dimethyl-2-methylenebicyclo(3.1.1)heptane.
CAS registry number: 127-91-3.
Melting point: -61,5°C.
UVmax: no data in the literature.
Solubility: alcohol, chloroform, ether, glacial acetic acid.



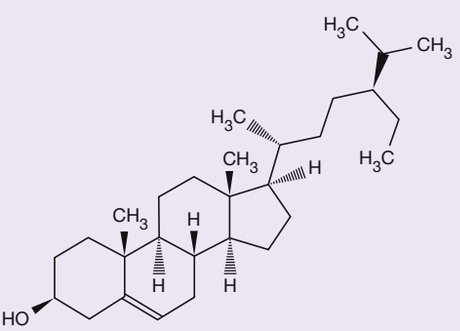
Name: p-cimene.
Molecular formula: $C_{10}H_{14}$ (molecular weight = 134.2).
Systematic name: 1-methyl-4-(1-methylethyl)-benzene.
CAS registry number: 99-87-6.
Melting point: -67,9°C.
UVmax: no data in the literature.
Solubility: practically insoluble in water. Miscible with alcohol, chloroform.



Nome: gonzalitosin I.
Molecular formula: $C_{18}H_{16}O_6$ (molecular weight = 328.3).
Systematic name: 5-hydroxy-7,3',4'-trimethoxyflavone.
CAS registry number: no data in the literature.
Melting point: no data in the literature.
UVmax: 213, 246, 270, 288, 333 nm (in ethyl alcohol).
Solubility: no data in the literature.



Name: tetraphylline B.
Molecular formula: $C_{12}H_{17}NO_7$ (molecular weight = 287.3).
Systematic name: 1-(β-D-glucopyranosyl-4-hydroxy-(1S-trans)-2-cyclopentene-1-carbonitrile.
CAS registry number: 34323-07-4.
Melting point: no data in the literature.
UVmax: no data in the literature.
Solubility: no data in the literature.



Name: β-sitosterol.
Molecular formula: $C_{29}H_{50}O$ (molecular weight = 414.7).
Systematic name: 3-β-stigmast-5-en-3-ol.
CAS registry number: 83-46-5.
Melting point: 140°C.
UVmax: no data in the literature.
Solubility: no data in the literature.

Name: apigenin.
Molecular formula: $C_{15}H_{10}O_5$ (molecular weight = 270.2).
Systematic name: 5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopiran-4-one.
CAS registry number: 520-36-5.
Melting point: 345-350°C.
UVmax: 269, 340 nm.
Solubility: Slightly soluble in warm alcohol. Soluble in dilute potassium hydroxide solutions.

Historical use

Turnera aphrodisiaca was already used by the ancient Mayan civilisation for the treatment of "dizziness and loss of balance" and as an aphrodisiac [12]. Traditionally, *Turnera aphrodisiaca* was the principal component of a prepared drink, advertised and sold as "Mexican tea", commonly used by the Mexican Indians for its presumed aphrodisiacal properties [13]. The Damiana based products advertised as having "energizing properties" and as "powerful aphrodisiacs", have been introduced into the American market for the first time in 1874 [14]. Since 1888, *Turnera aphrodisiaca* has been part of the National Handbook of the United States of America [15] and it has been approved by the Food and Drug Administration (FDA) as a food additive [1]. The activity of the extract is described in the British Pharmaceutical Codex of 1934 and in the British herbal pharmacopoeia (BHP) [16].

Turnera aphrodisiaca reached certain notoriety in the treatment of sexual impotence when used in combination with

strychnine, phosphorus or other presumed sexual stimulants [17]. The infusion of *Turnera aphrodisiaca* leaves has been used for the treatment of gastrointestinal and respiratory problems [18], reproductive issues [19] and for the treatment of gonorrhea [20].

Current use

At the present time, the products based on *Turnera aphrodisiaca* are available in the herbal shops, very often in combination with other plants such as Ginkgo, Ginseng and Saw palmetto (*Serenoa repens*) [15].

The *Turnera aphrodisiaca* mother tincture (ethyl alcoholic extract) is used as a homeopathic medicine for the treatment of decreased libido and as an antistress remedy [21].

The plant is part of the oral formulations of food additives used to improve lactation. In addition, it is included in different plant-based formulations used in the treatment of menopause symptoms, sexual dysfunctions and impotence [1].

Legislation

All the parts of *Turnera aphrodisiaca* and its extracts can be cultivated, purchased, possessed and distributed without any particular licence in the USA [22].

In Italy, neither the active principles nor the whole plant or parts of the plant are included in the tables containing narcotic or psychotropic substances subject to the supervision and control under Article 14 of Republic Presidential Decree 309/90 and subsequent updates. The Italian Ministry of Health included the flowers, the leaves and the tops of *Turnera aphrodisiaca* to the list of vegetable extracts admitted in dietary supplements. The products contained in the leaves are used as tonic, for mental and physical stimulation, for regulating the intestinal passage and digestive function, the urinary passage and liquid drainage from the body [23].

Pharmaco-toxicological properties

A large part of the *Turnera aphrodisiaca* uses can be attributed to its phytocomplex properties. The aqueous extract of the whole plant has a major hypoglycemic effect in the diabetic male mice [24]. The hypoglycemic activity has been shown in the decoction obtained from the leaves of *Turnera aphrodisiaca* which was administered orally to rabbits [25]. In addition, the aqueous extract stimulates the sexual activity in lazy male rats at a dose of 1 ml/kg, while it turns out to be inactive in the sexually active rats [26]. The extract acts mostly by increasing the central noradrinergic and dopaminergic effect and indirectly by increasing the oxytocinergic transmission.

According to the literature, from all the extracts of *Turnera aphrodisiaca* (petroleum ether, chloroform, methyl alcohol and water extracts), only the methyl alcohol extract (25 mg/kg, administered orally after alcohol evaporation and resuspension in saline) shows a significant anti-anxiety activity in the animal model [27]. In a further study, the anti-anxiety activity of apigenin at a dose of 2 mg/kg was highlighted [28]. In addition, the authors show that, at a higher dose (about 12 times the anxiolitic dose), the apigenin presents a mildly sedative effect absent at doses of 2.5 and 10 mg/kg. At these doses an analgesic dose-dependent effect has been demonstrated comparable with the effect of 5 mg/kg morphine sulphate. The maximum effect has been observed 30 minutes after the administration of 10 mg/kg of apigenin.

Even though traditionally *Turnera aphrodisiaca* has been used as an aphrodisiac, there are no pharmacological and clinical studies to confirm this presumed effect. In a study performed with different extracts (petroleum ether, chloroform, methyl alcohol and water extracts), in order to evaluate the aphrodisiacal effect in mice, the chloroform extract showed a remarkable effect, following oral administration at a dose of 200 mg/kg while, the methyl alcoholic extract has shown an aphrodisiac effect at a lower dose (50 mg/kg) [29]. Regarding the volatile *Turnera aphrodisiaca* oil, no aphrodisiac effect has been found.

In a study published in 2001, 34 women have been treated with a dietary supplement containing ginseng, ginkgo, and *Turnera aphrodisiaca* extracts as well as *l*-arginine, vitamins and mineral salts. After 4 weeks, 73.5 % of the treated women reported an improvement of the total sexual life, compared to 37.2 % of the placebo group. This result obtained with a product containing more active compounds, while interesting, does not allow the confirmation of the aphrodisiac properties of *Turnera aphrodisiaca* alone [30].

Toxicity

Turnera aphrodisiaca is comparatively safe, although possible toxicity can not be excluded given by the presence of cyanogenic compounds [1]. There is a case of a man who, after having consumed about 230 grams of a *Turnera aphrodisiaca* extract, presented with seizures and paroxysm similar to the symptoms of strychnine poisoning [31].

Data regarding the acute toxicity of 1,8-cineole [10]
In dog - LDLo following subcutaneous administration: 1500 mg/kg
In mouse - LD50 following subcutaneous administration: 1070 mg/kg
In mouse - LD50 following intramuscular administration: 1000 mg/kg
In guinea pig - LDLo following intramuscular administration: 2250 mg/kg
In rat - LD50 following oral administration: 2480 mg/kg

Data regarding the acute toxicity of α-pinene [10]
In guinea pig - LDLo following inhalation: 0.572 mg/m^3
In rat - LDLo following inhalation: 0.625 mg/m^3
In rat - LD50 following oral administration: 3700 mg/kg
In mouse - LD50 following intraperitoneal administration: > 500 mg/kg

Data related to the acute toxicity of β-pinene [10]
In rat - LD50 following oral administration: 4700 mg/kg

Data related to the acute toxicity of p-cimene [10]
In rat - LD50 following oral administration: 4750 mg/kg

Data related to the acute toxicity of β-sitosterol [10]
In mouse – LD50 following oral administration: > 25000 mg/kg

There is no data in the literature regarding the acute toicity of arbutin, gonzalitosin I, tetraphylline B and apigenin.

Adverse Effects

There are no reports of any toxicity or significant collateral effects and there are no specific contraindications for the plant use. Excessive doses of *Turnera aphrodisiaca* (more than 200 grams) can cause insomnia and headache and have a mild laxative effect [32,33].

Pharmacological interactions

Because of its hypoglycemic effect, the plant should be used with caution in conjunction with medicines causing hypoglycemia [32]. The Damiana in combination with Yerba Mate and Guaranà can be used for weight loss [34].

Effects in pregnancy

The alcoholic extracts of the *Turnera aphrodisiaca* root have shown oxitocin activity, therefore the use is not recommended in pregnancy [35].

Analytical determinations

No methodologies have been reported in the international literature concerning the analysis of *Turnera aphrodisiaca* active principles in biological fluids of eventual consumers. However, analytical methods have been published regarding the determination of these active principles on the aerial parts [36,37] and on the leaves, stem, flowers and fruits of the plant and also in commercial products containing the plant [37]. This latter method applied to quantify apigenin uses high performance thin layer chromatography.

The method below reported is a brief outline useful to the investigator to set up the analysis. For specific details, it is advisable to refer to the original text.

Determination of 1,8 cineole in the essential oil of *Turnera diffusa*

(From: GODOI AF, VILEGAS W, GODOI RH, VAN VAECK L, VAN GRIEKEN R. Application of low-pressure gas chromatography-ion-trap mass spectrometry to the analysis of the essential oil of *Turnera diffusa* (Ward.) Urb. J. Chromatogr A. 2004; 1027: 127-130) [36].

The analysis was performed on the aerial parts of *Turnera diffusa* using gas chromatography coupled with mass spectrometry.

Extraction of the compound

500 g of dried material (from the aerial parts of *Turnera diffusa*) is distilled with water for 4 hours. Subsequently, about 1 ml of the oil is separated from the aqueous phase by extraction with ethyl ether. The organic phase is evaporated and the residue is dissolved in hexane.

Analytical conditions

Chromatografic column : CP Wax 52 (0.53 mm x 10 m x 1 µm)
Injector temperature: 270 °C
Gas: helium at a rate of 1.5 ml/minute
Injection mode: splitless
Temperature program: 80 °C-230 °C a 60 °C/minute. The final temperature is maintained for 0.87 minutes
Detector: mass spectrometer with ion trap interface

Retention time of the tested compound

1,8-cineole: 0.63 minutes

Characteristic fragments of the tested compound

1,8-cineole: m/z 154, 139, 108, 93, 81, 43

Standards

The origin of the standards was not specified.

Calibration curve

The methodology for the preparation of the standard curve was not described.

Results

Since the presented methodology includes just a qualitative analysis, the concentration of the analytes was not determined.

References

1. KUMAR S, TANEJA R, SHARMA A. The genus *Turnera*: A review update. Pharm Biol. 2005; 43: 383-391.
2. GODOI AF, VILEGAS W, GODOI RH, VAN VAECK L, VAN GRIEKEN R. Application of low-pressure gas chromatography-ion-trap mass spectrometry to the analysis of the essential oil of *Turnera diffusa* (Ward.) Urb. J Chromatogr A. 2004; 1027: 127-130.
3. JACKSON BD. Index kewenisis, an enumeration of the genera and species of flowering plants. Oxford, Clarendon Press, 1946; 2: 1137-1138.
4. AUTERHOFF H, HÄUFEL HP. Contents of Damiana drugs. Arch Pharm Ber Dtsch Pharm Ges. 1968; 301: 537-544.
5. AUTERHOFF H, MOMBERGER H. Constituents of the volatile oil from damianae leaves. Arch Pharm. 1972; 305: 455-462.
6. DOMÍNGUEZ XA, HINOJOSA M. Mexican medicinal plants. XXVIII. Isolation of 5-hydroxy-7,3',4'-trimethoxyflavone from Turnera diffusa. Planta Med. 1976; 30: 68-71.

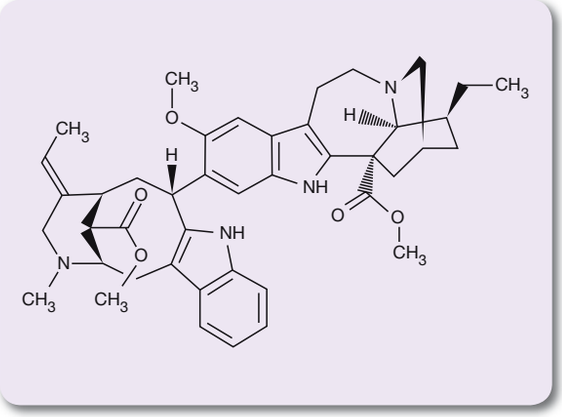
7. KUMAR S, SHARMA A. Apigenin: the anxiolytic constituent of *Turnera aphrodisiaca* Ward. Pharm biol. 2006; 44: 84-90.
8. SPENSER KC, SEIGLER DS. Tetraphyllin B from Turnera diffusa. Planta med. 1981; 43: 175-178.
9. STEINMTZ EF. Damianae folia. Acta Phytotherapeutic. 1960; 7: 1-2.
10. http://toxnet.nlm.nih.gov
11. THE MERCK INDEX An Enciclopedia of chemicals, drugs, and biologicals. 16th Ed. Merck & Co., Inc. 2006.
12. MARTINEZ M. Las Plantas Medicinales de Mexico; Ediciones Botas: Mexico, 1944; pp 116-119.
13. LOWRY TP. Damiana. J Psychoact Drugs 1984, 16, 267-268.
14. TYLER VE. Damiana - history of a herbal hoax. Pharm Hist. 1983, 25, 55-60.
15. ZHAO J, PAWAR RS, ALI Z, KHAN IA. Phytochemical Investigation of *Turnera diffusa*. J. Nat. Prod. 2007; 70: 289-292.
16. BRITHIS HERBAL PHARMACOPOEIA. West Yorks, Brithis Herbal Medicine Association1983, p. 29
17. OSOL A, FARRAR GF, LEUALLEN EE, YOUNGKEN HW, DETWEILER DK. Dispensatory of United States of America (24th edition). Philadelphia, J. B. Lippincott Company, 1947: pp. 1422-1423.
18. CACERES A. Turnera aphrodisiaca. In: Giron L, Caceres A, eds., Plantas de Uso Medicinal en Guatemala. Editorial Universataria San Carlos de Guatemala, 1996: pp. 160-162.
19. SAGGESE D. Medicinal Herbs of Argentina (10th edition). Rosario, Argentina, Antoghazzi & Co.,1959: pp. 1-189.
20. KOCH L. Drug collection from Bolivia systematically, anatomically and chemically examined. Arch Pharmacol. 1936; 274: 343-369.
21. BOERICKE W. Pocket Manual of Homoeopathic Materia Medica. New Delhi, India, B. Jain Publisher Private Limited, 1988; p. 659.
22. LEUNG AY, FOSTER S. Encyclopedia of Common Natural Ingredients Used in Food, Drugs and Cosmetics, 2nd ed., (New York: John Wiley & Sons, Inc. 1996) 204.
23. The list of vegetal extracts admitted in dietary supplements in Italy can be found at http://www.salute.gov.it
24. PEREZ RM, OCEGUEDA A, MUNOZ JL, AVITA JG, MORROW WW. A study of the hypoglycaemic effect of some Mexican plants. J Ethnopharmacol. 1984; 12: 253-262.
25. AGUILARA FJA, RAMOS RR, GUTIRREZ SP, CONTRETRAS AA, WEBER CCC, SAENZ JLF. Study of the anti-hyperglycaemic effect of plants used as antidiabetics. J Ethnopharmacol. 1998; 61: 101-110.
26. ARLETTI R, BENELLI A, CAVAZZUTI E, SCARPETTA G, BERTOLINI A. Stimulating property of *Turnera diffusa* and *Pfaffia paniculata* extracts on the sexual behavior of male rats. Psychopharmacol. 1999; 143: 15-19.
27. KUMAR S, SHARMA A. Anti-anxiety activity studies of various extracts of Turnera aphrodisiaca Ward. J Herb Pharmacother. 2005; 5: 13-21.
28. KUMAR S, MADAAN R, SHARMA A. Pharmacological evaluation of bioactive principle of Turnera aphrodisiaca. Indian J Pharm Sci. 2008; 70: 740-744.
29. KUMAR S, MADAAN R. SHARMA A. Evaluation of Aphrodisiac Activity of *Turnera aphrodisiaca*. IJPR. 2009; 1: 1-4.
30. ITO TY, TRANT AS, POLAN ML. A double-blind placebo-controlled study of ArginMax, a nutritional supplement for enhancement of female sexual function. J Sex Marital Ther 2001: 27: 541-549.
31. MARTINEZ M. Las plantas medicinales de Mexico. Edizione Botas, 1969: pp. 119-122.
32. HUI H, TANG G, GO VL. Hypoglycemic herbs and their action mechanisms. Chin Med. 2009; 4: 11.
33. SPIGNOLI G, MERCATI V, BONCOMPAGNI E. Guida bibliografica ai più noti fitoterapici. ABOCA edizioni, 1999, pp. 87-88.
34. ANDERSEN T, FOGH J. Weight loss and delayed gastric emptying following a South American herbal preparation in overweight patients. J Hum Nutr and Diet. 2001; 14: 243-250.
35. VIEIRA JEV, MATOS FJA, BARROS GSG, SOUZA MP, MEDEIROS M. pharmacological study of plants from north-eastern Brazil. Rev brasil Farm. 1968; 49: 67-75.
36. GODOI AF, VILEGAS W, GODOI RH, VAN VAECK L, VAN GRIEKEN R. Application of low-pressure gas chromatography-ion-trap mass spectrometry to the analysis of the essential oil of Turnera diffusa (Ward.) Urb. J Chromatogr A. 2004; 1027: 127-130.
37. KUMAR S, MADAAN R, SHARMA A. Estimation of apigenin, an anxiolytic constituent, in *Turnera aphrodisiaca*. Indian J Pharm Sci 2008; 70: 847-851.

Voacanga africana

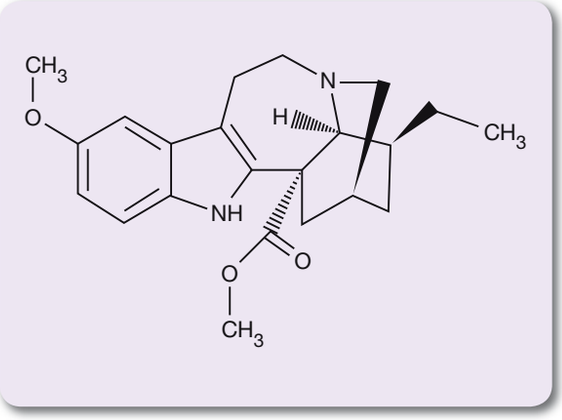
Name: *Voacanga africana*
Family: *Apocynaceae*
Genus: *Voacanga (Corynanthe)*
Species: *Voacanga africana* Staff.
Synonims: unknown
Origin: West Africa, Congo, Tanzania
Active compounds: voacamine (7.2%), voacangine (5.6%), voacristine (4.0%), voacorine (3.7%), vobtusine (0.4%), tabersonine (3.5%), ibogaine (0.4%), vobasine (1.6%).

There are different plants which belong to the *Voacanga* genus originally from Africa or Asia with a rather heterogenous alkaloid content. The mixture of alkaloids is also different in the different parts of the plant (roots, trunk, leaves, seeds). The alkaloids contained in this species are present at 5-10% in the bark of the root, 4-5% in the bark of the trunk, 0.3-0.45% in the leaves and 1.5% in the seeds [1]. Among the alkaloids contained in small quantities in *Voacanga africana*, it is worth mentioning the ibogaine, used clinically for the treatment of drug addiction [2]. The alkaloids voacamine, voacangine and ibogaine are also contained in the plant *Peschiera fuschiaefolia* of the *Apocynaceae* family.

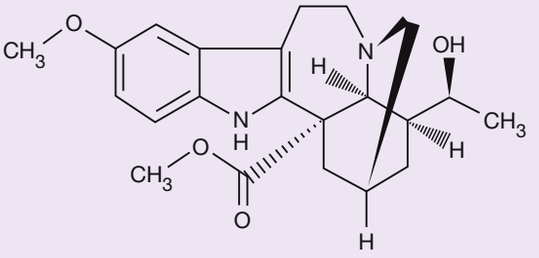
Chemical formula and physico-chemical properties of the active compounds



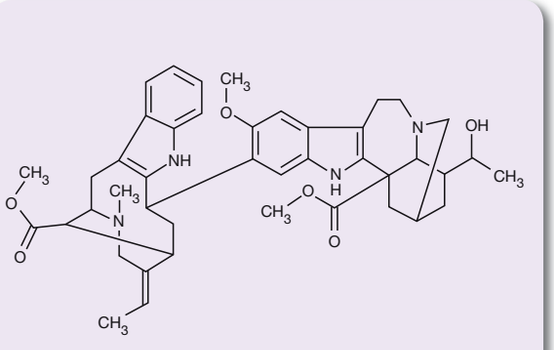
Name: Voacamine.
Molecular formula: $C_{43}H_{52}N_4O_5$ (molecular weight = 704.8).
Systematic name: 12-methoxy-13-((3-α)-17-methoxy-17-oxovobasan-3-il)-ibogamine-18-carboxylmethylester.
CAS registry number: 3371-85-5.
Melting point: 234°C.
UVmax: 225, 295 nm.
Solubility: chloroform and acetone and slightly soluble in methyl alcohol and ethyl alcohol.



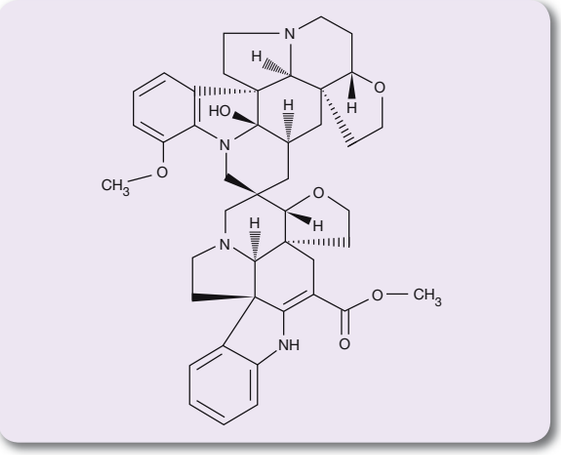
Name: Voacangine.
Molecular formula: $C_{22}H_{28}N_2O_3$ (molecular weight = 368.4).
Systematic name: 12-methoxyibogamine-18-carboxyl-methylester.
CAS registry number: 510-22-5.
Melting point: the compound sublimates without melting at 136-137°C.
UVmax: 225, 287, 300 nm.
Solubility: chloroform and acetone and slightly soluble in methyl alcohol and ethyl alcohol.



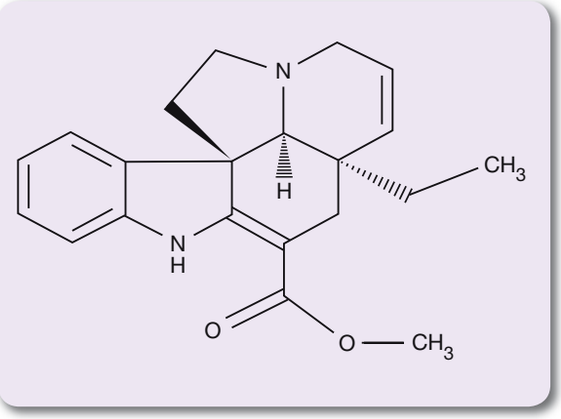
Name: Voacristine.
Molecular formula: $C_{22}H_{28}N_2O_4$ (molecular weight = 384.4).
Systematic name: 20-hydroxy-12-methoxyibogaine-18-carboxylmethylester.
CAS registry number: 545-84-6.
Melting point: 106°C.
UVmax: no data in the literature.
Solubility: no data in the literature.



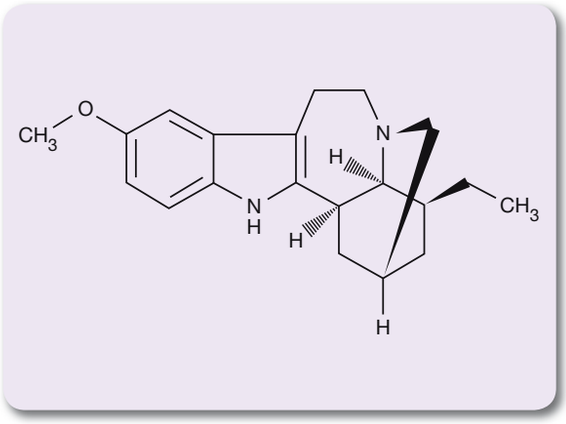
Name: Voacorine.
Molecular formula: $C_{43}H_{52}N_4O_6$ (molecular weight = 720.8).
Systematic name: (20S) 20-hydroxy-12-methoxy-13-((3-alpha)-17-methoxy-17-oxovobasan-3-il)-ibogamine-18-carboxylmethylester.
CAS registry number: 5130-80-3.
Melting point: 238°C.
UVmax: no data in the literature.
Solubility: methyl alcohol.



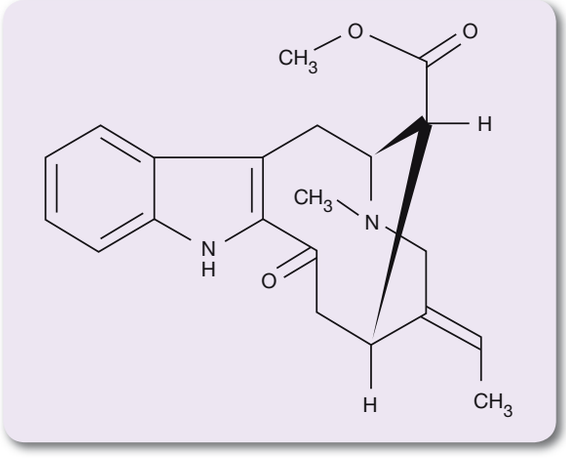
Name: Vobtusine.
Molecular formula: $C_{43}H_{50}N_4O_6$ (molecular weight = 718.3).
Systematic name: 2',2'a,4,4',5,5',6,6'a,8,13,14,14'c,15',16'-tetradecahydro-6'hydroxy-11-methoxy spiro[1H,15aH-furo[2',3':7,8]indolizin[8,1-cd]carbazole2(2aH),8'(9'H)1H,6H,7H,17aH]-furo[2',3':7,8]indolizin[8,1-cd]pirido[1,2,3-lm]carbazole]-7-carboxylmethylester.
CAS registry number: 19772-79-3.
Melting point: 300°C.
UVmax: (ethyl alcohol): 225, 265, 328 nm.
Solubility: chloroform.



Name: Tabersonine.
Molecular formula: $C_{21}H_{24}N_2O_2$ (molecular weight = 336.4).
Systematic name: (5α, 12β, 19α)-aspidospermidine 3-carboxylmethylester.
CAS registry number: 4429-63-4.
Melting point: no data in the literature.
UVmax: no data in the literature.
Solubility: no data in the literature.



Name: Ibogaine.
Molecular formula: $C_{20}H_{26}N_2O$ (molecular weight = 310.4).
Systematic name: 12-methoxyibogaine.
CAS registry number: 83-74-9.
Melting point: 148°C.
UVmax: (methyl alcohol): 226, 298 nm.
Solubility: ethyl alcohol, ether, chloroform, acetone and benzene. Insoluble in water.



Name: Vobasine.
Molecular formula: $C_{21}H_{24}N_2O_3$ (molecular weight = 352.0).
Systematic name: 3-oxomethylester-vobasan-17-oic acid.
CAS registry number: 2134-83-0.
Melting point: no data in the literature.
UVmax: no data in the literature.
Solubility: no data in the literature.

Historical use

In Africa the *Voacanga africana* it is known and used since ancient times in traditional medicine for the treatment of infectious diseases, mental disorders or as an analgesic. In the Ivory Coast, the natives used the plant to treat leprosy, diarrhoea, generalized edema, and convulsions in children [3]. The shamans of Western Africa used to ingest the bark as cerebral stimulant and the seeds for divinatory purposes.

Current use

In addition to the medical use of the plant in Africa, the *Voacanga africana* is sold today in the "smart shops" (or on the Internet sites for marketing of smart drugs) for recreational purpose because of its alleged psychoactive properties. People ingest the seeds looking for the hallucinogenic properties of the plant reported by consumers on web sites.

Legislation

With respect to Europe, in Switzerland the ibogaine is classified as an illicit drug while in England the ibogaine purchase and the possession for personal use is legal, while trafficking and administration to a third person is illegal.
In the United States, the ibogaine and its source, the plant of *Tabernanthe iboga*, are controlled substances (Schedule I drug in the Controlled Substances Act) like hallucinogens. It is illegal to sell them, buy them or to possess them without a DEA licence (Drug Enforcement Administration). Conversely, neither of the principal alkaloids of the *Voacanga africana* is subject to control in the United States of America, therefore the plant can be cultivated and it can be possessed.
In Italy, neither the whole plant nor part of it, nor voacamine, voacangine, voacristine, voacorine, vobtusine, tabersonine, ibogaine, vobasine, can be found in the in tables containing narcotic or psychotropic substances subject to the supervision and control under Article 14 of Republic Presidential Decree 309/90 and subsequent updates.
A clear legislation regarding the use and the purchase of *Voacanga africana* does not exists. There is a proposal concern-

ing the legislation on herbal medicine, dated 1996, which includes the *Voacanga africana* in Table A of plants not to be sold in herbal shops. This proposal never became a law. Nor it became a law proposal N. 2411, dated 2002 concerning the regulation of herbal medicine, in which the seeds of *Voacanga africana* have been listed in the Annex I which relates to the medicinal plants which can be exclusively used by pharmacists for the preparation of galenic products due to their high toxicity even at minimal concentrations.

Pharmaco-toxicological properties

Most the pharmacological effects of the *Voacanga africana* have been studied only on animal models. In rats, the extracts of the plant exercise an anti-ulcer activity with a mechanism of action presumably related to an citoprotective effect at the level of the gastric mucous membrane [3]. A minor plant alkaloid, the 7,8-dihydro-8-hydroxypalmitine, seems to be responsible for this pharmacological effect producing an antisecretory effect similar to anti-H2 and stimulating at the same time the production of mucus at the gastric level [4]. In association with ranitidine, this compound can increase the antisecretory effect [5].

In the mouse, the voacangine, in doses between 1 mg/kg and 10 mg/kg, has a slight hypotensive effect due to peripheral vasodilation. In very low doses, this alkaloid acts as an intestinal stimulant, a local anesthetic and as a central nervous system depressant [6].

The voacamine possesses cardiotonic properties. This compound has a positive inotropic effect on the heart with a mechanism of action different from the digitalic glucosides. Compared to digitoxin, the alkaloid is 100 to 250 times less toxic. The voacamine exercises also a depressing effect at the central nervous system level; at toxic doses it can cause death by depression of the bulbar respiratory centers [7].

Similar to voacamine, the voacorine behaves also as cardiotonic. In this case, however, the biological effect is more like the digitalis effect. In fact, in contrast to the voacamine that reduces the coronary blood flow, the voacorine increases it in gradual and lasting way. At the dose of 3 mg/kg, the alkaloid exerts a positive inotropic effect over the heart of the rabbit and a negative chronotropic effect. Also the voacorine can depress the central nervous system [8].

The voacristine has demonstrated *in vitro* dose-dependent cytostatic or cytotoxic properties on *Saccharomyces cerevisiae* cultures and *in vitro* cytotoxic activity on the ovarian cell line carcinoma A2780 [9].

A particularly interesting alkaloid from pharmacological point of view is ibogaine. This compound appeared to be effective for the treatment of the abstinence syndrome and of the craving associated with drugs of abuse [10].

The ibogaine inhibits the cholinesterase producing acetylcholine accumulation at the synaptic level that is translated in a reduced cardiac frequency and hypotension. However, an excessive cholinergic activity can cause convulsions, paralysis and respiratory arrest. The pharmacological effects, especially on the central nervous system (excitability, euphoria and visual and auditory hallucinations) are generally dose dependent. The hallucinations usually accompanied by anxiety, are shown only at high doses [10].

In the rat, it seems that the ibogaine modulates the neuronal excitability and the synaptic transmission at the level of the parabrachial nucleus changing in reversible manner the nervous transmissions that involve the dopaminergic and glutammatergic excitory systems. Such an effect has been observed also by the extracts of *Voacanga africana* with a hundred times lower efficacy compared to ibogaine [11].

Finally, the tabersonine seems to exercise a light hypotensive effect probably due to peripheral vasodilatation as well as a spasmolitic action at the intestinal level [12].

It has been attributed to *Voacanga africana* a possible anticonvulsant effect related to the antagonist action on NMDA receptors with an increase of sleep duration induced by diazepam [14].

Toxicity

Toxicity data relative to each active component can be found in studies performed in mouse. Voacamine at toxic doses induces short convulsions followed by dyspnea and suffocation.

Data regarding the acute toxicity of voacamine [7]

In mouse - LD50 following intravenous administration: 21.5 mg/kg

Data regarding the acute toxicity of voacangine [6]

In mouse - LD50 following intravenous administaraion: 41 mg/kg

Data regarding the acute toxicity of voacorine [12]

In mouse - LD50 following intravenous administration: 30 mg/kg

Data regarding the acute toxicity of tabersonine

In mouse - LD50 following intravenous administration 100-150 mg/kg

Data regarding the acute toxicity of ibogaine

In rat - LD50 following intraperitoneal administration: 145 mg/kg

In rat - LD50 following oral administration: 327 mg/kg

In guinea pig - LD50 following intraperitoneal administration: 82 mg/kg

Adverse Effects

There are no clinical studies in the literature regarding the effects of *Voacanga africana* or each single active component. Most of the known effects are the result of subjective experience from the recreational use of the plant. Approximately 20-30 minutes after the ingestion of at least 50 seeds, a change in the emotional state characterized by a sensation of extreme laxity occurs. About an hour later, spatial distortions and then vivid dreams for about eight hours may take place. These effects, with pronounced drowsiness and exhaustion are described as lasting up to the day following the ingestion of the seeds [14].

Effects in pregnancy

There is no data in the literature regarding the use in pregnancy or during lactation.

Pharmacological interactions

Voacangine, acting as a depressor at the central nervous sistem level, can reinforce the pharmacological effects of barbiturates [6].

Analytical determinations

No methodologies have been reported in the international literature concerning the analysis of *Voacanga africana* active principles in biological fluids of eventual consumers. Assays exist to detect ibogaine in different biological matrices and tissues [15,16] and ibogaine together with voacamine, voagangine in the bark of other plants containing these alkaloids such as *Peschiera fuschiaefolia* and *Tabernanthe iboga* [17]. The method of alkaloids detection in then bark of *Peschiera fuschiaefolia* implies the use of a liquid chromatograph coupled to a mass spectrometer [17].

The method below reported is a brief outline useful to the investigator to set up the analysis. For specific details, it is advisable to refer to the original text.

Determination of ibogaine in human biological fluids and tissues

(From: HEARN W, PABLO J, HIME G, MASH D. Identification and quantitation of Ibogaine and o-demethylated metabolite in brain and biological fluids using gas chromatography-mass spectrometry J Anal Toxicol 1995; 19: 427-431) [16].

The analysis is carried out on cerebral tissue and blood, plasma and urine using a gas chromatograph coupled to a mass spectrometer.

Extraction of the compounds

To 1 ml blood, plasma or urine, 100 µl ibogaine–d_3 (internal standard), 2 ml 1% sodium hydrochloride and 2 ml sodium carbonate at pH 10 are added. The solution is mixed on a horizontal shaker for one hour at ambient temperature and then extracted with 5ml ethyl acetate. The organic layer is evaporated under a stream of nitrogen, the residue dissolved in methyl alcohol and 1 µl is injected into the gas cromatograph.

Concerning cerebral tissue, 1 g homogenated tissue is added with 100 µl ibogaine–d_3 (internal standard), 3 ml 1% sodium hydrochloride and 3 ml sodium carbonate at pH 10. The mixture is then extracted as above reported for biological fluids.

Analytical conditions

Chromatographic column: DB5 (0,25 mm x 15 m x 0,1 µm)

Injector temperature: 270°C

Carrier gas: Helium at a flow rate of 1 ml/min

Injection mode: splitless

Temperature program: starting temperature at 50°C for 1 minute, then 50°C-230°C at 25°C/min, then 230°C for 30 minutes, then 230 °C-300°C at 5°C/min for 7 minutes.

Detector: mass spectrometer with electron impact interface

Retention times of the tested compounds

Ibogaine: 12 minutes

Ibogaine d_3: 12.5 minutes

Characteristic fragments for the tested compounds

Ibogaine: m/z 310, 295, 225

Ibogaine d_3: 313, 298, 228

Standards

The standard of ibogaine and ibogaine d3 used in the analyses was purchased from Omincgem Corporation (Belgium).

Calibration curves

The calibration solutions (range of concentration: 5-1000 ng/ml or ng/g) were prepared daily adding appropriate concentration of stock solutions in methyl alcohol to blank blood, plasma, urine and cerebral tissue.

Result

The determination of ibogaine with the above reported methodology evidenced an amount of active principle ranging from 100 to 495 ng/ml in blood and plasma, between 110 and 296 ng/ml in urine and between 53 and 200 ng/g in cerebral tissue from consumers.

References

1. LEEUWENBER G. Voacanga, (Apocynaceae), a review of it's taxonomy, phytochemistry, ethnobotany and pharmacology. Agric. Univ. Wagenigen papers. 1985: 83-85.
2. HITTNER JB, QUELLO SB. Combating substance abuse with ibogaine: pre- and posttreatment recommendations and an example of successive model fitting analyses. J psychoactive drugs. 2004; 36: 191-199.
3. TAN PV, PENLAP VB, NYASSE B, NGUEMO JDB. Anti-ulcer actions of the bark methanol extract of Voacanga africana in different experimental ulcer models in rats. J Ethnopharmacol. 2000; 73: 423-428.
4. TAN PV, NYASSE B. Anti-ulcer compound from Voacanga africana with possible histamine H2 receptor blocking activity. Phytomedicine. 2000; 7: 509-515.

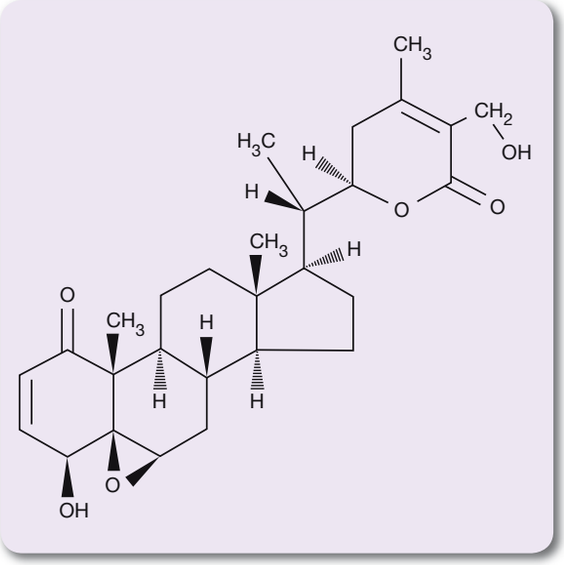
5. TAN PV, NYASSE B, DIMO T, WAFO P, AKAHKUH BT. Synergistic and potentiating effects of ranitidine and two new anti-ulcer compounds from Enantia chlorantha and Voacanga africana in experimental animal models. Pharmazie. 2002; 57: 409-412.
6. QUEVAUVILLER A, BLANPIN O. Pharmacodynamic study of voacamine, an alkaloid of Voacanca africana, Apocynaceae. Therapie. 1957; 12: 635-647.
7. QUEVAUVILLER MA, BLANPIN O. Pharmacodynamics in comparing voacamine & voacorine, alkaloids from Voacanga africana Stapi (Apocynaceae). Ann Pharm Fr. 1957; 15: 617-630.
8. MORIN H, LE MEN J, POURRAT H. Pharmacodynamic study of tabersonine, an alkaloid extracted from the seeds of Amsonia tabernaemontana Walt. (Apocyanaceae). Ann Pharm Fr. 1955; 13: 123-126.
9. KUNESCH N, MIET C, TROLY M, POISSON J. Alkaloids of Voacanga. 8. Alkaloids of leaves and seeds of Voacanga africana Stapf. Ann Pharm Fr. 1968; 26: 79-86.
10. JELLIN JM, GREGORY P, BATZ F. Pharmacist's letter/Prescriber's Letter Natural Medicines Comprehensive Database, 3rd ed, Therapeutic Research Faculty, Stockton, CA, 2000.
11. KOMBIAN SB, SALEH TM, FIAGBE NI, CHEN X, AKABUTU JJ, BUOLAMWINI JK, PITTMAN QJ. Ibogaine and a total alkaloidal extract of Voacanga africana modulate neuronal excitability and synaptic transmission in the rat parabrachial nucleus in vitro. Brain Res Bull. 1997; 44: 603-610.
12. CHATURVEDULA VSP, SPRAGUE S, SCHILLING JK, KINGSTON DG. New cytotoxic indole alkaloids from Tabernaemontana calcarea from the Madagascar rainforest. J Nat Prod. 2003; 66: 528-531.
13. BUM EN, TAIWE GS, NKAINSA LA, MOTO FC, SEKE ETET PF, HIANA IR, BAILABAR T, ROUYATOU, SEYNI P, RAKOTONIRINA A, RAKOTONIRINA SV. Validation of anticonvulsant and sedative activity of six medicinal plants. Epilepsy Behav. 2009; 14: 454-458.
14. BLANPIN O, QUEVAUVILLER A, PONTUS C. Sur la voacangine, alcaloïde du Voacanga Africana-Staff-Apocynacées. Thérapie. 1961; 16: 941-945.
15. CHEZE M, LEONAN A, DEVEAUX M, PEPIN G. Determination of ibogaine and noribogaine in biological fluids and hair by LC-MS/MS after Tabernanthe iboga abuse. Iboga alkaloids distribution in a drowing death case. Forensic Sci Inter 2008; 176: 58-67.
16. HEARN W, PABLO J, HIME G, MASH D. Identification and quantitation of Ibogaine and O-demethylated metabolite in brain and biological fluids using Gas Chromatography-Mass Spectrometry. J Anal Toxicol. 1995; 19: 427-431.
17. LEPINE F, MILOT S, ZAMIR L, MOREL R. Liquid chromatography/mass spectrometric determination of biological active alkaloids in extracts of *Peschiera fuschiaefolia*. J Mass Spectrom. 2002; 37: 216-222.

Withania somnifera
(Ashwagandha)

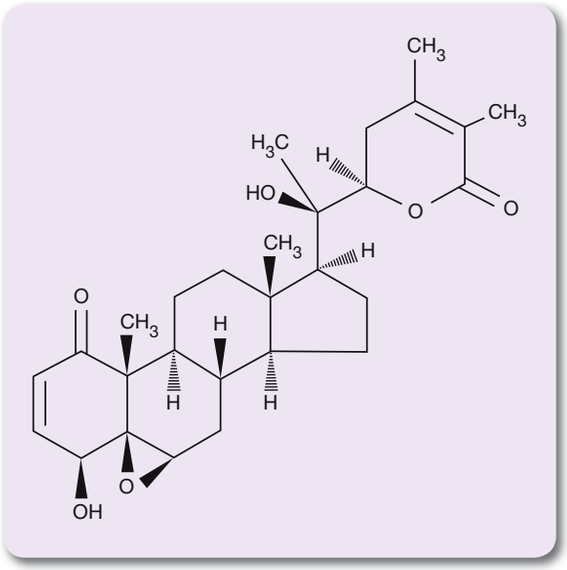
Name: *Withania somnifera* - ashwagandha
Family: *Solanaceae*
Genus: *Withania*
Species: *Whitania somnifera (L.)* Dunal
Synonims: ashwagandha, winter cherry, Indian ginseng
Origin: India, South Africa, Oriental Asia ,Mediterranean Basin
Active compounds: the majority of the active compounds are withanolides (steroidal lactones with ergostan skeleton) such as: withaferine A, withanolide I, II, III, A, D, E, F, G, H, I, J, K, L and alkaloids such as anaferine, isopelletierine.

There are 23 different plants belonging to the genus *Withania*, nevertheless only the *Withania somnifera* seems to have medicinal properties. The active compounds are concentrated in the roots and in the berries of the plant, but can also be found in the leaves and in the trunk. To date, 12 alkaloids have been characterized and isolated, 35 withanolides and different sitoindosides.
The majority of the described ashwagandha properties are due to the presence of two withanolides: withaferine A and withanolide D [(1)]. The concentration of the active compounds depends on the site of the extraction: from the roots (0.066% withaferine A, 0.193% withanolide D), the trunk (0.048% withaferine A, 0.007% withanolide D) or the leaves (0.238% withaferine A 0.003% withanolide D) [(2)]. In a study of five different plants of ashwaganda, the concentrations of withaferine varied between 0.3-0.8% in the leaves, 0.1% in the trunk and between 0.007 and 0.1% in the roots [(3)]. There is no data in the literature regarding the concentration of the active compounds in the berries.

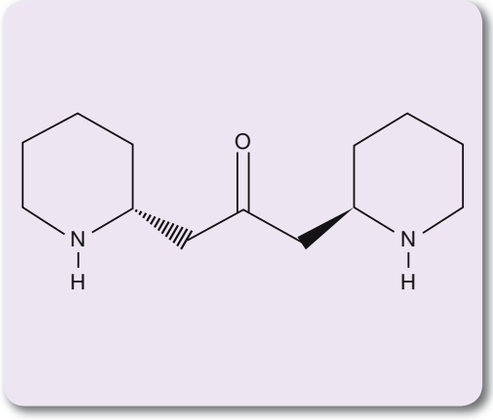
Chemical formula and physico-chemical properties of the active compounds



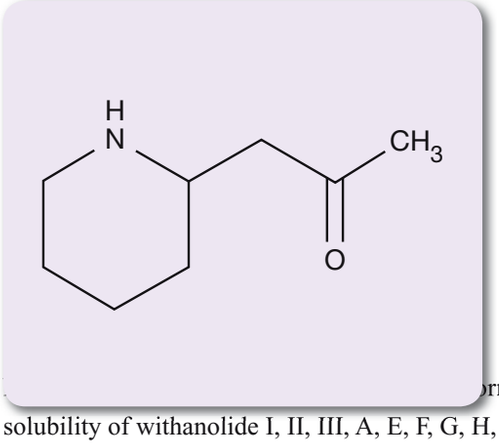
Name: Withaferine A.
Molecular formula: $C_{28}H_{38}O_6$ (molecular weight = 470.5).
Systematic name: delta-lactone (4β,5β,6β,22R)-5,6-Epoxy-4,22,27-tri hydroxy-1-oxoergosta-2,24-dien-26-oic acid.
CAS registry number: 5119-48-2.
Melting point: 243°C.
UVmax (ethyl alcohol): 214, 335 nm.
Solubility: ethyl alcohol, dimethyl sulfoxide.



Name: Withanolide D.
Molecular formula: $C_{28}H_{38}O_6$ (molecular weight = 470.5).
Systematic name: delta-lactone (4β,5β,6β,22R)-5,6-Epoxy-4,20,22-tri hydroxy-1-oxoergosta-2,24-dien-26-oic acid.
CAS registry number: 30655-48-2.
Melting point: 251-253°C (ethyl acetate).
UVmax: no data in the literature.
Solubility: no data in the literature.



Name: Anaferine.
Molecular formula: $C_{13}H_{24}N_2O$ (molecular weight = 224.3).
Systematic name: 1,3-bis[(2R)-piperidin-2-yl]propan-2-one.
CAS registry number: 19519-53-0.
Melting point: no data in the literature.
UVmax: no data in the literature.
Solubility: no data in the literature.



Nome: Isopelletierine.
Molecular formula: $C_8H_{15}NO$ (molecular weight = 141.2).
Systematic name: (9CI)1-(2-Piperidinyl)-(+-)-2-propanone.
CAS registry number: 539-00-4.
Melting point: no data in the literature.
UVmax: no data in the literature.
Solubility: no data in the literature.

rmula, systematic name, CAS registry number, melting point, UV max and solubility of withanolide I, II, III, A, E, F, G, H, I, J, K and L.

Historical use

For the last 3000 years, the *Withania somnifera* also known as Ashwagandha, Indian ginseng or winter cherry, has an important plant in the ayurvedic and traditional medicine. Historically, the plant has been used as an aphrodisiac, tonic for the liver, anti-inflammatory agent, astringent, and, more recently, for the treatment of bronchitis, asthma, ulcer, insomnia and senile dementia.

Current use

At present time, the ashwagandha is used in the ayurvedic medicine mostly as an adaptogen. The adaptogens represent a

class of compounds (plants) that, according to the ayurvedic tradition, are able to induce in the sick organism conditions of increased resistance to the sickness itself. The adaptogens are rather harmless, they do not have a specific action mechanism, they are told to normalise the pathological conditions and are represented generally by the glycosides and alkaloids of the plants. Different clinical studies, as well as research using animals, seem to support the use of the ashwagandha in the treatment of anxiety, neurological and cognitive disorders, inflammations [1,4].

Legislation

There are no known restrictive legislations for the sale of the ashwagandha and/or its active compounds in different countries of the European Community. In Canada, the ashwagandha is placed on a list of cosmetic products and the potential toxicity needs to be established on an individual basis [5]. In Italy, there are no restrictions for the use of ashwagandha and/or its active compounds although in May 10th, 1996 law proposal (Rules for herbs and officinal plants) the aswagandha seeds were placed on a list of products forbidden for sale as herbal preparation [6]. The Italian Ministry of Health included the root of *Withania somnifera* nut in the list of vegetal extracts admitted in dietary supplements [7].

Pharmaco-toxicological properties

The biological and pharmacological effects of the ashwagandha are attributable to the withanolides present in the plant.
The ashwagandha shows antistress, anti-inflammatory, immunomodulator, antitumor, antioxidant and hematopoietic properties. Besides these properties, the ashwagandha has an effect on the endocrine system, the cardiovascular and respiratory apparatus and on the central nervous system although the mechanisms of action are not yet completely clarified.
The mechanism of the antistress properties seems to be an inhibition of the up-regulation of the dopaminergic receptors induced by stress at the level of the corpus striatum [8]. The immunostimulatory effect seems to be correlated with the ability of the plant to induce the synthesis of nitrogen monoxide(NO) from the macrophages [9].
Between the active compounds of the ashwagandha with antineoplastic properties, the withaferine A, seems to be the most promising, although the mechanism of the above reported action is not completely clear [10]. The substance seems to possess powerful anti-angiogenic properties that make it particularly interesting in the research associated with the development of new antitumor drugs [11]. *In vitro*, the withaferine A inhibits the cellular proliferation acting on protein synthesis with cytotoxic effects [12]. Experiments made on human tumour cell lines (lung, breast, central nervous system) have confirmed these properties of the withaferine A and other compounds extracted from the ashwagandha [13].
Indeed, the withaferine E was also shown to have antitumor effects [14].The antitumor properties of the withaferine A and of the withanolide D have been studied also in the sarcoma-180 from mouse [15].
The withanolides behave as hormonal precursors able to be converted, as needed, into active hormones. In a double blind study, 42 patients suffering of osteoarthritis have been treated by a mixture of herbs containing ashwagandha and other plants or with placebo for a duration of three months. During the entire treatment, the pain, the degree of disability and the sedimentation rate have been monitored and , periodically, a radiological checkup was performed. The patients given the herb mixture have shown a significant reduction of the of severity of pain and of the degree of disability compared to the controls; nevertheless other evaluated parameters remained unchanged in the two groups [16].
The *Withania somnifera* also possesses anticonvulsant activity, a property which seems to be related to an action site for barbiturates present at the GABA receptor level [17].
The antiinflamatory activity of the *Withania somnifera* extracts has been studied in a granuloma experimental model induced by the administration of carragenine. These studies have demonstrated that the *Withania somnifera* reduces the synthesis of collagen and the amount of glucosaminoglycans contained in the granulomatous tissue [18].
In another study, inflammation has been produced by formalin injection in the posterior limb of the rat. Such a condition causes reduced glucose absorption in the intestine, which can be assessed *in vitro*. In the rats treated with ashwagandha or with the antiinflamatory drug oxyphenbutazone, the malabsorption of glucose does not take place, suggesting that the ashwagandha has an antiinflamatory effect similar to that caused by the oxyphenbutazone with a mechanism of action presumably tied to the inhibition of cyclooxygenase [19].
The ashwagandha increases the activity of the peritoneal macrophages producing an antimicrobial effect [20]. The antibac-

terial action of the plant has been demonstrated in a recent a study in which it has been observed that the oral administration of an aqueous ashwagandha extract reduces the bacterial effect on the vital organs and increases the survival time in mice infected with *Salmonella typhimurium* [21].

Other pharmacological effects observed in the laboratory include a slight inotropic and chronotropic effect (withanolides), cholesterol reducing properties (β-sitosterols) [13,22,23], and nootropic effects due to increase in the cholinergic activity [24]. Finally, it has been proved that a pretreatment with *Withania somnifera* in rat models seems to protect from the structural changes in *nucleus accumbens* by morphine withdrawal [25].

Toxicity

In a study of chronic toxicity in rats, the administration of a dose of 100 mg/kg of ashwagandha extract for 30 days has caused a significant reduction in the weight of the spleen, the thymus and the adrenal gland and a significant increase in the acid phosphatase level [26].

In mouse, with the intraperitoneal administration of an alcoholic plant extract, the LD50 is 1260 mg/kg while following the oral administration of the only alkaloids fraction, the LD50 is always 432 mg/kg.

The causes of death in animals are the respiratory paralysis and the clonic convulsions [27].

Data regarding the acute toxicity of withaferine A

In mouse - following intraperitoneal administration LD50: 54 mg/kg

There are no data of toxicity related to withanolide D.

Adverse Effects

Elevated ashwagandha doses can cause gastrointestinal troubles, vomiting and diarrhoea [14]. In the laboratory animals, the alkaloid fraction has a sedative effect which, by increasing the dose can lead to respiratory depression. Some authors advise the use of the plant extracts in conjunction with alcohol, barbiturates and ansiolitics in general [1]. A study led by Malhotra et al. [26] has highlighted the ability of the ashwagandha to develop a sedative effect similar to that of pentobarbital. The same study has demonstrated that in the mice the alkaloids of the ashwagandha can increase the toxicity of metamphetamine and metrazol.

Rats administered ashwagandha for a period of 10-14 days have developed renal problems (renal stones and tubular degeneration) hepatic problems (centrilobular degeneration) and respiratory troubles (peribronchial and perivenous edema) [28].

The case of a 32-year-old woman showing symptoms of thyrotoxicosis as a consequence of ashwagandha use for the treatment of chronic fatigue syndrome, has been described in the literature [29]. This case, even without others evidences in the literature, was confirmed by studies in animals in which the thyrotoxic effect following ashwagandha use has been attributed to an increase in the thyroid hormones.

Pharmacological interactions

The *Withania somnifera* can have a sedative effect.

Potential pharmacological interactions can occur with:

- anticonvulsants
- antipsychotics
- benzodiazepines
- barbiturates (phenobarbital)
- phenytoin
- primidone
- tricyclic antidepressants
- valproic acid
- zolpidem

Therefore, it is advisable not to mix derivatives of the plant with medicines that depress the central nervous system and to stop its use close to possible surgical interventions that may require general anaesthesia [30].

Effects in pregnancy

The American Herbal Products Association has assigned the ashwagandha to the class 2b (not to use in pregnancy) [31]. The plant can have a possible abortive effect [14].

Analytical determinations

No methodologies have been reported in the international literature concerning the analysis of *Withania somnifera* active principles in biological fluids of eventual consumers. However, analytical methods have been published regarding the determination of these active principles in the plant root , leaves and trunk powder [2].

The method below reported is a brief outline useful to the investigator to set up the analysis. For specific details, it is advisable to refer to the original text.

Determination of withanolides in the plant of *Withania somnifera*

(From: GANZERA M, CHOUDHARY MI, KHAN IA. Quantitative HPLC analysis of withanolides in *Withania somnifera*. Phytotherapy. 2003; 74: 68-76) [2].

The analysis is carried out on pulverized root, leaves and trunk of *Withania somnifera* and commercial products containing parts of the plant, using liquid chromatography coupled with diode array spectrophotometric detection and with electrospray mass spectrometry.

Extraction of the compounds

One gram of pulverized material ensuing from the plant or from commercial products is extracted three times with 3 ml of methyl alcohol and sonicated for 10 minutes. After centrifugation at 3000 rpm for 5 minutes, the supernatant is reconstituted to a final volume of 10 ml with methyl alcohol. The liquid commercial products are simply diluted 1:1 with methyl alcohol. All the samples are filtered before being analysed.

Analytical conditions

Chromatography column: Synergi MAX-RP 80 (150 x 4.6 mm x 4 µm)
Mobile phase A: Water
Mobile phase B: methyl alcohol: alcoholic reagent (ethyl alcohol, methyl alcohol, isopropanol 90,6:4,5:4,9, v/v/v) 1:1, v/v
Separation gradient for coupling with diode array spectrophotometric detection: gradient (mobile phase A 65% time zero to 55% in 25 minutes)
Separation gradient for coupling with with electrospray mass spectrometry: (mobile phase A 55% time zero to 45% in 25 minutes)
Flow rate for coupling with diode array spectrophotometric detection: 1 ml/min
Flow rate for coupling with with electrospray mass spectrometry: 0.5 ml/min
Detector 1: diode array set at 230 nm
Detector 2: mass spectrometer with positive mode electrospray (ESI) interface
Ionization voltage: 50 V
Source voltage: 3 Kv

Retention times of the tested compounds

Withaferine A: 14.2 minutes

Withanolide D: 17.2 minutes

Frammenti caratteristici delle sostanze ricercate

Withaferine A: m/z 488, 417, 399

Withanolide D: m/z 488, 453, 288

Standards

The withaferine A and the withanolide D used in the analyses have been purchased from Chromadex (LGC Promochem s.r.l.,Sesto San Giovanni, Milan, Italy).

Calibration curves

Two milligramms of each standard were dissolved in methyl alcohol (standard solution). Five calibration points (range: 400 ng/ml–1.6 µg/ml) were prepared by dilution of the standard solution in methyl alcohol.

Results

The analysis of the root, the trunk and the leaves of *Withania somnifera* have confirmed the presence of the withaferine A and of the withanolide D in all the parts of the plant but with a significant difference in their proportions. In the root, the withanolide D proved to be present in greater percentage (0.193% vs 0.066%),while in the leaves in smaller percentage (0.003% vs 0.238%) compared to the withaferine A. In the trunk, the percentage of both compounds is low (0.007% for the withanolide D and 0.048% for the withaferine A). Both compounds have been found in the analysed commercial products. In the solids, the quantity of the withaferine A varied from 0.003% to 0.051% while the quantity of withanolide D varied from 0.006% to 0.049%. In the liquid commercial products, the quantity of the withaferine A is in the range 0.027-0.065% and the withanolide D varies between 0.238% to 0.364%.

References

1. NOT REPORTED AUTHORS. Withania somnifera. Monograph Altern Med Rev. 2004; 9: 211-214
2. GANZERA M, CHOUDHARY MI, KHAN IA. Quantitative HPLC analysis of withanolides in *Withania somnifera*. Fitoterapia. 2003; 74: 68-76.
3. KHAJURIA RK, SURI K, GUPTA RK, SATTI NK, SURI OP, QAZI GN. Separation, identification, and quantification of selected withanolides in plant extracts of *Withania somnifera* by HPLC-UV(DAD) – positive ion electrospray ionisation-mass spectrometry. J Sep Sci. 2004; 27: 541-546.
4. MISHRA LC, SING BB, DAGENAIS S. Scientific basis for the therapeutic use of Withania somnifera (ashwagandha): a review. Altern Med Rev. 2000; 5: 334-346.
5. HEALTH CANADA- Substances in cosmetics and personal care products regulated under the food and drugs act (F&DA) that were in commerci between January 1, 1987 and September 13, 2001
6. SENATO DELLA REPUBBLICA- Legislatura 13°- Disegno di legge n. 249. Norme in materia di erboristeria e di piante officinali.
7. The list of vegetal extracts admitted in dietary supplements in Italy can be found at http://www.salute.gov.it
8. RAMARAO P, RAO KT, SRIVASTAVA RS GHOSAL S. Effects of glycowithanolides from Withania somnifera on morphine-induced inhibition of intestinal motility and tolerance to analgesia in mice. Phytotherapy Res. 1995; 9: 66-68.
9. IUVONE T, ESPOSITO G, CAPASSO F, IZZO AA. Induction of nitric oxide synthase expression by Withania somnifera in macrophages. Life Sci. 2003; 72: 1617-1625.
10. UMA DEVI P. Withania somnifera Dunal (ashwagandha): potential plant source of a promising drug for cancer chemotherapy and radiosensitization. Indian J Exper Biol. 1996; 34: 927-932.
11. MOHAN R, HAMMERS HJ, BARGAGNA-MOHAN P, ZHAN XH, HERBSTRITT CJ, RUIZ A, ZHANG L, HANSON AD, CONNER BP, ROUGAS J, PRIBLUDA VS. Withaferin A is a potent inhibitor of angiogenesis. Angiogenesis. 2004; 7: 115-122.
12. FUSKA J, FUSKOVA A, ROSAZZA JP NICHOLAS AW. Novel cytotoxic and antitumor agents. IV. Withaferin A: relation with its structure to the in vitro cytotoxic effects on P388 cells. Neoplasma. 1984; 31: 31-36.
13. JAYAPRAKASAM B, ZHANG Y, SEERAM NP, NAIR MG. Growth inhibition of human tumor cell lines by withanolides from Withania somnifera leaves. Life Sci. 2003; 74: 125-132.
14. LINDNER S. Withania somnifera. Aust J Med Herbalism. 1996; 8: 78-82.
15. CHOWDHURY K, NEOGY RK. Mode of action of Withaferin A and Withanolide D. Biochem Pharmacol. 1975; 24: 919-920.
16. KULKARNI RR, PATKI PS, JOG VP, GANDAGE SG, PATWARDHAN B. Treatment of osteoarthritis with a herbomineral formulation: a double-blind, placebo-controlled, cross-over study. J Ethnopharmacol. 1991; 33: 91-95.

17. KULKARNI SK, SHARMA A, VERMA A, TICKU, MK. GABA receptor mediated anticonvulsant action of Withania somnifera root extract. Indian Drugs. 1993; 30: 305-312
18. BEGUM V, SADIQUE J. Effect of Withania somnifera on glycosaminoglycan synthesis in carrageenan-induced air pouch granuloma. Biochem Med Metabol Biol. 1987; 38: 272-277.
19. SOMASUNDARAM S, SADIQUE J, SUBRAMONIAM A. Influence of extra-intestinal inflammation on the in vitro absorption of 14C-glucose and the effects of anti-inflammatory drugs in the jejunum of rats. Clin Exp Pharmacol Physiol. 1983; 10: 147-152.
20. DHULEY JN. Therapeutic efficacy of ashwagandha against experimental aspergillosis in mice. Immnopharmacol Immunotoxicol. 1998; 20: 191-198.
21. OWAIS M, SHARAD KS, SHEHBAZ A, SALEEMUDDIN M. Antibacterial efficacy of Withania somnifera (ashwagandha) an indigenous medicinal plant against experimental murine salmonellosis. Phytomedicine. 2005; 12: 229-235.
22. TRIPATHI AK, SHUKLA YN, KUMAR S. Ashwagandha (Withania somnifera Dunal - Solanaceae): A status report. J Med Aromatic Plant Sci. 1996; 1: 46-62.
23. ROJA G, HEBLE MR, SIPAHIMALANI AT Tissue cultures of Withania somnifera: morphogenesis and withanolide synthesis. Phytotherapy Res. 1991; 5: 185-187.
24. SCHLIEBS R, LIEBMANN A, BHATTACHARYA SK, KUMAR A, GHOSAL S, BIGL V. Systemic administration of defined extracts from Withania somnifera (Indian ginseng) and shilajit differentially affects cholinergic but not glutamatergic and gabaergic markers in rat brain. Neurochem Int. 1997; 30: 181-190.
25. KASTURE S, VINCI S, IBBA F, PUDDU A, MARONGIU M, MURALI B, PISANU A, LECCA D, ZERNIG G, ACQUAS E. Withania somnifera prevents morphine withdrawal-induced decrease in spine density in nucleus accumbens shell of rats: a confocal laser scanning microscopy study. Neurotox Res. 2009; 16: 343-355.
26. SHARADA AC, SOLOMON FE & UMA DEVI P. Toxicity of Withania somnifera root extract in rats and mice. Indian J Pharmacog. 1993; 31: 205-212.
27. MALHOTRA CL, MEHTA VL, DAS PK DHALLA NS. Studies on Withania- ashwagandha, Kaul (Part V): The effect of total alkaloids (ashwagandholine) on the central nervous system. Indian J Physiol Pharmacol. 1965; 9: 127-136.
28. ARSECULERATNE SN, GUNATILAKA AAL, PANABOKKE RG. Studies on medicinal plants of Sri Lanka. part 14: toxicity of some traditional medicinal herbs. J Ethnopharmacol. 1985; 13: 323-335.
29. VAN DER HOOFT CS, HOEKSTRA A, WINTER A, DE SMET PA, STRICKER BH. [Thyrotoxicosis following the use of ashwagandha]. Ned Tijdschr Geneeskd. 2005; 149: 2637-2638.
30. HARNESS R, BRATMAN S. Drug-Herb-Vitamin Interactions Bible. Ed. Prima.
31. MCGUFFIN M, HOBBS C, UPTON R. Botanical Safety Handbook. CRC Press, Boca.

Spices

New “Smart drugs” under the brand name “SPICE” were first sold on the Internet and in “ethno-shops” in 2006. “SPICE” as it reads on the product label is told to be a herbal mixture which , similar to tobacco, can be smoked. In fact “SPICE” products are advertised and sold as exotic incense blends which releases a rich aroma “not indicated for the human consumption” when burned. However, they are smoked and the effects of “SPICE” are reported by consumers to be similar to those of cannabis [1-2]. The same users define smoked “SPICE” as “legal highs” referring to their legal status [1-2].

At present time there are a number of products under the “SPICE” brand: Spice Silver, Spice Gold, Spice Diamond, Spice Arctic Synergy, Spice Tropical Synergy, Spice Egypt.

Reading the labels on the different “SPICE” products, it becomes evident that they contain the same plants but the quantities and their relative proportions differ.

The labels on the colourful “SPICE” packages indicate that the product contains between 0.4–3.0 g of a mixture said to consist of several potentially psychoactive plants. These plants seem to have been chosen because some of them are traditionally used by some South American or Asiatic ethnic groups like “substitutes for marijuana”. Therefore, users can expect similar effects to those obtained after having smoked cannabis.

Based on the knowledge of the chemical content of some of these plants, for at least two of them, the *Pedicularis densiflora* (Indian Warrior) and the *Leonotis leonurus* (Lion’ s Tail) there are reports in the literature on their psychoactive effect. For other plants, the reports are scarce and mostly anecdotical.The following table lists the herbal components of “SPICE” products [1].

Table 1.

COMMON NAME	SPECIES	FAMILY
Beach bean	*Canavalia maritima;* sin. *C. rosea*	Fabaceae
Lion’s tail	*Leonotis leonurus*	Lamiaceae
Honeyweed/Siberian motherwort	*Leonurus sibiricus*	Lamiaceae
Sacred lotus	*Nelumbo nucifera*	Nelumbonaceae
White and blue water lily	*Nymphaea alba* e *Nymphaea caerulea*	Nymphaeaceae
Indian warrior	*Pedicularis densiflora*	Orobanchaceae
Dwarf skullcap	*Scutellaria nana*	Lamiaceae
Maconha brava	*Zornia latifolia* o *Z. diphyilla*	Fabaceae

In 2009, a study initiated by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) identified the current developments in the on-line drug market. The majority of the on-line retailers were based in the United Kingdom (37%), Germany (15%), Netherlands (14%), and Romania (7%).

While still retailed on-line in the United Kingdom, Romania, Ireland and Latvia, “SPICE” products were no longer sold in on-line shops based in Germany, Austria and France due to legal actions to ban or otherwise control “SPICE” products.

The average Internet price of “SPICE” products varied, but was generally between 20 EUR and 30 EUR per 3 g package, depending on the country and the “strength” of the product. Provided that one 3 g package is sufficient for seven “ joints” (0.4 g per joint), the price is roughly comparable to that of cannabis in Europe, i.e. approximately 3–4 EUR per joint.

Compared to 2008, a greater range of alternative smoking blends to “SPICE” was being advertised by retailers. A total of 27 different herbal smoking blends were identified across all investigated retailers (e.g. Yucatan Fire, Sence, Genie, N-joy). They were advertised as containing plant-based ingredients, however, according to retailer-supplied information some also contain the hallucinogenic mushroom *Amanita muscaria* (e.g. Pep Spice Twisted).

The use of "SPICE" is alarming for the scientific community since not much is known about the pharmacology and toxicology of these herbs. Moreover, it is generally assumed that the biological and psychological effects described by users are due to the added synthetic cannabinoids, which were tested on animal models but not in humans.

In addition, not only there is little knowledge about the single herbal compounds of these mixtures but also there is no news on pharmacological interactions and properties of active compounds present in mixtures, which may result in non predictable additive or synergistic effects. Only clinical observation of the effects on users can fill the void of knowledge.

At the end of 2008, some European states (Germany, Austria, Denmark and Holland) and the US Drug Enforcement Administration (DEA) have reported the presence of synthetic psychoactive compounds in "SPICE" products which acted on the cannabinoid receptors [3-4]. Specifically, the Austrian National Focal Point on Drugs and Drug Addictions (NFP) formally notified the EMCDDA on the new psychoactive substance JWH-018 — a synthetic cannabinoid receptor (CB) agonist that had been identified in at least three "SPICE" products (Spice Gold, Silver and Diamond). Similarly, the German NFP notified the EMCDDA of the synthetic cannabinoid CP 47,497, while Holland and Denmark have signalled the presence in "SPICE" products of the synthetic cannabinoid JWH-073. Outside of Europe, the US DEA reported that another synthetic cannabinoid, HU-210 had been found in 'small but verifiable amounts' in "SPICE" products [3].

These molecules added to the herbal mixtures sold as "SPICE" were originally developed in research laboratories with the aim of decoding the molecular and biochemical mechanisms of the endocannabinoid system.

At international level, none of the above synthetic cannabinoids are controlled as far as their use or sale and there is no information on any of them having been authorised as a medicinal product in the European Union. There are no officially published safety data and almost nothing is known about their effects in humans. Their uncommon chemical structure added to some characteristics as volatility (and subsequently "smokability") and the activity at low doses, are likely to present further analytical and toxicological challenges.

It can be assumed that different amounts or combinations of synthetic cannabinoids may have been added to some "SPICE" products to produce cannabis-like subjective effects. There is no proof that JWH-018, JWH-073, HU-210, CP 47,497 and their analogues are present in all "SPICE" products, and even in the different batches of the same product.

Media information suggests that some "SPICE" products may have been produced in Asia (e.g. China), but it remains unclear where and how the actual production of the herbal mixtures, the synthetic cannabinoids and their addition to the herbal mixtures takes place.

References

1. EMCDDA. Understanding the 'Spice' phenomenon, Thematic papers, European Monitoring Centre for Drugs and Drug Addiction (2009). http://www.drugsandalcohol.ie/12597/1/Understanding_the_Spice_phenomenon.pdf
2. LINDIGKEIT R, BOEHME A, EISERLOH I, LUEBBECKE M, WIGGERMANN M, ERNST L, BEUERLE T. Spice: a never ending story? Forensic Sci Int. 2009; 191: 58-63.
3. DEA (US Drugs Enforcement Administration), *Microgram Bulletin* 2009; 42 (3).
4. UCHIYAMA N, KIKURA-HANAJIRI R, KAWAHARA N, HAISHIMA Y, GODA Y. Identification of a cannabinoid analog as a new type of designer drug in a herbal product. Chem Pharm Bull. (Tokyo) 2009; 57: 439-41.

Monographs of synthetic cannabinoids and plants present in the “SPICE” products

SYNTHETIC CANNABINOIDS

The synthetic cannabinoids are a large family of chemically unrelated molecules but functionally similar to Δ-9-tetraidrocannabinol (THC), the active ingredient of cannabis. Like THC, the synthetic cannabinoids bind to the same cannabinoid receptors in the brain and other organs as the endogenous ligand anandamide. Correctly designated as “cannabinoid receptor agonists” they were developed over the past 40 years as therapeutic agents for pain management. However, it proved difficult to separate the analgesic properties from the undesired psychoactive effects. Recently, the synthetic cannabinoids have been found in different herbal preparations and in incenses. A typical example are ”SPICE” products (Gold, Silver Yucatan, Fire), although subsequently many other products with similar characteristics have been identified.

1. CHEMISTRY

Even though they are defined often simply as “synthetic cannabinoids”, the greatest part of such molecules are not structurally correlated to the so called “classic cannabinoids” (analogues of THC based on a dibenzopyran ring). The “cannabinoid receptor agonists” constitute a heterogeneous group of substances, although they have common characteristics, such as: they are lipid -soluble, non-polar, with 22-26 carbon atom chains. They are fairly volatile (and hence, ‘smokable’). Common characteristic of several synthetic cannabinoids is the presence of a lateral chain that confers activity to the molecule: an “optimal activity” requires more than four and up to nine saturated carbon atoms [(1)]. The synthetic cannabinoids can be classified into seven structural groups [(2)]:

1. Naphthoylindoles; (JWH-018, JWH-073 e JWH-398);
2. Naphthylmethylindoles;
3. Naphthoylpyrroles;
4. Naphthylmethylindenes;
5. Phenylacetylindols (benzoylindols, e.g. JWH-250);
6. Cyclohexylphenols (CP 47,497 and their analogues);
7. Classical cannabinoids (HU-210).

The analogues of THC, called “classical cannabinoids”, are based on a dibenzopyran ring. Developed from 1960, they include HU-210 (the initials HU indicate the Hebrew University) [(3-7)], nabilone, dronabinol and many others. During the early 70’s, pharmaceutical companies developed a series of cyclohexylphenols (CP) with cannabinoid -like activity. Examples of these molecules include the CP 47,497 and its analogues [(8-11)]. In the scientific literature they are called “non-classical cannabinoids”. In the 1990s, J.W. Huffman et al. at Clemson University, USA created a large series of naphthoylindoles, naphthylmethylindoles, naphthoylpyrroles, naphthylmethylindenes and phenylacetylindoles (i.e. benzoylindoles) (known as aminoalkylindoles or JWH compounds — after the name of their inventor) [(12-14)]. An example of phenylacetylindole is JWH-250, identified in ‘SPICE’ products in Germany.

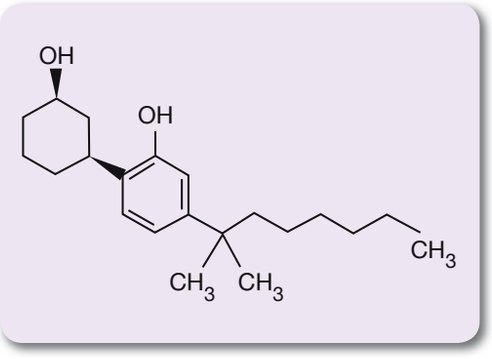
Table 2 illustrates some characteristics of six principal synthetic cannabinoids found in “SPICE” products, compared with the characteristics of Δ-9-THC.

Table 2. Δ-9-THC and the six synthetic cannabinoids with high affinity for CB1 receptors, found in"SPICE" products

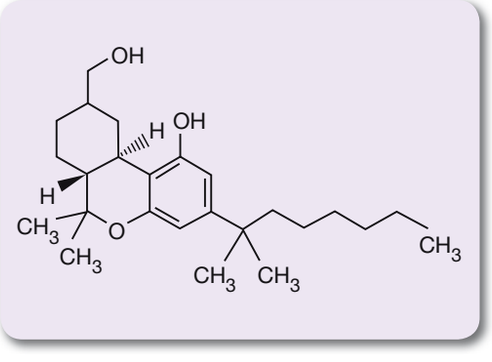
	Δ-9-THC	HU-210	CP 47, 497	JWH-018	JWH-073	JWH-398	JWH-250
Family	natural dibenzopyran	"classic" cannabinoid dibenzopyran	cyclohexyl phenol	naphthoylindol	naphthoylindol	naphthoylindol	Phenylacetylinde/ benzoylindol
Subgroup		Analog of Δ9-THC		1-alkyl-3-(1-naphtoyl) indol	1-alkyl-3-(1-naphtoyl) indol	3-(4-alo-1-naphtoyl)indol	1-pentyl-3-phenylacetylindol
Potency and selectivity	Reference substance Partial agonist of CB1 receptor	Non-selective agonist of CB1/CB2 receptors	Potent selective agonist of CB1 receptor	Very potent, selective agonist of CB2 receptor (potent agonist of CB1 receptor)	Potent agonist of CB1 receptor (weaker agonist of CB2 receptor)	Non-selective, very potent agonist of CB1/CB2 receptors	Very potent, selective agonist of CB1 receptor (weaker agonist of CB2 receptor)
Ligand affinity to CB1_Ki (nM)	**10,2**	**0,06**	**9,54**	**9**	**8,9**	**2,3**	**11**
Synthesised by	Natural origin	R. Mechoulam	Pharmaceutical company	JW Huffman	JW Huffman	JW Huffman	JW Huffman

1.1 Structure of the principal synthetic cannabinoids found in "Spice" products [15-19]

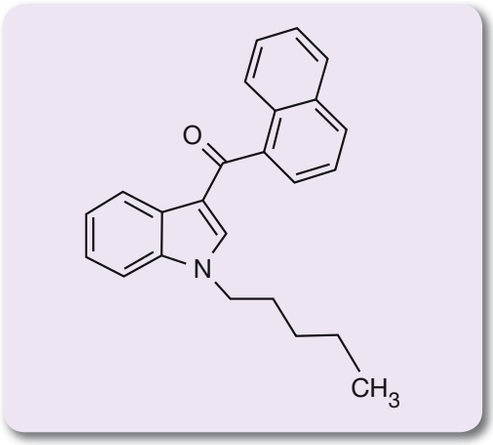
The synthetic cannabinoids have a molecular structure that resembles that of Δ-9-tetrahydrocannabinol, the active principle of cannabis. It is important to mention the presence of a lateral chain in all the molecules, responsible for the activity on the central nervous system.



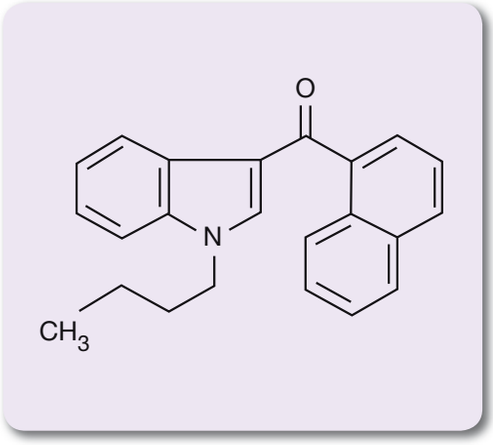
Name: CP 47,497.
Molecular formula: $C_{21}H_{34}O_2$ (molecular weight = 318.5).
Systematic name: cis-5-(1,1-dimethylheptyl)-2-(3-hydroxycyclohexyl)-phenol
CAS registry number: 70434-82-1.
Melting point: no data in the literature.
UVmax: no data in the literature.
Solubility: no data in the literature.



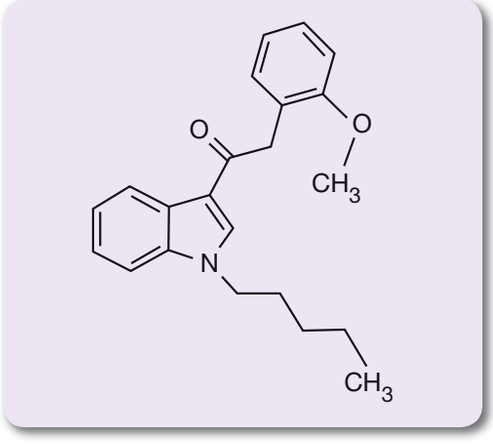
Name: HU-210.
Molecular formula: $C_{25}H_{38}O_3$ (molecular weight = 386.6).
Systematic name: (6aR-cis-3-(1,1-dimethylheptyl)-6a,7,10,10a-tetrahydro-1-hydroxy-6,6-dimethyl-6Hibenzo[b,d]piran-9-methyl alcohol.
CAS registry number: 112830-95-2.
Melting point: no data in the literature.
UVmax: no data in the literature.
Solubility: dimethylsulphoxide



Name: JWH-018.
Molecular formula: $C_{24}H_{23}NO$ (molecular weight = 341.5).
Systematic name: 1-naphthyl-(1-pentylindol-3-yl)methanone
CAS registry number: 209414-07-3.
Melting point: 49-54°C.
UVmax: no data in the literature.
Solubility: no data in the literature.



Name: JWH-073.
Molecular formula: $C_{23}H_{21}NO$ (molecular weight = 327.4).
Systematic name: 1-naphtyl(1-butylindol-3-yl)methanone
CAS registry number: 208987-07-3.
Melting point: no data in the literature.
UVmax: no data in the literature.
Solubility: no data in the literature.



Name: JWH-250.
Molecular formula: $C_{22}H_{25}NO_2$ (molecular weight = 335.4).
Systematic name: no data in the literature.
CAS registry number: no data in the literature.
Melting point: no data in the literature.
UVmax: no data in the literature.
Solubility: no data in the literature.

1.2 Physical Status

The pure synthetic cannabinoids can be found as solids or as oils. The herbal mixtures, sold as smokeable preparations, are usually placed in aluminum envelopes containing about 3 g of dried material to which one or more synthetic cannabinoids are added presumably by a vaporization process. Often the manufacturer does not disclose the true content of the mixture: some of the listed herbs are, in fact, not present, while the presence of the synthetic cannabinoids is not usually declared on the label. In addition, many times tocoferol (vitamin E) is added to the mixture in high concentration, probably to make the detection of the synthetic cannabinoids more difficult.

1.3. Synthesis and chemical precursors

Some synthetic cannabinoids can be easily bought. For many others, it is possible to find published methods for their synthesis, and their precursors can be obtained from chemical suppliers. However, some compounds are more difficult to find or to synthesize. For example , the synthesis of naphthoylindoles from precursors require many steps, while the preparation of the dibenzopyran is complicated by the fact that the synthesis is followed by the separation of enantiomers from the racemic mixture.

2. PHARMACOTOXICOLOGICAL PROPERTIES

The cannabinoid receptor agonists interact at the brain level with the CB1 receptors mimicking THC effects. *In vitro* studies demonstrated a greater affinity of syntetic cannabinoids (expressed as Ki) for these receptors when compared to THC. All the synthetic cannabinoids identified in the analyzed herbal mixtures have, like THC, high affinity for the CB1 receptors (Ki = 10.2nM). HU-210 has a particularly low Ki: it binds to the CB1 receptors with a 100 times higher affinity than THC.

The knowledge on the pharmacology and toxicology of the synthetic cannabinoids is scarce: there are only few studies done in humans which render the consumption of these substances potentially harmful. For example, it has been hypothesized that because of its structural characteristics, JWH-018, could have carcinogenic properties. Furthermore, it is possible that, because of the strong affinity to the CB receptors and strong effects on central nervous system, certain synthetic cannabinoids with a particular long half-life might have a prolonged psychoactive effect. A large batch to batch variability in cannabinoid content has been observed in the herbal mixtures (both in the type of cannabinoid and the amount). Subsequently, there is a potentially higher risk of overdose, compared to cannabis consumption and also potentially higher risk of dependence and abstinence syndrome [(16)].

3. ANALYTICAL DETERMINATIONS

The cannabinoids can be easily separated using gas or liquid chromatography and identified by mass spectrometric detection. Currently, international literature reports an assay for the identification of JWH-018 and CP 47,497 in some "SPICE" products [(18)], and a method for JWH-018 determination in blood from two consumers [(20)].

4. LEGISLATION

None of the synthetic cannabinoids is subjected to international control as per the conventions of the United Nations for the control of drugs. Conversely, in many member states of the European Union JWH-018, the JWH-073, HU-210, and CP 47,497 (with its homologues C6, C8 and C9) have been regulated. In Poland, JWH-018 and certain components of "SPICE" (*Leonotis leonorus* and *Nymphea caerulea*) are subjected to control. In Germany, JWH-018 and CP 47,497 have been fast-track regulated. In Austria, Estonia and France JWH-018, HU-210, and CP 47,497 have been placed on the list of controlled substances. In Sweden and Lithuania, in addition to the previous ones, also JWH-073 has been classified as narcotic. Luxembourg seems to have adopted a similar approach making reference to the synthetic agonists of the cannabinoid receptors. The United Kingdom has adopted generic definitions and plans to introduce control measures for a wide range of synthetic cannabinoids. Also other member states are taking in consideration the possibility of introducing similar control measures.

On April 7, 2010, the Italian Minister of Health signed, in agreement with the Drug Policy Department at the Presidency of the Ministers Council, an ordinance that prohibits the manufacture, the import, and trade (including on line selling on-line) of "SPICE" and related products, sold as flavored and scented mixtures or incenses. From june 16, 2010 the synthetic cannabinoids JWH-018, JWH-073 and CP 47, 497 have been included in tables containing narcotic or psychotropic substances subject to the supervision and control under Article 14 of Republic Presidential Decree 309/90 and subsequent updates.

In conclusion, there is a need for more information about the full chemical composition of "SPICE" and little is known about the pharmacology and toxicology of the herbs which are declared to be contained in the "SPICE" products. Therefore, at the moment, it is impossible to draw definitive conclusions regarding the safety of the consumption of these prod-

ucts and their impact on the public health.

Rather, the true content of these products is questioned, since it appears that some of the ingredients listed on labels are not actually present in the "SPICE" products, which instead contain undeclared active compounds. It is generally accepted by the scientific community that the pharmacological and psychoactive effects described by users of "SPICE" are due in fact to the addition of synthetic cannabinoids, not mentioned on the label. This fact raises the suspicion that there may be a deliberate marketing strategy to represent these products as "natural" and therefore an attempt to mislead the consumer with a false presentation.

References

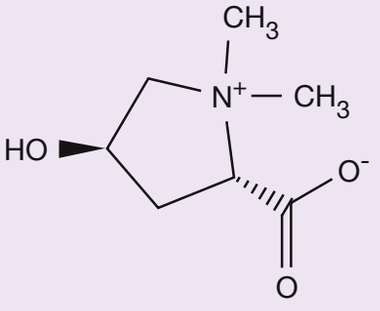
1. AUNG MM, GRIFFIN G, HUFFMAN JW, WU M, KEEL C, YANG B, SHOWALTER VM, ABOOD ME, MARTIN BR. Influence of the N-1 alkyl chain length of cannabimimetic indoles upon CB(1) and CB(2) receptor binding. Drug Alcohol Depend. 2000; 60: 133-40.
2. EMCDDA. Understanding the 'Spice' phenomenon, Thematic papers, European Monitoring Centre for Drugs and Drug Addiction (2009). http://www.drugsandalcohol.ie/12597/1/Understanding_the_Spice_phenomenon.pdf
3. MECHOULAM R, FEIGENBAUM JJ, LANDER N, SEGAL M, JÄRBE TU, HILTUNEN AJ, CONSROE P. Enantiomeric cannabinoids: stereospecificity of psychotropic activity. Experientia 1988; 44: 762-764.
4. GLASS M, NORTHUP JK. Agonist selective regulation of G proteins by cannabinoid CB1 and CB2 receptors. Mol Pharmacol. 1999; 56: 1362-1369.
5. OTTANI A, GIULIANI D. HU 210: a potent tool for investigations of the cannabinoid system. CNS Drug Reviews 2001; 7: 131-145.
6. JIANG W, ZHANG Y, XIAO L, VAN CLEEMPUT J, JI SP, BAI G, ZHANG X. Cannabinoids promote embryonic and adult hippocampus neurogenesis and produce anxiolytic- and antidepressant-like effects', J Clin Invest. 2005; 115: 3104-3116.
7. PERTWEE RG. The therapeutic potential of drugs that target cannabinoid receptors or modulate the tissue levels or actions of endocannabinoids. AAPS J. 2005; 24; 7: E625-54.
8. HUFFMAN JW, THOMPSON AL, WILEY JL, MARTIN BR. Synthesis and pharmacology of 1-Deoxy Analogs of CP-47,497 and CP-55,940', Bioorg Med Chem. 2008; 16: 322-335.
9. COMPTON DR, JOHNSON MR, MELVIN LS, MARTIN BR. Pharmacological profile of a series of bicyclic cannabinoid analogs: classification as cannabimimetic agents. J Pharmacol Exp Ther. 1992; 260: 201-209.
10. COMPTON DR, RICE KC, DE COSTA BR, RAZDAN RK, MELVIN LS, JOHNSON MR, MARTIN BR. Cannabinoid structure–activity relationships: correlation of receptor binding and in vivo activities. J Pharmacol Exp Ther. 1993; 26: 218-226.
11. WEISSMAN A, MILNE GM, MELVIN LS JR. Cannabimimetic activity from CP-47,497, a derivative of 3-phenylcyclohexanol. J Pharmacol Exp Ther. 1982; 223: 516-523.
12. HUFFMAN JW, DUNCAN SG. Synthesis and pharmacology of the 1',2'-dimethylheptyl-Δ8-THC isomers: exceptionally potent cannabinoids. Bioorg Med Chem Lett. 1997; 7: 2799-2804.
13. HUFFMAN JW, SZKLENNIK PV, ALMOND A, BUSHELL K, SELLEY DE, HE H, CASSIDY MP, WILEY JL, MARTIN BR. (2005), '1-Pentyl-3-phenylacetylindoles: a new class of cannabimimetic indoles. Bioorg Med Chem Lett. 2005; 15: 4110-4113.
14. HUFFMAN JW. Cannabimimetic indoles, pyrroles, and indenes: structure-activity relationships and receptor interactions. in Reggio, P. H. (ed.), The cannabinoid receptors, Humana Press, Totowa, NJ (2009).
15. ZIMMERMANN US, WINKELMANN PR, PILHATSCH M, NEES JA, SPANAGEL R, SCHULZ K. Withdrawal phenomena and dependence syndrome after the consumption of "Spice Gold". Dtsch Arztebl Int. 2009; 106: 464-467.
16. http://www.emcdda.europa.eu/publications/drug-profiles/synthetic-cannabinoids
17. HOWLETT AC, BARTH F, BONNER TI, CABRAL G, CASELLAS P, DEVANE WA, FELDER CC, HERKENHAM M, MACKIE K, MARTIN BR, MECHOULAM R, PERTWEE RG. International union of pharmacology. XXVII. Classification of cannabinoid receptors. Pharmacol Rev. 2002; 54: 161-202.
18. AUWÄRTER V, DRESEN S, WEINMANN W, MÜLLER M, PÜTZ M, FERREIRÓS N. Spice and other herbal blends: harmless incense or cannabinoid designer drugs?' J Mass Spectrom. 2009; 44: 832-837.
19. HUFFMAN JW, MABON R, WU MJ, LU J, HART R, HURST DP, REGGIO PH, WILEY JL, MARTIN BR. 3-Indolyl-1-naphthylmethanes: new cannabimimetic indoles provide evidence for aromatic stacking interactions with the CB1 cannabinoid receptor. Bioorg Med Chem. 2003; 11: 539-549.
20. TESKE J, WELLER JP, FIEGUTH A, ROTHÄMEL T, SCHULZ Y, TRÖGER HD. Sensitive and rapid quantification of the cannabinoid receptor agonist naphthalen-1-yl-(1-pentylindol-3-yl)methanone (JWH-018) in human serum by liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2010 Mar 18. [Epub ahead of print].

Canavalia maritima
(beach bean)

Name: *Canavalia maritima (sin. Canavalia rosea)*
Family: *Fabaceae*
Genus: *Canavalia*
Species: *Canavalia maritima*
Synonims: Bay Bean, Beach-bean, jackbean, maunaloa, puakauhi, wonderbean, Friol de la Playa
Origin: Spread on the sandy coasts of the tropical regions of the Asiatic, African and American continent
Active compounds: L-Betonicine, medicarpin

The *Canavalia maritima* is a perennial, herbaceous plant that can resist the dry conditions of the tropical coasts. Betonicine has been isolated from the *Canavalia maritima*, but there is no evidence that this compound has hallucinogenic activity. In addition, the plant containes a series of protein components, such as a specific lectin isolated from the seeds of the plant [1].

Chemical formula and physico-chemical properties of the active compounds [2,3]



Name: L-Betonicine.
Molecular formula: $C_7H_{13}NO_3$ (molecular weight = 159.2).
Systematic name: (2S-trans)-2-Carboxylate-4-hydroxy-1,1-dimethylpyrrolidinium
CAS registry number: 515-25-3.
Melting point: 246-248°C.
UVmax: no data in the literature.
Solubility: water or warm alcohol, slightly soluble in cold alcohol, insoluble in benzene, ether, chloroform.

Name: Medicarpine.
Molecular formula: $C_{16}H_{14}O_4$ (molecular weight = 159.2).
Systematic name: (6aR-cis)-6a,11a-dihydro-9-methoxy-6H-benzofuro(3,2c)(1)benzopiran-3-ol.
CAS registry number: 32383-76-9.
Melting point: 128°C.
UVmax: 207, 282, 287 and 310 nm.
Solubility: no data in the literature.

Historical use

The history of *Canavalia maritima* can be traced to XVIII century when it was described as food source for the British explorer, captain James Cook and his crew during their trip around the world between 1768 and 1771 [1].
There is no mention of its use as hallucinogen during the sacred rites of the primitive societies, even though its seeds have been found in Mexican graves (in Oaxaca and in Yucatan) and in Perù, in sites dated between 300 before Christ and 900 after Christ [4].

Current use

There are different direct and indirect uses of *Canavalia maritima*. The seeds are widely consumed by humans as well as animals as a source of protein in the West African countries and in Nigeria [5]. The fresh pods and the seeds (boiled or roasted) are consumed in the North of Australia. In the Indian coast of the Karnataka region, the fishermen consume occasionally the fresh pods. The leaves are used as food for animals such as hare, rabbits and cattle. The root infusion is used for the treatment of pain, rheumatism and leprosy. The decoction is used for the treatment of tuberculosis. The leaves help to a lleviate the pain and the healing of burns. The flowers, fresh and dry, are used for garnish and flavor [6]. In South America and on the coasts of the Gulf of Mexico the *Canavalia maritima* beans are swallowed and the dry leaves smoked as marijuana substitutes [1]. At present time, this plant is of interest as one of compounds contained in the "SPICE" mixture.

Legislation

There are no particular restrictive measures in Europe or in the USA with respect to the plant or its active compounds. In Italy, neither betonicine nor medicarpine and the whole plant or parts of it are subjected to any type of legislative control.

Pharmaco-toxicological properties

Lectine, a protein isolated from *Canavalia maritima* acts as a relaxant of the activity on the vascular smooth muscle [7].
Medicarpin inhibits the proliferation of the HeLa cells (cervical epithelial carcinoma cells) and induces apoptosis. This finding has opened interesting perspectives for further studies of pterocarpin-similar compounds extracted from *Canavalia maritima* as antitumoural therapeutic agents [8].
It has been suggested that, given the nutritive properties of its seeds, *Canavalia maritima* might be useful in counteracting the reduced protein intake in hyperlipidemic subjects [9].

Toxicity

There is no data regarding the toxicity of the active compounds of *Canavalia maritima*.

Adverse Effects

There is no data regarding the adverse effects of *Canavalia maritima*.

Pharmacological interactions

Pharmacological interactions were not reported.

Effects in pregnancy

There is no data regarding the effects of *Canavalia maritima* use in pregnancy or during lactation.

Analytical determinations

There are no analytical methods described for the detection and quantitation of the active compounds of *Canavalia maritima* in biological fluids of eventual consumers nor in the plant or parts of it.

References

1. SEENA S, SRIDHAR KR. Nutritional and microbiological features of little known legumes, *Canavalia cathartica* Thouars and *C. maritima* Thouars of the southwest coast of India. Current Sci. 2006; 90: 1638-1650.
2. http://toxnet.nlm.nih.gov/
3. THE MERCK INDEX An Enciclopedia of chemicals, drugs, and biologicals. 16Th Ed. Merck & Co., Inc. 2006.
4. www.drugs-forum.com/forum/archive/index.php/t-13352.html
5. ABBEY BW, IBEH GO. Functional properties of raw and heat processed brown bean (*Canavalia rosea* DC.) flour. J Food Sci. 1987; 52: 406-408.
6. BHAGYA B, SRIDHAR KR. Ethnobiology of coastal sand dune legumes of Southwest coast of India. IJTK. 2009; 8: 611-620.
7. GADELHA C, MORENO F, SANTIGADELHA T, CAJAZEIRAS J, MATIAS DA ROCHA B, ASSREUY A, MOTA M, VIEIRA PINTO N, MEIRELES A, BORGES J, FREITAS B, CANDURI F, SOUZA E, DELATORRE P, CRIDDLE D, FILGUEIRA DE AZEVEDO W, CAVADA B. Native crystal structure of a oxide-releasing lectin from seeds of *Canavalia maritima*. J Struct Biol. 2005; 152: 185-194.
8. XU MJ, HUANG XP, LI M, SUN W, CUI JR, LIN WH. Cytotoxic and proapoptotic activities of medicarpin from *Canavalia maritima* (Aubl.) via the suppression of NFκB activation in HeLa cells. J Chinese Pharm Sci. 2009; 18: 331-336.
9. BHAGYA B, SRIDHAR KR, RAVIRAJA NS, YOUNG CC, ARUN AB. Nutritional and biological qualities of the ripened beans of Canavalia maritima from the coastal sand dunes of India. C R Biol. 2009; 332: 25-33.

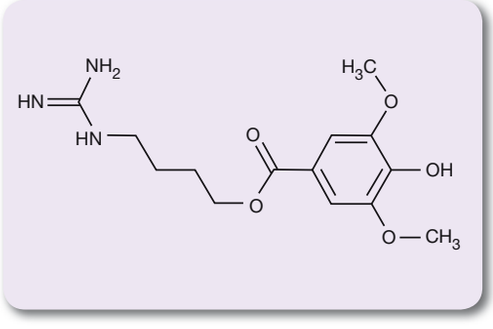
Leonotis leonurus
(lion's tail)

Name: *Leonotis leonurus*
Family: *Lamiaceae*
Genus: *Leonotis*
Species: *Leonotis leonurus*
Synonims: Lion's tail, Wild Dagga
Origin: South Africa
Active compounds: leonurine, marrubiine

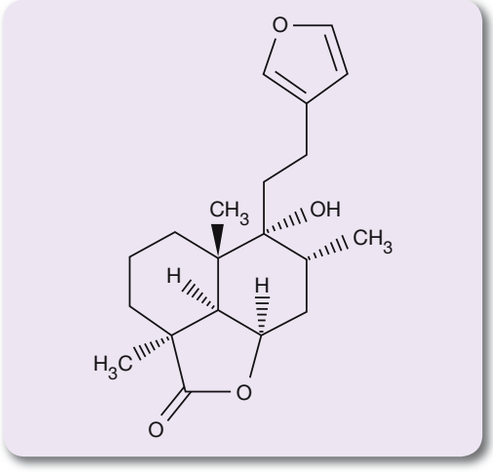
The *Leonotis leonurus* belongs to the Lamiaceae family and grows wild in South Africa. It is a shrub that grows between 2 and 5 metres in height with a strong smell [1]. The family *Leonotis* frequently associated with the *Cannabis* because of the African term "dagga", is often listed as a mild narcotic and hallucinogen, although such properties seem rather insignificant [2].

The *Leonotis leonurus* contains laudanum-type diterpenoids (marrubiine), tannins, chinones, saponine, alkaloids (leonurine) [1,4] and triterpenic steroids [1]. Premarrubiine- a precursor of marrubiine, has not been found [3], while there are volatile oils (0,15-0,18 %) which are responsible for the particular smell and a "nauseating vapour" that emanates from the dry leaves when they are smoked [5].

Chemical formula and physico-chemical properties of the active compounds [6,7]



Name: Leonurine.
Molecular formula: $C_{14}H_{21}N_3O_5$ (molecular weight = 311.3).
Systematic name: 4-(diaminomethylidenamino)butyl-4-hydroxy-3,5-dimethoxybenzoate.
CAS Registry number: 24697-74-3.
Melting point: 193-194°C (as hydrochloride monohydrate).
UVmax: 265, 343 nm.
Solubility: no data in the literature.



Name: Marrubiine.
Molecular formula: $C_{20}H_{28}O_4$ (molecular weight = 332.4).
Systematic name: 2H-naphto(1,8-bc)furan-2-one,6-(2-(3-furanil) ethyl)decahydro-6-hydroxy-2a,5a,7-trimethyl-,(2aS-(2a-α, 5a-β, 6α, 7α, 8a-α, 8b-α)).
CAS Registry number: 465-92-9.
Melting point: 159-162°C.
UVmax: no data in the literature.
Solubility: no data in the literature.

Historical use

There are many examples in the literature regarding the use of *Leonotis leonurus* by the African populations within the realm of traditional medicine [8]. The stalks, the leaves and flowers are the principal used parts [9,10]. Among the Zulu population, the infusion of leaves pounded in cold water was poured through the nostrils to alleviate febrile headaches. Infusions prepared by mixing in hot water the *Leonotis leonurus* roots with roots or pulp of the green fruit of *Strychnos spinosa* and other plants were used as emetics for snake bites [11]. The *Leonotis leonurus* leaves are traditionally smoked to alleviate epileptic seizures [1,3].

Current use

Infusions and decoctions of leaves and stalks, tinctures obtained from the flowers of *Leonotis leonurus* are used in the treatment of cough, cold, influenza, bronchitis, hypertension and headaches [3]. The decoctions are applied externally for the treatment of eczema, skin diseases, itches and muscular cramps [3]. Some consider the shrub as a weak drug with sedative effects of small therapeutic value [11].

The product is advertised on Internet as a substitute of hemp [12]. The leaves are smoked, even if smokers comment negatively on the harsh taste of the smoke. The side effects are reduced when the flowers are also smoked [13]. Following a moderate dose of smoked leaves (3-4 grams), the users report on being in a daze, dizzy, light euphoria and reduced stress. Similar effects can be observed when smaller doses of dry flowers are used. Higher doses of leaf material (8 grams or more) can induce light auditory visions and / or hallucinations and an increased euphoria [13].

Legislation

There is no knowledge of particular restrictive measures in Europe or in the USA to use the plant or its active compounds. In Italy, neither leonurine, nor the marrubiine or the plant itself, are subjected to any type of legislative control.

Pharmaco-toxicological properties

Although the mechanism of the pharmacological action of the diterpenoids (marrubiine) remains unclear [14], similar compounds which are part of *Marrubium vulgare* have been used in phytomedicine for the treatment of the wet cough and bronchial diseases [15].

The anticonvulsive activity of an aqueous extract of the dry leaves of *Leonotis leonurus* has been demonstrated *in vivo* in the mouse model. The activity seems to take place through a non-specific mechanism, acting more on the gabaergic system than the glutaminergic system. At the present time, it is impossible to identify one specific active component as being responsible for the anticonvulsive effect [3].

Following the intraperitoneal administration of 50-800mg/kg of the aqueous extract of *Leonotis leonurus* leaves the antinociceptive, antiinflamatory and hypoglycemiant activities have been studied. The results of this experimental study on animals indicate that the extract possesses all these properties, and they support the pharmacological findings that were suggesting the use of the plant in the management and / or control of the pain, in the articulation inflammations, and in other inflammatory conditions, such as, in adults at the treatment of the diabetes mellitus of type II [16].

The aqueous extracts of *Leonotis leonurus* leaves have shown to possess hypotensive [17] and antihelmintic activities [18].

The root extract of the plants has been studied in the rat, more *in vitro* (stimulating uterine activity) than *in vivo* (anti-implantation activity) for the effects that have a negative effect on fertility.A weak stimulating uterine activity has been demonstrated for the ethyl alcoholic extracts, but not for the aqueous extracts or extracted from n-butanol. An anti-implantation activity has been demonstrated more for the n-butanol extracts thanfor the etanol extracts, but not for the aqueous extracts [19].

Toxicity

Following administration of high doses (1600 and 3200 mg/kg) of an aqueous solution of *Leonotis leonurus* to rats, a decrease of the respiratory frequency, reduction of the motor activity, loss of the straightening reflexes and ataxia have been observed .Then administration of of 3200 mg/kg dose has been proven lethal in some animals. The symptoms observed before the death have been respiratory failure, convulsions, skeletal muscle paralysis and coma. Therefore, this extract

should be used with caution and doses higher than 3200 mg/kg should be avoided [1]. An oral sub-acute administration (400 and 800 mg/kg) of the extract does not cause any significant change of the hematologic parameters. Doses of 1600 mg/kg can cause a significant decrease of red cells, hematocrit, haemoglobin concentration, platelets, as well as white cells. The reduction of these parameters in the laboratory animals suggests that the use of this plant can cause anaemia [1]. There is no data present in the literature regarding the acute toxicity related to marrubiine and to leonurine.

Adverse Effects

There are no adverse effects reported in the literature.

Pharmacological interactions

There are no reports of possible pharmacological interactions with other compounds.

Effects in pregnancy

The use of the plant by pregnant or lactating women is not advisable.

Analytical determinations

There are no analytical methods reported for the determination of the *Leonotis leonurus* active principles neither in the plant nor in the biological fluids of eventual consumers.

References

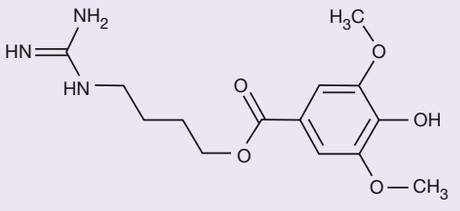
1. MAPHOSA V, MASIKA PJ, ADEDAPO AA. Safety evaluation of the aqueous extract of Leonotis leonurus shoots in rats. Hum Exp Toxicol. 2008; 27: 837-843.
2. ASCENSÃO L, MARQUES N, PAIS MS. Peltate glandular trichomes of Leonotis leonurus leaves - ultrastructure and histochemical characterization of secretions. Int J Plant Sci. 1997; 158: 249-258.
3. BIENVENU, E., AMABEOKU, G.J., EAGLES, P., SCOTT, G. AND E.P. Anticonvulsant activity of aqueous extract of Leonotis leonurus. Phytomedicine. 2002; 217: 217-223.
4. AUWÄRTER V, DRESEN S, WEINMANN W, MÜLLER M, PÜTZ M, FERREIRÓS N. 'Spice' and other herbal blends: harmless incense or cannabinoid designer drugs?. J Mass Spectrom. 2009; 44: 832-837.
5. WATT JM, BREYER-BRANDWIJK MG. The Medicinal and Poisonous Plants of Southern and Eastern Africa. [2nd Edition] 1962 London: Livingstone.
6. http://toxnet.nlm.nih.gov
7. THE MERCK INDEX An Enciclopedia of chemicals, drugs, and biologicals. 16th Ed. Merck & Co., Inc. 2006.
8. HUTCHINGS A, SCOTT AH, LEWIS G, CUNNINGHAM A. Zulu Medicinal Plants: An Inventory. University of Natal Press; 1996, pp 195-196.
9. VAN WYK, B, VAN OUDSHOORN B, GERICKE N. Medicinal Plants of South Africa. Pretoria: 2002 Briza Publications.
10. VAN WYK B, GERICKE, N. Peoples Plants - a guide to useful plants of South Africa. Pretoria: 2003 Briza Publications.
11. http://www.bolokids.com/2007/0433.htm.
12. http://en.wikipedia.org/wiki/Leonotis leonurus.
13. http://www.erowid.org/experiences/subs/exp_Leonotis_Leonurus.shtml.
14. VAN WYK BE, VAN OUDTSHOORN B, GERICKE N. Medicinal plants of South Africa, 2nd ed. Pretoria 2000 Briza Publications.
15. MARTINDALE: The complete drug reference. [34th Edition] (https://www.medicinescomplete.com/mc/martindale/current/login.htm?uri=http%3A%2F%2Fwww.medicinescomplete.com%2Fmc%2Fmartindale%2Fcurrent%2F).
16. OJEWOLE JAO. Antinociceptive, anti-inflammatory and antidiabetic effects of Leonotis leonurus (L.) R. Br. [Lamiaceae] leaf aqueous extract in mice and rats. Methods Find Exp Clin Pharmacol. 2005; 27: 257-264.
17. OJEWOLE JAO. Hypotensive effects of Leonotis leonurus acqueous extract in rats. Am J Hypertension. 2003; 16: P-2.
18. MAPHOSA V, MASIKA PJ, BIZIMENYERA ES, ELOFF JN. In-vitro anthelminthic activity of crude aqueous extracts of Aloe ferox, Leonotis leonurus and Elephantorrhiza elephantina against Haemonchus contortus. Trop Anim Health Prod. 2009 Aug 20 [Epub ahead of print].
19. DESTA B. Ethiopian traditional herbal drugs. Part III: anti-fertility activity of 70 medicinal plants. J Ethnopharm. 1994; 44: 199-209.

Leonurus sibiricus
(honeyweed)

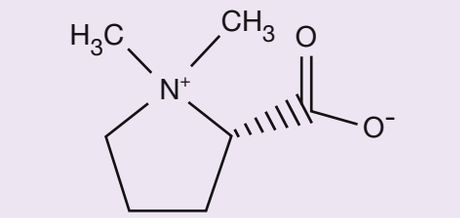
Name: *Leonurus sibiricus*
Family: *Lamiaceae*
Genus: *Leonurus*
Species: *Leonurus sibiricus*
Synonims: honeyweed, siberian motherwort, marihuanilla, I mu tsao, kacangma
Origin: native of Central Europe and South-West Asia including China, Mongolia and Russia.
Active compounds: leonurine, stachidrinae, leosibirine, isoleosibirine, leosibiricine.

Leonurus sibiricus is an herbaceous plant common in many parts of the world, including North America [1]. The plant contains alkaloids [2,3], iridoids, flavonoids, phenylpropanoid glycosides [4] and different diterpenoids [4-6]. In addition, the plant contains fatty acids (0.5 %), a resin (0.37 %) and resinic acid (0.83 %) [7].

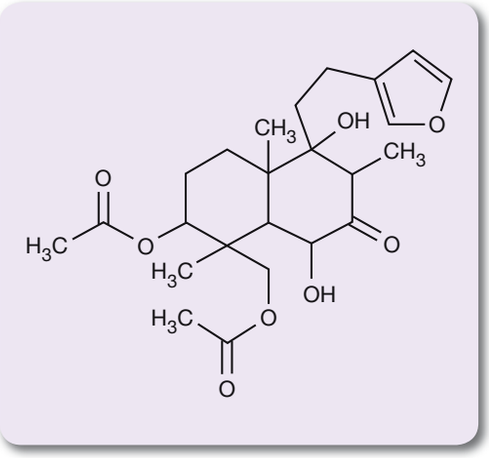
Chemical formula and physico-chemical properties of the active compounds [6-8]



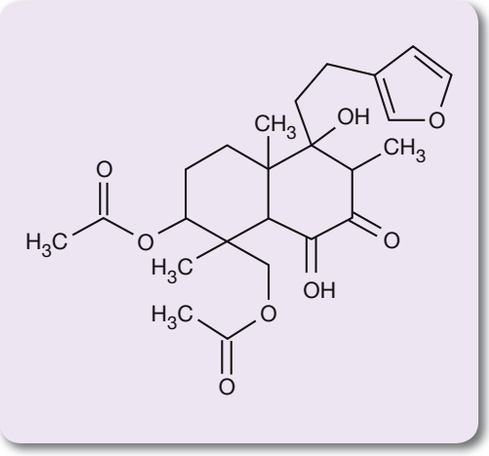
Name: Leonurine.
Molecular formula: $C_{14}H_{21}N_3O_5$ (molecular weight = 311.3).
Systematic name: 4-(diaminomethylidenamino)butyl-4-hydroxy-3,5-dimethoxybenzoate.
CAS Registry number: 24697-74-3.
Melting point: 193-194°C (as hydrochloride monohydrate).
UVmax: 265, 343 nm.
Solubility: no data in the literature.



Name: Stachydrine.
Molecular formula: $C_7H_{13}NO_2$ (molecular weight = 143.2).
Systematic name: 2-carboxy-1,1-dimethylpyrrolidinium salt.
CAS Registry number: 471-87-4.
Melting point: 235°C (anhydrous form).
UVmax: no data in the literature.
Solubility: the monohydrate is soluble in water, alcohol and dilute acids. Practically insoluble in ether, chloroform.



Name: Leosiberine.
Molecular formula: $C_{24}H_{34}O_8$ (molecular weight = 450.5).
Systematic name: 3β,19-diacetoxy-15,16-epoxy-6β,9α-dihydroxy-λ-13(16),14-dien-7-one.
CAS Registry number: no data in the literature.
Melting point: no data in the literature.
UVmax: no data in the literature.
Solubility: no data in the literature.



Name: Isoleosiberine.
Molecular formula: $C_{24}H_{34}O_8$ (molecular weight = 450.5).
Systematic name: 3β,19-diacetoxy-15,16-epoxy-6β,9α-dihydroxy-λ-13(16),14-dien-6-one.
CAS Registry number: 471-87-4.
Melting point: 235°C (anhydrous form).
UVmax: no data in the literature.
Solubility: no data in the literature.

Name: Leosibericine.
Molecular formula: $C_{22}H_{28}O_7$ (molecular weight = 404.4).
Systematic name: 8-acetoxy-9α,13,15,16-diepoxy-7-cheto-λ-14-en-19,6β-olid.
CAS Registry number: 471-87-4.
Melting point: 235°C (anhydrous form).
UVmax: no data in the literature.
Solubility: no data in the literature.

Historical use

The leaves of *Leonorus sibiricus* are traditionally picked when the plant is in flower, dried and smoked. It seems that it has a slight psychotropic and cannabis-like effect. The anecdotal literature suggests that the Indians of North America were using it in past centuries as a tonic to help them in daily work [10].

Current use

The plant is a stimulant of the airways and it has an effect on the motor endings: its roots and leaves are used as antipyretics although the leaves cause uterine contractions [11]. In the Chinese medicine the seeds are considered aphrodisiacs and the dried plant is prescribed as a tonic and as a remedy for puerperal and menstrual pathologies [12].

In the traditional medicine, the leaves are used in chronic rheumatism; their juice is antibacterial and widely applied in cases of psoriasis, scabies and chronic cutaneous eruptions. They are used also to alleviate the menstrual pain and the excessive bleeding [13].

The *Leonorus sibiricus* (Kacangma), because of its smell and taste, is also used largely as a cooking ingredient [14]. For recreational purposes, the herb and the flowers are dried to form a smokable resin. The users relate its effects somehow similar to those of cannabis [15].

Legislation

There is no knowledge of particular restrictive measures in Europe or in the USA to use the plant. In Italy, neither the active principles or the plant itself are subjected to any type of legislative control.

Pharmaco-toxicological properties

In vitro experiments have demonstrated that a decoction of products containing *Leonurus sibiricus* stimulates the H1 receptor for histamine and the α-adrenergic receptor of the uterus in mice [16]. The leonurine has a uterotonic effect already at the low concentration of 0.4 μg/ml [3].

The methyl alcoholic extract from the areal parts of *Leonurus sibiricus*, injected intraperitoneally in rats at a dose of 250 and 500 mg/kg, has a significant analgesic effect. In addition, when given orally at a dose of 200 and 400 mg/kg, it possesses anti-inflammatory activity [17].

The extracts of *Leonurus sibiricus* into different solvents (carbon tetrachloride, chloroform, acetone and methyl alcohol) have been studied for their antibacterial activity. Those in carbon tetrachloride and in chloroform have a wide spectrum antibacterial activity [18].

Toxicity

The toxicity of *Leonurus sibiricus* (kacangma) has been evaluated by administering it to male and female rats a low dose of 0.5g/kg , medium dose of 5g/kg and high dose of 25g/kg body weight [19]. The dose of 0.5 g/kg corresponds to the amount of active compounds (leonurine and stachydrine) in the type of *Leonurus sibiricus* present in homoeopathic remedies [3]. The products based on *Leonurus sibiricus* have not shown any evidence of acute toxicity, and even at high doses they did not cause death in the rat population. During the evaluation of sub-chronic toxicity, alterations of the body weight, of the organ weight and of the lipid profile parameters have been noticed, but these alterations did not reach toxicologic proportions. However, at higher doses administered to rats, a light anaemia characterised by decrease of haemoglobin, red blood cells and hematocrit has been observed.

Adverse Effects

There are no adverse effects reported in the literature.

Pharmacological interactions

There are no reports of possible pharmacological interactions with other compounds.

Effects in pregnancy

The plant stimulates uterine contractions and therefore it should not be used during pregnancy [7].

Analytical determinations

There are no analytical methods reported for the determination of the *Leonurus sibiricus* active principles neither in the plant nor in the biological fluids of eventual consumers.

References

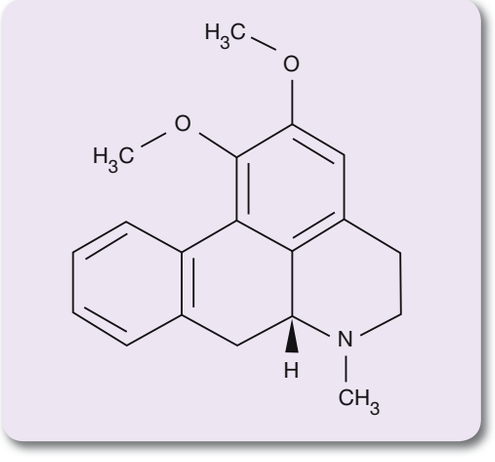
1. http://en.wikipedia.org/wiki/Leonurus_sibiricus.
2. HSU W. Chemical studies on the chinese drug, I-mu ts'ao. I. the structure of alkaloids A. Sci sinica. 1962; 9: 1341-1352.
3. YEUNG HW, KONG YC, LAY WP, CHENG KF. The structure and biological effects of leonurine. A uterotonic principle from the chinese drug, I-mu Ts'ao. Planta med. 1977; 31: 51-56.
4. MOON HT, JIN Q, SHIN JE, CHOI EJ, HAN HK, KIM YS, WOO ER. Bis-spirolabdane-Type Diterpenoids from *Leonurus sibiricus*. J Nat Prod. 2010 (in press).
5. SAVONA G, PIOZZI F, BRUNO M, RODRIGUEZ B. Diterpenoids from *Leonurus sibiricus*. Phytochem. 1982; 21: 2699-2701.
6. SATHOS M, SATHOS Y, ISOBE K, FUJIMOTO Y. Studies on the constituents of *Leonurus sibiricus*. Chem Pharm Bull. 2003; 51: 341-342.
7. KHARE CP. Indian Herbal Remedies: Rational Western Therapy, Ayurvedic and Other Traditional Usage. 2003. p 285
8. THE MERCK INDEX An Enciclopedia of chemicals, drugs, and biologicals. 14th Ed. Merck & Co., Inc. 2006.
9. http://toxnet.nlm.nih.gov
10. http://wiseplants.com/doku.php?id=siberian_motherwort
11. GHANI A. Medicinal plants of Bangladesh. Chemical constituents and uses. Dhaka: Asiatic Society of Bangladesh; 1998. p. 215.
12. KIRTIKAR KR, BASU BD. Indian Medicinal Plants, 2nd ed., vol. III. India: International Book Distributors; 1987. p. 2013.
13. ISLAM MA. Phytochemical and pharmacological screening of *Leonurus sibiricus*. B Pharm project report submitted to Pharmacy Discipline. Bangladesh: Khulna University; 2003. p. 14.
14. PIN CH, ABDULLAH A, MURUGAIYAH M. Toxicological Evaluation of Dried Kacangma Herb (*Leonurus sibiricus*) in Rats. Sains Malaysiana. 2009: 38; 499-509.
15. http://shaman-australis.com.au/shop/index.php?cPath=21_34_88.
16. SHI M, CHANG L AND HE G. Stimulating action of Carthamus tinctorius L. Angelica sinensis (Oliv.) Diels and *Leonurus sibiricus* L. on the uterus. Chin J Chin Materia Medica 1995; 20: 173-175.
17. ISLAMA MA, AHMEDA F, DASA AK, BACHAR SC. Analgesic and anti-inflammatory activity of *Leonurus sibiricus*. Fitoterapia. 2005; 76: 359-362.
18. AHMED F, ISLAM MA, RAHMAN MM. Antibacterial activity of *Leonurus sibiricus* aerial parts. Fitoterapia. 2006; 77: 316-317.
19. PIN CH, ABDULLAH A, MURUGAIYAH M. Toxicological evaluation of dried kacangma herb (*Leonurus sibiricus*) in rats. Sains Malaysiana. 2009; 38: 499-509.

Nelumbo nucifera
(indian lotus)

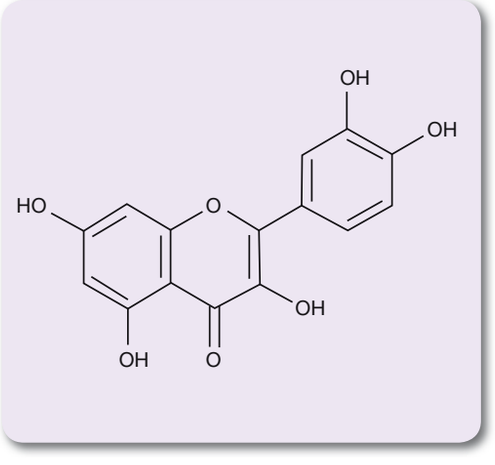
Name: *Nelumbo nucifera*
Family: *Nelumbonacee*
Genus: *Nelumbo*
Species: *Nelumbo nucifera*
Synonims: Indian lotus, Bean of India
Origin: Asia, Australia
Active compounds: nuciferine, quercetin

Nelumbo nucifera is an aquatic plant whose flower is considered sacred in the Hindu and Buddhist cultures. Very much used as cosmetic for its astringent and refrigerating properties, *Nelumbo Nucifera* has an activity similar to that of *Nymphaea caerulea*.

Chemical formula and physico-chemical properties of the active compounds [1]



Name: Nuciferine.
Molecular formula: $C_{19}H_{21}NO_2$ (molecular weight = 295.3).
Systematic name: 1,2-dimethoxy-6a-β-aporphine.
CAS registry number: 475-83-2.
Melting point: no data in the literature.
UVmax: no data in the literature.
Solubility: no data in the literature.



Name: Quercetin.
Molecular formula: $C_{15}H_{10}O_7$ (molecular weight = 295.3).
Systematic name: 3,3',4',5,7-pentahydroxyflavone.
CAS registry number: 117-39-5.
Melting point: 316°C.
UVmax: 258, 375 nm.
Solubility: glacial acetic acid, insoluble in water.

Historical use

There are no reports regarding the historical use of the plant.

Current use

The plant, known for its astringent and diuretic properties, is used in phytotherapy to control obesity [2].

Legislation

From April 2009, Russia prohibited the use of *Nelumbo Nucifera* and of any products that contain it in a mixture, such as SPICE. In the USA the use of the plant is not subjected to any restriction. In Italy, neither quercetin, nor nuciferine, or the whole plant or parts of it are subjected to any type of legislative control.

Pharmaco-toxicological properties

The (-)-nuciferine shows a pharmacological profile similar to that of chlorpromazine, although the two molecules are structurally different [3]. A study in rodents (rats and mice) has demonstrated that nuciferine (25-50 mg/kg ip) causes moderate to marked sedation, hypothermia, ptosis, reduced motility and it activates the "grooming" behavior (auto-cleaning with rubbing). The reflexes remain intact and the animals reply to the external stimuli. At elevated doses (100-150 mg/kg, ip) catalepsy sets in and the rats remain in uncomfortable positions induced artificially by the operator. Probably the nuciferine acts by blocking the dopaminergic receptors. It has been, in fact, demonstrated how the nuciferine is able to inhibit the stereotype induced by amphetamine, which, as it is well-known, is mediated by the stimulation of the dopaminergic receptors [4].

Regarding quercetin, its activity is attributed to the anti-inflamatory and antioxidant properties, as well as to the ability to protect from the free radicals. One of its principal activities noticed *in vitro* is the inhibition of catechol-O-methyltransferase, the enzyme which degrades noradrenaline; it is presumed that it could increase the thermogenesis and favor the weight loss. Other properties attributed to the quercetin are the allevation of asthma symptoms, and allergies, the prevention of low density lipoprotein (LDL) oxidation and of reducing the blood pressure [5].

Toxicity

Data regarding the acute toxicity of nuciferine [4]

In mouse: LD50 following intraperitoneal administration: 289 mg/kg.

Data regarding the acute toxicity of quercetin [1]

In mouse: LD50: following intraperitoneal administration 3000 mg/kg.
In mouse: LD50: following intravenous administration 18 mg/kg.
In mouse: LD50: following oral administration 159 mg/kg
In mouse: LD50: following subcutaneous administration 97 mg/kg
In rabit: LD50: following intravenous administration 100 mg/kg
In rats: LD50: following oral administration 161 mg/kg

Adverse Effects

There are no adverse effects for nuciferine described in the literature. For quercetin, there are no adverse effects regarding the oral administration, while if it is injected intravenously, it could cause nausea, vomiting, excessive sweating, and shortness of breath [5].

Pharmacological interactions

In the animal model, nuciferine (25mg/kg ip) significantly potentiates the sleep induced by hexobarbital (100 mg/kg ip), reduces significantly the mortality induced by amphetamine (30 mg/kg ip), and inhibits completely the stereotypic

manifestations induced by this latter drug. At last it increases by up to 50 % the anticonvulsive effect of low doses of diphenylhydantoin (2.5 mg/kg ip) [4]. Concerning quercetin, the concomitant use of fibromyelin and papain can increase its absorption [5].

Effects in pregnancy

There is no data regarding the use of the plant extracts or the active compounds during pregnancy or during lactation .

Analytical determinations

No methodologies have been reported in the international literature concerning the analysis of *Nelumbo nucifera* active principles in biological fluids of eventual consumers. However, analytical method has been published regarding the determination of nuciferine and related alkaloids in the vegetal extracts of the plant [6].

The method below reported is a brief outline useful to the investigator to set up the analysis. For specific details, it is advisable to refer to the original text.

Analysis of nuciferine, n-nornuciferine, o-nornuciferine, in leaves of *Nelumbo nucifera*

(From: LUO X, CHEN B, LIU JJ YAO S. Simultaneous analysis of n-nornuciferine, o-nornuciferine, nuciferine and roemerine in leaves of *Nelumbo nucifera Gaertn* by high-performance liquid chromatography-photodiode array detection-electrospray mass spectrometry. Anal Chim Acta 2005; 538: 129-133) [6].

The analysis was carried on the leaves of *Nelumbo nucifera* using a high-performance liquid chromatography coupled to a photodiode array and -electrospray mass spectrometer detector.

Extraction of the compounds

1 g of pulverized material is extracted twice with 20 ml of methyl alcohol-1 % hydrochloric acid mixture ((50:50 v/v) by 15 minutes sonication. After centrifugation, the supernatant is separated and reconstituted with 50 ml with the mobile phase. A volume of 2 ml of this solution is filtered (0.45 µm filter) before injection to the liquid chromatograph.

Analytical conditions

Chromatografic column : Shimadzu VP-ODS (150mm x 4,6 mm, 5 µm)
Mobile phase A: 0.1% aqueous solution of triethylamine
Mobile phase B: acetonitrile
Separation: gradient (mobile phase B: 0-15 min. 40-80%; 15-20 min. 80-95%; 20-21 min. 95-40%; 21-25 min. 40%).
Flow rate of nitrogen: 5l/min
Column temperature: 30°C
Evaporation gas temperature: 250°C
Solvation rate: 0.8l/min
Capillary voltage: 3500V
Fragmentor voltage: 20V
Detector 1: spectrophotometer with diode array detection
Detector 2: mass spectrometer with positive mode electrospray (ESI) interface.

Retention times of the tested compounds

N-nornuciferine: 5.02 minutes
O-nornuciferine: 7.70 minutes
Nuciferine: 10.79 minutes
Roemerine: 11.75 minutes

Characteristic fragments for the tested compounds

N-nornuciferine: m/z 282, 251, 219
O-nornuciferine: m/z 282, 265, 250
Nuciferine: m/z 296, 265, 250
Roemerine: m/z 280, 249

Standards

N-nornuciferine, O-nornuciferine, nuciferine and roemerine have been extracted and separated from the leaves of *Nelumbium nucifera* with 98 % purity.

Calibration curves

The stock solutions of the analytes (N-nornuciferine 35 µg/ml, O-nornuciferine 25 µg/ml, nuciferine 50 µg/ml and roemerine 12 µg/ml) were prepared in methyl alcohol and kept at-20°C. The working and calibration solutions (range of concentration: N-nornuciferine 0.35 - 35 µg/ml, O- nornuciferine 0.25-25 µg/ml, nuciferine 0.50-50 µg/ml and roemerine 0.12-12 µg/ml) were prepared daily by appropriately diluting the stock solutions with methyl alcohol.

Results

The authors of the above mentioned article produced the following quantitative results for the analyzed samples of *Nelumbo nucifera*:

N-nornuciferine: da 0.82 ± 0.02 mg/g a 1.43 ± 0.01 mg/g
O-nornuciferine: da 2.35 ± 0.01 mg/g a 4.47 ± 0.01 mg/g
Nuciferine: da 4.83 ± 0.03 mg/g a 7.61 ± 0.04 mg/g
Roemerine: da 0.31± 0.01 mg/g a 0.62 ± 0.01 mg/g

References

1. http://toxnet.nlm.nih.gov
2. http://www.erowid.org/plants/lotus/lotus_law.shtml
3. MACKO E, DOUGLAS B, WEISBACH JA. Studies on the pharmacology of nuciferine and related aporphines. Arch Int Pharmacodyn Ther. 1972; 197: 261-273.
4. BHATTACHARYA SK, BOSE R, GHOSH P, TRIPHATI VJ, RAY AB, DASGUPTA B. Psychopharmacological studies on (-) nuciferine and its Hofmann degradation product atherospermine. Psychopharmacol. 1978; 59: 29-33.
5. http://italiasalute.leonardo.it/dblog/articolo.asp?articolo=643
6. LUO X, CHEN B, LIU JJ YAO S. Simoultaneous analysis of N-nornuciferine, O-nornuciferine, nuciferine and roemerine in leaves of Nelumbo nucifera Gaertn by high-performance liquid chromatography-photodiode array detection-electrospray mass spectrometry. Anal Chim Acta 2005; 538: 129-133.

Nymphaea alba
(white lotus)

Name: *Nymphaea alba*
Family: *Nymphaeaceae*
Genus: *Nymphaea*
Species: *Nymphaea alba*
Synonims: European white waterlily, white lotus, nenuphar
Origin: commonly grows in Europe, in some North African regions and in the Middle East in fresh water [1]
Active compounds: ninfeine, nufarine [1]

There is no information about *Nynphaea alba* in the international scientific literature. The existing information, which still requires scientifical confirmations, is of anecdotal nature and obtained from unverified sources. This plant seems to have a modest medicinal interest: the active compounds are present mostly at the level of the rhizome and flowers. The rhizomes and the entire plant contain tannins, metarabic acid and two alkaloids: ninfeine and nufarine, which are told to act on the nervous system. The ninfeine seems to have sedative properties and can act as anaphrodisiac in small doses otherwise it is highly toxic and it can cause paralysis of the sensitive and motor nerves ending in death due to cardiac and respiratory arrest [2]. Even though the roots and the stalks are used in the traditional herbal preparations, the flower and the petals are more potent [1].

Chemical formula and physico-chemical properties of the active compounds

There is no data in the literature about the formula and physico-chemical properties of the active compounds.

Historical use

Plinio (23-79 After Christ) was recommending *Nynphaea alba* as a cure for erotic insomnia. The hermits of Egypt were using it as such and to preserve chastity. In the middle Ages, the *Nymphea* infusion was used to calm hysteria and nymphomania. Up to a few years ago, the plant infusion was used as an astringent in the treatment of diarrhea. Probably because of the beauty of its flowers, the *Nymphea alba* has always attracted attention and it has its place in many legends, fables and superstitions.

In the ancient Greece it symbolized the beauty and the oratorical art; it was the flower of the nymphs, of the naiads and of the spirits of the waters. For the Frisians, the emblem adorned with this flower meant glory and invincibility in war. For people of Slavonic origin, it was a talisman to fight against bad spirits and to bring good luck for long trips. Others were scattering the broken rhizomes on pastures, with the intention of protecting the cattle from harmful animals [3].

Current use

In herbal preparation, the flower infusion is used for male impotence, insomnia, nymphomania, night pollution, satyriasis, painful sexual erection, [4]. The plant is used as a component of “SPICE”.

Legislation

There are no particular restrictive measures in Europe or in the USA with respect to the plant or its active compounds. In Italy neither ninfeine, nor nufarine, or the whole plant or parts of it are subjected to any type of legislative control.

Pharmaco-toxicological properties

Ninfeine, nufarine, glycosides, resin, tannins and starch are the active compounds of the plant. The alkaloids ninfeine and nufarine seem to have the aphrodisiac properties, while the tannins act as astringents and anti-inflammatory compounds [3].

Leclerc writes: "….its active compounds belong to the class of nicotinic depressors. The majority of reactions following the use of the plant can be explained as inhibited gangliar potency. Its aphrodisiacal action might be a consequence of the medullar sedative power. The plant has strong anticonvulsive properties with the advantage of remaining a cardiac and respiratory stimulant" [4,5].

Toxicity

The use of the plant seems not to be free of danger, especially for the eventual effects onblood pressure, the cardiac and circulatory system as well as the respiratory system [6].

Adverse Effects

At very low doses, ninfeine has sedative and anaphrodisiac properties, otherwise it is highly toxic and it can cause nerve paralysis ending up in death by cardiac and respiratory arrest [6].

Pharmacological interactions

There are no documented pharmacological interactions.

Effects in pregnancy

The pregnancy effects have not yet been studied.

Analytical determinations

There are no reports describing the methodology for the analysis of the active compounds of *Nymphaea alba*.

References

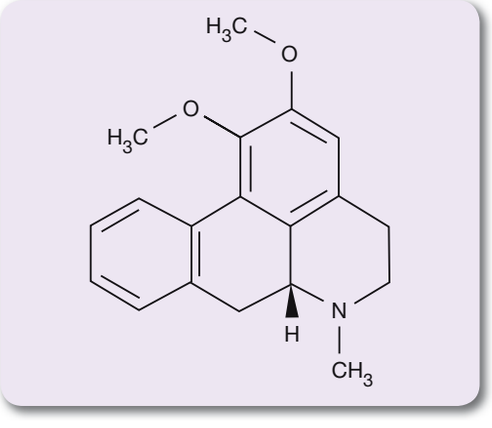
1. EMBODEN W. Transcultural use of narcotic water lilies in ancient Egyptian and maya drug ritual. J Ethnopharmacol. 1981; 3: 39-83.
2. http://toxnet.nlm.nih.gov/
3. http://en.wikipedia.org/wiki/Nymphaea_caerulea
4. http://www.statemaster.com/encyclopedia/Nymphaea-caerulea
5. http://www.erowid.org/plants/lotus/lotus_law.shtml
6. MACKO E., DOUGLAS B., WEISBACH JA. Studies on the pharmacology of nuciferine and related aporphines. Arch Int Pharmacodyn Ther. 1972; 197: 261-273.
7. BHATTACHARYA SK., BOSE R., GHOSH P., TRIPHATI VJ., RAY AB., DASGUPTA B. Psychopharmacological studies on (-) nuciferine and its Hofmann degradation product atherospermine. Psychopharmacol. 1978; 59: 29-33.

Nymphaea Caerulea
(Blue lotus)

Name: *Nymphaea caerulea*
Family: *Nymphaeaceae*
Genus: *Nymphaea* L.
Species: *Nymphaea caerulea* Savigny
Synonims: Sacred blue lily, Egyptian waterlily
Origin: along the Nile and other locations in East Africa
Active compounds: nuciferine

The flower of *Nynphaea caerulea* is constantly represented in the iconography of the ancient Egypt and many scholars think that the plant was used in sacred ceremonies to facilitate the contact between the priests and the invoked divinities [(1)]. The plant is sold as such, dried, or as a mother tincture. *Nymphaea Caerulea* shows an activity similar to that of *Nelumbo nucifera*, the Sacred Lotus, although more had been written about *Nelumbo nucifera* than about the *Nynphaea caerulea*. Both, the *Nymphaea caerulea* and the *Nelumbo nucifera* contain the alkaloid nuciferine.

Chemical formula and physico-chemical properties of the active compounds [(2)]



Name: Nuciferine.
Molecular formula: $C_{19}H_{21}NO_2$ (molecular weight = 295.3).
Systematic name: 1,2-dimethoxy-6a-β-aporphine.
CAS registry number: 475-83-2.
Melting point: no data in the literature.
UVmax: no data in the literature.
Solubility: no data in the literature.

Uso storico

The flowers of *Nymphaea Caerulea* open in the morning and fall in the water at sunset; this circadian rhythm of the plant, which follows the birth and the setting of the sun, had great importance in the ancient Egyptian mythology tying this flower to the birth of the solar divinities Atum and Ra. Its use in the ancient Egypt led to the conclusion that the plant could have narcotic properties (psychodysleptic) [(1)].The ancient Egyptians, indeed, were using the plant in sacred ceremonies. The psychoactive effects of the *Nymphea caerulea* made it probably a good candidate to be the mythical lotus plant eaten by the Lotus Eaters as described by Homer in Odyssey [(3)].

Current use

Used in aromatherapies, the *Nymphea caerulea* is considered a "divine essence", which causes euphoria, serenity and increased awareness [4].

Anecdotal sources state that doses of flowers of 5 to 10 grams induce a light stimulation, an alteration of the cognitive processes, an altered visual perception and visions with closed eyes [2]. In the modern culture, the flowers of the *Nymphea caerulea* are used to prepare different potions such as the tea and the wine of blue lotus. The recipes for such drinks suggest the maceration of the petals by soaking them up to 3 weeks. It can also be done by boiling the flowers for 10-20 minutes [4].

Legislation

From April 2009, Russia prohibited the use of *Nymphaea caerulea* and any products that contain it in a mixture, such as SPICE. In the USA the plant is not subjected to any restriction [5]. In Italy, the neither nuciferine, nor the whole plant or parts of it are subjected to any type of legislative control.

Pharmaco-toxicological properties

The (-)-nuciferine shows a pharmacological profile similar to that of chlorpromazine, although the two molecules are structurally different [6]. A study in rodents (rats and mice) has demonstrated that nuciferine (25-50 mg/kg ip) causes moderate to marked sedation, hypothermia, ptosis, reduced motility and it activates the "grooming" behavior (auto-cleaning with rubbing). The reflexes remain intact and the animals reply to the external stimuli. At elevated doses (100-150 mg/kg, ip) catalepsy sets in and the rats remain in uncomfortable positions induced artificially by the operator. Probably the nuciferine acts by blocking the dopaminergic receptors. It has been, in fact, demonstrated how the nuciferine is able to inhibit the stereotype induced by amphetamine, which, as it is well-known, is mediated by the stimulation of the dopaminergic receptors [7].

Toxicity

Data regarding the acute toxicity of nuciferine [7]

In mouse: LD50 following intraperitoneal administration; 289 mg/kg.

Adverse Effects

There are no adverse effects reported in the literature.

Pharmacological interactions

In animal model, nuciferine (25mg/kg ip) significantly potentiates the sleep induced by hexobarbital (100 mg/kg ip), reduces significantly the mortality induced by amphetamine (30 mg/kg ip), and inhibits completely the stereotypic manifestations induced by this latter drug. At last it increases by up to 50 % the anticonvulsive effect of low doses of diphenylhydantoin (2.5 mg/kg ip) [7].

Effects in pregnancy

There is no data regarding the use of the plant extracts or the active compounds during pregnancy or during lactation .

Analytical determinations

No methodologies have been reported in the international literature concerning the analysis of nuciferine in *Nymphea caerulea* or in biological fluids of eventual consumers. However, there is an analytical method for the same alkaloid extracted from *Nelumbo nucifera*. Details of the analytical methodology can be found in the monograph of *Nelumbo nucifera* [8].

References

1. EMBODEN W. Transcultural use of narcotic water lilies in ancient Egyptian and maya drug ritual. J Ethnopharmacol. 1981; 3: 39-83.
2. http://toxnet.nlm.nih.gov/
3. http://en.wikipedia.org/wiki/Nymphaea_caerulea
4. http://www.statemaster.com/encyclopedia/Nymphaea-caerulea
5. http://www.erowid.org/plants/lotus/lotus_law.shtml
6. MACKO E, DOUGLAS B, WEISBACH JA. Studies on the pharmacology of nuciferine and related aporphines. Arch Int Pharmacodyn Ther. 1972; 197: 261-273.
7. BHATTACHARYA SK, BOSE R, GHOSH P, TRIPHATI VJ, RAY AB, DASGUPTA B. Psychopharmacological studies on (-) nuciferine and its Hofmann degradation product atherospermine. Psychopharmacol. 1978; 59: 29-33.
8. LUO X, CHEN B, LIU JJ YAO S. Simoultaneous analysis of N-nornuciferine, O-nornuciferine, nuciferine and roemerine in leaves of Nelumbo nucifera Gaertn by high-performance liquid chromatography-photodiode array detection-electrospray mass spectrometry. Anal Chim Acta 2005; 538:129-133.

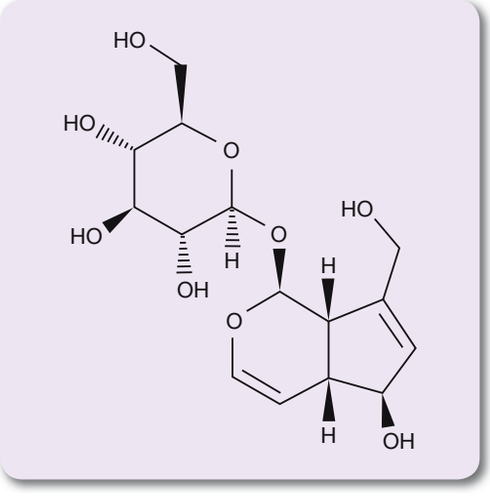
Pedicularis Densiflora

(Indian warrior)

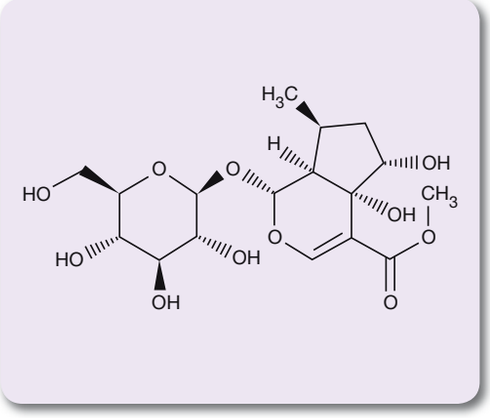
Name: *Pedicularis densiflora*
Family: *Orobanchaceae*
Genus: *Pedicularis*
Species: *Pedicularis densiflora*
Synonims: Indian warrior
Origin: California, Oregon
Active compounds: aucubin, pedicularioside

The *Pedicularis densiflora* is a perennial plant, one of the most powerful in the *Pedicularis* family.

Chemical formula and physico-chemical properties of the active compounds



Name: Aucubine.
Molecular formula: $C_{15}H_{22}O_9$ (molecular weight = 346.3).
Systematic name: (1S-(1α,4α,5α,7α))-1,4a,5,7a-tetrahydro-5-hydroxy-7-(hydroxymethyl)cyclopenta (c) pyran-1-il-β-D-glucopyranoside.
CAS registry number: 479-98-1.
Melting point: 181°C.
UVmax: no data in the literature.
Solubilità: water, methyl alcohol; insoluble in ether, chloroform, petroleum ether.



Name: Pedicularioside.
Molecular formula: $C_{17}H_{26}O_{11}$ (molecular weight = 406.3).
Systematic name: (1S-(1α,4α,5α,7α,7β)-Cyclopenta(c)pyran-4-carboxylic acid,1-(β-D-glucopyranosyl oxide)-1,4a,5,6,7,7a-hexahydro-4a,5-dihydroxy-7-methyl- methyl ester.
CAS registry number: 81203-55-6.
Melting point: no data in the literature.
UVmax: no data in the literature.
Solubility: no data in the literature.

Historical use

The native Americans (Washo) were using a mush obtained by macerating the plant on wounds, sores and swellings and were drinking a solution as a tonic obtained from the leaves. The use of the roots was very popular in the treatment of stomach pains, gastric ulcer and bloody stools. The *Pedicularis* was part of the group of vegetables used in traditional medicine for the treatment of cough and sore throat [3].

Current use

The websites which advertise " smart drugs" indicate the use of the plant as sedative when smoked. In addition, as a drink it can be used in the treatment of cough and respiratory problems. It can also have an aphrodisiac and muscular relaxation effect [4].

Legislation

There is no record of any restrictive measures in Europe and in the USA for the use of the plant or its active compounds. In Italy, neither the active ingredients of the *Pedicularis densiflora* nor the whole plant or parts of it are subjected to any type of legislative control.

Pharmaco-toxicological properties

Antitumoral properties are attributed to the Pedicularoside: studies *in vitro* have highlited the ability of the molecule to inhibit angiogenesis [5].

Toxicity

There is no data in the literature regarding the toxicity of the active compounds of *Pedicularis densiflora*.

Adverse Effects

There is no data in the literature regarding any adverse effects.

Pharmacological interactions

No pharmacological interactions have been reported.

Effects in pregnancy

The effects of the plant during pregnancy were not studied.

Analytical determinations

There are no reported methods of determination of the active compounds in *Pedicularis densiflora* as a whole plant or parts of it. However, there is a report concerning the determination of pedicularosides A and M in two plants belonging to the Pedicularis family [6].

The method below reported is a brief outline useful to the investigator to set up the analysis. For specific details, it is advisable to refer to the original text.

Determination of pedicularosides extracted from *Pedicularis densiflora*

(From: JIANG TF, OU QY, SHI YP. Separation and determination of phenylpropanoid glycosides from Pedicularis species by capillary electrophoresis. J Chromatogr A. 2003; 986: 163-167) [6].

The determination of phenylpropanoid glycosides (pedicularoside A and M) is carried out using capillary electrophoresis coupled to ultraviolet spectrophotometric detection.

Extraction of the compounds

1 gram of powder is extracted 3 times with 50 ml of methyl alcohol. The obtained solution is dried and the residue resuspended in 20ml of methyl alcohol.

Analytical conditions

Capillary: Yongnian (35 cm x 50µm I.D. x 365µm O.D.)
Mobile phase: 30 mM Borate buffer and 10% methyl alcohol (pH 9,0)
Applied voltage: 15 kV
Temperature: 25°C
Detector: spectrophotometer with ultraviolet detection (250 nm).

Retention times of the tested compounds

Pedicularoside A: 6.3 minutes
Pedicularoside M: 5.2 minutes

Standard

All the standards were bought from Beijing Chemical Reagent Plant.

Calibration curve

The standard solutions of the analytes have been prepared in methyl alcohol at a concentration of 5 mg/ml. The calibration solutions have been obtained by appropriate dilutions (range for the pedicularoside A: 20-2000 µg/ml, range for the pedicularoside M: 50-5000 µg/ml).

Results

The extracts of the analysed plants showed the following percentage of active principles: pedicularoside M 0.028 -0.13 % and pedicularoside A: 0.074 – 0.61 %.

References

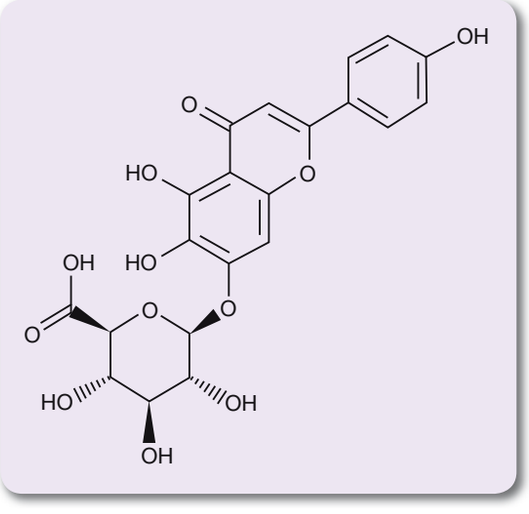
1. THE MERCK INDEX An Enciclopedia of chemicals, drugs, and biologicals. 16th Ed. Merck & Co., Inc. 2006.
2. http://toxnet.nlm.nih.gov/
3. Foster S, Hobbs C, Tory R. A field guide to Western medicinal plants and herbs. Peterson Field Guides 2002; 175.
4. http://wiseplants.com/doku.php?id=indian_warrior
5. MU P, GAO X, JIA ZJ, ZHENG RL. Natural antioxidant pedicularioside G inhibits angiogenesis and tumourigenesis in vitro and in vivo. Basic Clin Pharmacol Toxicol. 2008; 102: 30-4.
6. JIANG TF, OU QY, SHI YP. Separation and determination of phenylpropanoid glycosides from Pedicularis species by capillary electrophoresis. J Chromatogr A. 2003; 986: 163-167.

Scutellaria nana
(Dwarf skullcap)

Name: *Scutellaria nana*
Family: *Lamiacee*
Genus: *Scutellaria*
Species: *Scutellaria nana*
Synonims: Dwarf skullcap
Origin: California
Active compound: scutellarine

There is little known about the active compounds and other substances contained in this plant. Anecdotal information shows that *Scutellaria* is a plant with psychoactive properties that is used as a light sedative (anxiolytic) [1].

Chemical formula and physico-chemical properties of the active compounds



Name: Scutellarine.
Molecular formula: $C_{21}H_{18}O_{12}$ (molecular weight = 462.3).
Molecular formula: 2- Flavone, 4',5,6,7-tetrahydroxy-, 7-β-D-glucopyranoside.
CAS registry number: 27740-01-8.
Melting point: >300°C.
UVmax: 285, 335 nm.
Solubility: insoluble in water, soluble in alkaline solutions and in glacial acetic acid, slightly soluble in organic solvents.

Historical use

According to some internet sources the plant, native the South West of USA, has been used in the past centuries by the North American Indians as a sedative and in the treatment of insomnia and anxiety [3].

Current use

The plant is used as sedative and has been prescribed in some cases of epilepsy and insomnia [4]. Websites which market the smart drugs include the plant in the class of hallucinogenic herbs which, if swallowed in great quantities, can cause dazed sensations. As a matter of fact, the pharmacological properties of the active compound of the plant have not yet been clarified by systematic clinical studies [3]. Human clinical trials to prove the anxiolytic and sedative properties of the plant have not been done. In the official website of SPICE, the plant is described in a very generic way, simply specifying that it is a well known plant used traditionally by Cherokees and other tribes of native Indians in North America [4].

Legislation

In Europe and in the USA there are no restriction with respect to the use of the whole plant or its active component. In Italy, nor scutellarine, neither *Scutellaria* as a whole plant or parts of it is not subjected to any type of legislative control.

Pharmaco-toxicological properties

There is evidence in the scientific literature about a variety of plants belonging to the *Scutellaria* family to which antitumoural [5,6], antiangiogenic [7], hepatoprotective [8] , antimycotic, antibacterial and antiviral [9-11] properties are attributed. There are numerous *in vitro* studies on the antitumoural abilities of *Scutellaria litwinowii*, whose action is probably be due to the cytotoxic and apoptogenic properties of its components [12].

Toxicity

Unofficial internet sources report that an overdose of a concentrated solution of *Scutellaria* causes dizziness, stupor, confusion, spasms of the limbs, intermittent pulse, and symptoms of epilepsy [13].

Adverse Effects

Following *Scutellaria nana* intake, some cases of hepatic damage have been reported. Still, after more careful examination, it seems that the products containing the *Scutellaria* which have caused hepatic damages, were also containing *Teucrium chamaedrys*, a plant with well-known hepatotoxic properties [14-15]. In a documented case, the death of a 28-year-old young man following the ingestion of *Scutellaria*, Pau of Arch (a large native tree in the amazonic woods) and zinc, was reported. In addition, in this case it seemed that there was also a contamination of the herbal mixture taken by the patient with *Teucrium chamaedrys* [16]. The unofficial internet sources consider indeed scutellarine one of the active compounds present in *Teucrium chamaedrys* [17]. It should be mentioned that in Italy the use of any form of *Teucrium chamaedrys* is forbidden due to its hepatotoxicity [18].

Pharmacological interactions

Although not well documented, a synergistic effect with the drugs which cause drowsiness has been observed [13].

Effects in pregnancy

Unofficial Internet sources mention documented cases of toxicity in pregnancy, because of the fact that the plant can inhibit the release of corionic gonadotropine and of prolactin [13].

Analytical determinations

There are no described analytical methods for the identification of scutellarine in biological fluids of eventual consumers of *Scutellaria nana* nor in the whole plant or part of it.

References

1. http://www.tranceplants.net/Shop/product-info.php?pid146.html
2. THE MERCK INDEX An Enciclopedia of chemicals, drugs, and biologicals. 16th Ed. Merck & Co., Inc. 2006.
3. http://toxnet.nlm.nih.gov/
4. http://www.spice-gold.com/IT/spice_background_IT.html
5. LEE TK, LEE YJ, KIM DI, KIM HM, CHANG YC, KIM CH. Pharmacological activity in growth inhibition and apoptosis of cultured human leiomyomal cells of tropical plant Scutellaria barbata D Don (Lamiaceae). Env Toxicol Pharmacol 2006; 21: 70-79.
6. YU JQ, LIU HB, LEI JC, TAN WJ, HU XM, ZOU GL. Antitumor activity of chloroform fraction of *Scutellaria barbata* and its active constituents. Phytother Res. 2007; 21: 817-822.
7. WANG SS, ZHENG ZG, WENG YQ, YU YJ, ZHANG DF. FAN WH, DAI RH, HU ZB,. Angiogenesis and anti-angiogenesis activity of Chinese medicinal herbal extracts. Life Sci 2004; 74: 2467-2478.

8. LIN CC, SHIEH D, YEN MH. Hepatoprotective effect of the fraction of Banzhi-lian on experimental liver injuries in rats. J Ethnopharmacol. 1997; 56: 193-200.
9. BLASZCZYK T, KRZYZANOWSKA J, LAMER-ZARAWSKA E. Screening for antimycotic properties of traditional Chinese drugs. Phytother Res 2000; 14: 210-212.
10. YANG ZC, WANG BC, YANG, XS, WANG Q, RAN L. The synergistic activity of antibiotics combined with eight traditional Chinese medicines against two different strains of *Staphylococcus aureus*. Colloids Surf B Biointerfaces. 2005; 41: 79-81.
11. LI YL, OOI LSM, WANG H, BUT PPH, OOI VEC. Antiviral activities of medicinal herbs traditionally used in southern mainland China. Phytother Res 2004; 18: 718-722.
12. TAYARANI-NAJARAN Z, EMAMI SA, ASILI J, MIRZAEI A, MOUSAVI SH. Analyzing cytotoxic and apoptogenic properties of scutellaria litwinowii root extract on cancer cell lines. Evid Based Complement Alternat Med. 2009 in press.
13. http://www.drugs.com/npc/scullcap.html
14. MCGUFFIN M, HOBBS C, UPTON R, GOLDBERG A. American Herbal Product Association's Botanical Safety Handbook. Boca Raton, FL: CRC Press, 1997, 105.
15. HULLAR TE, SAPERS BL, RIDKER PM, JENKINS RL, HUTH TS, FARRAYE FA. Herbal toxicity and fatal hepatic failure [letter]. Am J Med 1999; 106: 267-268.
16. BROWN D. A case of fatal liver failure associated with herbal products. Healthnotes Rev Complement Integrative Med 1999; 6: 176-177.
17. http://it.wikipedia.org/wiki/Teucrium_chamaedrys
18. Italian Ministry of Health – Decree may 30 2003: Prohibition of use of *Teucrium Chamaedris*. (official gazette n. 185 from august 11, 2003)

Zornia Latifolia
(Maconha Brava)

Name: *Zornia Latifolia*
Family: *Fabaceae*
Genus: *Zornia*
Species: *Zornia Latifolia*
Synonims: Maconha Brava, Food of the Gods
Origin: South America (Argentina, Bolivia, Brasil, Columbia, Ecuador, Paraguay, Peru)
Active compounds: there are no active compounds reported in the literaure.

Zornia latifolia is a perennial plant, known to have an effect on the central nervous system. The common name Maconha Brava means "false marijuana" and indeed the dry leaves and the blossoms of *Zornia Latifolia* are used as hallucinogen by the Brazilian Indios. Local legends tell that this "wild herb" was smoked by the ancient gods [(1)].

Chemical formula and physico-chemical properties of the active compounds
Active compounds are not reported in the literature.

Historical use
The dried leaves were used by the Brazilian Indians to achieve visual hallucinations.

Current use
The websites that market this plant mention properties similar to those of cannabis. The dried leaves and the seeds are the part of the plant which are smoked to obtain the hallucinogenic effects [(1)].

Legislation
There is no knowledge of particular restrictive measures in Europe or in the USA to use the plant. In Italy, *Zornia Latifolia* is not subjected to any type of legislative control.

Pharmaco-toxicological properties
The international literature does not report any information regarding the pharmaco-toxicological properties of the plant, of its extracts or its active compounds. The consumers use the plant in mixtures of dried herbs that can be smoked (for example: SPICE) to obtain cannabis-like properties [(2)].

Toxicity
There is no data regarding the toxicity of the active compounds.

Adverse Effects
Some websites report perception changes, euphoria, burning eyes, an increase in cardiac frequency, dry mouth, etc.

Pharmacological interactions
There are no reported pharmacological interactions.

Effects in pregnancy
The effects in pregnancy have not been studied.

Analytical determinations

There is no reported methodology for the determination of the active compounds or the plant itself.

References

1. SCHULTES R.E. & A. HOFMANN, 1979, *Plants of the Gods*, McGraw-Hill, New York, NY. Reprinted in 1992, Healing Arts, Rochester, VT.
2. http:// herbalistics. com .au/shop
3. http://www.drugs-forum.com/forum/showthread.php?t=12937