

AFRICAN HERBAL PHARMACOPOEIA

Edited by :

Brendler T., Eloff J.N., Gurib-Fakim A., Phillips L.D.

AAMPS
Association for
African Medicinal Plants Standards
www.aamps.org

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Foreword

This book is dedicated to all the African healers, whose recorded and unrecorded wisdom and experience forms the basis of scientific enquiry into the manufacture and use of traditional herbal remedies in African Society. We would also like to pay homage to all those scientists and researchers who have dedicated their careers to the study of African medicinal plants and herbal medicine.

Although Sub-Saharan Africa and the Indian Ocean Islands contain about 60,000 of the world's higher plant species, roughly a quarter of the world's total, less than 8% of the 1,100 medicinal plants commercialised internationally are African in origin. This situation has largely arisen because information on the traditional uses of African plants has seldom been written down but rather transferred orally from generation to generation by story tellers and traditional healers.

Medicinal plants constitute an extremely important resource for the development of the global pharmaceutical, cosmetic, and fragrance industry. More than 40% of licensed drugs are originally of plant origin. In Africa, indigenous medicine is usually the most important form of treatment, a culturally accepted practice that forms part of a diverse local health system. WHO estimate that 80% of the world's population depends on medicinal plants for their primary healthcare. In much of rural Africa it is the only form of therapy that exists.

African herbal medicine relies more on wild harvested plants than any continent on earth yet the sustainability of this indigenous resource is increasingly endangered. It has been estimated that the continent has some 216 million hectares of closed forest with a calculated loss through deforestation of 1% per annum (The global average loss rate is 0.6%) This means Africa has the highest rate of deforestation in the world. The direct impact of this massive loss of bio-diversity is that many medicinal plants will become extinct even before they are documented.

Loss of plants also means loss of accompanying traditional knowledge. The value of countless generations of observations of the application of certain plants on human and animal disorders is impossible to value, especially in relation to present day global bio-prospecting activities. The preparation of the African Herbal Pharmacopoeia hence comes at a critical moment in the history of African herbal medicine.

In 2000 a Commonwealth Medicinal Plants Business Forum was held in Cape Town with delegates from across Africa. One of the major constraints to the growth of a modern African phytomedicines industry identified by the Forum is the lack of suitable technical specifications and quality control standards. This makes it extremely difficult for buyers whether national or international to evaluate the safety and efficacy of plants and extracts or compare batches of product from different places or from year to year. This is in marked contrast with Europe and Asia where traditional methods and formulations have been recorded and evaluated both at the local and national level. Consequently the level of world trade in European and Asian medicinal plants and extracts is far greater than those from Africa. This situation will not change unless Africa also publishes an internationally recognised herbal pharmacopoeia prepared by researchers of international standing and integrity.

It was against this background that the EU-ACP Centre for the Development of Enterprise in 2003 approved a project to identify the fifty most important African Medicinal Plants in commerce and to prepare a set of quality control standards/monographs for the chosen species. The complete set would include the key African plants presently traded regionally or internationally as well as plants considered

as having long term future potential. These standards were to combine data used for scientific plant monographs with information normally contained in trade specifications and quality control data sheets. The resulting monographs are designed for both local users of medicinal plants as well as the international pharmaceutical industry.

Six African institutions were short listed to prepare the standards. In 2004 the contract was awarded to the Phytomedicine Programme of the University of Pretoria on the understanding that scientists from across Africa were recruited to help prepare monographs in their respective regions.

There have been previous attempts to develop herbal pharmacopoeias in Africa. These had three main limitations. Firstly they were usually regional in scope, secondly plant selection was largely random, often including plants of non-African origin, and thirdly the research work was usually conducted by small groups of academic researchers with limited input from growers, processors and end users of African medicinal plants and extracts. If the AAMPS Pharmacopoeia was to be accepted throughout Africa and beyond it was vital that as many stakeholders as possible be involved in the preparation process and leading national and international experts on African herbal medicine be consulted.

With funding from the European Union a large number of role players were brought together to determine what components should be included in each monograph and to select the most important species to research. Despite large regional variations it was very encouraging to note that amongst the scientists, healers, harvesters, exporters and importers attending these meetings there was little disagreement as to which species to select and what components should be included in the individual standards/ monographs.

It was during one of these consultation meetings at Centurion in May 2005 that delegates decided that the preparation of the **African Herbal Pharmacopoeia (AfrHP)** was of sufficient importance to establish an organisation dedicated specifically to this task. As a result the **African Association of Medicinal Plants Standards (AAMPS)** was set up in Mauritius with a mandate, enshrined in the **Centurion Declaration**, to prepare and disseminate a comprehensive, internationally acceptable Pharmacopoeia and to build a living database to continuously upgrade and expand this work.

AAMPS monographs provide comprehensive and up to date botanical, biochemical, pharmacological and commercial information on more than fifty of the most important medicinal plants used in Africa. They have been prepared by many of Africa's leading scientists and subsequently reviewed by an international expert panel. Plant samples for each monograph were sourced from around the continent and subject to biochemical analysis and fingerprinting. After evaluation by the AAMPS Scientific Committee several additional features were suggested including the micro morphology of the plant material, distribution maps, HPLC traces and TLC chromatograms of adulterants. While the printed version of the Pharmacopoeia does not include all these features they will be added at a later stage. The scope and quality of AAMPS herbal monographs are, however, similar to those prepared in Europe, North America and Asia. Each monograph contains information required by producers, collectors and traders in medicinal plants and extracts as well as technical data needed by researchers, manufacturers and practitioners.

It has always been AAMPS intention that the African Herbal Pharmacopoeia is a living database which could be accessed on the World Wide Web. One important advantage of the living database is that gaps in our knowledge can be identified as research projects and new information and new plants added. This makes it possible to continuously expand and enhance the African Herbal Pharmacopoeia and keep it relevant and up to date.

The preparation of the African Herbal Pharmacopoeia has taken more than six years of dedicated work by thirty-one experts in African medicinal plants and herbal medicine. We are fully aware that there are gaps in our knowledge and understanding. Some plants have been the subject of intensive study over

many years; others have received little or no attention. For some monographs we obtained help from the undisputed world authorities in the field. In others such in depth expertise was simply not available to us. This made it very difficult to prepare monographs to a uniform standard. In some instances significant gaps meant we had to leave out certain monographs that were to be included in the AfrHP. These will now be included in the living database or in later versions of the printed Pharmacopoeia.

There is clearly much more work to be done both in expanding and upgrading the AfrHP and equally important promoting these standards nationally and internationally. Our dream is that this work will not only generate income and employment for all those working in Africa in this sector but also unlock the health benefits of these unrealised medicinal plants.

Thomas Brendler, Kobus Eloff, Ameenah Gurib-Fakim, Denzil Phillips
Editors. June 2010

THE CENTURION DECLARATION

“We, the undersigned, with a view to improving the health, safety, welfare and livelihood of the people of Africa, hereby declare the intention:

To establish an Association with a registered office in Mauritius to support the African herbal industry and regulatory authorities by developing quality control and quality assurance standards for African medicinal plants and herbal medicines.

To offer membership of the newly formed association to any individual or organisations dedicated to the establishment of such standards and to the creation of an African Herbal Pharmacopoeia

To jointly review and promote the 20 African herbal profiles currently being prepared by the Phytomedicine Programme, University of Pretoria. These herbal profiles include plants of African origin, which are considered of regional and international importance and which can be sustainably sourced in Africa.

To raise funds to prepare and disseminate a further 30 African herbal profiles selected by the founding members of the association at the Centurion Lake Hotel review meeting on May 2005.

To prepare and publish an African Herbal Pharmacopoeia as a living database drawn initially from the 50 herbal profiles and to promote its use nationally and internationally.

To help obtain international acceptance of these herbal standards and the subsequent herbal pharmacopoeia and to lobby health authorities throughout Africa to use such standards to facilitate licensing safe and effective herbal medicines in Africa

To promote capacity building in Africa for the establishment of regional training centres for certification, compliance and quality control of herbal medicines.

To promote the safe, sustainable national and international trade in the fifty-one profiled African medicinal plants

To carry out any other activities deemed by the members of the association to further the objectives of the Association.

Signed 29th May 2005

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Monographs



Acacia senegal

GENERAL DESCRIPTION

Scientific Name with Author: *Acacia senegal* Willd.

Synonyms: *Mimosa senegal* L., *Acacia verek* Guill. et Perr., *Senegalia senegal* (L.) Britton

Family: Leguminosae: Mimosoideae

Vernacular Names: Akovia, aiti, bulbi, gum acacia, gum arabic tree, gum tree, gommier, gommier blanc, kikwata, kouait, mgunga, patuki, shagar samgh arabi, three-thorned acacia, white gum-acacia.

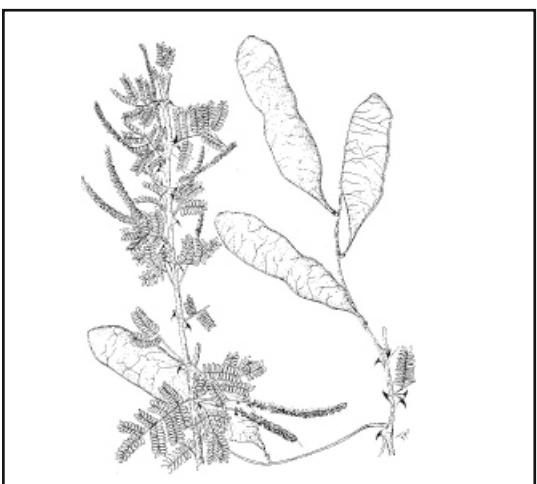
Botanical Description: This deciduous shrub or small to medium-sized tree can reach a height of up to 15 m tall. The bark is yellowish-brown to purplish-black, rough or smooth, papery and sometimes peeling off in strips, deeply fissured and blackish on old trees. The crown is slightly rounded and somewhat spreading. The branches are spindly; branchlets glabrous to densely pubescent, with prickles just below the nodes. Leaves alternate, bipinnate; petiole, rachis sparingly to densely dressed with spreading hairs but rarely glabrous. Inflorescence is an axillary spike, reaching up to 12 cm long, axis densely pubescent or glabrous. The bisexual flowers are white or cream in colour; corolla 3 – 4 mm long; stamens numerous. Fruit is an oblong pod, greyish brown in colour, dehiscent and containing up to 7 seeds. The latter are horseshoe-shaped. Sapwood pale to creamy yellow, heartwood pale to dark brown.

Origin and Distribution: *Acacia senegal* is widely distributed in the drier parts of tropical Africa, from Senegal and Mauritania in the west to Eritrea and Ethiopia in the northeast and to South Africa in the south. Of the 4 recognized varieties, var. *senegal* is the most widespread and is found throughout the area of distribution of *Acacia senegal* except along the west coast of central and southern Africa; outside Africa it occurs in Oman, Pakistan and India and has been introduced

into Egypt, Australia, Puerto Rico, and the Virgin Islands. This variety is the major source of gum arabic. *Acacia senegal* var. *kerensis* Schweinf. occurs in Ethiopia, Somalia, Uganda, Kenya, and Tanzania; var. *leiorhachis* Brenan throughout eastern Africa from Ethiopia to South Africa; var. *rostrata* (Sim) Brenan in the same area and in Namibia and Angola, and possibly also in Oman (Schmelzer & Gurib-Fakim, 2008).



Plant Part Used: Gum exudates. Gum arabic is defined as a dried exudation obtained from the stems and branches of *Acacia senegal* or related species of *Acacia* (Billaud et al., 1996).



ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses: The root decoction is used in East Africa against constipation and gonorrhoea while the decoction of the bark is drunk to treat diarrhoea and stomach disorders (Kokwaro, 1993). Bark, leaves, and gum are antitussive, expectorant and astringent, and used to treat colds, ophthalmia, diarrhoea, haemorrhages, coughs, dysentery, gonorrhoea, haemorrhage, sore throat, typhoid, urinary tract infection. In causing partial destruction of many alkaloids including atropine, hyoscyamine, scopolamine, homatropine, morphine, apomorphine, cocaine, and physostigmine, gum arabic might be viewed as a possible antidote (Burkhill, 1995). The seed contains a fat which is used both in medicine and for soap making.

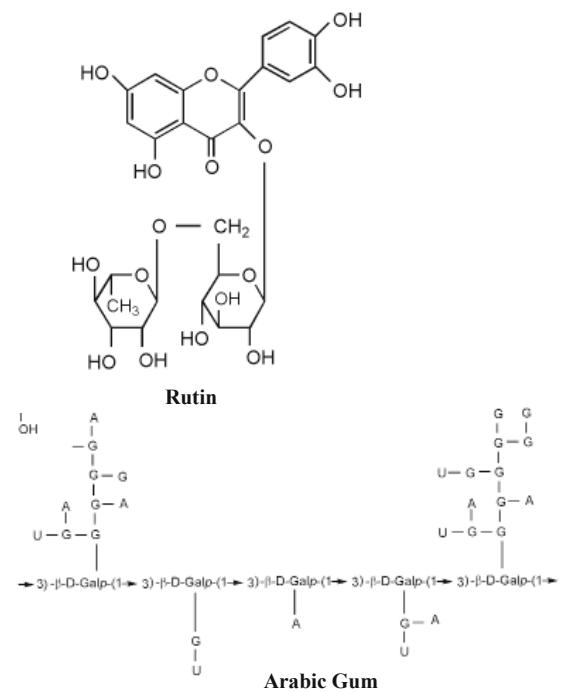
Acacia is commonly present in chewing sticks, mainly as an antimicrobial with activity against *Streptococcus fecalis*.

Other Relevant Uses: Food: Gum arabic from *Acacia senegal* is commonly used as an additive in foodstuffs. It is easily soluble in water and forms solutions over a wide range of concentrations. It has highly valued emulsifying, stabilizing, thickening and suspending properties and does not become viscous. The food industry uses 60–75% of the world production. In confectionery, gum arabic is used to prevent crystallization of sugar, as an emulsifier, and as a glaze or topping in bakery products; in soft drinks and alcoholic drinks it is used either as a vehicle for flavouring or as a stabilizer or clouding agent; in frozen dairy products gum arabic is used for encapsulating flavours such as citrus oils. Gum arabic is used locally in special dishes and as chewing gum.

Industry: Gum arabic is used in the printing industry for coating offset lithographic plates to prevent oxidation, to increase their hydrophilic properties, and to make them repellent to ink. It is also a base for photosensitive chemicals. In ceramics, gum arabic helps to strengthen the clay. Other technical applications include pyrotechnics and ink manufacturing. In textiles, paints, paper size and adhesives (including the traditional office glue and postage stamps) its use has decreased to very low levels in recent years.

CHEMICAL CONSTITUENTS

Compounds: Chemically, gum arabic is a slightly acidic complex composite of glyoproteins and polysaccharides and their calcium, magnesium and potassium salts. The main polysaccharide is arabic acid, a branched polysaccharide with a (1,3)-linked D-galactose backbone with (1,6)-linked ramified branches composed of L-arabinose, L-rhamnose and D-glucuronic acids. The proteins are characterized as hydroxyproline-rich arabinogalactan proteins. Commercial samples of gum arabic showed the following composition: arabinose 24–29%, galactose 32–41%, rhamnose 12–18%, uronic acid 14–17% and protein about 2%. The molecular weight is 47,000–3,000,000 Da, representing a number of basic monomeric sugars of 290–18,500. In human nutrition, gum arabic has less than 1 cal/g (Schmelzer & Gurib-Fakim, 2008).



QUALITY CONTROL

Identification: Gum from wild trees is variable and somewhat darker in colour than that from cultivated plants (Duke, 1981a).

Organoleptic Properties: Gum arabic is odourless with a bland taste, yellowish and some tears are veriform in shape. Ripened or bleached gum occurs in rounded or ovoid tears over 2.5 cm in diameter, and in broken fragments. Tears are nearly white or pale yellow and break readily with a glassy fracture. Kordofan (Sudan) Gum is yellow or pinkish, has fewer cracks, and is more transparent (Duke, 1981a).

Macroscopic Characteristics: The powdered gum is white to white-yellowish.

Microscopic Characteristics: The powdered gum is white to white-yellowish presenting angular particles on microscope, no starch, and few particles of vegetal tissues (Organisation de l'Unité Africaine, 1985).

Growth rings of the stem consists of flattened marginal parenchyma or thick-walled fibres. Vessels solitary, in pairs or radial groups, 70–200 µm; perforation plates simple; intervessel pits alternate, vestured; vessel-ray pits similar to intervessel pits. Fibres with simple pits. Axial parenchyma confluent (var. *leiorhachis*) or banded (var. *rostrata*). Rays 1–5-seriate, homogenous, average length is 270 µm (var. *leiorhachis*) to 420 µm (var. *rostrata*). Prismatic crystals in chambered axial parenchyma cells.

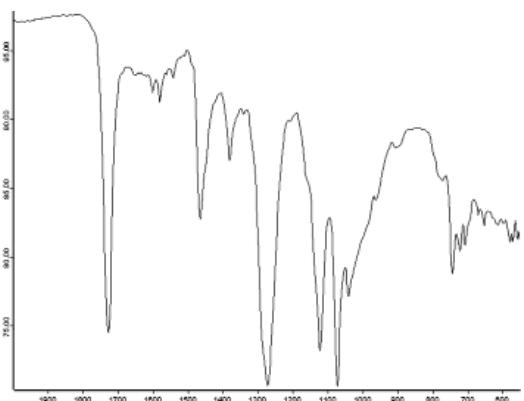
Solubility: The gum arabic is totally soluble in an equal volume of water and gives a translucent, viscous, slightly acid solution; 1g of gum arabic is dissolved readily in 2 ml water (Organisation de l'Unité Africaine, 1985). It is almost insoluble in ethanol (96%).

TLC / HPLC / GC



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

NIR Spectroscopy



Adulterants and Adulterations: Gums from other acacias, and sometimes from *Albizia* and *Combretum*, are also marketed as gum arabic. Although regulations for the admission of gum arabic no longer distinguish between gums from *Acacia senegal* and *Acacia seyal*, and although the gum of *Acacia seyal* is most often marketed as gum arabic, its properties are inferior to those of the gum from *Acacia senegal*. In exports from Sudan, the distinction is clearly made where gum from *Acacia senegal* is marketed as 'gum hashab', while gum from *Acacia seyal* is sold under the name 'gum talha'. In Zimbabwe, gum from *Acacia karroo* is locally traded as gum arabic. Synthetic substitutes for gum arabic are 'modified starches', such as xanthan and gellan, which increasingly replace gum arabic as food hydrocolloids (Schmelzer & Gurib-Fakim Ed., 2008).

Standard Preparations: *Mucilago Gummi Arabici.* *Pulvis Gummi Tragacanthera Compositus.* Gum arabic solutions can be sterilized by autoclaving at 121–122°C for 1 h (Organisation de l'Unité Africaine, 1985).

Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

Acacia is commonly present in chewing sticks, mainly as an antimicrobial with activity against *Streptococcus fecalis*. *Acacia* has also shown some cholesterol-lowering and antidiabetic properties, although there is insufficient evidence in support of these uses (Leung & Foster, 1980). *Acacia* gum has been shown to inhibit the growth of periodontic bacteria and the early deposition of plaque (Clark et al., 1993).

Treatment with gum arabic from *A. senegal* resulted in moderate but significant increases of creatinine clearance and altered electrolyte excretion in healthy wild-type 129S1/SvImJ mice. It may thus effect favourably renal insufficiency (Nasir et al., 2008).

Gum arabic has a trophic effect on the large intestine mucosa (and possibly also on the small intestine) and supply of cations in the large bowel, where some of them such as Mg²⁺ and Ca²⁺, are efficiently absorbed when studied in rats previously adapted to a high starch, fiber-free diet (Tulung et al., 1987).

Gum arabic reduced dietary lipid emulsification and lowered triacylglycerol lipolysis, suggesting a mechanism by which gum acacia may limit lipid absorption (Pasquier et al., 1996).

Acacia can be digested by rats to an extent of 71%; guinea pigs and rabbits also seem to utilize it for energy (Fötisch et al., 1998). Gum arabic may actually elevate serum or tissue cholesterol levels in rats.

Clinical Studies: Ali et al. (2008) assessed the effect of gum arabic (*Acacia senegal*) oral treatment on the metabolic profile of chronic renal failure (CRF) patients. By the end of the 3 months of treatment, serum urea, serum creatinine, serum phosphorus, and serum uric acid levels

significantly decreased for gum users compared with the baseline and control group ($p < 0.05$). Serum calcium levels increased for gum users, and these increases were significantly different ($0.05 < p < 0.001$) from baseline and control group. Thus, oral administration of gum arabic could conceivably alleviate adverse effects of CRF.

A further two studies have documented gum acacia consumption lowered serum cholesterol levels (Ross et al., 1983; Eastwood et al., 1986).

SAFETY DATA

Preclinical Safety Data: Toxicity data on gum arabic indicates little or no acute, short-term, or subchronic toxicity. Gum arabic is negative in several genotoxicity assays, is not a reproductive or developmental toxin, and is not carcinogenic when given intraperitoneally or orally. Clinical testing indicated some evidence of skin sensitization with gum arabic. Ingested orally, acacia is nontoxic. A recent study on the toxicity of gum arabic indicated the toxic level of SUPER GUM (naturally processed polysaccharide exudates from gum acacia trees) to be more than 5.0%, and the 'No Observed Adverse Effect Level' (NOAEL) was concluded to be 5.0% (3,117 mg/kg body weight/day for males, and 3,296 mg/kg body weight/day for males) (Doi et al., 2006). No teratogenic effect was observed in rats during investigating the teratogenic effect of Gum arabic (Collins et al., 1987).

Single Dose Toxicity cf. appendix 1

Sensitizing Potential: Sensitization to gum arabic carbohydrate structures may occur in atopic patients with pollen sensitization. Allergy to gum arabic is mediated preferentially by IgE antibodies directed to polypeptide chains of gum arabic (Sander et al., 2006).

KEY (PROPOSED) USAGE

Therapeutic Indications: Anti-inflammatory and demulcent.

TRADE INFORMATION

Nature of plant material: Gum exudes from cracks in the bark of wild trees, mostly in the dry season, with little or none in the rainy season when flowers are out. In some areas, a long strip of the bark is torn off and the gum is allowed to exude. In Africa, it is regularly tapped from trees, which are about 6 years old by making narrow transverse incisions in bark in February and March. Within a month, tears of gum form on surface and are gathered. Trees begin to bear between 4–18 years of age and are said to yield only when they are in unhealthy state due to poor soil, lack of moisture, or damaged. Attempts to improve conditions tend to reduce yield.

Flower from January to March; fruit from January to April, July, August or October (Duke, 1981a).

Conservation status: In Mali, the species is protected and in India, Nigeria, and Pakistan, it is under cultivation. In Sudan, the trees are cultivated over a very large area in special “gum gardens.” In Pakistan, the best period for afforestation is in the early monsoon between the months of April and June. The plant is best propagated from seeds, which are produced once every few years, and surface sowing is recommended in mildly alkaline sandy soils. However, the plant can also be reproduced by shoot cutting. Trees coppice well (N.A.S., 1980).

Nature of plant products: The plant is wildcrafted to some extent but quantities of cultivated material is available.

Gum arabic is traded in a large number of qualities but can grouped into 3 grades. The best quality is large or round vermiform tears, white, pale cherry, or brownish yellow. Second from best is in rounded, vermiform or branched tears, smaller in size than the top quality and generally darker in colour. The poorest grade is in small brown grains or lightly coloured vermiform tears with a tendency to coalesce in masses (Burkhill, 1995).

Processing and storage: The gum is collected from the cracked or cut bark. Collected gum is carefully freed of extraneous matter, sorted and sometimes ripened in sun before export.

The product is highly stable when dried. Conserved in tightly closed containers.

Gum arabic solutions can be sterilized by autoclaving at 121–122°C for 1h (Organisation de l’Unité Africaine, 1985).

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Adansonia digitata

GENERAL DESCRIPTION

Scientific name with author: *Adansonia digitata* L.

Family: Bombacaceae

Vernacular Names: Anaipuliya-marum, apebroodboom, arbre aux calebasses, arbre de mille ans, baobab, brahma-mlinka, cabacevre, calabaceira, calebassier du Sénégal, cream of tartar tree, dead-rat tree, Ethiopian sour gourd, gorakh-imli, hathi-khatiyan, imbondeiro, kremetartbome, mapou zombie, mbuyu, mkuu hafungwa, mkuu hapingwa, molambeira, monkey bread tree, muuyu, pain de singe (fruit), papparappuli, seemasinta, Senegal calabash (fruit), sumpura, upside-down tree.

Botanical Description: A deciduous medium- to very large-sized tree up to 20 (- 23) m tall, trunk often of vast girth with a comparatively short bole, and spreading branches. Branches stout near the trunk, young branches often tomentose; root system extending up to 2 m deep and horizontally wider than the height of the tree. Bark smooth, greyish, frequently with purple tinge or brown, which is often used in folk classification to distinguish between 'varieties'. Leaves digitate; leaflets 3 in young plants and 5 or 7 in older ones, obovate-oblong or lanceolate, 5.0 cm x 12.5 cm. Flowers hanging, solitary, on long, axillary peduncles, white, bat-pollinated. Fruit woody, gourd-like, 20 - 30 cm x 10 cm, grey with soft, yellow-green felt outside, having farinaceous, yellow or white, sometimes pink pulp. Seeds reniform, lustrous brown or black with thick testa. Seedling with hypogeal germination; cotyledons breaking free from testa; first leaves simple, narrowly linear; taproot swollen.

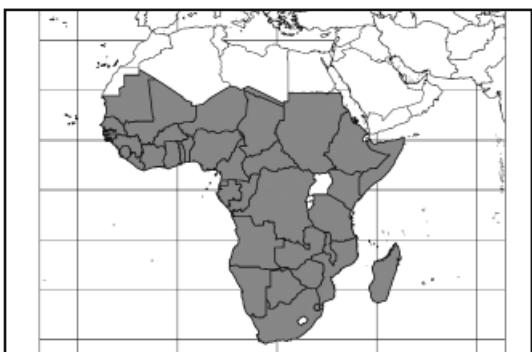
Origin and Distribution: The genus consists of 8 species, 1 originating in Africa, 6 in Madagascar, 1 in northwestern Australia. Baobab prefers sandy topsoil overlying a loamy subsoil. It tolerates poorly-drained soils with heavy texture, but is absent in deep sand. It is at its best at altitudes of 450 - 600 m with an annual rainfall of 300 - 500 mm; it is common in areas with an annual rainfall

of 200 - 800 mm, whereas extremes in annual rainfall of 90 mm and 1500 mm have been recorded. It is found from sea-level to 1000 (- 1500) m altitude.

Severe frost will kill even mature trees, and in the southern part of its area of distribution it is found mostly on north facing slopes, sheltered from cold southern winds. Seedlings and small trees are vulnerable to fire, but mature trees are fire-resistant. African baobab occurs naturally in most countries south of the Sahara.

Originally, it was absent from many Central African countries, but it has been introduced in most of them. In West Africa, it often occurs in baobab orchards around villages, and is thus considered to be a 'follower of man'. In some countries, its distribution is limited, e.g., in Chad, where it is not found in the east, and South Africa where it is mostly limited to the Transvaal. Essentially, baobab is associated with savannahs, especially the drier parts.

However, there are extensions of its distribution into forest areas, probably associated with human habitation. It appears to be introduced into more Equatorial areas, such as Gabon, and both Congos (Brazzaville and Kinshasa), and to countries with a marked dry season such as São Tomé, Madagascar, and the Comoros. Central African Republic represents a natural gap in the distribution, although some introduction has occurred. It has been introduced into Egypt and Yemen. Outside Africa, it has been widely introduced in tropical and subtropical regions.



Plant Parts Used: Fruit, seeds, leaves and bark.



ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses: Baobab leaves, bark, pulp and seeds are used as food and for multiple medicinal purposes in many parts of Africa (Etkin & Foss (1982) cited in Yazzie et al., 1994; Diop et al., 2005). Some 300 traditional uses of baobab were documented alone in Benin, Mali, and Senegal across 11 ethnic groups and 4 agro-ecological zones.

Baobab fruit pulp has traditionally been used as an immunostimulant (El-Rawy et al. (1997) cited in Al-Qarawi et al., 2003), anti-inflammatory, analgesic (Ramadan et al. (1993) cited in Al-

Qarawi et al., 2003), pesticide (Tuani et al. (1994) cited in Al-Qarawi et al., 2003), antipyretic, febrifuge, and astringent in the treatment of diarrhoea and dysentery (Ramadan et al. (1993) cited in Al-Qarawi et al., 2003) and in the treatment of smallpox and measles as an eye instillation (Burkhill (ined.) cited in Wickens, 1979). Fruit pulp and powdered seeds are used as a diaphoretic (Sidibe & Williams, 2002). Further, the gum obtained from the plant is used to treat gastritis and enteritis.

Seeds are used for diarrhoea and hiccough. The seedoil is used for inflamed gums and toothache, and also to treat a variety of skin complaints (Sidibe & Williams, 2002).

Baobab leaves are used as a diaphoretic, an expectorant, as a prophylactic for fever, as an astringent (Watt & Breyer-Brandwijk (1962), Woodruff (1969) and Beltrame (1974) all cited in Wickens, 1979). They are also used to treat fatigue, insect bites, Guinea worm, internal pains, dysentery, kidney and bladder diseases, ophthalmia, otitis, diarrhea and inflammations (Sidibe & Williams, 2002; Burkhill (ined.) cited in Wickens, 1979). Powdered leaves are used as an anti-asthmatic, as they have antihistaminic and antispasmodic properties. Young baobab leaves are crushed into a poultice for painful swellings (Sidibe & Williams, 2002). Topical application reduces inflammation, relieves pain, promotes healing and reduces secondary bacterial infections. Baobab bark is widely used as a substitute for quinine in case of fever or as a prophylactic (Shukla et al., 2001; Oliver (1959) cited in Wickens, 1979). The activity of baobab bark as a febrifuge, however, has not been detected in experimental malaria treatments, although it is both diaphoretic and antiperiodic. In Congo Brazzaville, a bark decoction is used to bathe rickety children and in Tanzania as a mouthwash for toothache. Moreover, the bark contains a white, semi-fluid gum that can be obtained from bark wounds and is used for cleansing sores (Burkhill (ined.) cited in Wickens, 1979). In East Africa, the bark is used as an antidote to *Strophanthus* poisoning. According to Watt & Breyer-Brandwijk (1962, cited in Wickens, 1979) the bark contains the alkaloid 'adansonin', which has a *Strophanthus*-like action. In Malawi, a baobab extract is poured onto the wound of an

animal killed with a *Strophanthus*-based arrow poison to neutralize the poison before the meat is eaten (Wickens, 1979; Wickens (1982) cited in Sidibe & Williams, 2002). An infusion of roots is used in Zimbabwe to bathe babies to promote smooth skin (Wickens (1982) cited in Sidibe & Williams, 2002).

Other Relevant Uses: Baobab fruit pulp has ten times the vitamin C content of an orange. It is used in seasoning, as an appetizer and to make juices. The soft, white flesh or pulp is the so-called monkey bread. It is used to curdle milk, in ice-cream, and as a sweet. In Sudan, it is made into a milk-like drink called 'gubdi'. An emulsion of the fruit pulp is used to adulterate milk. The dried pulp is used as a substitute for cream of tartar in baking (only the fruit of the Madagascan species, *A. madagascariensis* yields 'cream of tartar'). Seeds contain appreciable quantities of crude protein, digestible carbohydrates and oil, and have high levels of lysine, thiamine, Ca and Fe. They can be eaten fresh or dried, roasted, ground into flour and added to soups and stews. In coastal Kenya and Tanzania, the pulp-coated seeds are coloured, sugarcoated, and sold as sweets. The seeds are used to adulterate groundnuts and as a coffee substitute. A fatty oil can be extracted from the seed kernels. It is semi-fluid, golden yellow, gently scented, non-drying and has a long shelf life. It is used for cooking and in the cosmetics industry. Baobab leaves are superior in nutritional quality to fruit pulp, and contain significant levels of vitamin A. Leaves are a staple for many populations in Africa, and are eaten fresh, dried, cooked as a spinach-like vegetable, or dried and powdered as an ingredient of soups and sauces. The shoots and roots of seedlings are also eaten, in Benin, young seedling roots are considered a sweet for children. The roots are boiled and eaten in West Africa in times of famine (hunger food). The flowers are eaten raw (De Caluwé et al., 2009).

Bark and root bark fibre is stripped from the lower part of the trunk and is used to make rope, string, cords for musical instruments, fishing nets, loin cloths, sacking, baskets, mats and waterproof hats. Fibres remain from decayed wood that are used as packing material. The wood itself is useless as timber and makes poor firewood and charcoal. It is, however, used locally for canoes,

wooden platters and floats for fishing nets. Fruit husks are used as fuel and are made into containers and fishing floats. Pollen mixed with water yields a glue for carpentry. The bark is used for tanning and dyeing. A red dye is obtained from the roots. The powdered peduncle and the husk of the fruit are used as a substitute for tobacco. Leaves and fallen flowers are eaten by livestock, fruit pulp, seeds and residues of oil extraction are fed to cattle during the dry season (De Caluwé et al., 2009).

Baobab also has a number of important socio-cultural uses. Trees with hollow trunks are used for water storage, having capacities of up to 7500 L. Hollow trees are also used as tombs, meeting places, prisons, stables, bus shelters, storage rooms, watchtowers, bathrooms, cool rooms and dairies. The extraor-dinarily shaped baobab tree is surrounded by a wealth of legends, superstitions, folktales and anecdotal references throughout Africa, and beyond.

CHEMICAL CONSTITUENTS

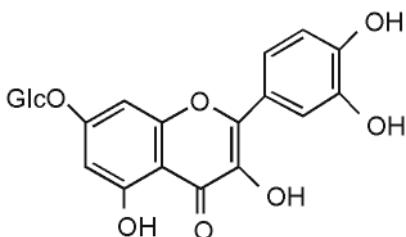
Leaves contain (dry weight): 13 - 15% protein, 60 - 70% carbohydrate, 4 - 10% fat and around 11% fibre and 16% ash. Leaves are known to be significant sources of minerals, especially iron, magnesium, potassium, manganese, molybdenum, phosphorus, copper, zinc and calcium. Mineral content shows wide variation. The plant's state of maturation, genetic variances, and environmental factors may account for the reported discrepancies. Leaves contain tryptophan, lupeol acetate, β -sitosterol, scopoletin, friedelin, baueronol, and number of fatty acids, especially palmitic and linolenic acid (De Caluwé et al., 2009).

Fruit pulp is rich in carbohydrates, energy, calcium, potassium, thiamine (0.038 mg/100 g), nicotinic acid, riboflavine (0.06 mg/100 g), vitamin B6 (2.13 μ g /100 g), niacin (2.16 mg/100 g), total carotene (200 μ g/100 g) and vitamin C (34 - 499 mg/100 g). It is dry, acidulous, mealy, and rich in mucilage, pectins, tartarate and tartaric, citric, malic, succinic and ascorbic acid. Sweetness is provided by fructose, saccharose and glucose contents. Campesterol, stigamsterol, isofucasterol and avenasterol have also been reported. Fruit pulp can further be considered a good source of aspartic and glutamic acids, arginine, tyrosine,

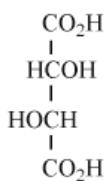
isoleucine, leucine, phenylalanine, lysine, valine and cysteine (De Caluwé et al., 2009)

Seed kernels have an energy value of 1803 kJ/100 g. Levels of carbohydrates vary. Lipid content is 155 mg/g of dry weight with 1 - 2 mg/g linoleic acid, and slightly more oleic acid contents. Seeds contain 4-demethylolsterols (esp. sitosterol), tocopherols (esp. γ -tocopherol) and hydrocarbons (esp. squalene). Seed protein contains a high amount of lysine. The acceptability and optimal utilization of baobab seed as a protein source is limited by the presence of anti-nutritional factors such as trypsin and protease inhibitors (trypsin inhibitor activity of 5.7 TIU/mg sample), tannins (23% catechin), phytic acid (73 mg/100 g), oxalate, alkaloids, phytate and amylase inhibitors (De Caluwé et al., 2009).

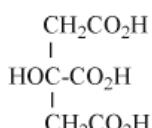
Baobab bark was found to be high in fat, calcium, copper, iron, and zinc. The alkaloid adansonin ($C_{48}H_{36}O_{33}$) is thought to be the active principle for treatment of fevers. Bark additionally contains betulinic acid and various flavonols such as quercetin-7-O- β -D-xylopyranoside and triterpenoids such as 7-bauer-en-3-acetate. Root bark contains β -sitosterol and two glycosides (De Caluwé et al., 2009).



Quercetin-7-O- β -D-xylopyranoside



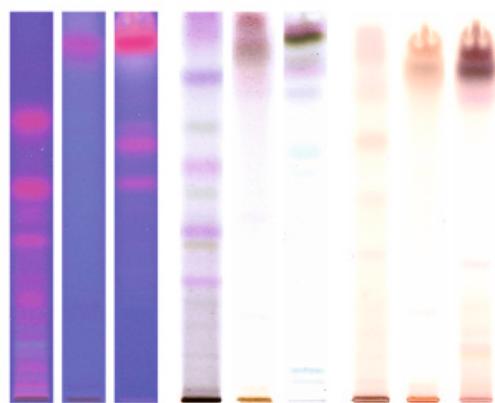
Tartaric acid



Citric acid

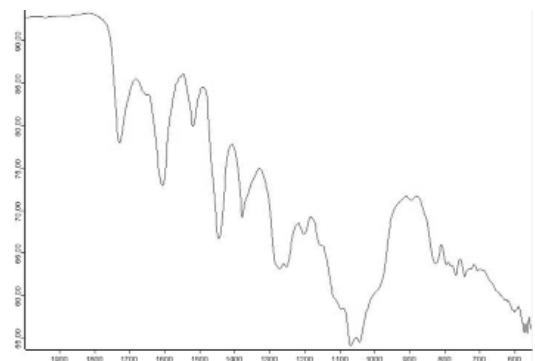
QUALITY CONTROL

TLC / HPLC / GC



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

NIR Spectroscopy



Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

Dietary antioxidants, including polyphenolic compounds, vitamins E and C, and carotenoids, are believed to be effective nutrients in the prevention of oxidative stress-related diseases (Kaur & Kapoor (2001) cited in Besco et al., 2007), such as inflammation, cardiovascular disease, cancer and aging-related disorders (Willet (2001) cited in Besco et al., 2007). The daily recommended dose of vitamin C can be obtained from 23 g of baobab powder (Sidibe et al., 1996). In view of the high antioxidant capacity, baobab fibre has been proposed as a new value-added ingredient for food preparation and/or

nutraceutical applications. The overall antioxidant capacity (IAC), corresponding to the sum of the corresponding water- and lipid-soluble antioxidants capacity, of baobab has been compared with those of orange and kiwi (Vertuani et al., 2002; Besco et al., 2007). The IAC values were: baobab red fibre (1617.3) >>> baobab fruit pulp (240.5) >> baobab fresh leaves (89.0) >> baobab seeds (51.4) > orange fresh fruit pulp (24.3) > kiwi fruit pulp (2.4). Aqueous extracts of *A. digitata* pulp also inhibit ethanol-induced gastric ulceration in rats. Oral pretreatment with *A. digitata* (150 - 600 mg/kg) caused significant dose-dependent increase both in preventive ratio and percentage ulcer reduction. This effect might in part be due to its astringent and antioxidant properties (Karumi et al., 2008). The aqueous extract of baobab fruit pulp exhibited significant hepatoprotective activity (Al-Qarawi et al., 2003). Root bark and leaf methanol extracts have shown antiviral activity against Herpes simplex, sindbis and polio (Anani et al., 2000), also viricidal and intracellular antiviral activity, which may indicate the presence of multiple antiviral compounds, or a single compound with multiple actions (Hudson et al., 2000).

Aqueous extracts of baobab fruit pulp have a marked anti-inflammatory and antipyretic activity. This effect could be due to the presence of sterols, saponins and triterpenes in the fruit pulp. Antipyretic activity resembled that of acetylsalicylic acid (ASA) in hyperthermic rats (Ramadan et al., 1993). Analgesic and antipyretic activities were also mentioned by UN (2005, cited in Masola et al., 2009).

Adding baobab pulp powder to fermenting tempe (main component is soybean) may prevent the growth of pathogenic bacteria such as *Salmonella* spp., *Bacillus* spp. and *Streptococcus* spp. Also, increasing the concentration of baobab pulp powder led to an increase in the population of lactic acid bacteria. This is beneficial since lactic acid bacteria produce an enzyme that breaks the oligosaccharides in soybean down to their mono- and disaccharide constituents. The presence of lactic acid bacteria in tempe will also extend the shelf life of the product because of the preservative attributes of lactic acid bacteria (Afolabi et al., 2005). Some antibacterial activity against *Staphylococcus aureus*, *Streptococcus faecalis*,

Bacillus subtilis, *Escherichia coli* and *Mycobacterium phlei* has also been documented (Anani et al. (2000) cited in Masola et al., 2009). Extracts of baobab roots eliminate the motility in *Trypanosoma congolense* within 60 minutes and drastically reduce motility in *T. brucei brucei*. *T. brucei brucei* and *T. congolense* are unicellular parasites transmitted by the bites of tsetse fly and are the causative agent of sleeping sickness in humans and related diseases in animals (Atawodi et al., 2003).

In order to study potential hypolipidaemic properties of the baobab leaf extract, normal and ethanol-fed rats were administered intragastrically by intubation 0.75 g/kg body weight methanolic extract of the leaves of *A. digitata* for two weeks. The extract was found to lower the lipid levels in rats fed with the extract. The possible hypolipidaemic effect could be attributed to the presence of saponins and fibre in the extract, which has been shown to bind to serum lipids especially cholesterol, thereby easing their excretion from circulation (Geidam et al., 2004). The pulp also showed considerable antisickling activity (Adesanya et al., 1988).

Aqueous and alcoholic extracts of the leaves produced a slight drop in blood pressure after administration to dogs. Adansonia-flavonoside is able to reduce capillary permeability in rabbits. A marked decrease of carotid pressure after application was also seen in dogs as was a countering of asthmatic crisis induced by histamine aerosol in guinea pigs.

SAFETY DATA

Preclinical Safety Data: Alcoholic and aqueous extracts of the leaves showed low toxicity in mice. Tartaric acid can be mildly irritating in high concentrations. EU Novel Food Approval was granted for Baobab Fruit Pulp in July 2008 (Herbal Science International Ltd, 2006). The file reports no allergenic effects from direct consumption of the pulp. Other toxicity tests confirmed that fruit pulp was no toxic based on 6 - 10 g doses in drinks or 10 - 15 g in a fruit bar. Baobab Dried Fruit Pulp has also received GRAS approval in the USA in 2009 as an ingredient in blen-ded fruit drinks at a level of up to 10 percent and fruit cereal bars at a level of up to 15 percent.

Single Dose Toxicity cf. appendix 1**KEY (PROPOSED) USAGE****Therapeutic Indications:** Febrifuge, antibacterial.

Evaluation of Efficacy: Efficacy pharmacologically proven.

TRADE INFORMATION

Nature of plant material: Not threatened, except in some very specific regions. Natural rejuvenation is slowed or even prevented by increasing human population pressure.

Processing and storage: Sun drying, roasting and fermentation are traditional processing techniques that improve the chemical composition of both baobab pulp and seed. Fermentation decreases the tannin levels in baobab seed. Fermentation of the seeds for 6 days was shown to offer many advantages over roasting as evidenced by crude protein, moisture and minerals contents. Some of the commonly used processing techniques for baobab include soaking in water, or boiling in alkaline or acidic solutions. A decrease in tannin contents of baobab seeds was observed after a cold-water, hot-water and hot-alkali treatment. De-hulling and cold-water treatment of seeds was shown to greatly reduce the activity of amylase inhibitors. Alkali treatment appeared to be the most effective method for reducing trypsin inhibitor and tannin contents, and had the additional advantage of improving protein digestibility (De Caluwé et al., 2009).

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Aframomum melegueta

GENERAL DESCRIPTION

Scientific Name with Author: *Aframomum melegueta* K.Schum.

Family: Zingiberaceae

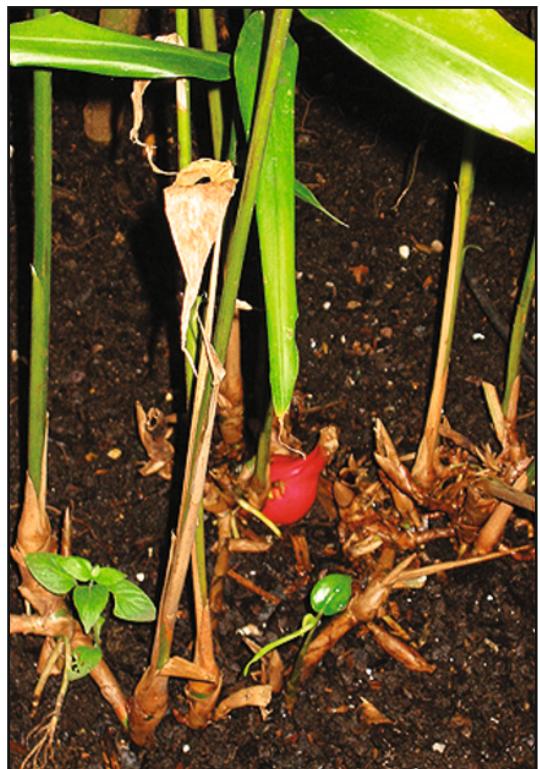
Vernacular Names: Alligator pepper, chitta, ginny grains, ginny papper, graines, grains of paradise, grani de melegueta, grani de paradise, greater cardamom, grenes, Guinea grains, Guinea pepper, Guinea seeds, Malagettapfeffer, malagueta, malaguette, ma-niguetta, maniguette, melaguata, meligetta pepper, obro, Paradieskörner, paradise grains, paradise nuts, poivre de Guinée.

Botanical Description: A herbaceous plant growing from a rhizome, reaching 1 m. The leaves are narrow, 25 x 2.5 cm, arranged on tufted stems. Inflorescences are white or pink, borne singly at the base of the plant. Fruits are reddish-brown ovoid capsules, up to 30 mm long, enclosed in leafy bracts and containing many red to brown angular seeds in a jelly-like pulp.

Origin and Distribution: Grains of paradise are native to tropical West Africa, occurring in Ghana, Liberia, Ivory Coast, Togo and Nigeria, eastwards to Uganda. Ghana is the main exporting country. It is also found in Cameron, Gabon and the Democratic Republic of Congo.



Plant Part Used: Seed



Possible Alternative Source Species: Other aromatic plant species with a flavour similar to cardamom are also known as grains of paradise (Burkill, 2000). Grains of paradise are closely related to Alligator pepper (also known as mbongo spice, hepper pepper) a north African spice which corresponds to the seeds and seed pods of *Aframomum danielli*, *Aframomum citratum* or *Aframomum exscapum*.

Grains of paradise should not be confused with Malagueta pepper (*Capsicum frutescens* var. *malagueta*, Solanaceae) a type of chili used in Brazil, Portugal, and Mozambique.

ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses: The medicinal uses of *Aframomum* include as an aphrodisiac, against measles and leprosy, for excessive lactation and postpartum haemorrhage, as a purgative, galactagogue and anthelmintic, and as a haemostatic agent. Use of the seeds appears in old pharmacopoeias. Gerard (1597) says: “The graines chewed in the mouth draw forth from the head and stocke waterish pituitous homors.... They also comfort and warme the weake, cold and feeble stomacke, helpe the ague, and rid the shaking fits, being drunke with Sacke”.

The seeds and rhizomes are used in West African herbal medicines as a cough medicine and as a remedy to encourage fertility in women. A decoction of the leaves is used for rheumatism and as an antiemetic, and a decoction of the fruits for dysenteric conditions.

The efficacy of some traditional Persian treatments for erectile dysfunction, such as the aphrodisiac property of *Aframomum melegueta*, has been supported by modern pharmacological investigation (Tharakan & Manyam, 2005).

Other Relevant Uses: Common as a flavouring for beer.

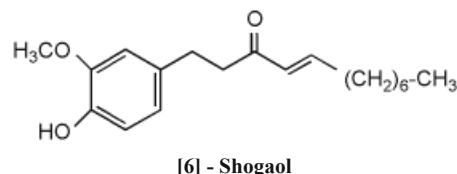
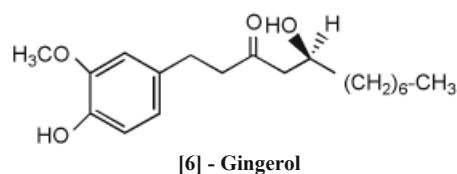
CHEMICAL CONSTITUENTS

Compounds: The presence of the following bioactive constituents in *A. melegueta* seeds have been reported by Okwu (2005): flavonoids (5.76 mg/100 g), phenols (0.09 mg/100 g), saponins (1.24 mg/100 g), tannins (0.38 mg/100 g), ascorbic acid (12.32 mg/100 g), niacin (0.05 mg/100 g), riboflavin (0.26 mg/100 g), and thiamin (0.24 mg/100 g). The plant is also a good source of minerals.

The essential oil from grains of paradise is dominated by the sesquiterpene hydrocarbons humulene, α - and β -caryophyllene (together 83%) and their oxides (together 9%). In the

acetone extract of grains or paradise from Ghana, the following hydroxyarylalkanones were found: 1-(4-hydroxy-3-methoxyphenyl)-decan-3-one ([6]-paradol), 1-(4-hydroxy-3-methoxyphenyl)-3-hendecan-3-one ([7]-paradol) and 1-(4-hydroxy-3-methoxyphenyl)-3-deca-4-ene-3-one ([6]-shoagol) in approximately equal parts. Other works report [6]-paradol, [6]-shoagol and [6]-gingerol (5-hydroxy-[6]-paradol) as the main hydroxyarylalkanones.

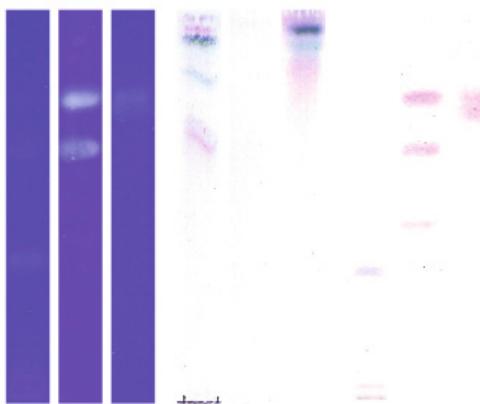
The compositions of absolute and supercritical CO_2 extracts of *A. melegueta* seeds were analyzed by Fernandez et al. (2006), who identified 33 components of the absolute and 43 components of the supercritical fluid extracts, namely monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes and phenolic alkenones. Major components in both extracts were, respectively, [6]-paradol (35.1 and 13.3%), [6]-shogaol (21.5 and 6.1%), [6]-gingerdione (9.8 and 28.0%), α -humulene (10.5 and 7.2%) and [6]-gingerol (1.3 and 10.0%).



QUALITY CONTROL

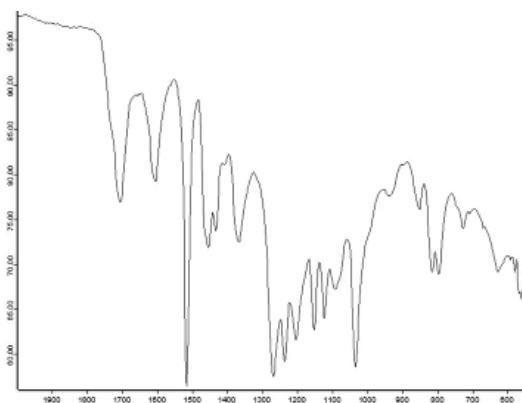
Organoleptic Properties: All parts of the plant are aromatic, the seeds with a pungent flavour like ginger, and the roots similar to cardamom.

TLC / HPLC / GC



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

NIR Spectroscopy



Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

The essential oil from the seeds of *A. melegueta*, as well as various cream preparations thereof, was significantly active against *Staphylococcus aureus*, *Bacillus subtilis* (MIC 0.001% v/v), *Candida albicans* (MIC 0.5% v/v), *Aspergillus niger* and *Pseudomonas aeruginosa* (1.0% v/v) (Igwila et al., 1991, Konning et al., 2004). Oladunmoye et al. (2007) investigated the effect of an ethanol leaf extract of *A. melegueta* on the following pathogens: *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Klebsiella pneumoniae*, with *K. pneumoniae* and *S. aureus* being the most susceptible.

The hexane extract of seeds of *A. melegueta* yielded [6]-paradol and [6]-shogaol, which were found to be active against *Mycobacterium chelonei*, *M. intracellulare*, *M. smegmatis* and *M. xenopi* (MIC 10-15 mg/ml). The desmethyl derivative of [6]-paradol retained the antimycobacterial activity against *Candida albicans* (Galal, 1996).

An aqueous seed extract of *A. melegueta* possesses membrane-stabilizing activity, along with antioxidant properties, and may also have a modifying effect on the responses of white blood cells to tissue injury (Umukoro & Ashorobi, 2008).

An ethanol extract of *A. melegueta* seeds exhibited antioxidant effects on lard (57%) and groundnut oil (54.3%) at 300 ppm (Adegoke et al., 2003).

Aqueous and ethanol leaf extracts from *A. melegueta* were tested for their antifungal activity against *Aspergillus niger*, *A. flavus*, *Fusarium oxysporum*, *Rhizopus stolonifer*, *Botryodiplodia theobromae* and *Penicillium chrysogenum* both in vitro and in vivo. The ethanolic extract proved more effective in inhibition of mycelia and spore germination (Okigbo & Ogbonnaya, 2006).

An ethanol extract of *A. melegueta* proved effective in an investigation regards its inhibitory effect on gastric secretion and its protective activities on gastroduodenal mucosa against pyloric ligation, hypothermic restraint stress, indomethacin, cysteamine, and cytotoxic agents in rats (Rafatullah et al., 1995).

A saline extract of Guinea pepper was found to induce gastric acid secretion in rats. This effect may be caused by stimulation of both muscarinic and histaminic receptors (Enyikwola, 1994).

An aqueous seed extract of *A. melegueta* (115 mg/kg) modified the sexual behaviour of male rats by increasing sexual arousal (Kamtchouing et al., 2002).

An aqueous seed extract of *A. melegueta* (intraperitoneal dose of 25-100 mg/kg) showed peripheral analgesic activity in formalin-induced paw licking, Randall-Selitto paw pressure, and hot plate models of pain in rodents (Umukoro & Ashorobi, 2007).

Capra et al. (2008) have been investigating the anti-inflammatory properties of an *A. melegueta* preparation by assessing the effect of this drug on neuronal injury in the rat hippocampus after parasagittal fluid percussion injury. They observed a significant reduction in the number of Fluoro-Jade (a marker for neuronal injury)-positive neurons with neuroprotective properties for *A. melegueta*.

Clinical Studies: A study of the effects of the seeds of *Aframomum melegueta* on cardiovascular function in both normal and hypertensive human subjects showed a peripheral vasodilatation effect via the nitric oxide-cGMP pathway. The rate of efficacy indicates potential usefulness in managing hypertension (Lawal et al., 2007).

SAFETY DATA

Preclinical Safety Data

Single Dose Toxicity cf. appendix 1

Acute toxicity tests showed no toxic symptoms and mortality over a period of 14 days with doses of 0.25-4 g/kg (Rafatullah et al., 1995).

KEY (PROPOSED) USAGE

Therapeutic Indications: Aphrodisiac, antibacterial.

Evaluation of Efficacy: Efficacy traditionally proven.

TRADE INFORMATION

Nature of plant material: Grains of paradise is a herbaceous perennial propagated by seed or rhizome. The first crop can be harvested around 10-12 months after transplanting. Harvesting is done when the green pods turn to red and produce dark brown mature seeds with a characteristic pungent taste. Green immature pods produce seeds with no flavour.

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Agathosma betulina

GENERAL DESCRIPTION

Scientific Name with Author: *Agathosma betulina* (P.J.Bergius) Pillans

Synonyms: *Barosma betulina* (P.J. Bergius) Bartl. & H.L. Wendl.

Family: Rutaceae

Vernacular Names: Boegoe, bucco, buchu, ibuchu, rondeblaarboegoe, round leaf buchu (Smith 1966).

Botanical Description: The plant is a resprouting shrub of up to 1.5 m in height. The leaves are about 20 mm long, characteristically very broad, less than twice as long as wide (and widest in the upper half), with a rounded apex which curves backwards (Spreeth, 1976). Conspicuous oil glands are present along the margins and lower surfaces of the leaves (under high magnification visible as round glands in the mesophyll). The flowers are solitary, star-shaped, and have five white or pale purple petals. This species is sometimes confused with *A. crenulata*, but in the latter the leaves are more than twice as long as they are wide (and widest in the lower half). Detailed descriptions are given by Pillans (1950) and Spreeth (1976).

Origin and Distribution: *A. betulina* is restricted to the Cederberg Mountains of the Western Cape Province of South Africa, from Porterville and Tulbagh in the south to Citrusdal and Clanwilliam in the north (Pillans, 1950; Spreeth, 1976).



Plant Part Used: Fresh or dried leaves



ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses: Buchu has a long history of use against inflammation, and kidney and urinary tract infections (as diuretic, diaphoretic, and urinary tract disinfectant) (Dijkman, 1891; Laidler, 1928; Watt & Breyer-Brandwijk, 1962; Forbes, 1986). The product is indeed a diuretic and a mild urinary antiseptic. It is taken to stimulate kidney function and to treat mild cystitis and also prostatitis. In small doses it is an appetite stimulant and is used as a digestive, carminative, and antispasmodic. It is a stimulant, useful to treat hangovers, and is also used to treat colds and influenza, cough, rheumatism and gout (Van Wyk & Gericke, 2000). Numerous other uses have been recorded, including in bath water for rheumatism and topically (often as buchu vinegar)

for wounds and bruises (Watt & Breyer-Brandwijk, 1996; Van Wyk et al., 1997; Van Wyk & Gericke, 2000; Van Wyk & Wink, 2004). It is still commonly used as an ingredient of over-the-counter medicines to treat cystitis (Newall et al., 1996).

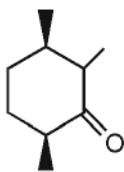
Buchu was an important part of the Khoi culture in the Cape (Laidler, 1928; Forbes, 1986) and still enjoys a great reputation throughout South Africa as a general health tonic and medicine.

CHEMICAL CONSTITUENTS

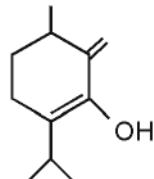
Compounds: The plant contains essential oil components (about 1.8% on a dry weight basis). The presence of two isomers of diosphenol (= buchu camphor) at about 40% of the total oil is highly characteristic for *A. betulina* (more or less absent in *A. crenulata* and hybrids). The level of pulegone is low (< 5%) in *A. betulina* but high (ca. 50%) in *A. crenulata*. Sulphur-containing minor compounds are responsible for the characteristic blackcurrant smell and flavour of buchu oil (Kayser et al., 1975; Collins et al., 1996).

Also reported are flavonoids, especially diosmin (based on early reports; also claimed to have diosmetin, quercetin-3,7-diglucoside and rutin). No reliable recent primary references giving details of the flavonoid composition of buchu could be found. The presence of diosmin as the main compound (Wichtl & Bisset, 2000) seems to be confirmed by a recent paper detailing quality control of commercial buchu tablets (El-Shafae & El-Domiati, 2001). Diuretic effects are attributed to diosmin (a flavonoid) (Wichtl & Bisset, 2000; Wichtl, 2002) and perhaps terpinen-4-ol (also present in juniper berries).

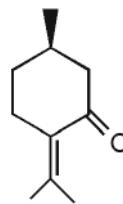
Also present are mucilages and resins.



Isomenthone



α -Diosphenol

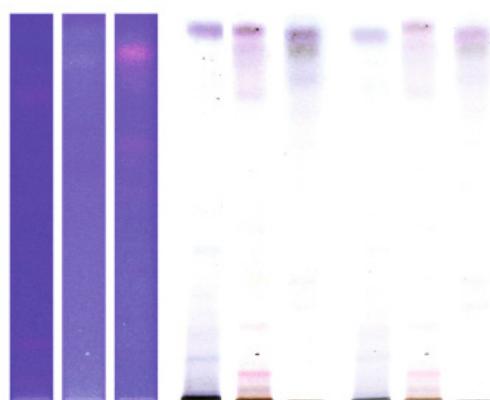


Pulegone

QUALITY CONTROL

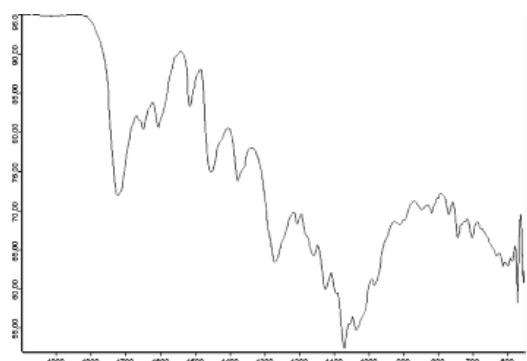
Organoleptic Properties: Aromatic dried leaves and stems, greenish-yellow in colour. Sweet, honey-like scent.

TLC / HPLC / GC



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

NIR Spectroscopy



Purity Tests / Requirements: The plant is erroneously stated to contain pulegone as major ingredient of the volatile oil, and as a result it is considered to be potentially toxic. Pulegone is a known hepatotoxin present in pennyroyal oil (*Mentha*

pulegium). The level of pulegone is less than 5% of the total oil in *A. betulina*.

It is therefore important to insist on *A. betulina* as source material and that quality control is rigorously applied to test for the presence of pulegone and the level of diosphenol.

Adulterants and Adulterations: *Agathosma betulina* is easily mistaken for *A. crenulata*, and these two species are often used interchangeably (NB: Only *A. betulina* is considered safe and effective for medicinal use). Hybrids between the two species are also known to occur (they are chemically intermediate but are low in diosphenol). The leaf length:width ratio is a way to identify the two species (2.34 in *A. crenulata*, 1.95 in *A. betulina*), but for accurate characterization the essential oil components have to be analyzed by TLC or preferably GC (high level of diosphenol – ca. 40% – in *A. betulina*, little or nothing in *A. crenulata*) (Collins et al., 1996; Posthumus et al., 1996).

Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

A spasmolytic activity on guinea-pig ileum (and apparent blocking of calcium channels) was demonstrated for *A. betulina* (but not significant in *A. crenulata*) (Lis-Balchin et al., 2001).

The essential oil has little or no antimicrobial activity.

Diosmin is known to have anti-inflammatory activity at high doses (Farnsworth & Cordell, 1976).

SAFETY DATA

Preclinical Safety Data

Single Dose Toxicity cf. appendix 1

KEY (PROPOSED) USAGE

Therapeutic Indications: Diuretic and a mild urinary antiseptic.

Dosage, Method, and Duration of Administration: Dried leaf: 1-2 g by infusion three times daily. Liquid extract (1:1 in 90% alcohol): 0.3-1.2 ml. Tincture (1:5 in 60% alcohol): 2-4 ml (Newall et al., 2001).

Special Warnings and Precautions for Use: Can be safely consumed when used appropriately. Only *A. betulina* should be used (pulegone, concentration < 5%). *A. crenulata* contains high levels (> 30%) of pulegone (a known liver toxin) and should be avoided.

Evaluation of Efficacy: Diuretic: efficacy traditionally proven; Urinary tract disinfectant: efficacy traditionally proven; Cystitis: efficacy traditionally proven; Appetite stimulant: efficacy traditionally proven; Topically (for wounds and bruises): efficacy traditionally proven.

The absence of documented proof of efficacy of biological activity relating to the traditional claims has resulted in a negative monograph by the German Commission E (Blumenthal et al., 1998).

TRADE INFORMATION

Nature of plant material: Plants flower in spring. Harvesting can be done at any time of the year, depending on the need for seed harvesting.

Material is mainly wild-harvested. Commercial plantations have been established and there is considerable interest in crop development, which is currently under way. Louisa Blomerus of the Agricultural Research Council at Elsenburg coordinates a crop development programme.

Conservation status: Not listed.

Processing and storage: Leaves are air-dried and may be steam-distilled to obtain buchu oil.

Personal experience showed that the dry product is stable but that tinctures are not. No quantitative data on stability have been published.

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Aloe ferox

GENERAL DESCRIPTION

Scientific Name with Author: *Aloe ferox* Mill.

Synonyms: *Aloe candelabrum* A. Berger

Family: Asphodelaceae

Vernacular Names: Aloès, aloe del Capo, bitteraalwyn, Cape aloe, Kap-Aloe, kaapse aalwyn, umhlaba.

Botanical Description: A robust, single-stemmed succulent with broad, spiny leaves and usually bright red or orange flowers in erect racemes (Reynolds, 1950; Van Wyk & Smith, 2004). It differs from *Aloe marlothii* in the erect, non-slanting flower clusters and from *A. arborescens* (and hybrids) in the single-stemmed growth form (not branched). It is also similar to *A. africana*, but the latter has recurved leaves, upcurved flowers, and blooms mainly in July to September (usually May to August in *A. ferox*). (All these species and hybrids are chemically distinct from *A. ferox*).

Origin and Distribution: Eastern parts of South Africa, from Swellendam in the south to the southern parts of KwaZulu-Natal in the north (Reynolds, 1950; Van Wyk & Smith, 2004).



Plant Part Used: Cape aloe: Dried juice from the secretory ducts of the leaves ("aloin cells") obtained by traditional or modern methods of

"tapping" and concentrated by boiling or spray-drying to form a solid (traditional product) or a yellow-brown powder.

Cape aloe gel powder: The inner, non-bitter polysaccharide fraction of aloe leaves obtained by alcohol precipitation and spray-drying.

Cape aloe whole leaf powder: The total leaf, dried and powdered.



ETHNOBOTANICAL INFORMATION

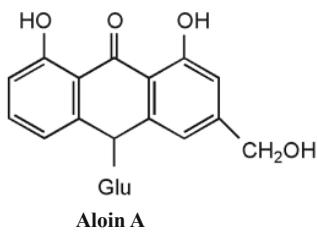
Major Ethnopharmacological Uses: Cape aloe is an important laxative and is included in bitter tonics (Marloth, 1915; Hodge, 1953; Watt & Breyer-Brandwijk, 1962; Bruce, 1975; Forbes, 1986; Van Wyk et al., 1997; Van Wyk & Gericke,

2000; Bruneton, 1999; Neuwinger, 2000; Van Wyk & Smith, 2004; Van Wyk & Wink, 2004). Minor uses include the treatment of arthritis, eczema, and conjunctivitis.

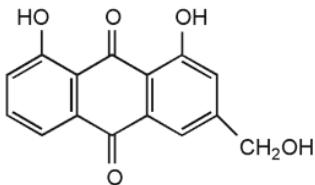
Other Relevant Uses: The gel has become popular as a health drink. Extracted and spray-dried leaf gel is used in skin- and hair-care products. Cape aloe whole leaf powder has various poorly documented uses.

CHEMICAL CONSTITUENTS

Compounds: Hydroxyanthracene derivatives, mainly aloin (12-27%) occurs in *Aloe ferox* (Van Wyk et al., 1995). Aloin is found as an equal mixture of the two stereoisomers aloin A and aloin B. In addition, small amounts of aloinosides A and B and 5-hydroxyaloin A are found, as well as chromone derivatives, mainly aloesin and aloeresins A and B, with small amounts of aloeresin C. (Aloenin and derivatives, characteristic of *Aloe arborescens*, are sometimes stated to occur in Cape aloe, but this is an indication of adulteration or the use of *Aloe ferox* x *Aloe arborescens* hybrids).



Aloin A



Aloe - emodin anthrone

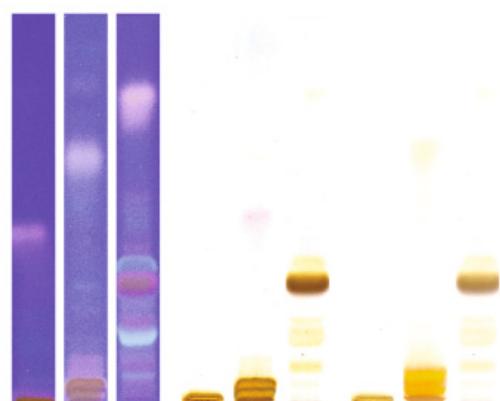
QUALITY CONTROL

Identification: Bright yellow to orange powder.

Organoleptic Properties: Extremely bitter taste.

TLC / HPLC / GC: Quality can easily be controlled with TLC, using standard procedures

(Wagner & Bladt, 1995). The anthracene content is determined by HPLC. The HPLC method comprises a standard reverse-phase C18 column with a linear gradient of methanol and water.



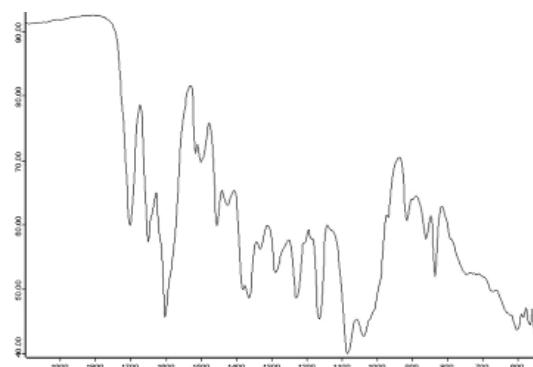
Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

Markers and Quantitative Methods: Cape aloe: The purgative (laxative) activity is due to aloin A, aloin B and other anthracene components. The combined aloin content should not be less than 18% (calculated as anhydrous aloin). If it is lower than 18%, then this should be specified.

Cape aloe gel powder: Upon acid hydrolysis the polysaccharide fraction yields glucose as the main hydrolysis product (Mabusela et al., 1990) and not mannose, as in the case of *Aloe vera*.

Aloe ferox whole leaf powder: Variable quantities of aloin and polysaccharide components may be present.

NIR Spectroscopy



Adulterants and Adulterations: Aloe products may be adulterated by or mistaken for various other species: *Aloe africana*, *A. speciosa*, *A. arborescens*, *A. marlothii* and numerous others. Cape aloe can be identified by the unique combination of anthracene and chromone derivatives (and the absence of homonataloin and other components not present in this species) using routine TLC and HPLC methods.

Cape aloe may be adulterated with stones and other solid objects.

Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

Cape aloe is a stimulant laxative that irritates the mucous membranes of the colon, resulting in an increase in the secretion of mucus and hence a stimulation of peristalsis (Watt & Breyer-Brandwijk, 1962; Bruce, 1975; British Pharmacopoeia, 1993; European Pharmacopoeia, 1993; Martindale, 1993; Newall et al., 1996; ESCOP, 1996-1999; Blumenthal et al., 1998; Burger & Wachter, 1998; Bruneton, 1999; WHO, 1999; Wichtl & Bisset, 2000; Goodman et al., 2001; Schulz et al., 2001; Wichtl, 2002).

There is no documented scientific evidence for the biological activity of Cape aloe gel powder or Cape aloe whole leaf powder.

Clinical Studies: The laxative effect of Cape aloe is well documented (see references). There is no evidence of efficacy for other products (Cape aloe gel powder, Cape aloe whole leaf powder). Hydrating, insulating, and protective effects can be expected with topical products.

SAFETY DATA

The use of Cape aloe is not without risk. It may upset the electrolyte balance and cause damage to the heart or kidneys (Newall et al., 1996). The lethal dose is said to be 1 g/day, taken for several days. Insufficient evidence exists to make an accurate assessment of risk (Schultz et al., 2001).

Preclinical Safety Data

Single Dose Toxicity cf. appendix 1

Carcinogenicity: No reliable data exist for the claimed carcinogenicity of Cape aloe (Westendorf et al., 1988; WHO, 1999) and no genotoxic risks have yet been demonstrated. No teratogenic or fetotoxic effects were seen in animal safety studies done on aloe extracts and aloin A (WHO, 1999).

KEY (PROPOSED) USAGE

Therapeutic Indications: Laxative

Dosage, Method, and Duration of Administration: For laxative use, the minimum quantity required to produce a soft stool is 0.06-0.17 g in adults and children over 10 years. This corresponds to a dose of 10-30 mg hydroxyanthraquinones per day, or 100 mg taken as a single dose in the evening (Newall et al., 1996; Blumenthal et al., 1998; ESCOP 1996-1999). The product is used as a component of "Swedish bitters" and other bitter tonics such as "Lewensesens".

Contraindications: Contraindicated during pregnancy, menstruation, breastfeeding and in persons with kidney complaints. Also contraindicated for children under the age of 10 years (WHO, 1999).

Special Warnings and Precautions for Use: The laxative use should be restricted to acute constipation (not suitable for chronic conditions). Cape aloe should not be used for periods exceeding 1-2 weeks (danger of electrolyte imbalance).

Cape aloe: Can be safely consumed when used appropriately with specific use restrictions.

Cape aloe gel powder: Can be safely consumed when used appropriately.

Cape aloe whole leaf powder: Insufficient data for classification.

Interactions: The absorption of orally administered drugs may be reduced (owing to decreased intestinal transit). The use of Cape aloe with cardiotonic glycosides and antiarrhythmic drugs may cause an aggravation of electrolyte imbalances.

Pregnancy and Lactation: Not to be used during pregnancy, not to be used while nursing.

Evaluation of Efficacy: Cape aloe (bitters): Laxative: efficacy clinically proven, plant material with toxic potential. Hypoglycaemic: efficacy clinically proven, plant material with toxic potential (Ghannam et al., 1986). Panacea (e.g. "Swedish bitters"): efficacy traditionally proven. Arthritis: efficacy traditionally proven.

Cape aloe gel powder: Wound healing: insufficient information for classification. Sun protection: insufficient information for classification. Immune stimulation: insufficient information for classification.

TRADE INFORMATION

Nature of plant material: Flowering/harvesting time: Harvesting is mainly during the growing (wet) season in winter.

Cultivated/wild crafted: Practically all product is wild crafted from natural populations. A report by TRAFFIC indicated that the industry is sustainable (Newton & Vaughan, 1996). Commercial plantations have been established on a small scale.

Conservation status: Not protected in the Western and Eastern Cape Provinces; protected elsewhere (Newton & Vaughan, 1996).

Nature of plant products: Origin: Southern Cape (mainly Mosselbay-Albertinia region); Eastern Cape (mainly the Uniondale, Grahamstown and Seymour areas).

Processing and storage: Cape aloe is obtained by concentrating (boiling, spray-drying) of the yellow juice tapped from *Aloe ferox* leaves.

Cape aloe gel powder is obtained by alcohol precipitation, followed by spray-drying.

Cape aloe whole leaf powder is obtained by drying and powdering the whole leaf. The bitter fraction (anthracene derivatives) is present or partly removed.

Cape aloe is highly stable in dry form. In solution, the aloin content rapidly decreases (converted to aloe-emodin).

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Antidesma madagascariense

GENERAL DESCRIPTION

Scientific Name with Author: *Antidesma madagascariense* Lam.

Family: Euphorbiaceae

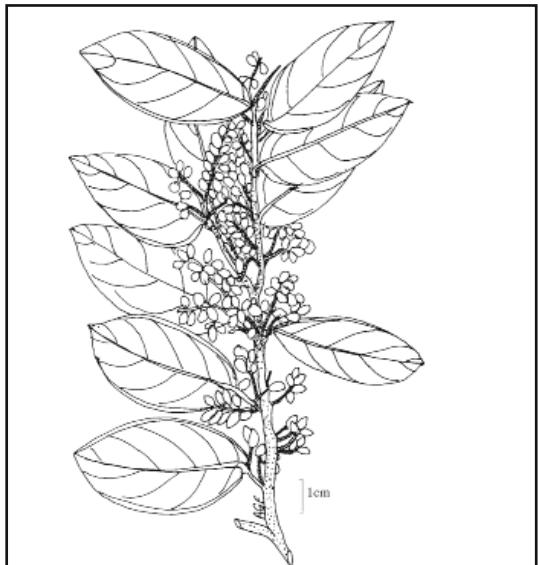
Vernacular Name: Bois bigaignon

Botanical Description: Shrub or low tree reaching 5 m tall. Leaves very variable in size and shape, often oval to elliptic, 4-10 cm long and 3-5 cm in width, the margins entire or slightly sinuous. Presence of domatia in the axils of the primary nerves. Plant dioecious. Flowers minute, greenish or red. Fruit fleshy, more or less flattened, 6-7 mm long, shiny, purple to black when ripe.

Origin and Distribution: The genus consists of about 170 species growing in tropical and warm habitats. *A. madagascariense* is native to the Mascarene Islands where it occurs in Mauritius and Reunion Islands. It is probably also present in Madagascar. Common in humid forests in Mauritius and shows ample variations in the texture and shape of the leaves which can be coriaceous to papery, narrowly elliptic to broadly oval and the domatia very visible or missing in some cases.



Plant Parts Used: Leaves and bark



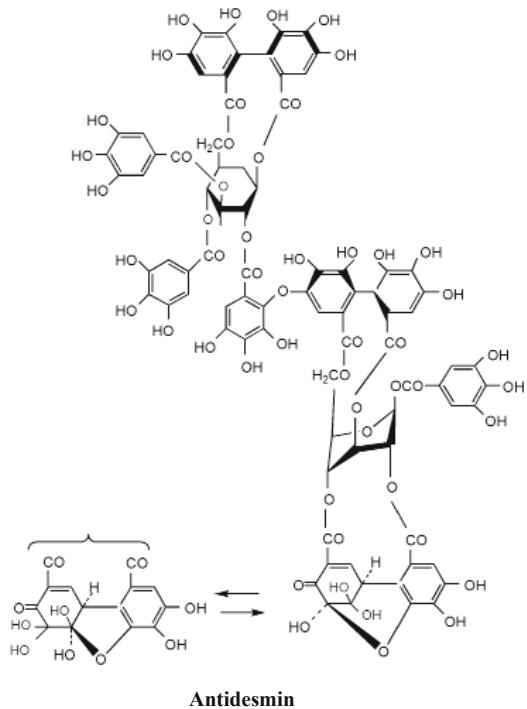
ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses: In Mauritius, a decoction made from the leaves is used against dysentery. To treat albuminuria, 10 leaves are boiled in 1 litre of water and the tea is drunk regularly. The leaves as well as the bark are used as diuretic, against fever, as an astringent and a remedy against diabetes. A decoction of the leaves is used as a bath against boils and against muscle pains. A decoction of the leaves of *Antidesma madagascariense* mixed with those of Fandamane (*Aphloia theiformis*) and those of Patte Poule Piquant (*Toddalia asiatica*) helps against jaundice (Gurib-Fakim et al., 1996).

CHEMICAL CONSTITUENTS

Compounds: *Antidesma* species are characterised by the presence of triterpenes and hydrolysable tannins – carpusin and a dimer – antidesmin (Namba et al., 1991). The crude aqueous leaf and stem extract is reported to contain phenols, tannins, terpenes and leucoanthocyanins. The hexane fraction of the stem contains phenols and terpenes. The chloroform:methanol extract of the stem contains alkaloids, phenols, tannins, terpenes, flavonoid glycosides, and leucoanthocyanins. The hexane fraction of the leaf gave positive tests for terpenes. The chloroform:methanol extract tested positive for phenols, tannins, terpenes (Narod, 2001).

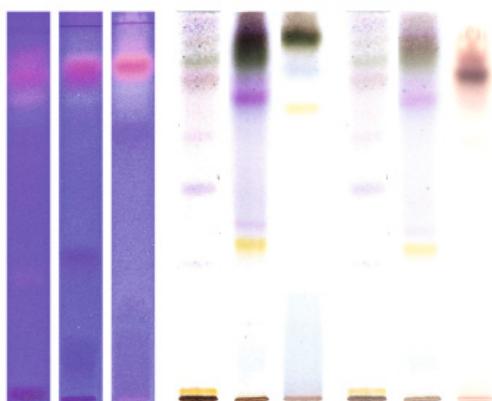
Recently several species of the tribe of Antidesmeae have been screened for antidesmone occurrence by LC-MS and quantitative HPLC (UV). The leaf material has been screened and the content was then compared with the bark and roots. It has been observed that antidesmone has been found at up to 65 mg/kg of plant dry weight except for *Antidesma madagascariense*, growing in Mauritius (Buske et al., 2002).



Antidesmin

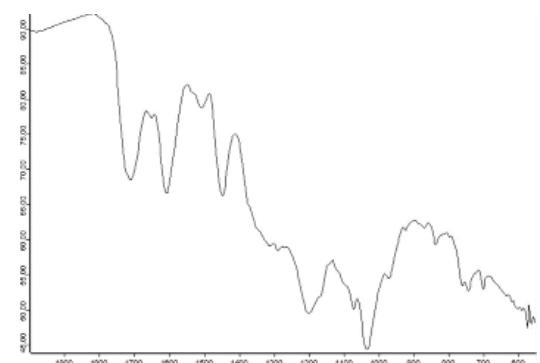
QUALITY CONTROL

TLC / HPLC / GC:



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

NIR Spectroscopy



Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

Antidesmone has been reported to have strong fungitoxic activity against *Cladosporium cucumerinum* (Buske et al., 1999). The fractionated extracts as well as their aqueous extracts of the stem and leaves of *A. madagascariense* growing in Mauritius have been tested for their antimicrobial activities. The test concentration for the aqueous and the organic extracts was 8 mg/ml. The aqueous and methanol fractions manifested activity against the following bacteria: *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Staphylococcus aureus* at a concentration of 8 mg/ml. No

activity was reported against *Candida albicans* nor *Aspergillus niger*. The hexane reaction manifested activity only against *P. aeruginosa* at a test concentration of 32 mg/ml and against *S. aureus* at a concentration of 16 mg/ml. The (1:1) methanol:chloroform fraction manifested activity against *P. aeruginosa*, *S. aureus*, *Aspergillus niger* and *Candida albicans* all at test concentration of 16 mg/ml.

The hexane leaf fraction was found to be active only against *A. niger* at a test concentration of 16 mg/ml. The methanol:chloroform (1:1) extract was active against *P. aeruginosa* (32 mg/ml) and *A. niger* (16 mg/ml). The methanol fraction of the leaf was found to be active against the following bacteria: *E. coli* (8 mg/ml), *P. aeruginosa* (8 mg/ml) and *S. typhimurium* (8 mg/ml), *S. aureus* (2 mg/ml) and *A. niger* (16 mg/ml).

The fraction of the stem has also been investigated for the ability to induce contraction or relaxation in isolated ileal and aortal strips of rats. The methanol fraction manifested weak initial contractile response followed by significant and sustained contractile responses on the rat ileal strip. There was however, no response on rat aortal strips.

The tannin-free aqueous extract of the leaf and stem has shown sustained activity against *S. typhimerium* and *S. aureus* at a test concentration of 32 mg/ml. The chloroform:methanol (1:1) mixture of the stem showed sustained activity against *P. aeruginosa*, *S. aureus*, and *A. niger* at test concentration of 32 mg/ml. The methanol activity of the stem showed activity against all of the four test bacteria at 32 mg/ml and no reduced activity against *A. niger*. The methanol extract of the leaf showed no activity against *S. typhimerium*, *S. aureus* and *A. niger* (Narod, 2001).

Rangasamy et al. (2007) investigated the activity of several plant species from Mauritius. The antimicrobial activity of *Antidesma madagascariense* methanol extract was determined by the disk diffusion method. The extract was active against the following pathogens: *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enteritidis*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Bacillus*

subtilis, and *Candida albicans*. There was no activity against *Colletotrichum gloeosporioides*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Guignardia* sp., and *Fusarium oxysporum*.

When minimum inhibitory concentrations were determined *A. madagascariense* methanol extracts had the best activity against *Salmonella enteritidis* (125 µg/ml) and *Staphylococcus aureus* (500 µg/ml). The total activity, i.e. the volume to which the compounds present in 1 g of plant material could be diluted and still inhibit the bacteria, was 326 ml for *Salmonella enteritidis* and 81 ml for *Staphylococcus aureus* (Eloff, 2000).

Mahomoodally et al. (2006) found that *Antidesma madagascariense* extracts stimulated D-glucose, L-tyrosine, fluid and electrolyte in vitro transport across rat everted intestine, comparable to the action of insulin.

SAFETY DATA

Preclinical Safety Data

Single Dose Toxicity cf. appendix 1

KEY (PROPOSED) USAGE

Therapeutic Indications: Antibacterial, diuretic.

Pregnancy and Lactation: Not to be used during pregnancy. Not to be used while nursing.

Evaluation of Efficacy: Efficacy traditionally proven.

TRADE INFORMATION

Nature of plant material: Conservation status: Not threatened.

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Rangasamy O, Raoelison G, Rakotoniriana FE, Cheuk K, Urverg-Ratsimamanga S, Quetin-Leclercq J, Gurib-Fakim A, Subratty AH (2007) Screening for anti-infective properties of several medicinal plants of the Mauritians flora. *J. Ethnopharmacol.* 109: 331–337.

Aphloia theiformis

GENERAL DESCRIPTION

Scientific Name with Author: *Aphloia theiformis* Benn.

Synonyms: *Aphloia mauritiana* Baker, *A. integrifolia* (Vahl.) Benn., *Lightfootia theiformis* Vahl., *Ludia heterophylla* auct. non Lam.

This species is known to have high morphological variations due to ecological factors. There are several homonyms and infraspecific taxa for *Aphloia theiformis*: *Aphloia theiformis* fo *andringitensis*, *Aphloia theiformis* fo *obcuneata* H.Perrier, *Aphloia theiformis* fo *tsaratananae* H.Perrier, *Aphloia theiformis* subsp. *deltoidis* Poir., *Aphloia theiformis* subsp. *madagascariensis* Clos, *Aphloia theiformis* subsp. *stylosa* Boivin, *Aphloia theiformis* var. *angustissima* H.Perrier, *Aphloia theiformis* var. *caerulescens* H.Perrier, *Aphloia theiformis* var. *closii* (Tul.) H.Perrier, *Aphloia theiformis* var. *latifolia* B.Boivin, *Aphloia theiformis* var. *micrantha* (Tul.) H.Perrier, *Aphloia theiformis* var. *micranthera* H.Perrier, *Aphloia theiformis* var. *minima* (Baker) H.Perrier, *Aphloia theiformis* var. *racemosa* H.Perrier, *Aphloia theiformis* var. *sechellensis* (Clos.) F.Friedmann.

Family: Flacourtiaceae

Vernacular Names: Bois d'anemone, bois fandamane, bois goyave, bois merle, bwa merl, change ecorce, fandamane, fandramanana, hazondrano, kirandrambehivavy, kirandrambiavy, maramanana, miaramanana, ravimboafotsy, tsilomay, voa-fotsy, zompy.

Botanical Description: Small tree reaching 15 m tall; trunk approaching 30 cm in diameter, with a characteristic bark which is black or blackish brown in colour, deciduous in patches, the underlying bark being pale brown and smooth. Young branchlets reddish, striated. Adult leaves variable in shape, with blade narrow to elliptic, narrowly oboval or oboval-elliptic, 3 - 8 (-10) x 1.2 - 4.5 (-6) cm, obtuse to acuminate at the summit, obtuse to cuneiform, rarely slightly

cordate or amplexicaul at the base, subcoriaceous, glabrous, sometimes bluish-green in the dry state, with denticulate to dentate margins, often entire towards the base, with 8-10 pairs of secondary veins; petiole (2-) 3-8 mm long, 1 mm in diameter. Flowers axillary, solitary, and in fascicles or bunches of few flowers; pedicels 6-20 (-34) mm long. Sepals white, turning pale yellow, circular, 5-6 (-7) mm in diameter. Filaments of the stamens 4-6 mm long. Berry subglobular to ovoid-pyriform, 6-8 mm long and 4-6 mm in diameter.

Origin and Distribution: Indigenous to Madagascar, the Comoros, the Mascarenes, the Seychelles and tropical Africa, the species is still common in Mauritius and Reunion Island. It has become extremely rare or is already extinct on Rodrigues. Common in remnants of the native forest in the humid upland zone of Mauritius.



Plant Parts Used: Fruits, leaves, bark and roots.



ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses: A tea made from the leaves of *A. theiformis* with the bark of *Terminalia bentzoe* treats dysentery. The leaf decoction is used for fever (Mad., Mru.) (Adjanohoun et al., 1983) and a diuretic (Sussman, 1980; Wong Ting Fook, 1980). It also helps against rheumatism, ulcers, jaundice (Mad., Mru.) and gastrointestinal infections (Fakim, 1990). Daruty (1886) reports on the use of this plant as an emetic (Mad., Mru.). The leaves, mixed with the bark of *Erythroxylum laurifolium* and the roots of *Cocos nucifera* help against fever. In the Comoros, a leaf decoction is used for stomach pains; in the Seychelles, a root and leaf decoction helps against impetigo. Mixed with those of *Cinnamomum camphora* or those of *Piper nigrum*, a refreshing drink is obtained. A decoction of the leaves mixed with those of *Dracaena reflexa* and those of *Centella asiatica* is given to teething children. The root decoction is used to wash skin infections, while a leaf poultice

is applied to nail infection. A decoction of the root is reported to be a good purgative (Gurib-Fakim et al., 2004).

In Madagascar, Boiteau (1999) reports that an infusion of the leaves is diuretic, used against malarial fevers, haematuria, and seems to protect the red blood corpuscles against lysis in vitro. In 1990, Descheemaeker reported that bile-related problems are treated by drinking an infusion made from the following mixture of plants: leaves of *A. theiformis*, maize stigma, pumpkin leaves, leaves of *Cyperus* sp., and plants of *Euphorbia hirta*.

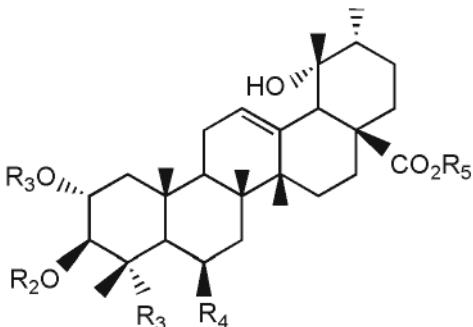
CHEMICAL CONSTITUENTS

Compounds: The main constituent of *A. theiformis* is mangiferin, also known as aphloiol and whose structure has been established as being C-glucoside of 1,3,6,7-tetrahydroxyxanthone (Paris et al., 1964).

Preliminary screenings of the leaf extract showed the presence of saponins and tannins (Smadja & Vera, 1991). Lavergne (1990) reported on the composition of the leaves and stems: they were found to contain phenols, saponins, terpenes, triterpenes, flavones, flavonoids and traces of tannins.

The plant growing in Madagascar is reported to be rich in leucoanthocyanins, flavonoids, tannins, steroids, 2-desoxy-2 sugars, saponins and traces of polyphenols (Gurib-Fakim & Gueho, 2000).

The methanolic extract of the leaves from Mauritius is reported to contain three triterpenes, namely tormentic acid glucoside ester, 23-hydroxytormentic acid glucoside ester and 6 β -hydroxytormentic acid (Gopalsamy et al., 1988). Previous works have reported the presence of aphloiol, a tetrahydroxyflavone (Pernet, 1957).

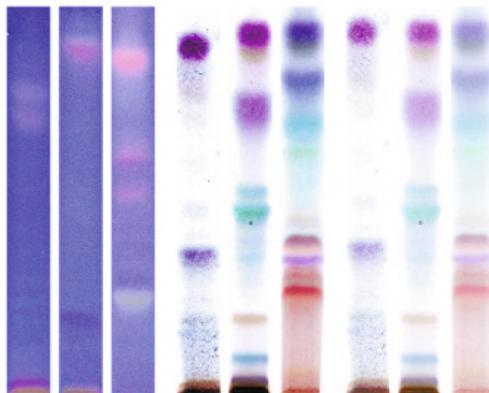


- 23-Hydroxytormentic acid ester glucoside : $\text{R}_1=\text{R}_2=\text{R}_4=\text{H}; \text{R}_3=\text{CH}_2\text{OH}, \text{R}_5=\text{Glc}$
- 6β -Hydroxytormentic acid ester glucoside : $\text{R}_1=\text{R}_2=\text{H}; \text{R}_3=\text{Me}, \text{R}_4=\text{OH}, \text{R}_5=\text{Glc}$

QUALITY CONTROL

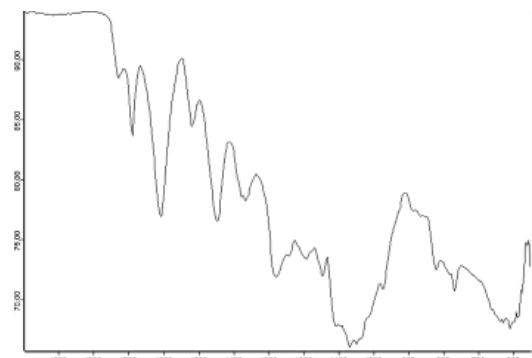
Identification: Crushed, dried green leaves and twigs.

TLC / HPLC / GC



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

NIR Spectroscopy



Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

The anti-infective properties of *A. theiformis* were studied against different strains of pathogenic bacteria and fungi, namely *Salmonella enteritidis*, *Enterobacter cloacae*, and *Bacillus subtilis*, which were all found to be susceptible to the crude methanolic extract (Rangasamy et al., 2007).

The methanolic extracts of the leaves kill the snail *Biomphalaria glabra* at a concentration of 400 ppm. This snail is a vector for *Schistosoma mansoni*, the latter being responsible for the transmission of the disease bilharzia (Gopalsamy et al., 1988).

SAFETY DATA

Preclinical Safety Data

Single Dose Toxicity cf. appendix 1

KEY (PROPOSED) USAGE

Therapeutic Indications: Antimicrobial, anti-inflammatory.

Pregnancy and Lactation: Not to be used during pregnancy or while nursing.

Evaluation of Efficacy: Efficacy traditionally proven.

TRADE INFORMATION

Nature of plant material: Conservation status: Not threatened (Mru.), Endangered (Rod.), Abundant (Mad.).

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Artemisia afra

GENERAL DESCRIPTION

Scientific Name with Author: *Artemisia afra* Jacq. ex Willd.

Family: Asteraceae

Vernacular Names: African wormwood, Afrikanischer Wermut, als, alsem, armoise d'Afrique, lengana, umhlonyane, wildeals.

Botanical Description: *Artemisia afra* is an aromatic, multi-stemmed perennial suffrutex of up to 2 m high. The branches are somewhat woody, but die back each year. It has bipinnately compound, feathery leaves that are dark green above and conspicuously silvery below. The flowers are borne in small rounded heads (ca. 5 mm in diameter) in slender racemes in late summer. The disc florets are cream-coloured. Ligulate florets are absent (Van Wyk et al., 1997; Van Wyk & Wink, 1994).

Origin and Distribution: Widely distributed in southern and eastern Africa, from South Africa to Ethiopia (Van Wyk et al., 1997; Van Wyk & Wink, 2004).



Plant Parts Used: Dried leaves and twigs, or the essential oil distilled from fresh above-ground parts.



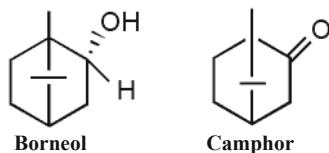
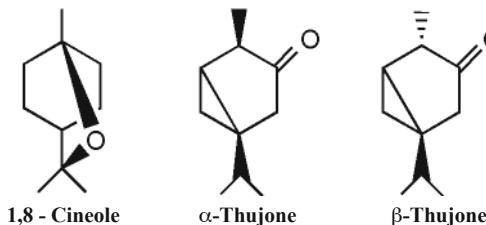
ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses: *Artemisia afra* is one of the most popular and widely used of all traditional medicines from South Africa (where it has a well-recorded history of use) up to Kenya, Tanzania and Ethiopia. A very wide diversity of

uses has been recorded, so that the plant may be considered as a general tonic in its own right. According to numerous authors (Dykman, 1908; Watt & Breyer-Brandwijk, 1962; Hutchings et al., 1996; Van Wyk et al., 1997; Neuwinger, 2000; Van Wyk & Gericke, 2000; Von Koenen, 2001; Van Wyk & Wink, 2004), the traditional uses include the treatment of colds, influenza, cough, sore throat, asthma, pneumonia, blocked nose, stomach ailments, colic, flatulence, indigestion, constipation, gastritis, poor appetite, heartburn, internal parasites, measles, headache, earache, gout, diabetes, malaria and wounds. The plant is used as a bitter tonic and appetite stimulant in the Cape region of South Africa (Dykman, 1908; Rood, 1994; Thring & Weitz, 2006).

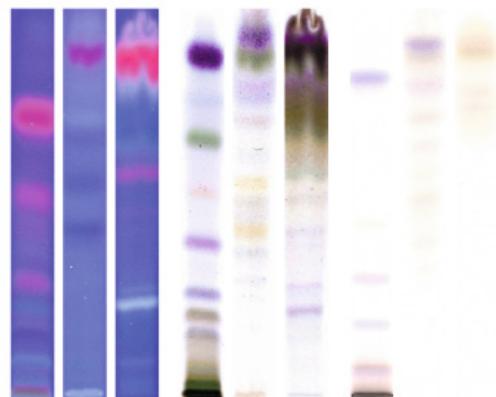
CHEMICAL CONSTITUENTS

Compounds: *Artemisia afra* is exceptionally variable in its essential oil components (Graven et al., 1990; Lawrence, 1996; Viljoen et al., 2006), depending on the geographical origin as well as the chemotypes (which may differ within populations). The main compounds of the oil are usually 1,8 cineole (eucalyptol), α -thujone, β -thujone, camphor and borneol, together with some sesquiterpenoids such as chrysanthenyl acetate. Ethiopian oil yielded yomogi alcohol and artemisyl acetate as dominant constituents (Worku & Rubiolo, 1996). Several sesquiterpene lactones (guaianolides and glaucolides) have been isolated from above-ground parts (Jakupovic et al., 1988). Non-volatile constituents also include triterpenes (α -amyrin, β -amyrin and friedelin) and alkanes (ceryl cerotate and N-nonacosane) (Silbernagel et al., 1990), as well as surface flavonoids (methyl ethers of luteolin) (Wollenweber et al., 1989).



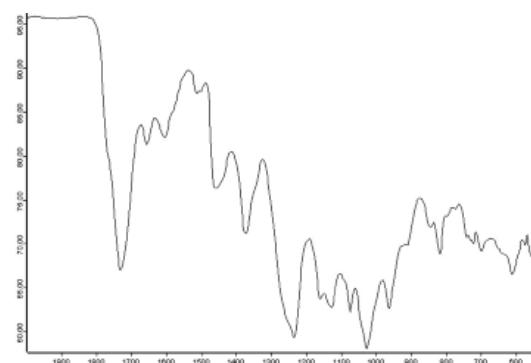
QUALITY CONTROL

TLC / HPLC / GC: Standard TLC methods for the analysis of essential oils can be used (Wagner and Bladt, 1995). The presence and level of α - and β -thujone should be determined by gas chromatography using standard methods (e.g. Viljoen et al., 2006). Van der Kooy et al. (2008) showed that metabolomics is a viable option for quality control purposes.



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

NIR Spectroscopy



Adulterants and Adulterations: No adulteration has yet been recorded. Owing to the variable chemical profile, adulteration with other aromatic members of the Asteraceae is possible.

Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

The activity is usually ascribed to the essential oil components, of which α - and β -thujone are usually amongst the main compounds, with variable levels of 1,8-cineole, borneol, camphor and other monoterpenoids and variable amounts of sesquiterpenoids, depending on the clone and provenance.

Recent in vitro and some in vivo studies have shown that *Artemisia afra* has the following activities: antimicrobial (Graven et al., 1992; Gundidza, 1993; Rabe & Van Staden, 1997; Mangena & Muyima, 1999; Huffman et al., 2002; Jäger, 2003; Motsei et al., 2003; Viljoen et al., 2006; Van Vuuren & Viljoen, 2006; Vagonas et al., 2007), antioxidant (Graven et al., 1992; Burits et al., 2001), antimalarial (Weenen et al., 1990; Kraft et al., 2003; Clarkson et al., 2004; Gathirwa et al., 2007), anti-nematodal (McGaw et al., 2000), cardiovascular (hypotensive) (Guantai & Addae-Mensah, 1999), cytotoxic (Jenett-Siems et al., 2002) and sedative (Nielson et al., 2004; Stafford et al., 2005).

SAFETY DATA

Preclinical Safety Data: The chemotype of Artemisia and the level of thujone were unfortunately not recorded. In humans, high doses of thujone can cause confusion, convulsions, and coma, but selected chemotypes (especially low thujone clones) are already grown on a small scale.

Single Dose Toxicity cf. appendix 1

Acute and chronic toxicity of *Artemisia afra* and extracts thereof were studied by Mukinda and Syce (2007), who recorded acute intraperitoneal and oral LD₅₀ values in mice of respectively 2.45 and 8.96 g/kg (for aqueous extracts, representing 10% of the herb, w/w).

Repeated Dose Toxicity: In rats, a low chronic toxicity potential was demonstrated over a period of 3 months, with daily doses of 0.1 and 1.0 g/kg of aqueous extract.

KEY (PROPOSED) USAGE

Therapeutic Indications: Bitter tonic.

Dosage, Method, and Duration of Administration: Unknown. In view of the potentially harmful effects of thujone, Van Wyk and Wink (2004) recommended a maximum daily dose of 3 g dry herb per day. In commercial product development, the level of thujone and its control (through the cultivation of selected clones) should be given a high priority.

Special Warnings and Precautions for Use: *Artemisia afra* can be safely consumed when used appropriately. In humans, high doses of thujone are toxic, so that the use of thujone-containing herbs such as wormwood to flavour absinthe and other alcoholic beverages has been banned in most countries (Van Wyk & Wink, 2004; Wink & Van Wyk, 2008). Although thujone is a convulsant poison at high doses and may produce delirium, hallucinations and seizures when taken chronically, the sporadic ingestion of small quantities is probably not a health risk (Bruneton, 1999; Van Wyk & Wink, 2004; Wink & Van Wyk, 2008).

Pregnancy and Lactation: Not to be used during pregnancy. Not to be used while nursing.

Evaluation of Efficacy: Antimicrobial efficacy pharmacologically proven, plant material with toxic potential (Graven et al., 1992; Gundidza, 1993; Rabe & Van Staden, 1997; Mangena & Muyima, 1999; Huffman et al., 2002; Jäger, 2003; Motsei et al., 2003; Viljoen et al., 2006; Van Vuuren & Viljoen, 2006; Vagonas et al., 2007). Antioxidant efficacy pharmacologically proven, plant material with toxic potential (Graven et al., 1992; Burits et al., 2001). Antimalarial efficacy pharmacologically proven, plant material with toxic potential (Weenen et al., 1990; Kraft et al., 2003; Clarkson et al., 2004; Gathirwa et al., 2007). Anti-nematodal efficacy pharmacologically proven, plant material with toxic potential (McGaw et al., 2000). Cardiovascular (hypotensive) efficacy pharmacologically proven, plant material with toxic potential (Guantai & Addae-Mensah, 1999). Cytotoxic efficacy pharmacologically proven, plant material with toxic potential (Jenett-Siems et al., 2002). Sedative efficacy pharmacologically

proven, plant material with toxic potential (Nielson et al., 2004; Stafford et al., 2005). Bitter tonic (amarum) efficacy traditionally proven. Abdominal cramps efficacy traditionally proven.

TRADE INFORMATION

Nature of plant material: Plants are harvested at any time of the year, but mainly towards the end of the growing season (summer to late summer).

The plant has been mainly wild-harvested because it is abundant in nature. Small-scale cultivation has been initiated. In the long term, selected clones will have to be cultivated, as the product is chemically exceptionally variable. The level of thujone in the essential oil, for example, varies from 0% to 95%, depending on the clone and the provenance (Van Wyk et al., unpublished data).

Conservation status: Not threatened. The plant is exceptionally common in its natural distribution range.

Processing and storage: Stems are simply cut and dried.

No reliable stability data are available. The product is likely to be stable when dried but relatively unstable in solution. The volatile essential oils may evaporate, especially if the product is stored under warm conditions.

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Aspalathus linearis

GENERAL DESCRIPTION

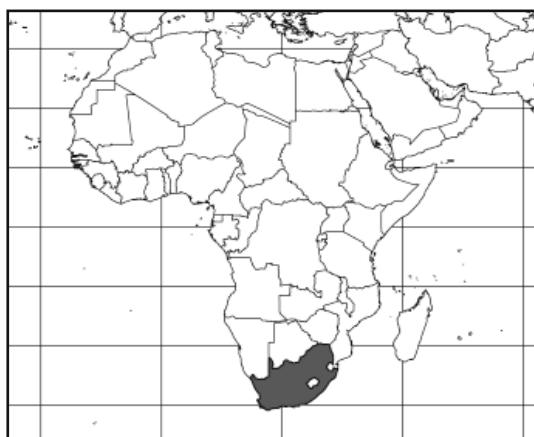
Scientific Name with Author: *Aspalathus linearis* (Burm.f.) R.Dahlgren

Family: Fabaceae

Vernacular Names: *Aspalathi linearis herba*, aspalathus, *Herba Aspalathi*, rooibos, Rotbusch

Botanical Description: *Aspalathus linearis* is a shrub 0.5 - 2 m in height, with bright green, simple, needle-shaped leaves which turn a rich reddish-brown colour upon fermentation. The small, yellow, typically pea-shaped flowers are produced in spring and early summer. The fruit is a flat, obliquely falcate, glabrous, single-seeded pod. The species is exceptionally variable with regard to morphology (Dahlgren, 1968, 1988), chemistry (Van Heerden et al., 2003), and genetics (Van der Bank et al., 1995). Only one type is commercially cultivated: the so-called Red type or Rocklands type, originally from the Pakhuis Pass area (Van Wyk et al., 1997).

Origin and Distribution: Western parts of the Western Cape Province of South Africa, mainly in the Citrusdal, Clanwilliam, and Nieuwoudtville regions. The production area stretches from Darling and Piquetberg in the south to Gifberg and Nieuwoudtville in the north (Dahlgren, 1968, 1988).



Plant Parts Used: Leaves and twigs

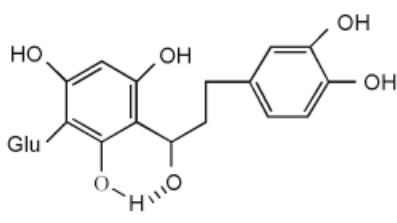
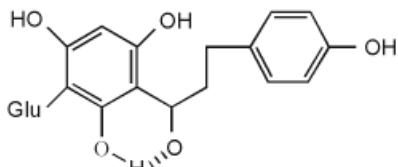


ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses: Rooibos tea has been widely used as a milk substitute for infants who are prone to colic (Rooibos Tea Control Board, 1973). It is considered to have significant antispasmodic activity (Snyckers & Salemi, 1974). Rooibos has become very popular as a health beverage because it is free from harmful stimulants and devoid of caffeine (Blommaert & Steenkamp, 1978). Rooibos tea is widely used as an ingredient in cosmetics and is believed to be beneficial in cases of eczema. The main traditional use is as a health drink (Watt & Breyer-Brandwijk, 1962; Van Wyk et al., 1997; Burger & Wachter, 1998; Van Wyk & Gericke, 2000; Van Wyk & Wink, 2004), and the recent popularity of the beverage is a result of innovative marketing (Van der Walt & Machado, 1992).

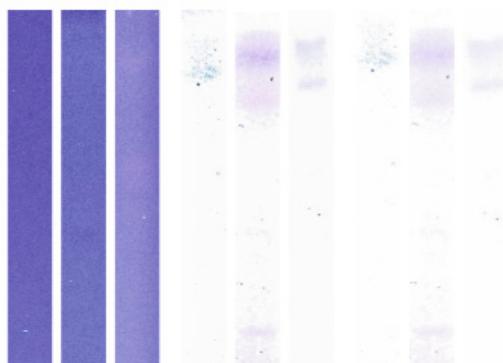
CHEMICAL CONSTITUENTS

Compounds: *Aspalathus linearis* contains several flavonoid glycosides, including orientin, isoorientin, and quercetin, but the dihydrochalcones aspalathin and nothofagin are the main constituents (Snyckers & Salemi, 1974; Koeppen & Roux, 1965; Rabe et al., 1994; Joubert, 1996; Van Heerden et al., 2003)

**Aspalathin****Nothofagin**

QUALITY CONTROL

TLC / HPLC / GC: From the low extraction yields it appears that there are hardly any non-polar compounds present. Hot water extraction would have yielded more compounds separating in EMW. The chalcone aspalathin is easily detected on TLC using solvent systems for flavonoid glycosides. The compound can also be analysed by HPLC with standard reverse-phase C18 columns and methanol-water gradients.

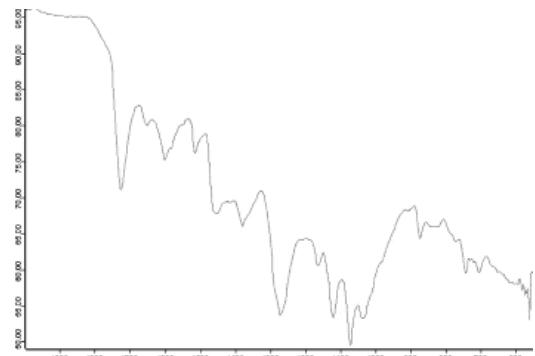


Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

Markers and Quantitative Methods: The chalcone aspalathin is the main phenolic compound and is used for quality control (Joubert, 1996; Bramati et al., 2002, 2003). The level varies

greatly, from 1 mg/g for fermented tea to 50 mg/g for green (unfermented) tea. Smaller quantities of nothofagin are also present.

NIR Spectroscopy



Adulterants and Adulterations: Very rare. Various indigenous herbal teas are produced from the various ecotypes and forms of rooibos tea. Some are of a poor quality. The red colour and appearance of commercial rooibos tea are characteristic. The presence of aspalathin is absolute proof of identity (the compound is known only from rooibos tea).

Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

Recent studies have shown significant activity of rooibos against negative effects related to mutagenesis and oxidative damage (Neethling et al., 1988; Marnewick, 2000, 2003a, 2003b, 2005; Standley et al., 2001; Joubert et al., 2003). The antioxidant activity and antiradical properties have been investigated and are linked to aspalathin (which showed significant activity when compared with other teas and commercial antioxidants (Von Gadow et al., 1997).

Effects on cell division (Lamošová et al., 1997) and dermatological conditions (Shindo & Kato, 1991) suggest a rationale for the cosmetic use of rooibos tea extracts.

A recent animal study suggests the prevention of age-related accumulation of lipid peroxidases in the brain (Inanami et al., 1995).

SAFETY DATA

Ethnic Use Safety Data: Rooibos has been widely used and enjoyed like ordinary tea since 1904. No side effects are known.

Preclinical Safety Data

Single Dose Toxicity cf. appendix 1

KEY (PROPOSED) USAGE

Therapeutic Indications: Antioxidant.

Dosage, Method, and Duration of Administration: Rooibos is made and enjoyed in the same way as ordinary tea (ca. 2.5 g is taken as an infusion, or more often as decoction in a cup of water, several times per day).

Contraindications: None.

Special Warnings and Precautions for Use: None. Can be safely consumed when used appropriately.

Interactions: None.

Adverse Effects: None.

Evaluation of Efficacy: Antioxidant efficacy pharmacologically proven (Von Gadow et al., 1997, Standley et al., 2001, Joubert et al., 2003, Marnewick et al., 2003a). Antimutagenic efficacy pharmacologically proven (Standley et al., 2001, Marnewick et al., 2000, 2003b, 2005). General tonic efficacy traditionally proven. Antispasmodic efficacy traditionally proven.

TRADE INFORMATION

Nature of plant material: Flowering/harvesting time: Mainly in the summer months (October to March).

Cultivated/wild crafted: Practically all product is from cultivated material (the so-called Rocklands type or red tea type). About 1% of annual production is wild crafted as speciality products.

Conservation status: not listed (cultivated crop).

Processing and storage: The tea is mostly harvested with sickles and tied into bundles. It is then chopped into 3 mm-long segments, moistened, bruised and left in heaps to “sweat” or “ferment” for several hours until a sweet smell develops (Dahlgren, 1968; Van Wyk et al., 1997; Van Wyk & Gericke, 2000; Van Wyk & Wink, 2004). So called “fermentation” is actually an oxidation process, during which the phenolic compounds in the plant are enzymatically oxidised (Joubert, 1996). When the tea maker is satisfied with the colour and aroma, the tea is spread out thinly to dry in the sun. Large quantities are available for export, including an organically produced product, “green” (unfermented) tea, and also a spray-dried extract (powder).

Highly stable in dry form. Farmers often store the product on farms for several years. The main compound, aspalathin, is relatively unstable.

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Balanites aegyptiacus

GENERAL DESCRIPTION

Scientific Name with Author: *Balanites aegyptiacus* Delile

Synonyms: *Balanites maughamii* Sprague, *Balanites ferox* G. Don, *Ximenia aegyptica* L. (excl. *Balanites roxburghii* Planch), *Agialida senegalensis* van Tiegh., *Agialida barteri* van Tiegh., *Agialida tombuctensis* van Tiegh., *Balanites ziziphoides* Milbr. et Schlechter, *Balanites latifolia* (van Tiegh.) Chiov.

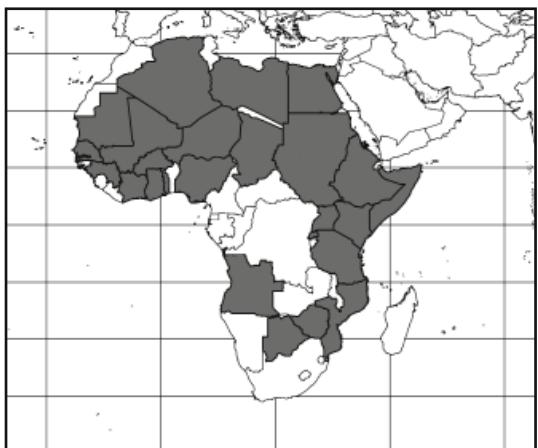
Family: Balanitaceae

Vernacular Names: Bito, bito tree, corona di Jesus, datte du desert, dattier du desert, desert date, heglig, higlig, jerico balsam, korak, kurio, laloab, lalobe, loba, lolab tree, myrobolan d’Egypt, q-og-g-ogat, saronga, shashoba, simple-thorned torchwood, soapberry tree, tau, thorn tree, zachun.

Botanical Description: Multibranched, spiny shrub or tree between 10 and 12 m tall, crown spherical, in one or several distinct masses. Trunk short and often branching from near the base. Bark dark brown to grey, deeply fissured. Branches armed with stout yellow or green thorns up to 8 cm long. Leaves with two separate leaflets; leaflets obovate, asymmetric, 2.5 - 6 m long, bright green, leathery, with fine hairs when young. Flowers in fascicles in the leaf axils, fragrant, yellowish-green. Fruit is a rather long, narrow drupe, 2.5 - 7 cm long, 1.5 - 4 cm in diameter. Young fruits green and tomentose, turning yellow and glabrous when mature. Pulp bitter-sweet and edible. Seed: the pyrene (stone) is 1.5 - 3 cm long, light brown, fibrous and extremely hard. It makes up 50 - 60% of the fruit. One kilogram of fruit can give around 500 - 1500 dry, clean seeds.

Origin and Distribution: The plant is believed to be indigenous to all dry lands south of the Sahara (Sahel), Sudan, East and West Africa, extending southwards to Malawi in the Rift Valley and to the Arabian Peninsula. The natural distribution is obscured by cultivation and naturalization. The plant has been introduced into cultivation in Latin America and India; it has wide ecological

distribution, but is mainly found on alluvial sites with deep sandy loam and free access to water.



Plant Part Used: Fruit



ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses: The plant is widely used in traditional medicine. The fruit, root, and stem bark sap are used as a purgative, as an anthelmintic, and for diarrhoea and colic, and in some countries (Sudan) against bilharzia. The fruit juice is also indicated for chronic cough and stomach pain. A powder made from the leaf and bark is applied to wounds and abscesses, especially on the soles and undersides of the feet. The fruit pulp macerate is used to bring down a fever. For suppurating urinary tract inflammation, a root macerate or bark powder in water is drunk. A root macerate is drunk in case of snake bites, and the bite area must be bathed with the decoction.

Other Relevant Uses: *Balanites* is an efficient fishing poison. To catch fish, the bark is scraped into small pieces and put into the water, or the bark is buried in the sand under the water. Alternatively, the fruit is pulverized and the powder is sprinkled on the surface of the water. Large fish such as tilapia can resist the poison for a long time and are seldom killed. *Balanites* is used locally for many products: the wood is used for making tools and furniture, the fruit for sweets and alcoholic beverages, and the kernels for cooking oil. Extracts of the fruit and bark are lethal to snails (host for schistosomiasis) and water fleas (host for guinea worm). *Balanites* is also a food plant.

CHEMICAL CONSTITUENTS

Compounds: Seed kernel yields the saponins (diosgenin and yamogenin). The fat content of the kernels is high (40–46%). Among the major fatty acids present are saturated acids (24.0%), oleic acid (31.0%), linoleic acid (43–45%), and palmitic acid (15%).

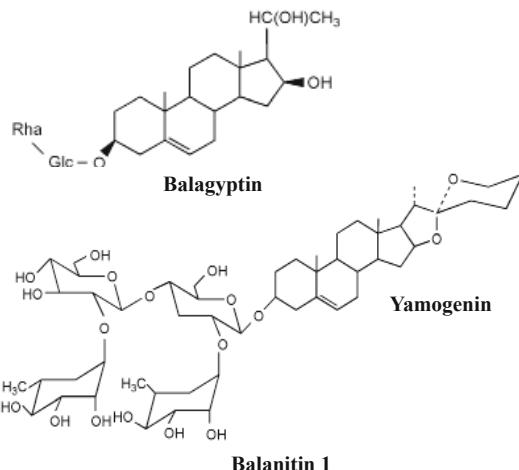
Phytochemical investigation of the mesocarp yielded saponins (3%) and glyceride oils (5%). The saponin mixture contained 25 α - and 25 β -spirostadiene, diosgenin and yamogenin as well as higher fatty acids. Also, from the mesocarp two pregnane glycosides were isolated: balagypitin and pregn-5-ene-3 β ,16 β ,20(R)-triol, 3-O- β -D-glucopyranoside.

The fruit pulp contained bis-(5-formylfurfuryl)ether and 5-hydroxymethylfurfurol. Other components identified are: phosphatidylinositol, phosphatidylcholine

(lecithin), phosphatidylethanolamine (cephalin) and phosphatidic acid. Flavonoid glycosides have also been identified in the fruit: isorhamnetin-3-rutinoside and isorhamnetin-3-rhamnoglucoside.

The methanol extracts of the roots, bark, wood and leaves have yielded saponins, namely balanitins I, II, and III. These are tri- and tetraglycosides with yamogenin as the aglycone, followed by diosgenin and a sugar. The stem bark of the plant of Indian origin has shown the presence of the furanocoumarin bergapten (5-methoxysoralen). The root and bark contain steroidal saponins balanitin 1-7. A new saponol, 6-methyldiosgenin and a new furostanol saponin, balanitoside, have been isolated from the fruit (mesocarp).

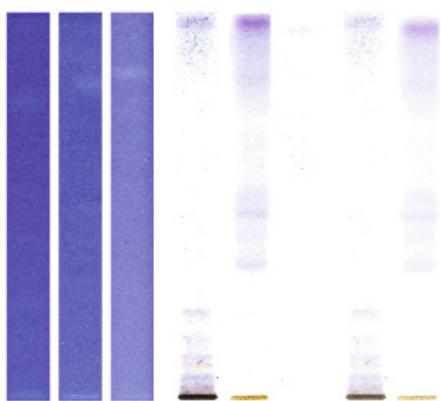
The leaves and seeds contain a mixture of six diosgenin glycosides, including di-, tri-, and tetraglycosides. The stem bark of the plant of Indian origin has shown the presence of the furanocoumarin bergapten (5-methoxysoralen). The root and bark contain steroidal saponins balanitin 1-7. A new saponol, 6-methyldiosgenin and a new furostanol saponin, balanitoside have been isolated from the fruit (mesocarp). A further furostanol saponin was isolated from the mesocarp, balanitesin. Also, from the mesocarp two pregnane glycosides were isolated: balagypitin and pregn-5-ene-3 β ,16 β ,20(R)-triol 3-O- β -D-glucopyranoside.



QUALITY CONTROL

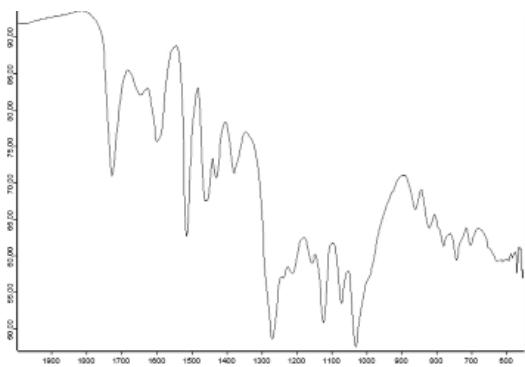
Identification: The ripe fruit resembles a date in both size and appearance. The brown outer skin consists of a sticky pulp within which lies the oil-bearing seed or nut. The kernel yields a highly stable golden-yellow oil suitable for cooking. Analysis has shown that the fruit typically consists of 21.8% outer skin, 30.7% pulp, 36.7% shell and 10.8% kernel on a dry basis.

TLC / HPLC / GC



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

NIR Spectroscopy



Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

An aqueous extract of the mesocarps of the fruits of *Balanites aegyptiacus* exhibited prominent antidiabetic activity with oral administration in streptozotocin-induced diabetic mice. It was

revealed that the individual saponins did not show antidiabetic activity, whereas the recombination of these saponins resulted in significant activity. The efficacy of *Balanites aegyptiacus* fruit mesocarp was compared with praziquantel in mice infected with the Sudanese strain of *Schistosoma mansoni*. Infected mice were given a single dose of 200 mg/kg body weight of *B. aegyptiacus* fruit mesocarp and 200 mg/kg body weight of praziquantel 6 weeks after the onset of the infection. A significant reduction was observed in EPG (egg count per gram of faeces), eggs burden in tissues and recovery of adult worms ($P < 0.05$) for both plant- and drug-treated animals.

The aqueous extract of *Balanites aegyptiacus* bark, which is used in Sudanese folk medicine in the treatment of jaundice, was without effect when studied on rabbit intestine, rabbit aortic strip, rat stomach strip, rat uterus, and rat phrenic but significantly reduced the contractility and the rate of the isolated perfused rabbit heart. Administration of the aqueous extract to biliary ductligated rats showed a dose-dependent significant decrease in serum bilirubin. The chronic and sub-chronic toxicity investigations indicate the safety of the aqueous extract at a dose level, which showed a significant decrease in serum bilirubin level in experimental obstructive jaundice in rats.

The anti-inflammatory and anti-nociceptive activities of methanol (ME) and butanol (BE) extracts and of two new saponins isolated from *Balanites aegyptiacus* bark were evaluated. The study was carried out both in vivo and in vitro. The samples, extracts and pure substances were administered to animals intragastrically. Two different animal models: carrageenin-induced oedema in the rat, and the acetic acid-induced writhing test in mice, were adopted. Moreover, the antioxidant power of extracts, fractions, and individual constituents from *Balanites aegyptiacus* has been evaluated in vitro, using a method based on the Briggs-Rauscher (BR) oscillating reaction. Results obtained demonstrate that both ME and BE have a significant effect at the highest dose on the number of abdominal writhes induced by acetic acid, with respectively 38% and 54% inhibition, but no significant difference was observed for extracts at the lowest dose and for the pure compounds compared with control animals.

The same extracts exhibit a significant reduction on rat paw oedema. The inhibition produced by ME is about the same (28 +/- 3% lowest dose, 32 +/- 3% highest dose) after administration. A more evident effect is obtained by BE (41 +/- 3% and 68 +/- 6%, respectively) and single saponins B1 and B2 (62 +/- 5% and 59 +/- 6% respectively) after oral administration. The antioxidant activity obtained seems to be in accordance with the pharmacological results. The histological sections of rat paw confirm the antiphlogistic activity of the plant extracts.

SAFETY DATA

Preclinical Safety Data

Single Dose Toxicity cf. appendix 1

A 50% methanolic extract of the stem bark at 180 - 188 mg/kg mice ip produced hypothermia in dogs, 50 mg/kg iv caused hypotension. On the isolated guinea-pig ileum it caused spasmolytic effects. LD₅₀ = 375 mg/kg mice ip. However, for guinea-pigs 5g/kg body weight there was no toxicity when given orally.

Repeated Dose Toxicity: The toxicity of an aqueous extract of *Balanites aegyptiacus* was tested on *Oreochromis niloticus* and on the freshwater snail *Biomphalaria pfeifferi*. The LC₅₀ of the lethal concentrations of the extract on the fish and the snails was 1.12 mg/l and 2.0 mg/l, respectively.

KEY (PROPOSED) USAGE

Therapeutic Indications: Antimicrobial, antidiabetic, antimarial, anthemintic, insecticidal, molluscicidal.

Special Warnings and Precautions for Use: A toothpaste made of the bark caused erosive cheilitis. A patch test with the bark of the plant was negative, but strongly positive when mixed with the patient's saliva.

Adverse Effects: Potentially capable of inducing photoirritant dermatitis.

Evaluation of Efficacy: Efficacy traditionally proven, plant material with toxic potential.

TRADE INFORMATION

Nature of plant material: The species has a diffuse flowering and fruiting habit: flowers and fruits occur during a prolonged season, although a peak is always encountered. In areas with a pronounced seasonal climate (northern and southern part of the distribution range) fruit maturation occurs before the rainy season. In most of the Sahelian region the main flowering season is between October and March, the main fruiting season between December and April. In southern Africa (Zambia to Zimbabwe) flowering is in September to December, fruiting is between April and August. Flowering in Nigeria varies between November and April, with ripe fruits becoming available in December and January and occasionally later, from March to July. Elsewhere, fruiting and foliage production occur at the height of the dry season.

Conservation status: Not threatened

Processing and storage: Fruits are harvested when they turn yellow and the flesh becomes soft and sweet. The outer fruit pulp must be removed as soon as possible to avoid fermentation. If extraction is not possible in the field, the fruits should be kept dry and spread in a thin layer during temporary field storage. The fruit pulp can be removed after soaking the fruits in water. Every ton of whole fruit processed yields half a ton of hard woody shell, which is highly combustible and produces a high-quality charcoal. Oil is obtained by simple expression methods.

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Boswellia sacra

GENERAL DESCRIPTION

Scientific Name with Author: *Boswellia sacra* Flueck.

Synonyms: *Boswellia carteri* Birdwood (The epithet '*carteri*' is spelt differently as '*carterii*' in several papers and books. However in the lead botanical paper by Thulin & Warfa (1987) '*carteri*' is used, and that is the preferred spelling for this monograph).

Family: Burseraceae

Vernacular Names: In Somali Mohor (tree), Beyo (resin). Often the name is combined with adjectives to indicate habitat or quality of the tree, such as Mohor Add (Add = white) mohor madow (madow = black). Several words are used in Somali and also in Arabic for the resin of *B. sacra*. In Arabic the general word is Sheehaz, although it is also known as Shabi, Shazri, Luban etc. The general name for the resin in Somali is Beyo, as opposed to Meydi for the resin of *B. frereana*. In English Arabian frankincense.

Botanical Description: A full botanical description of *Boswellia sacra* is given by Thulin & Warfa (1987). The tree may be 1.5 - 8 m tall, branching from the base or with a distinct trunk, bark pale brown with some outer flaking, papery layers, and a thick reddish-brown inner resiniferous layer, young shoots tomentose or rarely glabrous, resin copious, milky, drying yellowish-brown. Leaves densely crowded at shoot apices, or alternate on young long shoots. Flowers produced with the leaves, in racemes or little-branched panicles 6 - 24 cm long, clustered at ends of short shoots. Fruit 3 - 4, 8-12 x 3.5 - 9 mm, narrowly to broadly pyriform, reddish-brown, glabrous.

Origin and Distribution: There are two geographic areas where this plant occurs. For many years botanists considered the frankincense plant occurring in Arabia (i.e. South Yemen and Dhofar region of Oman) as the distinct species *B. sacra* Flueck., while considering the similar plant found at the tip of the Horn of Africa in North

Somalia as a different species named *B. carteri* Birdw. However, recent botanical reports indicate that these two groups should be classed together as one species, namely *B. sacra* Flueck. (Thulin & Warfa, 1987). If there are differences between the species found on opposite sides of the Red Sea, these should be considered not as species but as variety or geographic differences. Although the synonym *B. carteri* is still widely used in scientific papers and in trade, one should refrain from using this and instead use *B. sacra*, the name that is accepted by botanical authorities (www.ipni.org/index.html).



Plant Part Used: Resin collected from the trunk and larger branches of the tree using a special scraping knife.



Possible Alternative Source Species: There are six most common *Boswellia* species whose gum-resins are widely traded and these are: *B. frereana* Birdw. known only from Somalia; *B. sacra* Flueck. (syn *B. carteri*) from Somalia, Yemen, and Oman; *B. papyrifera* Hochst. from Ethiopia and Sudan; *B. rivae* Engl. from Ethiopia; *B. neglecta* S. Moore from Ethiopia and Kenya; and *B. serrata* Roxb. from India. Though each gum-resin is unique, they are all known as frankincense or olibanum.

ETHNOBOTANICAL INFORMATION

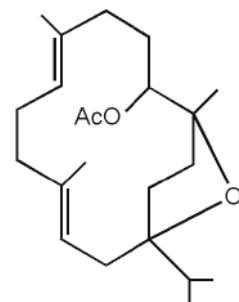
Major Ethnopharmacological Uses: *Boswellia* resin has been used as a major anti-inflammatory agent and for wound healing for centuries. Further ethnomedicinal uses include acne, amenorrhoea, analgesic, antifungal, anti-inflammatory, antiseptic, astringent, bursitis, cancer, carminative, cervical spondylosis, cystitis, digestive, diuretic, dyspepsia, emmenagogue, expectorant, genital area infections, sedative, skin ulcers/sores and uterine infections.

CHEMICAL CONSTITUENTS

Compounds: Over the years the study of the chemistry of frankincense has attracted the interest of many research groups in different parts of the world. The main limitation of these reports is the fact that many workers did not pay much attention to the precise botanical origin of the resin they studied. This is partly because people worked on resins obtained from trade circles (Cairo, Turkey, Israel, Hamburg etc.) rather than on resins collected from properly identified trees.

Abdel-Wahab (1987), working on a sample believed to be *B. carteri*, obtained from Cairo, Egypt, reported the chemical composition of the essential oil. It contained, in addition to several minor monoterpenes, two prominent compounds, namely octyl acetate and 1-octanol. However, it became very evident from our work (Dekebo et al. 1999) that this profile is typical of *B. papyrifera*, clearly showing that the material these workers investigated was *B. papyrifera* gum resin and not *B. sacra* (syn. *B. carteri*). A similar problem arose in the PhD dissertation of Basar (2005), which was conducted under the guidance of the late Professor Koenig of Hamburg University. The dissertation gives a comprehensive account of

the resin of *B. papyrifera*, although the botanical origin of the resin was erroneously reported to be *B. carteri*. One should therefore substitute *B. papyrifera* in place of *B. carteri* when reading this dissertation. Indeed, it is in this study that the unique compound verticilla-4(20)-7,11-triene was discovered for the first time as one of the constituents of *B. papyrifera*, which also contains incensole, incensole acetate, *n*-octanol, and octyl acetate. This group of compounds now enables one to distinguish with ease *B. papyrifera* not only from *B. sacra* (syn *B. carteri*), but also from all other *Boswellia* species.



Incensol acetate

QUALITY CONTROL

Identification: *B. sacra* resin is barely translucent, yellowish or brownish in colour, partly brittle.

Organoleptic Properties: Softens when chewed and tastes bitter but aromatic. It has a characteristic sweet, balsamic aroma and smells pleasant when placed on hot charcoal.

Macroscopic Characteristics: Frankincense forms almost spherical pea- or walnut-sized, tear-shaped, or irregularly shaped grains or stalactite-like masses which are yellowish, yellowish-red or brownish, with a dusty white on the outside. It is barely translucent, slightly brittle, with a waxy gloss at the shell-like breaks; thin splitters are usually transparent and clear.

Solubility: Frankincense decomposes in water and forms a turbid liquid. It is only partly soluble in ethanol and ether.

Species name	Common name	Water	Ethanol	Acetone	Hexane
B. papyrifera	Tigray-Type	90 mg/ 18%	360mg/70%	240mg/48%	275mg/54%
B. rivae	Ogaden Type	195 mg/ 39%	307mg/60%	287mg/57%	390mg/80%
B. sacra	Beyo (56 -178N)	93 mg/ 19%	370mg/74%	470mg/95%	220mg/45%
B. sacra	Beyo Hilary	88 mg/ 18%	315 mg/63%	325mg/65%	249mg/50%
B. frereana	Meydi Hilary	2 mg/ >0.5%	395mg/80%	386mg/77%	450mg/90%
B. frereana	Meydi 56- 178P	0.5 mg >0.5%	495mg/99%	220mg/44%	490 mg/98%

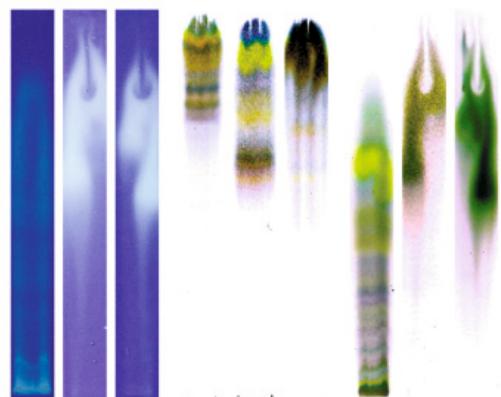
Very useful information about the nature of a given gum resin can be obtained by determining its solubility in different solvents. The procedure utilized is as follows: In each case 500 mg of the frankincense was powdered and extracted with the solvent by using a sonic bath for 20 min, filtered, and then the solvent removed using a rotary evaporator. The residue was weighed, giving a soluble fraction yield in mg and % as shown in the table above. As can be easily deduced, the results are indeed most interesting and have far-reaching implications for determining the identity and quality of a given resin. The Meydi sample is almost totally soluble in hexane (90-95%), whereas the solubility of Beyo in hexane is much lower, at 45-50%. Thus there is a clear distinction between Meydi and Beyo. The table above clearly shows that Meydi is essentially a pure resin, whereas the other types of frankincense can be considered as gum resin.

It is interesting to note that an anonymous author came to the above conclusion as early as 1941: "The samples of Mohor contained considerable amts. of H₂O-sol. gum, whereas the Madi samples were almost free from gum. Judging from the analyses, the difference in the composition of the exudations from the 2 species of trees is such that only that from *B. carteri* can be truly designated as a gum resin or gum oleoresin; the product of *B. frereana*, being practically free from gum, would be classified as an oleoresin of the elemi type, and not an incense gum." (Anon, 1941).

It is a pity that subsequent workers did not pay attention to the findings of this paper, which if followed would have helped to avoid the unnecessary confusions that permeated the literature during subsequent years. This simple solubility test could also be used as a quick test to distinguish Beyo gum resin (*B. sacra*) from

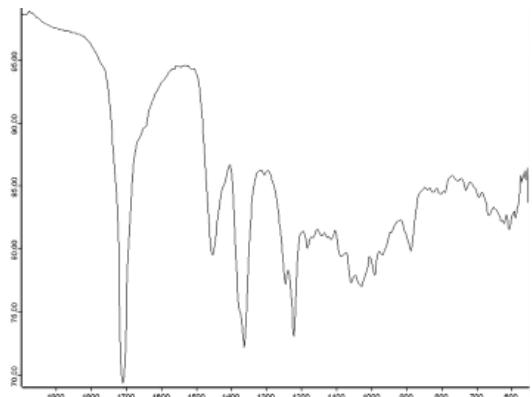
Meydi resin (*B. frereana*). Guenther (1943) also stated *B. sacra* (syn. *B. carteri*) gum resin to be "about 75% soluble in alcohol".

TLC / HPLC / GC



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

NIR Spectroscopy



Adulterants and Adulterations: Real frankincense resin is most commonly adulterated with colophonium and turpentine.

Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

The antimicrobial activities of *Boswellia* essential oils were individually evaluated against different microorganisms including fungi, Gram-positive and Gram-negative bacteria strains. The essential oils with the best activity against fungal strains were those obtained from *B. carteri* and *B.*

papyrifera with MIC values as low as 6.20 microg/ml (Camarda et al., 2007).

Sixteen triterpene acids and two cembrane-type diterpenes from *Boswellia carteri* were examined for their inhibitory effects on the induction of Epstein-Barr virus early antigen (EBV-EA) by 12-O-tetradecanoylphorbol-13-acetate (TPA) in Raji cells and on activation of (+/-)-(E)-methyl-2[(E)-hydroxyimino]-5-nitro-6-methoxy-3-hexamide (NOR 1), and cytotoxic activities against three human neuroblastoma cell lines, IMR-32, NB-39, and SK-N-SH in vitro. Seven compounds showed potent inhibitory effects on EBV-EA induction. Upon evaluation against activation of NOR 1, five compounds showed potent inhibitory effects. Fifteen compounds exhibited potent cytotoxic activities with IC₅₀ values of 4.1-82.4 μM against all of the three human neuroblastoma cells tested (Akihisa et al., 2006). Sixteen compounds exhibited marked anti-inflammatory activity against 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced inflammation (1 microg/ear) in mice with a 50% inhibitory dose (LD₅₀) of 0.05-0.49 mg/ear (Banno et al., 2006).

BC-4, an isomeric compound isolated from the plant *Boswellia carteri* Birdw. containing α- and β-boswellic acid acetate in 1:1, was cytostatic and could induce the differentiation of B16F10 mouse melanoma cells, blocked the cell population in G1 phase, and inhibited topoisomerase II activity. BC-4 inhibited the migration activity of B16F10. It induced apoptosis of human fibrosarcoma HT-1080 cells and also inhibited the secretion of matrix metalloproteinases from HT-1080 cells (Zhao et al., 2003). Boswellic acid acetate (BC-4) can further induce differentiation and apoptosis of leukemia cells (Jing et al., 1999).

Acetyl-11-keto-β-boswellic acid isolated from the gum-resin of *Boswellia carteri* targets the androgen receptor signalling and thus could offer significant protection against the development and progression of prostate cancer (Yuan et al., 2008). It also inhibits the growth of chemotherapy-resistant human PC-3 prostate cancer cells in vitro and induces apoptosis by activation of caspase 3 and the induction of DNA fragmentation. It further inhibits proliferation and induces apoptosis in PC-3 prostate cancer cells xenotransplanted onto the chick chorioallantoic membrane in vivo (Büchele et al., 2006).

Incensole acetate, isolated from the resin of *B. carteri*, was shown to inhibit the production of inflammatory mediators in an in vitro model system of C6 glioma and human peripheral monocytes (Moussaieff et al., 2008).

An extract from *B. carteri* gum exudate induces time- and concentration-dependent apoptosis in Jurkat cell line (Liu & Qi, 2000) and induces apoptosis in acute non-lymphocytic leukemic cells and HL60 cells (Qi et al., 1999).

SAFETY DATA

Preclinical Safety Data

Single Dose Toxicity cf. appendix 1

The LD₅₀ of boswellic acids is > 2g/kg in rats and mice; doses of 2g/kg in mice, rats, and monkeys have not caused death; high doses have reportedly not yielded significant effects on behavior or clinical, haematological, biochemical, and pathological data.

Repeated Dose Toxicity: Subacute toxicity studies in rabbits over three months, and chronic toxicity studies in rats and monkeys over six months, have found no toxic effects of boswellic acids at high doses.

KEY (PROPOSED) USAGE

Therapeutic Indications: We can now confidently state that several biological evaluation reports in the literature on so called *B. carteri* resins may not be from trees of this species. For instance in the recent paper by Akihisa et al., over 15 boswellic acids were isolated along with incensole and incensole acetate from gum-resin supplied to them by an American company, Scents of Earth. The workers were led to believe that the gum-resin was derived from *B. carteri* trees. The workers found the isolated compounds to possess strong anticancer and cytotoxic properties. However it is now evident that incensole and incensole acetate are unique markers present only in *B. papyrifera* originating from Ethiopia and are not present in authentic *B. sacra* (syn. *B. carteri*) gum-resins. Hence, the conclusions of the above important paper may be valid for *B. papyrifera* gum-resin and not for *B. sacra* (syn. *B. carteri*).

Boswellic acids are known to possess marked anti-inflammatory properties (Safayhi et al., 1997) and are useful in treating patients with rheumatoid arthritis, chronic colitis, bronchial asthma, etc.

Special Warnings and Precautions for Use:

Can safely be consumed when used appropriately.

Interactions: Extracts of the gum-resin of *Boswellia carteri*, *Boswellia frereana*, *Boswellia sacra*, and *Boswellia serrata* were demonstrated as being equally moderate to potent, non-selective inhibitors of the major drug metabolising CYP enzymes 1A2/2C8/2C9/2C19/2D6 and 3A4 (Frank & Unger, 2006).

Pregnancy and Lactation: Safety of boswellia during pregnancy has not been systematically studied, and therefore cannot be recommended.

Evaluation of Efficacy: Efficacy proven, but too few results to be conclusive.

TRADE INFORMATION

Nature of plant material: Conservation status: *B. sacra* occurs in *Acacia-Commiphora* woodland at altitudes not exceeding 1200 m, usually in rocky slopes and gullies in particular on limestone boulders. The areas where these trees are found are arid and mostly inaccessible making conservation work quite a difficult undertaking. The tree is sought after for its highly aromatic resin.

Nature of plant products: There are six most common *Boswellia* species whose resins are widely traded and these are: *B. frereana* Birdw. known only from Somalia *B. sacra* Flueck. (syn *B. carteri*) from Somalia, Yemen and Oman *B. papyrifera* Hochst. from Ethiopia and Sudan *B. riva* Engl. from Ethiopia *B. neglecta* S. Moore also from Ethiopia *B. serrata* Roxb. from India period.

The trees and resins of each of these frankincense producing species are known in different localities by different names. However in English the resins are all lumped under the general name frankincense or olibanum, although in most cases the geographic origin is given some emphasis.

Processing and storage: Extraction: approx 10 to 30 cuts are made on the trunk and larger branches of the tree using a special scraping knife. Although the resin can be collected at any time of the year, extraction usually begins in March or April and continues throughout the summer months. The trees can be exploited for a maximum of three years followed by a resting period of several years.

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Bulbine frutescens

GENERAL DESCRIPTION

Scientific Name with Author: *Bulbine frutescens* Willd.

Synonyms: *B. caulescens* L., *B. rostrata* Willd.

Family: Asphodelaceae (subfamily Asphodeloideae)

Vernacular Names: Balsemkopieva, bulbine, bulbinella, burn jelly plant, cat's tail, elimpofu, geelkatstert, ibhucu, intelezi, ithethe rankkopieva, khomo-ya-ntsukammele, sehlare-sa-mollo, sehlare-sa-pekan, snake flower.

Botanical Description: A variable perennial herb up to 0.5 m high (rarely up to 1.2 m), with woody stems bearing succulent leaves; leaves linear, terete or somewhat flattened, 30 - 250 mm long, 4 - 10 mm in diameter, bright green, yellowish green, or glaucous, glabrous. Flowers small, mostly yellow, rarely partly orange, in multiflowered racemes on slender peduncles; pedicel thin, usually curved, tepals six, yellow, rarely orange, erect to recurved, somewhat persistent. Stamens 6; filaments densely hairy. Fruit a small capsule. *B. frutescens* is unique in the genus because of the woody stems. It also differs from related species in details of the floral bracts (Baijnath, 1977).

Origin and Distribution: South Africa and Lesotho (widespread, but mainly in the Eastern Cape Province) (Baijnath, 1977). A very common garden plant throughout southern Africa.



Plant Part Used: Fresh or freeze-dried leaf gel



ETHNOBOTANICAL INFORMATION

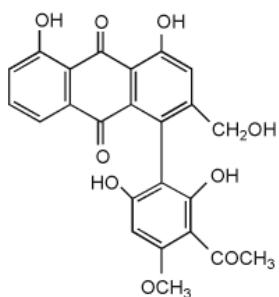
Major Ethnopharmacological Uses: The fresh leaf gel of *Bulbine frutescens* and the closely similar *B. asphodeloides* is widely used to treat wounds, cuts, grazes, burns, sores, rashes, itches, cracked lips, mosquito bites, ringworm, and herpes (Smith, 1895; Watt & Breyer-Brandwijk, 1962; Roberts, 1990; Van Wyk et al., 1997; Van Wyk & Gericke, 2000; Rood, 2008). Early records (Smith, 1895) exist for the use of the rhizomes and roots to treat scrofula – a form of tuberculosis affecting the lymph nodes – and the leaf juice as a

styptic. The fresh leaf juice of *B. lagopus* is applied to wounds, sores and skin conditions (Thring & Weitz, 2006). The fresh whole plant, including the roots of *B. abyssinica*, is used to make a tea, for use by women to treat unspecified ailments, vaginal and bladder problems, infertility, back pain, and cough (Van Wyk et al., 2008).

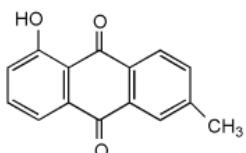
Infusions of the roots of several species, including *B. latifolia*, *B. natalensis*, *B. alooides*, *B. asphodeloides* and *B. narcissifolia* are taken against vomiting, diarrhoea, and to treat venereal diseases, urinary complaints, diabetes, rheumatism, convulsions, and blood disorders (Watt & Breyer-Brandwijk, 1962; Van Wyk et al., 1997; Felhaber & Mayeng, 1997; Van Wyk & Gericke, 2000; Rood, 2008).

CHEMICAL CONSTITUENTS

Compounds: The components of Bulbine gel and the structure of the gel polysaccharides appear to be unknown. Available studies have focused on knipholone-type phenylanthraquinones, including gaboroquinones A and B (Abegaz et al., 2002), as well as knipholone, 4'-O-demethylknipholone and sulfated analogues (Mutanyatta et al., 2005).



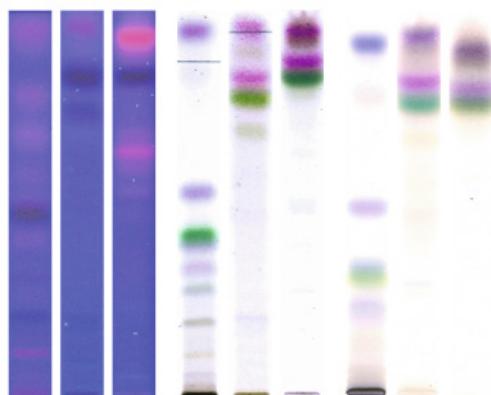
Knipholone



Chrysophanol

QUALITY CONTROL

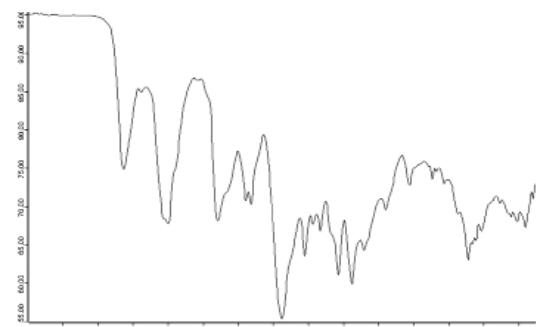
TLC / HPLC / GC: Standard TLC methods can be used for the detection of free sugars. The anthranoid metabolites in the stems can be studied using standard methods (reverse phase C18, methanol-water or acetone-water gradients) but pure gel is practically devoid of phenolic compounds. Methods proposed for aloe raw material (Standards South Africa, 2007) can be used, including the determination of alcohol-precipitable solids.



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

Markers and Quantitative Methods: Unknown. Gel polysaccharides and/or glycoproteins are likely to be responsible for the reported soothing and wound-healing properties. Stems and roots are rich in anthraquinones (Van Wyk et al., 2005) and contain several knipholone-type sulfated phenylanthraquinones and their sulfate-free analogs (Abegaz et al., 2002, Mutanyatta et al., 2005).

NIR Spectroscopy



Adulterants and Adulterations: Bulbine gel may be adulterated or confused with the gel from various Aloe species.

The fresh gel may be adulterated with aloe gel or the gel powder with various substances (as is common in the Aloe vera gel industry), e.g. malic acid or lactic acid. Preservatives or additives must be specified by the supplier (Standards South Africa, 2007).

Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

Bulbine frutescens gel is used in treating postoperative scars. A patent (Widgerow & Chait, 1998; Widgerow et al., 2000) claims that the inclusion of the gel of *B. frutescens* (with pantheol and asiaticoside) into microporous paper tape results in enhanced wound healing, with hastened scar maturation. The beneficial effects are ascribed to glycoproteins in the gel, as well as hydrating effects. Bulbine gel should comprise between 12.5 and 25% (preferably 20%) of the contact medium (Widgerow & Chait, 1998; Widgerow et al., 2000). The topical healing effect is likely to be due to polysaccharides and/or glycoproteins in the leaf gel. An extract has been incorporated into the formulation of a scar management programme.

The traditional use of Bulbine leaf juice suggests anti-inflammatory and wound-healing activity, but no published information on the pharmacological activity is available. Extracts of the dried leaves showed no activity against a range of bacteria (Rabe & Van Staden, 1997). Pure knipholone also showed no antibacterial activity (Van Staden & Drewes, 1994).

Knipholone and related compounds are strongly antiplasmodial (Bringman et al., 1999; Abegaz et al., 2002).

Clinical Studies: Clinical evidence of improved postoperative scar management and scar maturation was presented by Widgerow & Chait (1998) and Widgerow et al. (2000), who treated nearly 300 patients.

SAFETY DATA

No formal safety studies are available.

Preclinical Safety Data

Single Dose Toxicity cf. appendix 1

Widgerow & Chait (1998) reported temporary sensitivity to the topical application of *Bulbine gel* (with asiaticoside and pantheol) in two out of a total of nearly 300 patients.

KEY (PROPOSED) USAGE

Therapeutic Indications: The gel is used in skin-care products and wound-healing ointments.

Contraindications: No information is available.

Special Warnings and Precautions for Use: Bulbine leaf gel – For external use only.

Interactions: No information is available.

Adverse Effects: No information is available.

Evaluation of Efficacy: Enhanced wound healing/improved postoperative scar management efficacy clinically proven (Widgerow & Chait, 1998; Widgerow et al., 2000).

Topical use (burns, wounds, and inflammatory skin conditions) efficacy traditionally proven. Anti-inflammatory efficacy traditionally proven.

TRADE INFORMATION

Nature of plant material: Origin: South Africa

Flowering/harvesting time: Plants can be harvested at any time of the year.

Cultivated/wild crafted: The plant is exceptionally easy to grow and has been cultivated on a small scale for gel production. It is a common garden plant (garden succulent) in South Africa. Rapid scale-up is possible.

Conservation status: Not listed.

Processing and storage: Leaf gel is obtained by pressing or liquidizing the fresh leaves and stems. Stability of product: No reliable data are available. The product is stable but hygroscopic when freeze-dried, and unstable when fresh.

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Cajanus cajan

GENERAL DESCRIPTION

Scientific Name with Author: *Cajanus cajan* (L.) Millsp.

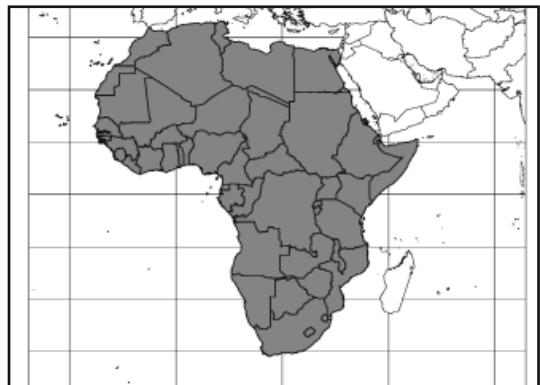
Synonyms: *Cajanus bicolor* DC., *C. flavus* DC., *C. indicus* Spreng., *C. luteus* Bello, *Cystisus cajan* L..

Family: Fabaceae

Vernacular Names: Adua, aduwa, agadagbul, agugu, alev, alev a batur, alverja, ambrevade, amdzimtua, atii, atiyi, biandobwe, blofoyo, Congo pea, dan mata waaken tantabaraa, dhal, feijao, fiofio, fjo, gandul, gungo pea, gungs pea, hjojho, kichepo, kongo-binch, konjoi, konsho, konshong, konso, konso-toge, konsoba, konson-tugo-na kudua, konyoi, koso, kunse, kukunchi, no eye pea, otili, pigeon pea, pigeon pea bush, pois d'Angole; pois d'ambrevade, pois du Congo, pois pigeon, Puerto Rico pea, red gram, shingwazo, shoo, shoo-shoo, stil, tiyi, waaken tuuraawaa, waaken yan maataa, yawendo, yo, yoo.

Botanical Description: Perennial woody shrub, mostly grown as an annual for the legume; stems strong, woody, up to 4 m tall, freely branching; root system deep and extensive, to about 2 m, with a tap root. Leaves spirally arranged on the stem, alternate, pinnately trifoliate, stipulate; stipels small, subulate; leaflets lanceolate to elliptic, entire, acute apically and basally, penninerved, resinous on lower surface and pubescent, to 15 cm long and 6 cm wide. Inflorescence in terminal or axillary racemes in the upper branches of the bush. Flowers occur in terminal or axillary racemes, are 2 - 3 cm long, multicolored, predominantly with yellow, red, purple, orange occurring in streaks or fully cover the dorsal side of the flag; zygomorphic. Pods compressed, 2 - 9-seeded, not shattering in the field. Seeds lenticular to ovoid, to 8 mm in diameter, about 10 seeds per gram, separated from each other in the pod by slight depressions.

Origin and Distribution: Pigeon pea is cultivated throughout the tropics. In Africa, the main countries growing pigeon pea are Kenya, Malawi, Nigeria, Uganda, and Tanzania.



Plant Parts Used: Seeds, leaves, and roots.



ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses: The leaves are prepared in an infusion for anaemia, hepatitis, diabetes, urinary infections, and yellow fever. The flowers are also prepared in an infusion for dysentery and menstrual disorders, and the seeds are infused to use as a diuretic. It is useful in the swelling of internal organs. Some traditional health practitioners claim that appropriate preparations containing *C. cajan* reduce the swelling of internal organs such as the stomach, liver, intestines, etc. They further claim that such a preparation can reduce cancer of these organs. The recommended method of preparation includes the use of about 10 g of the green leaves of *C. cajan* added to seven black peppers, finely ground, and mixed in water, and then taken as a drink. The green leaves of *C. cajan* ground in water and added to partly boiled water should

be applied externally on the affected body part. Pigeon peas should be cooked in water, the water given to the patient. Fresh seeds are claimed to help urinary incontinence in males, whereas the immature fruits are believed to benefit patients with liver and kidney ailments. In addition, the seeds of *C. cajan* are used in sickle cell anaemia preparations. Furthermore, extracts of *C. cajan* are used in traditional medicine practice for the management of diabetes, hepatitis, and dysentery, among others.

CHEMICAL CONSTITUENTS

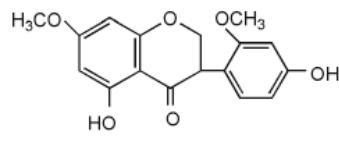
Compounds: Bioactivity-guided fractionation of extracts of roots and leaves of *C. cajan* yielded eight compounds: betulinic acid, biochanin A, cajanol, genistein and 2-hydroxygenistein, longistylin A and C, and pinostrobin.

Protocatechuic, *p*-hydroxybenzoic, vanillic, caffeic, *p*-coumaric and ferulic acids were identified and quantified in bark, stem and leaf. Glycerol, erythritol, threitol, arabinitol, xylitol, mannitol, galactitol, glucose, myoinositol, glucose, fructose and sucrose were the alditols, cyclitols, and free sugars present in varying amounts in the three parts of the plant. Uronic acid, ash, lignin and polysaccharides in the plant fractions were determined on the extractive-free dried materials. The stem and bark contained respectively 40.1% and 39.2% of α -cellulose.

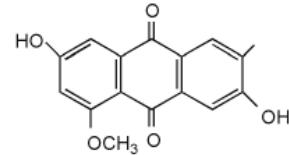
Analysis of the seeds (without husk) gave the following values per 100 g: 15.2% moisture, 22.3 g protein, 1.7 g fat (ether extract), 3.6 g mineral matter, 57.2 g carbohydrate, 9.1 mg Ca; 220 IU carotene evaluated as vitamin A and 150 IU vitamin B1. Sun-dried seeds of *C. cajan* are reported to contain per 100 g: 345 calories, 9.9% moisture, 19.5 g protein, 1.3 g fat, 65.5 g carbohydrate, 1.3 g fibre, 3.8 g ash, 161 mg Ca, 285 mg P, 15.0 mg Fe, 55 μ g beta-carotene equivalent, 0.72 mg thiamine, 0.14 mg riboflavin, and 2.9 mg niacin. Immature seeds of *C. cajan* are reported to contain per 100 g: 117 calories, 69.5% moisture, 7.2 g protein, 0.6 g fat, 21.3 g total carbohydrate, 3.3 g fibre, 1.4 g ash, 29 mg Ca, 135 mg P, 1.3 mg Fe, 5 mg Na, 563 mg K, 145 mg β -carotene equivalent, 0.40 mg thiamine, 0.25 mg riboflavin, 2.4 mg niacin, and 26 mg ascorbic acid. Of the total amino acids, 6.7% is arginine, 1.2% cystine, 3.4%

histidine, 3.8% isoleucine, 7.6% leucine, 7.0% lysine, 1.5% methionine, 8.7% phenylalanine, 3.4% threonine, 2.2% tyrosine, 5.0% valine, 9.8% aspartic acid, 19.2% glutamic acid, 6.4% alanine, 3.6% glycine, 4.4% proline, 5.0% serine, with 0 values for canavanine, citrulline, and homoserine. Methionine, cystine, and tryptophane are the main limiting amino acids.

The oil of the seeds contains 5.7% linolenic acid, 51.4% linoleic, 6.3% oleic, and 36.6% saturated fatty acids. Seeds are reported to contain trypsin and chymotrypsin inhibitors. Fresh green forage contains 70.4% moisture, 7.1 crude protein, 10.7 crude fibre, 7.9 N-free extract, 1.6 fat, and 2.3 ash. The whole plant, dried and ground, contains 1.2% moisture, 14.8% crude protein, 28.9% crude fibre, 39.9% N-free extract, 1.7% fat, and 3.5% ash. Four flavonoids, namely quercetin, luteolin, apigenin, and isorhamnetin, are contained in pigeon pea leaves.



Cajanol

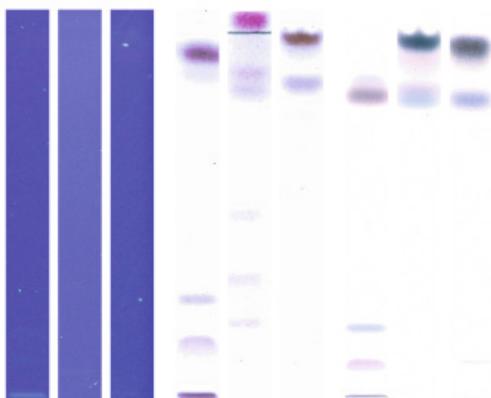


Cajaquinone

QUALITY CONTROL

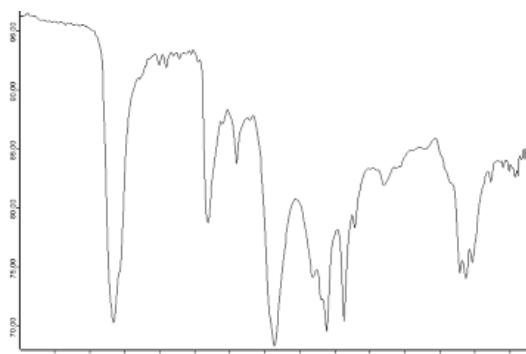
Identification: Seeds are variable in colour (white to brown to green), have a hard coat with hourglass-shaped cells and sometimes bear a U-shaped line.

TLC / HPLC / GC



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

NIR Spectroscopy



Adulterants and Adulterations: The closest wild relative *Atylosia cajanifolia* Haines and *Cajanus kerstingii*.

Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

Red cell glutathione S-transferase (EC 2.5.1.18) in volunteer sicklers, fed regularly with controlled doses of the antisickling extracts of *Cajanus cajan* seeds, was found to decrease progressively with time (78.92 +/- 8.94% drop by the third month). The in vivo effect of the extract also included a progressive increase in whole blood haemoglobin (38.43 +/- 8.62% rise by the third month). Phenylalanine, an antisickling amino acid, also exhibited a progressive in vitro inhibition of the activity of this erythrocyte enzyme (assayed at 37

C, pH 6.5) in a concentration-dependent manner (59.0 +/- 5.58% at 0.5 mM). A similar but more pronounced in vitro inhibitory effect was also exhibited by cajanus extract (84.0 +/- 4.01% at 0.5 mM equivalent). These findings suggest an influence of the antisickling nutrient/extract on the redox status of HbSS erythrocytes.

Amino acid analysis showed that solvent extracts of *C. cajan* seeds (white species) contain, as free amino acid, as much as 26.3% phenylalanine. Antisickling experiments based on the estimated amount of free phenylalanine in the methanol (water-soluble) extract of the seeds showed that the presence of this amino acid alone could account for about 70% of the antisickling potency of *C. cajan* seed extract. The amounts of phenylalanine and hydroxybenzoic acid in a *C. cajan* methanolic extract were estimated. Results showed that the amount of phenylalanine and hydroxybenzoic acid per gram weight of bean was 4.92 mg +/- 0.13 mg and 21.0 mg +/- 3.0 µg, respectively. Sickling inhibition was observed to be efficient with the extract, which contains a mixture of phenylalanine (0.69 mg/ml) and *p*-hydroxybenzoic acid (10.5 µg/ml), equivalent to those found in bean extract. The additive antisickling effect of both compounds can be therapeutically exploited for the treatment of sickle cell anaemia. Ekeke and Shode (1985) reported that extracts of *C. cajan* reversed sickled red blood cells to normal morphology.

Ashidi, Houghton and Hylands (2006) reported the cytotoxic effects of extract of *C. cajan* leaves. The authors used both human cancer and non-cancer cell lines in vitro. The extraction was undertaken with methanol continuously for five days using air-dried leaves of the plant. Subsequently, the extract was concentrated under reduced pressure. 60 g of the extract was adsorbed on silica gel GF254 and fractionated by vacuum liquid chromatography (VLC). The data showed that the dichloromethane (DCM) fraction manifested modest cytotoxicity against human amelanotic melanoma C32, human breast adenocarcinoma MCF-7, and human large cell lung carcinoma cell lines COR-L23 and human fetal lung fibroblast MRC-5 (IC_{50} = 12.0, 10.0, 10.0, and 15.0 µg/ml, respectively). Bioactivity-guided fractionation of DCM fraction was done by flash chromatography and then purified on preparative Thin-Layer Chromatography. Two

prenylated stilbenes (longistylin A and C) were isolated following the fractionation process. Ashidi and his colleagues were the first to report the cytotoxic effects of these compounds against human amelanotic melanoma C32, human breast adenocarcinoma MCF-7, and human large cell lung carcinoma COR-L23 cell lines. The authors further reported that the IC₅₀ of the compounds ranges between 20 and 35 µM. These findings provide preliminary (in vitro) scientific evidence to justify the inclusion of *C. cajan* in traditional herbal medicines used for the treatment of cancer in southwestern Nigeria.

In 2004, Ducker-Eshun and his colleagues isolated betulinic acid, biochanin A, cajanol, genistein, 2'-hydroxygenistein, longistylin A and C, and pinostrobin from the roots and leaves of *C. cajan*. They also reported that the two stilbenes, longistylin A and C, and betulinic acid exhibited a moderately high in vitro activity against chloroquine-sensitive *Plasmodium falciparum* strain 3D7.

Clinical Studies: One hundred patients with steady-state sickle-cell anaemia (SCA) were randomized into treatment and placebo arms. The extract/placebo were administered twice daily to the subjects. Weight, hepatosplenomegaly, blood levels of bilirubin, urea, creatinine, and packed cell volume (PCV) were monitored over a 6-month period. Recall episodes of pain 6 months before enrolment were compared with episodes of pain recorded during the treatment period. Twenty-six cases (55.3 %) had hepatomegaly on enrolment. This reduced significantly to 33.3% at 6 months ($p = 0.03$), but increased in the placebo arm ($p > 0.05$). The total number of recall painful episodes in cases was 207 (mean 4.4 ± 10.3 (SD), range (0 - 60) and fell to 191 (mean 4.2 ± 4.4 (SD), range 0 - 16); $p = 0.03$. Episodes of pain increased from 109 in controls (mean 2.6 ± 5.0 (SD), range 0 - 26) to 164 (mean 3.9 ± 4.3 (SD), range 0 - 22); $p = 0.01$. Mean PCV in the cases showed no appreciable changes ($p = 0.1$), but there was a significant increase in the controls ($p = 0.02$). In conclusion, the extract may cause a reduction of painful crises and may ameliorate the adverse effects of sickle cell anaemia on the liver.

SAFETY DATA

Preclinical Safety Data

Single Dose Toxicity cf. appendix 1

KEY (PROPOSED) USAGE

Therapeutic Indications: Sickle-cell anaemia.

Evaluation of Efficacy: Efficacy clinically proven, but too few results to be conclusive.

TRADE INFORMATION

Nature of plant material: Conservation Status: Pigeon pea is cultivated in more than 25 tropical and subtropical countries, either as a sole crop or intermixed with such cereals as sorghum (*Sorghum bicolor*), pearl millet (*Pennisetum glaucum*), or maize (*Zea mays*), or with legumes, e.g. peanut (*Arachis hypogaea*). Pigeon peas are very drought resistant and can be grown in areas with less than 650 mm annual rainfall.

Growth and Management: Pigeon pea is normally sown directly into prepared ground. Seeding depths of 2.5 - 5 cm are recommended. No pre-germination treatment of the seed is needed. Although some varieties mature seed in 5 - 6 months, longer-lived, tall varieties, including those that are more competitive in the wild, take 10 - 12 months to mature seed.

Processing and storage: The mycoflora, moisture content, and aflatoxin contamination of pigeon pea (*C. cajan* (L.) Millsp) stored in jute sacks and iron bins were determined at monthly intervals for a year. The predominant fungi on freshly harvested seeds were *Alternaria* spp, *Botryodiplodia theobromae*, *Fusarium* spp., and *Phoma* spp. These fungi gradually disappeared from stored seeds with time, and by 5 - 6 months most of them could no longer be isolated. The fungi that succeeded the initially dominant ones were mainly members of the genera *Aspergillus*, *Penicillium*, and *Rhizopus*. Populations of these fungi increased up to the end of one year's storage. Higher incidences of mycoflora and *Aspergillus flavus* were recorded in jute-sack samples throughout the storage period. The moisture content of stored seeds was found to

fluctuate with the prevailing weather condition, being low during the dry season and slightly high during the wet season. The stored seeds were free of aflatoxins for 3 and 5 months in jute sacks and iron bins, respectively. The level of aflatoxins detected in jute-sack storage was considerably higher than that occurring in the iron bin system. Of 196 isolates of *A. flavus* screened, 48% were toxigenic in liquid culture (54% from jute sacks and 41% from iron bins).

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Carissa edulis

GENERAL DESCRIPTION

Scientific Name with Author: *Carissa edulis* Vahl.

Synonyms: *Carissa pubescence*, *Arduina edulis* Spreng., *Azima pubescens* Suesseng., *Carissa edulis* var. *tomentosa* (A.Rich.) Stapf., *Carissa pilosa* Schinz., *Jasminonarium edule* (Vahl.) Kuntze., *Jasminonarium tomentosum* (A.Rich.) Kuntze.

Family: Apocynaceae



Vernacular Names: Agam, amatungula, arabian num-num, carandas-plam, ciruela de Natal, cizaki, Egyptian carissa, emir, enkeldoring-noemnoem, mkokolo, mlanoa-mboo, mpambala, mtanda-mboo, mudyabveni, mudzambaa, muhlababunzi, mumbingwa, muruguru, mutsamviringa, muyonza, mu-kawa, Natal plum, simple spined carissa, simple spined num-num, umlugulu.

Botanical Description: *Carissa edulis* is a spiny, much branched, small tree, shrub or scrambler, up to 5 m in height, with a milky sap. The bark is grey, smooth, young branchlets with or without hairs; spines simple, straight, 2 - 5 cm long, usually single. Leaves ovate to ovate-elliptic; opposite; occasionally almost circular, 2.5 - 6 x 1.8 - 3 cm, leathery, dark green above, paler green below, with or without short, soft hairs; lateral veins obscure; apex tapering, often with a bristlelike tip; base rounded to shallowly lobed; margin entire; petiole 1 - 4 mm long. Flowers white tinged with purple, red, or pink, up to 1.8 cm long, about 2 cm in diameter, slender, tubular, with corolla lobes overlapping to the right, sweetly scented, in terminal heads about 4 cm in diameter. Fruits ovoid to almost spherical, up to 1.1 cm in diameter, red to black, ripening to purplish black, containing 2 - 4 flat seeds (Ibrahim et al., 2005).

Origin and Distribution: Botswana, Cambodia, Cameroon, Eritrea, Ethiopia, Ghana, Guinea, Japan, Kenya, Myanmar, Namibia, Nigeria, Saudi Arabia, Senegal, South Africa, Sudan, Tanzania, Thailand, Uganda, Vietnam, and Yemen.

Plant Parts Used: Leaves and root bark.



ETHNOBOTANICAL INFORMATION

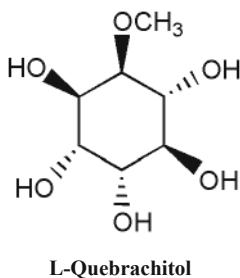
Major Ethnopharmacological Uses: The ripe black fruits are edible. The vapors of the boiled pungent roots are inhaled to treat chest congestion. The plant parts are used in ethnomedicine for a wide variety of illnesses, such as the following: the leaves, stem, and root barks are used for the treatment of sickle cell anaemia, oedema, toothache, also as abortifacient, and as purgative. The root is used for chest complaints, cough, gastric ulcer, and in expelling worms, especially *Taenia* spp. In addition, the roots contain an active ingredient (carisstone) that may prove useful in the treatment of cancer. The twigs contain quebrachitol and cardiotropins that are useful as an antihelminthic against tapeworm. The leaves are used in the treatment of hernia or cyst and the

bark is used aphrodisiac (Ibrahim et al., 2005). Other folkloric uses of *C. edulis* include treating fever, epilepsy, headache, gonorrhea, syphilis, rheumatism, rabies, and acts as a diuretic.

Other Relevant Uses: Additional uses include the following: (a) Food: fruits are sweet and pleasant to eat; in Ghana, they are normally added to the food of invalids as an appetizer. Vinegar can be made from them by fermentation; in Sudan and Kenya, they are made into a jam. The roots are put into water gourds to impart an agreeable taste and are added to soups and stews for the same reason; (b) Fodder: goats and camels in the dry parts of Sudan browse on *C. edulis*; (c) Fuel: the species is a source of excellent firewood; (d) Poison: in Kenya, a piece of the root is fixed into a hut roof as a snake repellent. The plant is also used as a source of dye (Ya'u et al., 2008).

CHEMICAL CONSTITUENTS

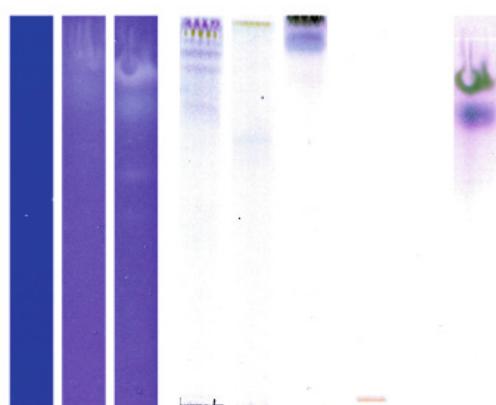
Compounds: Secondary metabolites isolated from this plant consist of monoterpenes sesquiterpenes, pentacyclic triterpenes, steroids, (Achenbach et al., 1985), benzenoids (12 - 15), Lignans, catalponol, coumarins, and the sugar derivative quebrachitol (Achenbach et al., 1983; Bentley et al., 2004), and essential oils (Moudachirou et al., 1998).



L-Quebrachitol

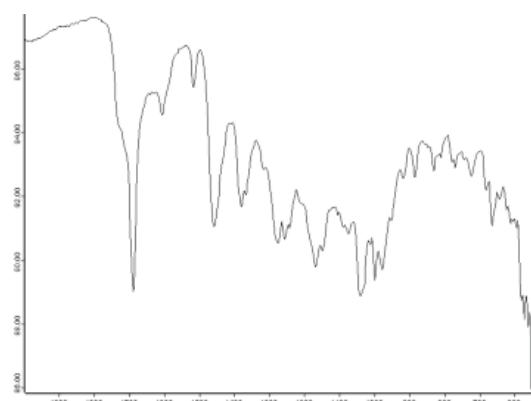
QUALITY CONTROL

TLC / HPLC / GC



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

NIR Spectroscopy



Adulterants and Adulterations: *C. edulis* closely resembles *C. bispinosa*, the obvious feature separating them being that *C. edulis* has straight thorns while those of *C. bispinosa* are Y-shaped.

Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

Both in vivo and in vitro studies on *C. edulis* indicate the following activities: anticonvulsant (Ya'u et al., 2008), anti-viral (Tolo et al., 2006), amoebicide, cardiotonic, and antileukaemic (Abebe et al., 2003), hypoglycaemic (El-Fiky et al., 1996), and diuretic properties (Nedi et al.,

2004). The authors believe that these results have provided a rationale for the use of *C. edulis* in traditional medicine for the management of such diseases as epilepsy, infections due to herpes simplex virus, and fever.

SAFETY DATA

Preclinical Safety Data

Single Dose Toxicity cf. appendix 1

KEY (PROPOSED) USAGE

Therapeutic Indications: Antimicrobial, analgesic.

TRADE INFORMATION

Processing and storage: Leaves were allowed to dry in the shade; root bark was dried in a fan-circulated oven at 40°C.

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Tolo FM, Rukunga GM, Muli FW, Njiagi ENM, Njue W, Kumon K, Mungai GM, Muthaura CN, Muli JM, Keter LK, Oishi E, Koffi-Tsekpo MW (2006) Anti-viral activity of the extracts of a Kenyan medicinal plant *Carissa edulis* against herpes simplex virus. J. Ethnopharmacol. 104: 92-99.

Ya'u J, Yaro AH, Abubakar MS, Anuka JA, Hussaini IM (2008) Anticonvulsant activity of *Carissa edulis* (Vahl) (Apocynaceae) root bark extract. J. Ethnopharmacol. 120: 255-258.

Catharanthus roseus

GENERAL DESCRIPTION

Scientific Name with Author: *Catharanthus roseus* (L.) G.Don.

Synonyms: *Vinca rosea* L., *Pervinca rosea* (L.) Moench, *Lochnera rosea* (L.) Reichenb., *Ammocalis rosea* (L.) Small, *Hottonia littoralis* Lour., *Vinca speciosa* Salisb.

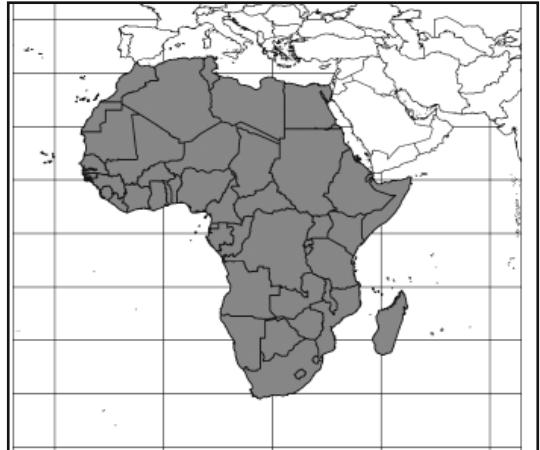
Family: Apocynaceae

Vernacular Names: Arivotambelona, befela, felabaratra, felatananamba, heladolo, maintsorininina, pervenche de Madagascar, rivotambelona, rose amere, salotra, saponaire, sarita, tonga, tongatse, tsimatinirinina, tsingevika, vonenina.

Botanical Description: An erect or bushy straggling herb to about 80 cm high. Leaves opposite in pairs, oblong-elliptic, 2-7 cm long and up to 3 cm broad. Flowers solitary or in small axillary clusters. Corolla with a narrow, straight tube expanded immediately below the limb; limb flat, of 5 obovate lobes about 2 cm long, of one of three colour forms: pink with a crimson eye, white with a crimson eye, or white with a yellow eye; follicles paired, cylindrical, 1.5-3.5 cm long.

Origin and Distribution: Originating from Madagascar, *C. roseus* now has a pantropical distribution. It is naturalized in continental Africa, the Americas, Asia, Australia, and Southern Europe, and on some islands in the Pacific Ocean. This distribution may have been caused by sailors who took the plants on board during their travels. The leaves of the plants were chewed to suppress the sensations of hunger and fatigue. Major areas for cultivation are found in the southern states of the USA, Mexico, South America, Middle East, China, and India.

There are four varieties of the plant: *C. roseus* var *roseus* Markgr.; *C. roseus* var *albus* G. Don; *C. roseus* var *angustus* Bakh.f., and *C. roseus* var *nanus* Markgr.



Plant Parts Used: Leaves, flowers, and the whole plant.



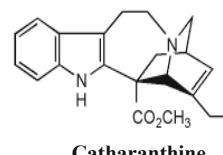
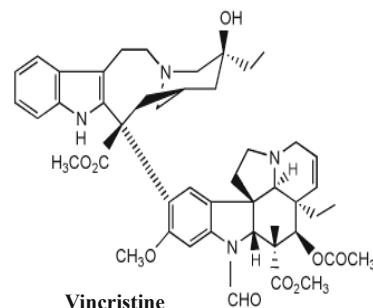
ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses: Medicinal uses include treatment of leukaemia, Hodgkin's disease, and diabetes. Its hypotensive effect was reported as early as the 17th century. *Catharanthus* is used as a bitter tonic, a galactogogue, and an emetic. In Madagascar the leaves are used to suppress hunger, and the decoction is used when a child is suffering from measles and 'Angalahy' – a disease which particularly afflicts the younger generation during a bout of fatigue. Use in the treatment of rheumatism, skin disorders, and venereal diseases has also been reported. In Benin a decoction of the leaves of *Cassia occidentalis* and *C. roseus* is used for hepatitis and dyspeptic disorders. The plant is used to treat stomach pains, loss of appetite, slow digestion, hypertension, flatulence, abdominal pains, and liver problems. A decoction of a few leaves brings down a fever. *C. roseus* var *alba* also treats stomach colics. A leaf decoction is useful against stomach problems, diabetes, and diarrhoea. A stem decoction taken orally is reputed for its antipyretic and antidiabetic properties.

CHEMICAL CONSTITUENTS

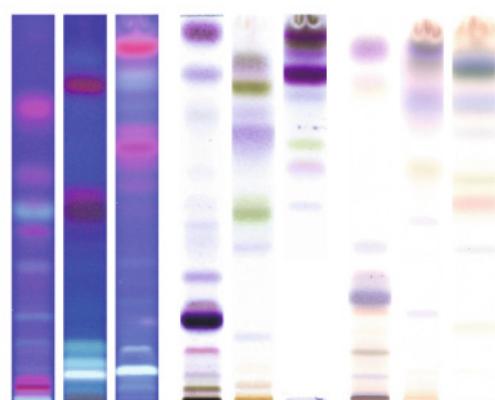
Compounds: With about 130 different compounds isolated, *C. roseus* can be regarded as a rich source of alkaloids. The alkaloids belong to the class of terpenoid indole alkaloids (TIAs). *Catharanthus* is particular rich in bisindole alkaloids (about 40 compounds). The aerial parts contain between 0.2% and 1% alkaloids. Most of the isolated compounds are the monomeric C20 and dimeric C40 compounds. The leaves contain the triterpenes amyrin, lupeol, ursolic acid, and anthocyanins. These monomers are the major components: vindoline (majoritaire), catharanthine, lochnerine, akuammicine, ajmalicine, tetrahydroalstonine, etc. Nonetheless, the truly active compounds are the dimers, which are the bis-indolic alkaloids found in yields as low as a few ppm. The compounds showing cytotoxic properties are vincaleucoblastine (VLB), vincristine (VCR), leurosine, and leurosidine. These four molecules have very closely related structures. The roots of the plant are rich in the monomeric derivatives of the heteroyohimbine type (serpentine, ajmalicine). Other compounds have been isolated from *C. roseus*, including

monoterpenoid glucosides (loganin, secologanin, sweroside, deoxyloganin, dehydrologanin), steroids (catasteron, brassinolides), phenolics, flavonoids and anthocyanins. From the roots, β-yohimbine, tetrahydroalstonine, yohimbine, and serpentine have been isolated. The anthocyanins hirsutidin, malvidine, and petunidine are also present.

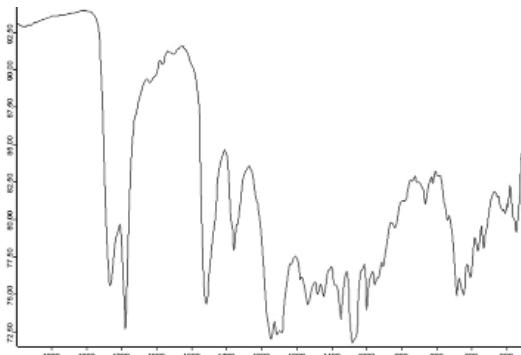


QUALITY CONTROL

TLC / HPLC / GC



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

NIR Spectroscopy**Standard specifications cf. appendix 2****PHARMACOLOGICAL PROPERTIES**

The dimeric alkaloids, in particular vinccaleucoblastine (VLB) and vincristine (VCR), have antitumoural and antileukaemic properties. Like other molecules having antitumoural properties, VLB and VCR are reasonably toxic. Reserpine and ajmalinine have been reported as having hypotensive and tranquillising properties. Leurosine sulphate, vindoline hydrochloride, and vindolinine have hypoglycaemic properties and their effect is much more pronounced than that of tolbutamide at similar doses. The hypoglycaemic activity begins slowly and its effect is longer lasting. Hydroalcoholic extracts of flowers, leaves, stems, and roots of *C. roseus* L. were tested for anti-hyperglycaemic and hypoglycaemic activities. Anti-hyperglycaemic activity was tested in glucose-overloaded hyperglycemic rats and hypoglycaemic activity in fasted normal rats at two dose levels, 100 and 200 mg kg G-1, respectively. Glimepride 0.1 mg kgG-1 was used as the reference drug for both the activities. Results showed that the hydroalcoholic extracts of every part tested exhibited significant anti-hyperglycaemic and hypoglycaemic activity. By comparison, the hydroalcoholic extract of leaves exhibited better activity; the stems and flowers were equally effective, followed by the roots. The flower extract is insecticidal. Decoction of the whole plant shows hypoglycaemic properties. Cytotoxic activity for small cell lung cancer LXFS 650 has been shown for some new hydroxymethyl derivatives 13 and 14 of the antitumour indole-indoline alkaloids vinblastine 2 and leurosine 5. Antimicrobial activity of aqueous extracts has been shown.

SAFETY DATA**Preclinical Safety Data****Single Dose Toxicity cf. appendix 1****KEY (PROPOSED) USAGE**

Therapeutic Indications: Ajmalicine is used for the treatment of hypertension. Vinblastine is used in the treatment of Hodgkin's disease, non-Hodgkin's lymphoma, testiscarcinomas, and sometimes against breast cancer and choriocarcinomas. Vincristine is an oxidised form of vinblastine and is used against acute leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, rhabdomyosarcomas, Wilms' tumors in children, and breast cancer. Anhydrovinblastine is used as an antineoplastic agent in the treatment of cervical and lung cancer.

Evaluation of Efficacy: Efficacy clinically proven.

TRADE INFORMATION

Nature of plant material: Conservation status: Not threatened.

Processing and storage: At present, bisindole alkaloids are mainly obtained by extraction and purification from the leaves of *C. roseus*. Extraction methods have been extensively optimised. Ground *C. roseus* tissue is extracted with water and concentrated. Subsequently, an extraction with an organic solvent, e.g. ethyl acetate, is performed. The concentrate is rich in catharanthine, vindoline, and anhydrovinblastine (AVLB). The yield of AVLB can be enhanced by addition of acid, salt, or hydrogen peroxide to the aqueous extraction medium. The yield can be further enhanced by the addition of sodium borohydride.

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Centella asiatica

GENERAL DESCRIPTION

Scientific Name with Author: *Centella asiatica* (L.) Urb.

Synonyms: *Centella coriacea* Nannfd., *Hydrocotyle asiatica* L., *Hydrocotyle lunata* Lam. and *Trisanthus cochinchinensis* Lour.

Family: Apiaceae

Vernacular Names: Artaniyae-hindi, asiatic pennywort, azijinés centelés žolé, azsiai gázlófű, barmanimuni, barmi, bevilaqua, bhram buti, boabok, bodila-ba-dinku, boileau, bokkudu, brahma manduki, brahmi, brahmi ghi, brahmi-butti, bua bok, centella, chhotra mani-muni, chi-hsueh-ts'ao, cochlearia, coq claria, coq lariat, coquelariat, ghi, ghod tapre, gotu kola, herba kakikuda, herba pegagan, herbe boileau, hydrocotyle, hydrocotyle asiatique, idrocotile, imsen korokla, Indian pennywort, Indian water navelwort, Indischer Wassernabel, karinga, karivana, kudangal, lièn tièn thảo, luei gong gen, mandooka parni, mandukparni, manimuni, marsh pepperwort, matoyahuho, mrang-khua, mtwigahuwu, nat' centely asijské, pa-na-e-khaa-doh, phác chèn, phaknok, phalwaen, rau má, rohtosammakonputki, sallatsspikblad, saraswathiaaku, takip-kohol, talapetraka, thalkuri, thankuni, thol-kuri, tilkushi, titjari, tono'itahi, tsubo-kusa, tungchian, vallarei, vallari, vilakwa, vitovitolenge, water pennywort, watervavel, yahon-yahon, yerba de chavos.

Botanical Description: Slender, prostrate perennial herb, the stems creeping, rooting at the nodes. Leaves alternate, simple, rounded and palmately veined, entire to repand-dentate; petiole 2 - 15 cm long, thin. Inflorescence as pseudo-capitules, 2 - 4 small flowers borne on short peduncles. Fruit orbicular to ellipsoid, with rounded mericarp on the upper part.

Origin and Distribution: The plant is indigenous to the warmer regions of both hemispheres, including Africa, Australia, Cambodia, Central America, China, Indonesia, the Lao People's

Democratic Republic, Madagascar, the Pacific Islands, South America, Thailand, southern United States of America, and Vietnam. It is especially abundant in the swampy areas of India, the Islamic Republic of Iran, Pakistan, and Sri Lanka.



Plant Parts Used: Leaves, whole plant.



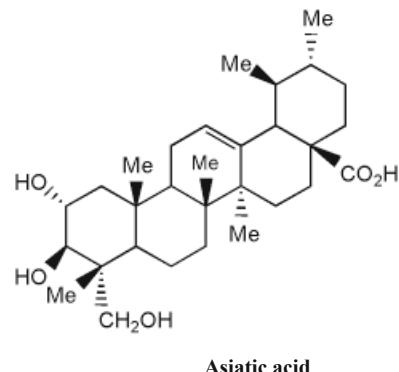
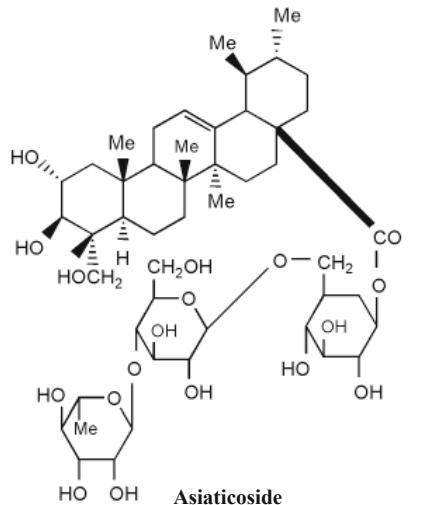
ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses: Applications include a variety of skin diseases, dyspeptic complaints, worms, fevers, insomnia, wound healing, etc. In small doses *Centella asiatica* acts as a stimulant, but in large doses it is used as a narcotic.

Other Relevant Uses: The leaves are also used in salads, curries, and cooked as a vegetable.

CHEMICAL CONSTITUENTS

Compounds: The whole plant contains alkaloids, tannins, coumarins, triterpenes, and traces of saponins (Gurib-Fakim et al., 1995). It further contains several triterpenes, saponins, and sapogenins. The alcoholic extract contains fatty acids: oleic, linoleic, lignoceric, palmitic and stearic glycerides, sitosterol, tannin, and resinous substances. The alkaloid hydrocotyline ($C_{22}H_{33}NO_8$, M.P. 210–212°C) has also been identified in the dry plant. It should be noted that the composition of the plant varies with its geographical distribution. The plant growing in Madagascar contains asiaticoside, but the same plant growing in Sri Lanka does not contain asiaticoside but rather a similar compound, centelloside, and other triterpenic acid derivatives: centoic and centellic acids. The plant also contains the saponins isothankuniside, thankuniside and the triterpenes brahmic, isobrahmic, isothankunic, madasiatic, medicassic, and thankunic acids. *C. asiatica* from Madagascar also contains the triterpenes madasiatic and madecassic acids. The latter has also been identified in *C. asiatica* growing in India and has thus been named brahmic acid. Another saponin very closely related to madecassoside has been identified in *Centella* species growing in China. These triterpenes are derived from ursenoic acid with an ursane nucleus. Of the triterpenes, mono- and sesquiterpenes, which have been identified in the plant, the most prominent are α -pinene, β -pinene, myrcene, γ -terpinene, bornyl acetate, α -copaene, β -elemene, β -caryophyllene, trans- β -farnesene, and germacrene-D. The flavonoids quercetin-3-glucoside and kaemferol-3-glucoside have also been identified in the plant.



QUALITY CONTROL

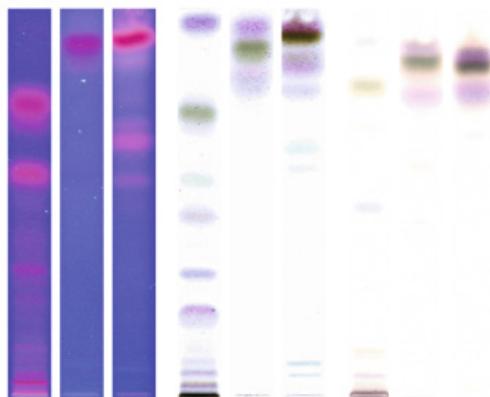
Organoleptic Properties: Tastes mildly bitter with a faintly tobacco-like odour.

Macroscopic Characteristics: The dried herb is a compressed mass of leaves, stem, and sometimes fruits. Leaves kidney-shaped or cordate, with a crenate margin and an obtuse apex, up to 5 cm in diameter but usually much smaller; petioles up to 0.5 mm in width, long, flattened and twisted; umbelliferous fruits of 2 - 4 bicarpellate schizocarps, flattened, longitudinally ridged and wrinkled, 3 - 5 mm in length.

Microscopic Characteristics: A grey-green powder consisting mainly of leaf fragments showing an uneven densely striated cuticle; upper and lower epidermis of polygonal cells with anisocytic stomata, more frequent on lower epidermis; thick-walled, uniseriate, covering trichomes on both surfaces, tapering towards the apical cell, up to 2 mm long but mainly

broken; infrequent large prism or cluster crystals of calcium oxalate. Petiole fragments show collenchyma of the cortex, secretory ducts in the cortical parenchyma, and bundles of narrow, septate fibres. Fragments of fruits infrequent, showing epidermis of polygonal cells, long uniseriate trichomes similar to those on the leaf, a parquetry layer, and parenchyma cells containing calcium oxalate prism crystals.

TLC / HPLC / GC



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

NIR Spectroscopy



Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

The pharmacological activity of *Centella asiatica* is thought to be due to several saponin constituents, including asiaticoside, asiatic acid, and madecassic acid. In vitro, each of these compounds stimulated the production of human

collagen I, a protein involved in wound healing. The plant is used in India for the healing of wounds and it is prescribed dermatology against burns, skin marks, wounds, and other cutaneous lesions. The saponins exhibit profound sedative properties, while extracts of the whole plant are insecticidal. Asiaticoside is also active against leprosy bacteria, *Mycobacterium leprae*, both in vivo and in vitro. It acts on the bacteria membrane and renders it susceptible to attack by drugs. Asiaticoside, as well as its oxide, acts on several forms of tuberculosis. The acids madecassic, asiatic and asiaticoside show important activity as wound-healing agents following oral application. The aqueous, ethanolic, basic, and ethanolic extracts of the plant show antifungal properties against *Trichophyton rubrum* and *Aspergillus niger* in vitro. The ethanolic extract of the plant is antiviral against the murine cytomegalovirus (MCMV) and the Sinbis virus (SV) at a minimum concentration of 125 and 65 (mg/ml), respectively. The plant extract significantly reduces gastric ulcerations in rats. The extracts of the whole plant are cytotoxic against Ehrlich (EAC) tumour cells as well as Dalton's ascites lymphomas (DLA) in mice. The anti-nociceptive activity of the water extract of *C. asiatica* (10, 30, 100 and 300 mg/kg) was studied using acetic acid-induced writhing and the hot-plate method in mice. The anti-inflammatory activity was studied in rats by prostaglandin E2-induced paw oedema. The water extract revealed significant anti-nociceptive and anti-inflammatory activity with both models.

Clinical Studies: In clinical trials, an extract of *C. asiatica* in a 1% salve or 2% powder accelerated healing of wounds. Oral administration of *C. asiatica* or asiaticoside and potassium chloride capsules was reported to be as effective as dapsone therapy in patients with leprosy. Clinical trials of the drug have demonstrated its anti-ulcer activity after oral administration. Clinical studies in the treatment of various venous disorders have demonstrated a positive therapeutic effect.

SAFETY DATA

Preclinical Safety Data

Single Dose Toxicity cf. appendix 1

KEY (PROPOSED) USAGE

Therapeutic Indications: Wound healing.

Adverse Effects: Contact dermatitis has been reported with the topical use of *Centella*. There is also a report of three cases of hepatotoxicity associated with ingestion of *Centella*, all presenting with jaundice, painful hepatomegaly, and granulomatous hepatitis with areas of necrosis.

Evaluation of Efficacy: Efficacy clinically proven.

TRADE INFORMATION

Nature of plant material: Conservation status: Not threatened.

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Combretum micranthum

GENERAL DESCRIPTION

Scientific Name with Author: *Combretum micranthum* G.Don

Synonyms: *C. altum* Perr., *C. floribundum* Engl. & Diels, *C. raimbaultii* Heck

Family: Combretaceae

Vernacular Names: Kinkeleba is the common name used in many Western African countries. Burkhill (1985) list a large number of common names used in Dahomey, Guinea Bissau, Ivory Coast, Mauritania, Niger, Nigeria, Senegal, Sierra Leone, Gambia and Upper Volta.

Botanical Description: *C. micranthum* is a shrub on rocky outcrops, but in woodlands may be a small tree with a stem diameter of up to 10 cm, or a liana reaching heights of up to 20 m (Burkhill, 1985). Leaves opposite, oval acuminate, lamina covered with reddish scales on the inner side, with downy tufts at the axis of lateral ribs. Inflorescences short axillary clusters on scaly stalks; calyx covered with ferruginous scales; whitish corolla; fruit four winged, smaller 1.5 x 1.5 cm.

Origin and Distribution: Western Africa, in savannah and forests.



Plant Part Used: Mainly dried leaves, but to a lesser extent also bark and roots.



ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses: So widely used as a general panacea and known for its diuretic, febrifugal, and digestive properties that the word kinkeleba has become synonymous with medicine. A leaf infusion was once drunk daily by Europeans in francophone Africa. A bush tea was taken in Gambia and Nigeria apparently as prophylaxis. An infusion was also drunk especially during Ramadan by Muslim people in Sierra Leone (Burkhill, 1985).

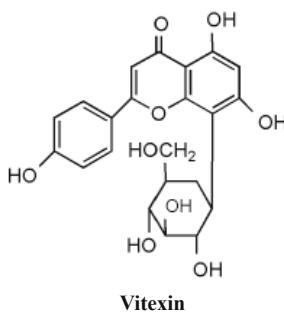
Other uses include diuretics and astringents, coughs, bronchitis, malaria, bilious haematuric fevers, and all ailments of the liver and gallbladder, for colic and nausea to prevent vomiting, in vapour baths or washes for fevers and lumbago, in association with other drug plants for beriberi, infantile and adult diarrhoea, haemorrhages, leprosy, enuresis, blenorragia, etc., sores and abscesses (Oliver-Bever, 1986).

Leaf infusions of this species are drunk for the treatment of colds, fever, colic, vomiting, and gastrointestinal problems (Prost, 1971).

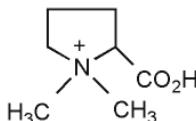
Root decoctions are used as anthelmintics as well as for washing wounds (Irvine, 1961). *C. micranthum* is also reported to be used for the treatment of malaria on the Ivory Coast in West Africa (Benoit et al., 1996).

CHEMICAL CONSTITUENTS

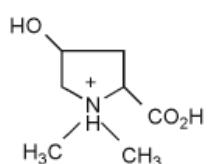
Compounds: In addition to general plant constituents such as organic acids, flavones, and tannins, the presence of maleic, glyceric and glycolic acid and catechin may explain the diuretic action. Quaternary alkaloids combretins A and B, catechins, glycosides, choline, organic acids, tannins and resin, flavonoids (vitexin and saponaretin), inositol, sorbitol, organic acids, and minerals have also been found (Ogan, 1962, Bassene et al., 1981, 1986). Bassene et al. (1986) published a method to determine vitexine and isovitexine in extracts. A recent review lists the chemical composition of *Combretum* species from southern Africa (Eloff et al., 2008).



Vitexin



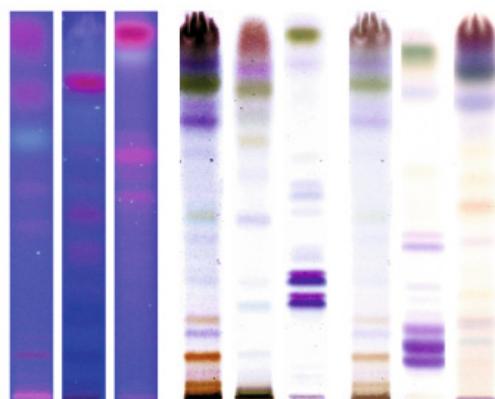
Stachydrine



Hydroxy - 4 - Stachydrine

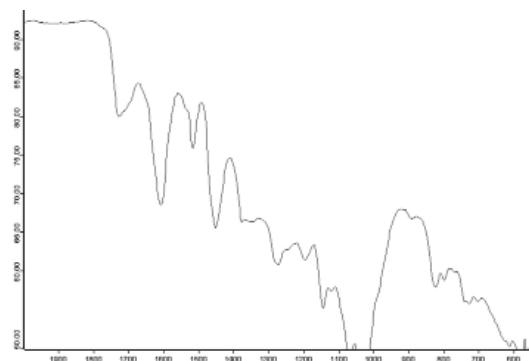
QUALITY CONTROL

TLC / HPLC / GC:



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

NIR Spectroscopy



Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

In accordance with the traditional uses, *C. micranthum* has antibacterial activities (no MIC data found), but the compounds producing these activities are unknown. The leaf extracts of *C. micranthum* were bactericidal against *Shigella dysenteriae*, *Staphylococcus paratyphi* B, and *Klebsiella ozenai*, and extracts made of fresh plant material were more active than those made from dried leaves (Karou et al., 2005). Karou et al. (2005) also demonstrated that the leaf extracts of *C. micranthum* are rich in polyphenols, and show good antioxidant activities. They speculated that the polyphenols might be the antibacterial

compounds. A recent review lists the biological activities of *Combretum* species present in southern Africa (Eloff et al., 2008).

Ferreia et al. (1993) demonstrated in vitro antiviral activity of a methanolic extract of *C. micranthum* leaves against HSV-1 and HSV-2.

An extract (50 - 100 mg/kg) significantly ($P < 0.05$) inhibited oedema induced by carrageenan in rats. Increased vascular permeability caused by acetic acid injection was also inhibited by the extract within the same dose range. *C. micranthum* extract (100 mg/ kg) inhibited granuloma formation in rats to a similar degree as indomethacin (5 mg/kg) (Olajide et al., 2002).

SAFETY DATA

Preclinical Safety Data

Single Dose Toxicity cf. appendix 1

KEY (PROPOSED) USAGE

Therapeutic Indications: The symptomatic treatment of hepatic insufficiency, colds, hepatitis and dyspeptic disturbance, and constipation. It is also a diuretic and antibacterial.

Dosage, Method, and Duration of Administration: The aqueous extract of one 10 g bag has to be taken twice daily for 7 days.

Special Warnings and Precautions for Use: Can be safely consumed when used appropriately. The extremely widespread use and absence of adverse effect reports for children and adults indicates that it is probably safe to use.

Do not use in the case of serious hepatocellular insufficiency, biliary tract obstruction, or serious kidney insufficiency.

Evaluation of Efficacy: Efficacy traditionally proven.

TRADE INFORMATION

Volume of production in the country:

Where cultivated, the density is 100 - 200 trees per hectare. In 2003, DMT Bamako, Mali, production was 1,139,040 kg, all used domestically.

Average price: The average price was 125 FCFA/ kg (0.191 euro/kg).

Hepatisane presentation (14 bags of 10 g) at DMT 588 F CFA (0.89 Euro); in pharmacy 825 FCFA (1.25 euro).

Nature of plant material: *C. micranthum* may form thickets near the coast, and in Gambia it is recorded as occurring mainly on termite mounds. *C. micranthum* may invade cultivated land and is drought and fire resistant. It can provide shade and forage mainly to goats, donkeys, and sheep. It is the second most favourite browse species of sheep (Sanon et al. 2007).

Cultivated/wild crafted: The plant is wild crafted, but apparently cultivated in Mali.

Conservation status: Not threatened.

Nature of plant products: Origin: Western Africa

Processing and storage: Infusions.

Stability of product: No information. Leaves of other *Combretum* spp. have extraordinary antibacterial activity, with no loss of activity after nearly 100 years (Eloff, 1999).

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Commiphora myrrha

GENERAL DESCRIPTION

Scientific Name with Author: *Commiphora myrrha* Engl.

Synonyms: *C. myrrha* (Nees) Engl. (1883), *C. rivae* Engl. (1897), *C. molmol* Engl. (1899), *C. molmol* (Engl.) Engl. (1925), *C. cuspidata* Chiov. (1941)

Family: Burseraceae

Vernacular Names: Dhidin (tree), echte Myrrhe, gomma mirra, gummi myrrh, gummi myrrha, gummi-resina myrrha, heerabol myrrh, hirabol myrrh, kerbe, mirra, molmol (gum-resin), myrrh, myrrha vera, Myrrhe, somalian myrrh, true myrrh.

Botanical Description: *C. myrrha* is a shrub or thorny tree reaching up to 4 meters. Hairless toothed leaves often grayish green. The bark has a silvery luster and peels off in small pieces. Strongly aromatic gum-resin oozes from natural cracks of the trunk of the tree (Vollesen, 1989). Male flowers are about 5 mm long and come out just before the rains. Fruits are about 1.2 cm long.

Origin and Distribution: The Myrrh tree is native to Ethiopia, Somalia, Kenya, and Arabia. Cufodontis had argued in favor of using the name *C. myrrha* for the Arabian and *C. molmol* for the African plant, however Vollesen (1989) has determined that both names are synonymous and that the name *C. myrrha* has precedence.



Plant Part Used: The most important product from *C. myrrha* is its gum-resin known as myrrh, which comes out from the trunk of the tree. It is called gum-resin because it is partly soluble in water (50% or more) and also soluble in hydrocarbon solvent such as hexane (15% or more), i.e. it has both the quality of gum (water soluble) and also of resin (hydrocarbon solvent soluble). Myrrh can be processed through extraction using water and other organic solvents, or subjected to steam distillation to give essential oil.



ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses: Myrrh has been used since ancient times as medicine and for ceremonial and religious purposes. In ancient times, myrrh was used by the Egyptians for embalming. The Commission E (Blumenthal et al., 2000) approved myrrh for topical treatment of mild inflammations of the oral and pharyngeal mucosa. The British Herbal Pharmacopoeia (1996) indicates myrrh tincture as a mouthwash for gingivitis and ulcers. Myrrh is also an important drug in Chinese Traditional Medicine (Yen, 1992). In Somalia and Ethiopia, a decoction of myrrh gum-resin is used to treat stomachache; it is mixed with powdered charcoal to make ink for writing on parchments, and burnt in houses and in the bush to chase away snakes (Dekebo, 2002). Myrrh resin is a common traditional medicine that is taken internally to treat stomach ailments.

Modern uses include flavoring flavor foods, drinks, and confectionary items, as additive of products for personal use such as perfumes, deodorants, shampoos, bath lotions, toilet soaps, toothpastes, mouth washes, air fresheners, etc.

Other Relevant Uses: The essential oil and alcoholic extracts are employed as fixative additives in the perfume industry.

CHEMICAL CONSTITUENTS

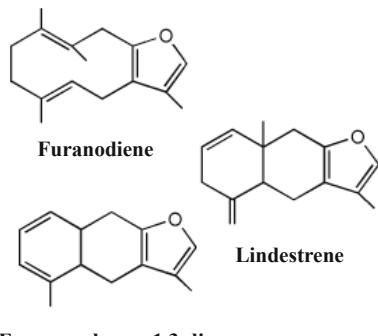
Compounds: The compounds that are really found in myrrh are by far smaller in number than those reported in the literature. This is because some of the chemical studies were based on commercial materials rather than on gum-resins obtained from properly identified trees. A study undertaken by Dekebo et al. (2002) and Baser et al. (2003) on true myrrh gum-resin collected from botanically authentic *C. myrrha* tree showed the most significant six compounds present in the essential oil of myrrh to be furanoeudesma-1,3-diene (the most abundant component), lindestrene, furanodiene, 2-methoxyfuranodiene, 2-acetoxyfuranodiene, and isofuranogermacrene (synonym: curzerene).

The gum-resin is of complex composition and can usually be separated into three fractions: two alcohol-soluble fractions (40 - 60%) and an

alcohol insoluble one (about 40% and not less). The alcohol-soluble compound group contains furanoeudesma-1,3-diene. A complex triterpenoid acid, -alcohols and -esters and volatile oil (about 2 - 10% but not less than 6%), with predominantly a mixture of sesquiterpenes. It also contains the eudesmane derivatives furanoeudesma-1,3-diene and lindestrene, amongst others, as well as the elemene-type compounds curzerenone, furanogermacrene derivatives, and furanoguaianes (including 2-methoxy-furano-guaia-9-2n2-8-one and 5-acetoxy-2-methoxy-4,5-dihydrofuranodiene-6-one) which is responsible for odour and taste.

Furanoeudesma-1,3-diene is the major compound (40%) in myrrh essential oil responsible for the analgesic properties (Dolara, 1996). A single compound is certainly not responsible for the other myriad of activities displayed by myrrh.

The water-soluble fraction consists primarily of a mixture of proteoglycans and uronic acid rich fractions. The dominant constituent (about 70%) is a 4-O-methyl-glucorono-galactan protein.



Furanoeudesma-1,3-diene

QUALITY CONTROL

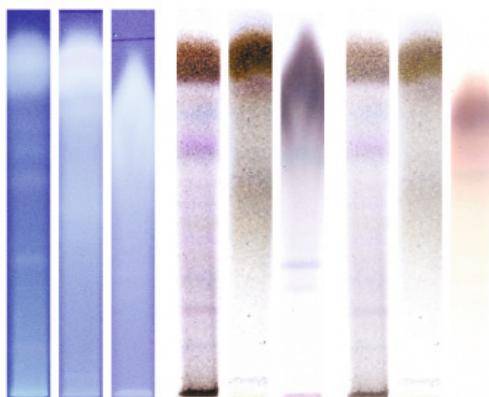
Identification: In commerce, it is common to separate myrrh into three or four grades, the best quality being grade 1, which comprises large lumps.

Organoleptic Properties: Myrrh resin is dark brown in colour with a characteristic deep balsamic aroma, not particularly pleasant, but not repulsive either. It also has a unique bitter taste that may serve as a quick aid to distinguish it from many other related resins.

Macroscopic Characteristics: When the resin is taken up in water using a sonic bath and freeze dried, it gives rise to a fluffy white powder.

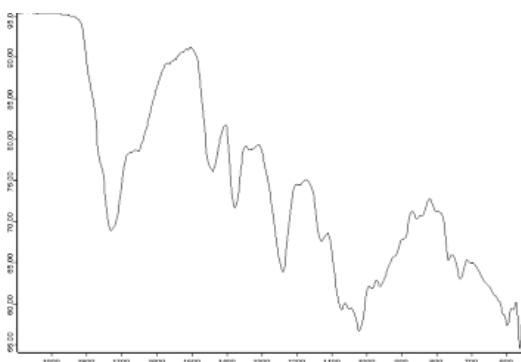
Solubility: Contrary to other gum-resins, myrrh is quite soluble in water (at least 50%).

TLC / HPLC / GC



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

NIR Spectroscopy



Adulterants and Adulterations: Although true myrrh is obtained from *C. myrrha*, sometimes traded gum-resin materials under the name of myrrh may contain gum-resins from other related species such as *C. africana*, *C. erythrae*, *C. habessinica*, *C. hodai*, *C. kua*, *C. schimperi*, etc. This has resulted in a great deal of confusion on the reported chemical constituents of myrrh (Dekebo et al., 2002a). Pharmacopoeial quality is difficult to obtain for this plant. Strictly speaking, genuine myrrh resin, with the typical aroma and characteristic chemical composition, is derived

only from *C. myrrha*. However, in commerce it is not uncommon to have resins of other *Commiphora* species present as adulterants in myrrh, thereby leading to reports in the literature of a large number of myrrh compounds that are not present in true myrrh. This is because most previous chemical studies on myrrh were based on resins from commerce rather than on materials obtained from properly identified trees. Myrrh from *C. myrrha* resin is distinctly different from that of other species of the genus *Commiphora* (Dekebo et al., 2002a; Baser et al., 2003). The common adulterants of myrrh are resins derived from *C. sphaerocarpa*, *C. holtziana*, *C. kataf*, *C. guidotti*, *C. mukul*, etc. The presence of resins from these species in myrrh reduces the quality significantly.

Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

Dolora et al. (1996) were able to validate the analgesic activity of myrrh, and also determine the two responsible constituents of myrrh: furanoeudesma-1,3-diene and curzerene.

Local anaesthetic, antibacterial, and antifungal activities have also been described for sesquiterpene fractions from myrrh.

A significant protective effect of the gastric mucosa against various necrotising agents has been observed with oral administration of an aqueous suspension of myrrh in rats. In tests in mice, the sesquiterpenes furanoeudesma-1,3-diene and curzerenone showed analgesic effects, which were reversed by the concomitant administration of naloxone. This interaction indicates that both substances react with brain opioid receptors.

SAFETY DATA

Preclinical Safety Data:

Single Dose Toxicity cf. appendix 1

Mekonen et al. (2003) showed the petrol extract of myrrh was not toxic to mice at a dose of 0.96 g/kg while Rao et al. (2001) also found myrrh is not toxic to mice at doses of up to 3 g/kg. These results show the high degree of safety if myrrh is

taken internally. However this may not be true of gum-resins from other *Commiphora* species. For instance, the gum-resin of *C. erlageriana* is known for its toxicity ascribed to unique podophylotoxin type compounds (Dekebo et al., 2002b).

KEY (PROPOSED) USAGE

Therapeutic Indications: The wound healing and anti-inflammatory properties of myrrh are well known, making it an important component of mouthwashes and toothpastes. For therapeutic use, myrrh is used exclusively in tincture form; this (used in the form of paints, mouthwash and rinses) is preferred for inflammatory conditions of oral and pharyngeal mucosa as it possesses disinfectant, deodorizing, and granulation-promoting properties.

Dosage, Method, and Duration of Administration: Infusion, decoction of the gum-resin, as well as tinctures (1 part gum-resin in 5 parts alcohol) are commonly used. A quick mouthwash is typically made by adding 5 - 10 drops of myrrh tincture to a glass of water. Other herbal ingredients such as clove, peppermint, rosemary, or sage may be added. Full-strength myrrh tincture can also be applied to sore gums, lips, or mouth tissue up to three times a day. Diluted myrrh tincture may be used as a skin wash or a vaginal douche. Amounts to use vary. A wide array of creative myrrh containing herbal products exist for painful and swollen tissues, tonsillitis, sore throat, against menstrual cramps, etc.

Evaluation of Efficacy: Analgesic (Dolara et al., 1996) efficacy pharmacologically proven. Anti-diabetic myrrh preparation (Al-Rowais, 2002) efficacy traditionally proven. Anti-tumour (Qureshi, 1993) efficacy pharmacologically proven. Gastric anti-ulcer (Al-Harbi et al., 1997) efficacy pharmacologically proven. Local anaesthetic, antibacterial, and antifungal (Dolara et al., 2000) pharmacologically proven.

TRADE INFORMATION

Volume of domestic consumption: Quite high but no reliable data.

Volume of export: Huge volumes are exported from the eastern African region to Europe, the Middle East, and China.

Average price: US\$ 10/kg.

Nature of plant material: The most valued product of this plant is its gum-resin.

Cultivated/wildcrafted: *C. myrrha* trees occur in semi-arid regions. It is not cultivated, other than being used to make hedges. The highly sought after resin, known as myrrh, is wildcrafted mainly by nomadic people who collect and sell the resin in nearby markets, which is then passed on through a supply chain, eventually reaching large enterprises which organise clean-up, sorting, and grading for eventual export to international traders. Main buyer countries are Germany and France, in Europe, and India and China, in Asia.

Flowering/harvesting time: The resin is harvested in the dry season, which varies from region to region. In Kenya, the best time for harvest is during the dry season in May to October. In the Ogaden region of Ethiopia, it is during December to June.

Conservation status: *Boswellia* and *Commiphora* species are economically and ecologically important plant species found mainly in the horn of Africa, particularly in Ethiopia, Somalia and Kenya (Lemenih & Teketay, 2003). Little is written and known about the distribution, potential production, development opportunities, and ecological conservation in the vast dry lands of these countries. Almost all production of myrrh and frankincense depends on wildcrafting. Unfortunately, the areas where these trees are found are arid, mostly inaccessible, and currently conflict prone, making conservation work highly challenging.

Nature of plant products: The material of commerce is imported from countries such as Eritrea, Ethiopia, Somalia, Yemen, and Sudan. Various commercial varieties are differentiated

according to their origin, including Somalian, Yemeni, and Hirabol myrrh.

Processing and storage: Best quality myrrh gum-resin is obtained when the exudates come out of the trunk naturally without tapping. The exudates harden gradually to give rise to a highly aromatic gum-resin, which is deep brown in color. Tapping results in light brown gum-resin with inferior quality. Tapping of *C. myrrha* is not common in Ethiopia, but is in Wajir and Mandera regions of Kenya (Curry, 2000). Tapping is done using an axe to remove surface of the bark from a small area about 3 cm wide and 7 - 10 cm long in a number of places on the trunk and main branches of each tree. After 7 days, the myrrh is harvested and a similar tapping made adjacent to the first. After a further 7 days, the myrrh from the second cut is harvested and a third made adjacent to the first two. This continues throughout the dry season.

Myrrh gum-resin is often processed to give value added products. It can be extracted by organic solvents such as hexane or petroleum ether, which after removal of solvent gives a viscous mass, known in commerce as "resinoid" and is darkish orange-brown in colour. If the solvent used for extraction is alcohol, one obtains the so-called "absolute". If one part of gum-resin is mixed with 5 parts of 50% alcohol in water, the solution that is obtained is used for various medicinal and other purposes and it is known as "tincture". Among the most popular value added products of myrrh is its essential oil, which is obtained by steam or hydro distillation. Myrrh oil is usually a dark brown viscous oil with specific gravity of nearly 1.0, forming a thick emulsion after distillation, which can be separated by vacuum filtration and other techniques.

Myrrh gum-resin is highly stable when kept in dry condition, but degrades fast if powdered to fine particles. Therefore product must be stored in well-closed containers, protected from fire (contains 85% vol. alcohol).

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Cryptolepis sanguinolenta

GENERAL DESCRIPTION

Scientific Name with Author: *Cryptolepis sanguinolenta* (Lindl.) Schltr.

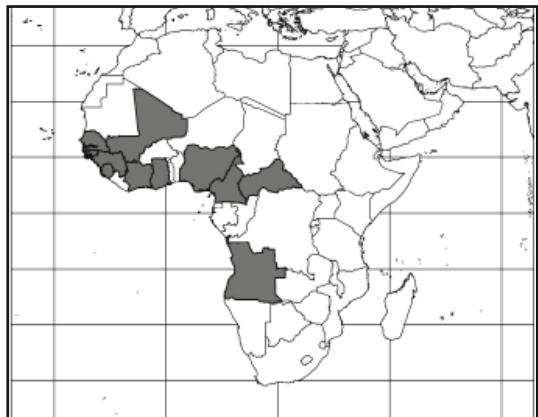
Synonyms: *Pergularia sanguinolenta* Lindl., *Cryptolepis triangularis* N.E.Br.

Family: Asclepiadaceae

Vernacular Names: Gangamau, Ghana quinine, kadze, nibima.

Botanical Description: *C. sanguinolenta* is a thin-stemmed twining and scrambling shrub. The leaves are elliptic or oblong-elliptic, with an acute apex and a symmetrical base, and petiolate, up to 7 cm long and 3 cm wide, glabrous. The inflorescence cymes, lateral on branch shoots, are few flowered, with a yellow corolla tube up to 5 mm long. Fruits are paired in linear follicles and are horn-like. The seeds are oblong, small (averaging 7.4 mm in length and 1.8 mm in the middle), pinkish, and embedded in long silky hairs.

Origin and Distribution: *Cryptolepis* grows wild, but can be cultivated. Indigenous to Africa, the plant is found as a climbing liana in Central, Eastern, and West Africa (Silva et al., 1996; Oliver-Beaver, 1986; Tona et al., 1998; Irvine 1961; Watt & Breyer-Brandwijk, 1962). It is found in forest clearings, as it commonly grows in dispersed open areas.



Plant Part Used: Root



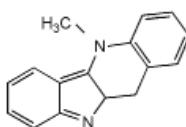
ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses: In Guinea-Bissau, a dried root decoction of *C. sanguinolenta* is used to treat hepatitis (Gomes et al., 1991). In Zaire and the Casamance district of Senegal, infusions of the roots are used in the treatment

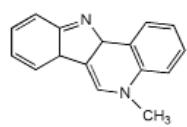
of stomach and intestinal disorders (Kerharo & Adam, 1974; Sofowora, 1982). In Ghana, the plant is used in traditional medicine to treat various fevers, malaria, urinary and upper respiratory tract infections, rheumatism, and venereal diseases (Boakye-Yiadom, 1979; Boye & Ampofo, 1983; 1990). The plant is used in Congolese Traditional Medicine for the treatment of amoebiasis (Tona et al., 1998).

CHEMICAL CONSTITUENTS

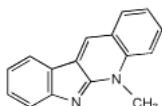
Compounds: Cryptolepine is the major alkaloid in *C. sanguinolenta*, but it is not the only one with biological/pharmacological activity. Other alkaloids present in minor quantities include cryptoheptine, hydrochloride, and 11-hydroxy derivatives of cryptolepine, iso- and neo-cryptolepine, quindoline, and the dimers biscryptolepine, cryptoquindoline, and cryptospirolepine (Cimanga et al., 1997; 1996; Sharaf et al., 1996a; 1996b; Crouch et al., 1995; Paulo and Gomes, 1995; Paulo et al., 1994b; Pousset et al., 1995; Sharaf et al., 1995a; 1995b; Pousset et al., 1994). The anti-plasmodial activity measured using the incorporation of 3H-hypoxanthine into the malaria parasite indicates that the hydrochloride and hydroxy derivatives, as well as neocryptolepine, are more active than quindoline (Cimanga, 1997). The dimers have been found to be less active than the monomers. Other alkaloids isolated from the plant are cryptosanguinolentine, cryptotackieine, and cryptomisrine, a novel indolo[3,2-b] dimeric alkaloid.



Crytolepin



Isocryptolepin



Neocryptolepin

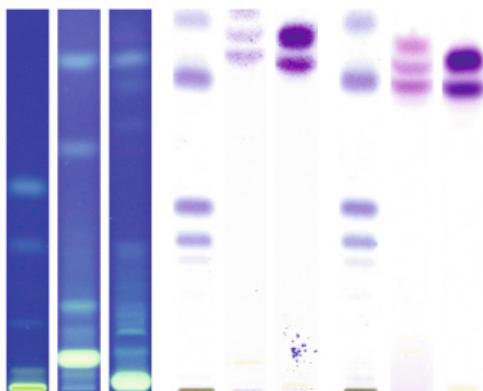
QUALITY CONTROL

Organoleptic Properties: Dried *C. sanguinolenta* has a sweet odour. The root varies from 0.4 cm to 6.6 cm long and 0.31 to 1.4 cm wide, and has a bitter taste. The root surface is light to mid brown in colour. The texture is hard and brittle, longitudinally rigid, with occasional cracks and striations. Rootlets are not present. Transverse cut surface bright yellow; odour woody. Powder: Colour yellow, taste bitter.

Macroscopic Characteristics: Root tortuous, branching with few or no rootlets; outer surface yellowish-brown, longitudinally ridged, occasional cracks or exfoliations; fracture smooth.

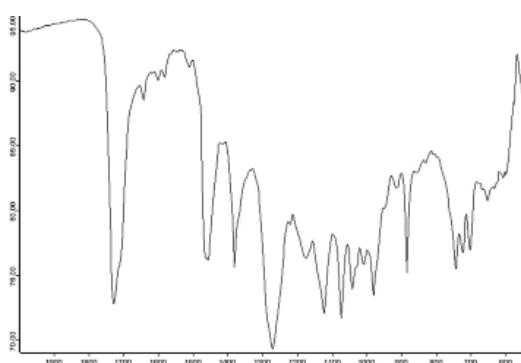
Microscopic Characteristics: Transverse section shows 5 - 8 rows of thin-walled cork cells with yellowish-brown contents; secondary cortex about two-thirds the diameter of the root, thin-walled polygonal parenchyma cells up to 1.0 µm, with simple and compound starch grains 0.05 - 0.18 µm; phloem parenchyma and sieve tubes separate cortex from wood; wood consists of lignified thickened vessels, fibres, and tracheids; vessels 0.23 - 1.27 µm diameter, fibres 0.05 - 0.27 µm diameter. Powder: Abundant cork cells and parenchyma starch grains. Lignified xylem elements of vessels and fibres also abundant. Colour varies, ranging from light to mid brown to bright yellow. When examined under a microscope using chloral hydrate R solution, the powder should show the following diagnostic characteristics: cork cells with mid to dark brown content; thin-walled polygonal phloem parenchyma containing compound starch grains; simple starch grains; calcium oxalate crystals; parts of loose fibres of varying lengths and diameters. Lignified thickened vessels are present.

TLC / HPLC / GC



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

NIR Spectroscopy



Purity Tests / Requirements: Absence of soil particles, calcium oxalate crystals, and sclereids; water extractive not less than 20% (w/v); alcohol extractive not less than 12%. Total alkaloids not less than 0.5%.

Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

In a study to investigate the antibacterial potentials of three selected medicinal plants against multiple antibiotic-resistant bacteria isolated from female patients suffering from urinary tract infections (UTIs), the antibiograms of two pathogens of the urinary tract, *E. coli* and *S. saprophyticus*, were determined and used to investigate the potentials of the plants *Cryptolepis sanguinolenta*, *Psidium guajava*, and *Tridax procumbens* as anti-UTI agents. The bacterial strains were identified according to standard microbiological methods, the isolates were tested for susceptibility to selected antibiotics using the disk diffusion technique, and the agar diffusion method was used to determine the antibacterial potentials of the three plant materials, expressed as the minimum inhibitory concentrations (MIC) - the lowest concentration of the herbal extract that showed no more than one or two colony-forming units. Although all three medicinal plants had antibacterial activities against *E. coli* and *S. saprophyticus*, *C. sanguinolenta* was found to be the most potent, with an MIC of 0.8 mg of freeze-dried material per ml of solution, compared to 3.2 mg and 1.6 mg for the other two plant materials. Thus *C. sanguinolenta* has anti-UTI properties against multiply resistant *E. coli* and *S. saprophyticus* (Mills-Robertson et al., 2006).

In a study to evaluate traditional remedies from Guinea-Bissau with antimicrobial effects, out of 12 plants used to treat infectious diseases, only *C. sanguinolenta* was found to be effective against *E. coli* and against nine of the 10 test organisms used (Silva et al., 1996). The plant extract was ineffective against *Pseudomonas aeruginosa*. Ineffectiveness against *P. aeruginosa* was also reported in another antimicrobial screening study in vitro, using alcoholic and aqueous crude extracts as well as five alkaloids isolated from the plant (Paulo et al., 1994). The major alkaloid, cryptolepine, was found to be effective against both Gram-positive and Gram-negative bacteria, the ethanolic extract being more effective.

Studies have been carried out to evaluate the antimicrobial properties of extracts of, and compounds isolated from, *C. sanguinolenta*. In a programme of biological evaluation to justify traditional uses of herbal remedies, *C. sanguinolenta* was studied because of its successful use in treating diarrhoea caused by intestinal amoebiasis, and found to be effective in vitro against *Entamoeba histolytica* (Tona et al., 1998). Over 100 strains of *Campylobacter* species, which are causative agents for gastroenteritis, have been used to study the effect of *C. sanguinolenta* and compounds isolated from it on diarrhoeal bacteria (Paolo et al., 1994). The findings were that cryptolepine was more effective than co-trimoxazole and sulfamethoxazole, just

as effective as ampicillin, and less effective than erythromycin and streptomycin, the antibiotics usually used against diarrhoeal diseases. The isolated alkaloid was the most effective. Although the ethanolic extract was effective, it was not as effective as the isolated alkaloid, and the aqueous extract was not effective against these diarrhoeal bacteria. The effect of the plant material was not so dramatic when *Vibrio cholerae*, the causative agent for enteric infection, was used as the test organism.

Of all the isolated alkaloids, cryptolepine is the most active antibacterial agent, and it is more active against Gram-positive bacteria than the Gram-negative ones (Boakye-Yiadom, 1979; Sawer et al., 1993). Some of the minor alkaloids are also effective antibacterial agents, including the hydrochloride cryptoheptine, neocryptolepine and biscryptolepine (Boakye-Yiadom & Herman Ackah, 1979) Cryptoquindoline was not active (Sawer et al., 1993). The antibacterial actions of neocryptolepine appear to mirror those of the major alkaloid, cryptolepine (Cimanga et al., 1998). Cryptolepine also has some antifungal activity against *Saccharomyces cerevisiae*, but not against *Candida* species (Sawer et al., 1995). Its antifungal activity seems to be limited compared to its antibacterial activities.

In vitro antiplasmodial activities, which are indicative of antimalarial activity, have been carried out using inhibition of the incorporation of the malaria parasite into red blood cells (Grellier et al., 1996; Kirby et al. 1995; Noamesi et al., 1991; Wright et al., 1996; Cimanga et al., 1997).

In one study, in which both the chloroquine-sensitive D6 strain and the chloroquine-resistant K-1 and W-2 strains of the malaria parasite were used, the aqueous, alcoholic and total alkaloidal extracts, and compounds isolated from the plant material, were found to be effective against all three strains of parasite to varying degrees. Of the extracts, the total alkaloid was the most active, with mean IC₅₀ values of 47, 42, and 54 µm for the three strains, respectively, compared to values of 2.3, 72, and 68 µm for chloroquine. The aqueous extract was the least active. Of the isolated compounds, cryptolepine was the most effective, with mean IC₅₀ values of 27, 33, and 41 µm for the D6 chloroquine-sensitive

and K-1 and W-2 chloroquine-resistant strains, respectively. Hydroxyl-cryptolepine was the next best compound with IC₅₀ values of 31, 45, and 59 µm, respectively, followed by neocryptolepine. Quindoline, or norcryptolepine without the methyl group, was the least active antimalarial of the isolated compounds (Cimanga et al., 1997). This is an indication that the methyl group contributes to antimalarial activity, at least in part. The result of this study with respect to the K-1 strain is in agreement with the work of Noamesi et al. (1991), who reported the antiplasmodial activity of cryptolepine against the multidrug-resistant K-1 strain of *P. falciparum*.

Wright et al. (1996), using a method based on inhibition of incorporation of multidrug-resistant K1 strain of *P. falciparum* into drug-treated infected red blood cells (rbc) compared with untreated infected and uninfected rbc, showed that among a number of anhydronium bases, only cryptolepine, the major alkaloid in *C. sanguinolenta*, had antiplasmodial activity similar to that of chloroquine. Kirby et al., (1995) also found cryptolepine to be highly active in vitro against the multidrug-resistant (K1) strain of *P. falciparum*. Using a method of assessing inhibition of parasite growth based on measurement of lactate dehydrogenase activity, Wright et al. (1990) showed that among a number of anhydronium bases, only cryptolepine, the major alkaloid in *C. sanguinolenta*, had antiplasmodial activity similar to that of chloroquine. The mean IC₅₀ value, determined from linear regression analysis of dose-response curves, was 0.114 µm for cryptolepine, compared to a mean value of 0.2 µm for chloroquine diphosphate.

Inhibition of β-hematin formation in a cell free system is another in vitro test for antiplasmodial activity. Reduction or elimination of the characteristic peaks of β-hematin at 1663 and 1210 cm⁻¹ in an infrared spectrum indicates efficacy. Cryptolepine has been shown to be effective in this model, the peaks disappearing when the reaction mixture was pre-incubated with the alkaloid (Wright et al., 2001) suggesting that the antiplasmodial effect of cryptolepine depended, at least in part, on a quinine-like mode of action.

In a programme of biological evaluation to justify traditional uses of herbal remedies, *C. sanguinolenta* was studied because of its successful use in treating diarrhoea caused by intestinal amoebiasis, and found to be effective in vitro against *Entamoeba histolytica* (Tona et al., 1998).

Bonjean et al. (1998) have reported cryptolepine as a potent topoisomerase inhibitor, and hence a promising anti-tumour agent. They showed that DNA was the prime target to which cryptolepine binds tightly, like an intercalating agent, interacting preferentially with GC-rich sequences. The alkaloid was also shown to cross the cell membrane easily, accumulating in the nucleus rather than in the cytoplasm.

Some pharmacological effects of *C. sanguinolenta*, quite unrelated to the use of the plant in folk medicine, are its anti-inflammatory and anti-hyperglycaemic properties. The anti-inflammatory properties were established in a study which showed the plant material as an inhibitor of carageenan-induced edema (Bambose & Noamesi, 1981) and that of platelet aggregation (Oyekan et al., 1988). An anti-hyperglycemic property has been shown as enhanced insulin-mediated glucose disposal in a mouse model of diabetes, and in an in vitro system using the 3T3-L1 glucose transport assay, indicating an effect on type 2 diabetes (Bierer et al., 1998; Luo et al., 1998). Hypotensive properties have also been reported, including effects on cholinergic nerve transmission, alpha-adrenoceptors, and muscarinic receptors (Noamesi & Bambose, 1980; Rauwald et al., 1992).

In a study to evaluate the anti-inflammatory and analgesic effects of *C. sanguinolenta*, Asiedu-Larbi et al. (2006) showed a moderate anti-inflammatory effect of the plant compared to aspirin. The method used was the percentage inhibition of carrageenan-induced oedema of the rat paw. Using the analgesia coefficient, they showed that the antinociceptive effect of the plant was comparable to that of morphine. The plant material was administered orally to rats as a decoction.

Clinical Studies: *C. sanguinolenta* has traditionally been used for the treatment of malaria and various

other infectious diseases (including urinary and upper respiratory tract infections), amoebiasis, rheumatism, venereal diseases and stomach/intestinal disorders.

In a study aimed at comparing the efficacy of the herbal substance with that of chloroquine, using the WHO extended 7 days in vivo test to measure *P. falciparum* response, a number of malaria patients with parasitaemia of 10 000-100 000 *P. falciparum* per 8000 white blood cells were used. The results indicated that the efficacy of an aqueous extract of the herbal substance was comparable to that of chloroquine. In this open randomised comparative study, 12 patients were treated with an aqueous extract of the herbal preparation and 10 with chloroquine. A dose of chloroquine or an aqueous extract of the herbal substance was given on three successive days. Patients on the herbal preparation received a daily dose of extract prepared from 75 mg of plant material per kg body weight (average weight 70 kg) after meals. All patients in both groups responded clinically, and asexual parasitaemia was cleared within 7 days. No recrudescence of parasitaemia occurred after a follow-up period of 21 days. Clinical symptoms such as fever, headache and body pains cleared faster in the group treated with the herbal preparation. There was no complaint of pruritus by patients in this group who also did not require antipyretics, whereas patients in the other group did, and 20% of them complained of pruritus.

Asiedu-Larbi (2004) reported on a pilot clinical trial carried out to compare the clinical efficacy of Nibima, an antimalarial herbal extract prepared from *C. sanguinolenta* roots, and Nasra, another antimalarial herbal preparation, both widely used in Ghana. Nibima has been used as therapy for malaria at the Centre for Scientific Research into Plant Medicine in Ghana since 1974. The study was designed as an open trial with primary endpoints, including clinical cure, i.e., the absence of clinical signs and symptoms, parasite clearance and safety. Clinical examination, blood films, and measurements of axillary temperature were taken on days 0, 1, 2, 3, 7 and 14. Clinical chemical parameters were measured on days 0, 3, 7, and 14, and ECG on days 0, 3 and 14. A clinical cure was defined as negative blood film, i.e. no parasites, afebrile state, and no symptoms of malaria by day 7. A reduction in parasitaemia (but no clearance), even with afebrile state and

asymptomatic by day 7, was regarded as partial response. No change or increase in parasitaemia by day 7, and rising body temperature during the first 3 days of treatment, was interpreted to mean no response. The results from the patients on *C. sanguinolenta* indicated that the effective dose was 150% of a recommended daily dose of 50 mg/kg of the freeze-dried aqueous extract taken over a period of 6 days. Sixty-six percent of the patients on this dose were cured, and 27% had a partial response. The rest had no cure. The total number of patients taking part in the study was 37. In the safety assessment, all parameters for liver function tests and renal function tests were within normal ranges. No abnormalities were detected in the urinalysis and all haematological parameters remained unchanged. No abnormalities in vital signs were detected, and although mild sinus bradycardia (53 - 59/min) was seen in some patients on day 3, the effect had disappeared by day 14.

A clinical study conducted to demonstrate the clinical efficacy of Phyto-Laria®, a tea-bag formulation of the herbal substance, involved 31 adult patients with simple uncomplicated malaria confirmed by microscopy. The WHO extended 7-day in vivo test was used. Most of the initial symptoms resolved within the first week. Mean parasite clearance time was 82.3 hours (24 - 144 hours). All patients evaluated had cleared their parasitaemia by day 5. The mean fever clearance time was 25.4 hours (12 - 96 hours). The overall cure rate was 93.5%, with two cases of recrudescence on days 21 and 28. The presenting symptoms of chills and fevers cleared rapidly in all patients, and were completely resolved by day 7 when the parasitaemia cleared. Other symptoms related to pain persisted in some patients even when they had no parasite in the blood, an indication that the herbal substance has no analgesic property. Evaluated on evidence of fever clearance and disappearance of parasitaemia by day 7 according to the WHO criteria, this tea-bag formulation of *C. sanguinolenta* is effective in the treatment of acute uncomplicated malaria (Boye et al., 2002).

SAFETY DATA

Preclinical Safety Data

Single Dose Toxicity cf. appendix 1

There have been reports of toxicity of the aqueous extracts of *C. sanguinolenta* and compounds isolated from the plant material when the method involved cell lines, such as HL-60 human leukemia cells, used to assess anti-tumour activity, or when in vitro methods of risk assessment were used (WHO, 1984). Cytotoxicity in antiviral test systems has also been reported (Ansah & Nigel, 2001). In one study, cytotoxicity, measured as anti-tumour activity, did not correlate with toxicity in the in vivo mouse model for malaria used in the same study (Wright et al., 2001). When Phyto-laria®, a *C. sanguinolenta* product formulated as a tea, was evaluated in vivo using oral administration and the conventional acute toxicity and clinical chemistry tests, the results indicated the product to be safe (Nyarko, 2002). No value for LD₅₀ was provided because there was no mortality in the test animals when a dose as high as 2000 mg/kg had been given. According to Luo and co-workers (Luo et al., 1998), there was no evidence of side effects or toxicity when the extract of *C. sanguinolenta* was used as a tonic, often taken for years.

KEY (PROPOSED) USAGE

Therapeutic Indications: Treatment of malaria and other infectious diseases.

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Cyclopia spp.

GENERAL DESCRIPTION

Scientific Name with Author: *Cyclopia* spp.: *C. genistoides* (L.) R.Br., *C. intermedia* E.Mey., *C. subternata* Vogel and *C. sessiliflora* Eckl. & Zeyh. are the most frequently commercially used species.

Family: Leguminosae

Vernacular Names: Cyclopia, heuningbostee, honeybush tea, Honigbusch, mountain tea.

Botanical Description: Joubert et al. (2008) mentioned that apart from *C. intermedia*, and *C. subternata*, other species such as *C. sessiliflora* (Heidelbergtree) and *C. genistoides* are also commercially used. Other species were also used in the past to prepare tea including *C. latifolia* and *C. longifolia*. *C. maculata* has also been reported as a source of honeybush tea, so it appears that all the *Cyclopia* species are suitable for making tea, though with variation in flavor (Dharmananda, 2009).

Originally the name heuningbostee or heuningtee referred to *C. genistoides*, and this species is making a comeback as an important source of high quality cultivated material. *C. intermedia* (so-called bergtee, or mountain tea), currently the main commercial source, is a multi-branched woody shrub of up to about one metre high.

The young twigs have a characteristically golden colour. Each leaf comprises three separate, subsessile leaflets. The attractive yellow flowers have characteristically grooved standard petals and are followed by flat, brown seed pods containing several seeds with conspicuous fleshy arils. *C. genistoides* (the original honeybush tea) may be distinguished by its smaller size and narrow, needle-like leaflets. *C. subternata*, (so-called vleitee) is also used commercially nowadays. The leaves and flowers are very similar to those of *C. intermedia* but the plant is an erect, sparsely branched shrub of 1.5 m or more. It regenerates from seed after fire, while *C. intermedia* resprouts from its woody base (Schutte et al., 1995; Schutte, 1997; Van Wyk et al., 1997; Van Wyk & Gericke, 2000).

Origin and Distribution: Fynbos region of the Western and Eastern Cape Provinces of South Africa. *Cyclopia genistoides* is restricted to the vicinity of Cape Town, whereas *C. intermedia*, *C. subternata*, and *C. sessiliflora* are found in the Eastern parts (George to Port Elizabeth) (Schutte, 1996).



Plant Parts Used: Stem and leaves





Possible Alternative Source Species: *C. intermedia* E. Mey., *C. subternata* Vogel, and *C. sessiliflora* Eckl. & Zeyh. can also be accepted as alternative sources of honeybush tea.

ETHNOBOTANICAL INFORMATION

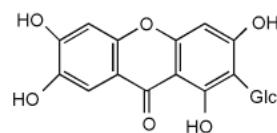
Major Ethnopharmacological Uses: Honeybush tea is a health drink that is mainly used as a tea substitute because it contains no harmful substances such as caffeine. It has also been used since early times for its direct positive effects on the urinary system and is valued as a stomachic that aids weak digestion without affecting the heart (Marloth, 1925; Watt & Breyer-Brandwijk, 1962; Van Wyk et al., 1997; Van Wyk & Gericke, 2000; Van Wyk & Wink, 2004). Traditionally, has also been used to stimulate milk flow in lactating mothers (Van Wyk & Gericke, 2000).

CHEMICAL CONSTITUENTS

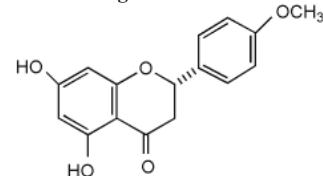
Compounds: The processed herb (all four crop species) contains mangiferin as one of the major constituents, together with smaller amounts of isomangiferin (De Nysschen et al., 1996). Other phenolic compounds include 4-hydroxycinnamic acid, flavones (naringenin, eriodictyol, hesperitin, isosakuranetin, hesperidin), isoflavanones (formononetin, afromosin, calycosin, pseudobaptigen, fujikinetin), and coumestans (medicagol, flemichapparin, sophora-coumestan B) (Ferreira et al., 1998). Small amounts of inositol, also known as (+)-pinitol, are present (Ferreira et al., 1998). Several other xanthones, flavonoids, other phenolic compounds, and polyphenolics have been identified in extracts of *C. intermedia* (Ferreira et al., 1998; Kamara et al., 2003). The various *Cyclopia* species are chemically similar and have similar mixtures of phenolic compounds.

The aroma components were dominated by monoterpene alcohols, of which α -terpineol (28%) was the major component, with minor amounts of linalool (7%), nerol (2%), and geraniol (8%). These monoterpenes are responsible for the sweet, floral, and fruity notes of the tea, while other components such as phenylethyl alcohol (3%) and 5-methylfurfural (2.1%) imparted also sweet and honey notes (Wang et al., 2006).

The raw material was also found to be free of caffeine according to High Pressure Liquid Chromatography (HPLC) analysis (Juliani et al., 2009). The analysis of total ashes has shown that the honeybush tea contained low levels of total minerals (1.4-1.5%). This is supported by the fact that the raw material contained low levels of macroelements such as calcium (0.16-0.22%), potassium (0.26-0.3%), phosphorus (0.03%), and magnesium (0.09%). Honeybush also contained low levels of the micro elements such as iron (6 mg/100 g dried material), thus not providing significant amounts of this macro- or microelements to the diet.



Mangiferin



Isosakuranetin

QUALITY CONTROL

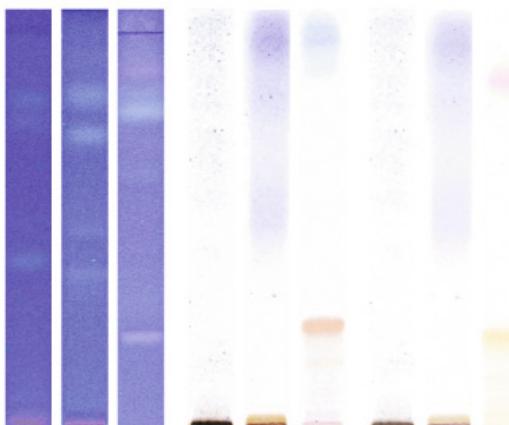
Identification: Small 1-5 mm pieces of stem and leaves with a brown to gold colour and a rich sweet aroma.

Identification by diosphenol content (TLC or GLC) is the only reliable quality control measure to date.

Organoleptic Properties: The fermented raw material is characterized by a light red color, with the characteristic sweet and honey-like, somewhat woody aroma. Free from foreign odors.

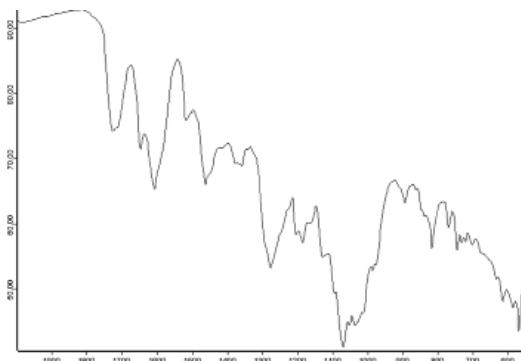
Moisture Content: Moisture 10% (m/m), max.

TLC / HPLC / GC



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

NIR Spectroscopy



Adulterants and Adulterations: *Agathosma crenulata*, *A. serratifolia*, *Agathosma* hybrids, and other members of the Rutaceae. Adulteration with leaves of *Embleum unicapsulare* and *E. serrulatum* has been reported (Burger & Wachter, 1998).

Adulteration very rare.

Standard specifications cf. appendix 2

Botanical dust 0.5% (m/m), max.

Total ash 2% (m/m), max.

Acid insoluble ash 0.5% (m/m), max.

Total phenolics 2% (gallic acid equivalents, m/m), min.

PHARMACOLOGICAL PROPERTIES

Antimutagenic and antioxidant effects have been demonstrated, especially for the untreated (unfermented or green) honeybush tea (Marnewick et al., 2003a; 2003b; 2005). *C. genistoides* methanol extracts display phytoestrogenic activity and act predominantly via ERbeta. HPLC and LC-MS analysis, however, suggests that the observed phytoestrogenic activity cannot be ascribed to polyphenols known to be present in other *Cyclopia* species (Verhoog et al., 2006)

SAFETY DATA

Ethnic Use Safety Data: No indications of any toxicity. Honeybush tea has been widely enjoyed as a health drink for a century or more and no adverse side effects have thus far been recorded.

Some studies have shown that honeybush flavonoids may affect enzymes that affect metabolism (Mertens-Talcott et al., 2006).

Preclinical Safety Data

Single Dose Toxicity cf. appendix 1

KEY (PROPOSED) USAGE

Therapeutic Indications: Diuretic, stomachic.

Dosage, Method, and Duration of Administration: 2.5 g of the dry product per cup of water is prepared by infusion or decoction and enjoyed in the same way as normal tea.

Contraindications: None.

Special Warnings and Precautions for Use: None. Can be safely consumed when used appropriately.

Interactions: None.

Adverse Effects: None.

Evaluation of Efficacy: Antioxidant effects: efficacy pharmacologically proven (Marnewick et al., 2003a; 2003b). Antimutagenic effects:

efficacy pharmacologically proven (Marnewick et al., 2000; 2003a; 2003b; 2005). Urinary system: insufficient information for classification. Stomach: insufficient information for classification.

TRADE INFORMATION

Nature of plant material:

Flowering/harvesting time: Traditionally, honeybush plants were harvested in spring (October and the first half of November), as the flowers were considered important in adding flavour and aroma to the tea. Nowadays plants are harvested over a much longer period.

Cultivated/wildcrafted: Several species have been domesticated, of which *C. genistoides* appears to have the best long term outlook from an agronomic and quality point of view. *C. subternata* is also cultivated to some extent while *C. intermedia* (so-called berg tea or mountain tea) is almost exclusively wildcrafted in the Langkloof region of the Eastern Cape Province and is still the most important commercial source of raw material.

Conservation status: Not threatened

Nature of plant products: The dried honeybush raw material consists of the leaves and stems that has been cut in small pieces (some cases flowers) that had underwent the process of fermentation and then drying.

Processing and storage: Preparation/processing: Branches are harvested in bundles and often have to be carried over long distances in mountainous terrain. The stems and leaves are chopped into small sections using a modified silage cutter, after which it is bruised and moistened. The heaps of material are left to oxidize spontaneously, or it is heated in an oven to about 60°C to enhance the process. Oxidation turns the chopped material into a rich brown colour and enhances the characteristic sweet smell of the herb. After a few hours, when the teamaker decides that the desired quality and aroma has been achieved, the tea is spread out in the sun to dry (Marloth, 1925; Watt & Breyer-Brandwijk, 1962; Van Wyk et al., 1997; Van Wyk & Gericke, 2000; Van Wyk & Wink, 2004). Nowadays, unfermented (green)

honeybush is also available. It was observed that the presence of flowers is not essential for the development of the characteristic sweet and honey like flavor, though the presence of flowers were found to improve flavor (Joubert et al., 2008). Some manufacturers of the honeybush tea in South Africa will steam pasteurize the raw material to decreased the contamination with mould and bacteria, particularly after the fermentation process.

Stability of product: Unknown. In the dry state, the product appears to be highly stable but no published information is available. Initial studies suggest a shelf life of at least two years, and a longer one if stored under good conditions (Juliani et al., 2009).

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Danais fragrans

GENERAL DESCRIPTION

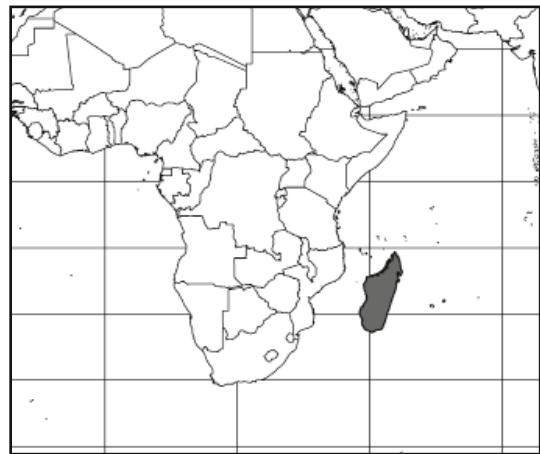
Scientific Name with Author: *Danais fragrans* C.F.Gaertn.

Family: Rubiaceae

Vernacular Names: Bongonomby, haizantoloho, hazonalafotsy, hezavahatra, laingomantsina, liane de boeuf, liane de bois jaune, liane jaune, lingue noire, rikiatra, tamboromanitra, tamboromantrina, tamboronaombe, tsivahabahatra, vahimantsy, zamboromantsina.

Botanical Description: Woody, scandent plant with stems 1-4 (-25) m long, more or less glabrous. Leaves opposite; leaf blades lanceolate, elliptic or oval, 1.5-15 x 1-8 cm, coriaceous; the nerves sometimes yellow, reticulate on the lower surface; petiole 0.5-2 (-3) cm long. Flowers fragrant, (4-) 5-merous, in axillary thyrses, peduncles 0.6-2 (-4) cm long; lobes orange red or reddish brown, 2-5 x 1-1.5 mm, acute or obtuse. Fruit is a yellowish green capsule, turning brownish, 2.5 - 5 mm in diameter, with resistant valves. Seeds black or pale, shiny, round, 0.8 mm in diameter, compressed, with reticulate wings.

Origin and Distribution: The genus consists of some 40 species originating in Tanzania, Madagascar, and the Mascarenes.



Plant Part Used: Bark and juice of roots



ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses: The root decoction is drunk against malaria (Neuwinger, 2000). In Mauritius, the bark is used to treat skin infections, while the juice extracted from the roots helps in the healing of wounds, abscess and general skin diseases (Daruty, 1886; Gurib-Fakim et al., 1993, 1997). A decoction of the bark is used as a refreshing drink and against fever (Lavergne & Vera, 1989).

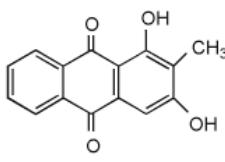
The root is known to be effective against fever. Often a decoction is administered especially when the patient is feeling nauseous after a malarial attack. The root is also used against mouth and gum infections. In spite of belonging to the same family as the *Cinchona* species, this plant is devoid of alkaloids and contains instead a red-orange dye highly prized in the dye industry. Descheemaeker (1979) reported that this plant was useful against secondary infections linked to syphilis. Boiteau

(1999) reported that this plant was a useful antidote to poisonous plant intoxication, namely Komanga. As early as 1910, Heckel [in Boiteau (1999)] had reported that the stem was useful in the treatment of skin infections and often the stem was replaced by the root for the same purposes. Pernet (1957) much later reported on the same properties of the plant as mentioned previously by Heckel in 1910.

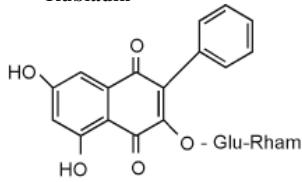
Other Relevant Uses: Apart from the reported medicinal uses, some *Danais* species yield dyes and fibres.

CHEMICAL CONSTITUENTS

Compounds: The plant is reported to contain alkaloids, phenols, saponins, and traces of sterols and triterpenes (Vera et al., 1990). Phytochemical analysis of the plant parts, namely stem and leaves, growing in Mauritius gave the following profile: alkaloids, phenols, flavones, tannins, sterols, terpenes, triterpenes, anthraquinones, and saponins (Gurib-Fakim et al., 1997). Andre et al. (1976) and Glasby (1991) report that the following compounds are present in the plant: anthraquinone 1-hydroxydimethylanthraquinone, the flavonoids kaempferol 3-O-rhamnoglucoside, kaempferol 3-O-rhamnoglucoside or nicotifloroside, quercetin 3-O-rhamnoglucoside, rubiadin and rubiadin xyloglucoside.



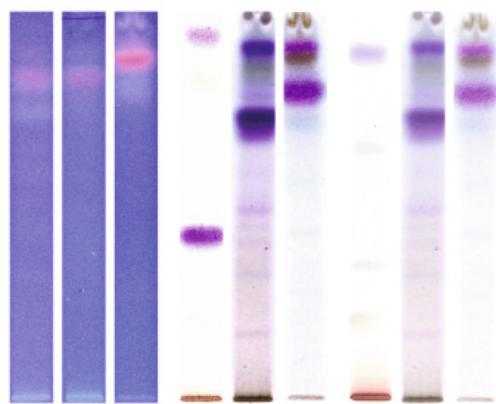
Rubiadin



Nicotifloroside

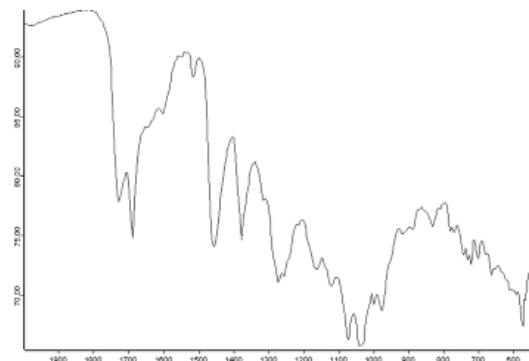
QUALITY CONTROL

TLC / HPLC / GC



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

NIR Spectroscopy



Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

The antibacterial properties of this plant may be attributed to the presence of quinones and flavonoids, as these compounds have established antimicrobial profiles.

SAFETY DATA

Preclinical Safety Data

Single Dose Toxicity cf. appendix 1

KEY (PROPOSED) USAGE

Therapeutic Indications: Analgesic, skin infections.

Pregnancy and Lactation: Not to be used during pregnancy. Not to be used while nursing.

Evaluation of Efficacy: Efficacy traditionally proven.

TRADE INFORMATION

Nature of plant material: Conservation status:
Not threatened.

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Euphorbia hirta

GENERAL DESCRIPTION

Scientific Name with Author: *Euphorbia hirta* L.

Synonyms: *Chamaesyce hirta* (L.) Millsp., *Euphorbia pilulifera* auct. non L., *E. capitata* Lam.

Family: Euphorbiaceae

Vernacular Names: Asthma herb, Australian asthma herb, burra leiteira, cat's hair, erva andorinha, erva de Santa Luzia, euphorbe pilulifère, hairy spurge, Jean Robert, kinywele, kitadali, kitapiaroho, kiziwa, luzia, malnommée, mwache, mziwaziwa, pill-bearing spurge, Queensland asthma herb, snakeweed, wiza.

Botanical Description: Annual, branched herb, prostrate to ascending, reaching up to 50 cm long, with latex; all parts short-hairy with sparse yellow hairs. The leaves are opposite, distichous, simple; blade ovate, 1-4 cm × 0.5-2 cm, base very unequal, one side cuneate, the other side rounded, apex almost acute, margin finely toothed, often with a purple blotch near the mid-vein. Inflorescence a terminal or axillary cluster of flowers, called a 'cyathium', with several cyathia densely clustered into a cyme. Flowers unisexual; male flowers sessile, female flowers with short pedicel. Fruit just exserted, acutely 3-lobed capsule. Seeds oblong-conical, slightly wrinkled, pinkish brown in colour.

Origin and Distribution: An anthropogenic ruderal herb, decumbent or erect to 40 cm tall, occupying open waste spaces, a weed of cultivation, roadsides, path sides, and a diversity of situations; occurring widespread throughout the African continent, and dispersed pantropically and subtropically around the world. The genus consists of about 1,600 species worldwide.



Plant Part Used: Leaves



ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses: Traditional knowledge about this popular traditional medicine is quite extensive and reflects its importance and popularity in Africa (Anon, 1985; Ayensu, 1978). In West Africa, a decoction of the whole plant is used by women as a galactagogue (Burkhill, 1994; Schmelzer & Gurib-Fakim Ed. 2008). It is sold in the local market for precisely this purpose. It has been reported that in Ghana, when the plant is chewed with palm kernel oil, the flow of milk increases within 24 hrs. The plant is also reported to have diuretic and purgative actions. It is also widely used in the treatment of venereal discharges. It is considered locally to be good for the treatment of diarrhoea and dysentery. Amoebic dysentery has been reported to be cured within 48 hrs after administration of a decoction of the plant. It is also regarded as an outstanding medication to treat respiratory system disorders, including asthma (Kokwaro 1993), bronchitis, hay fever, laryngeal spasms, emphysema, coughs, and colds. The leaves are mixed with those of *Datura metel* L. in preparing ‘asthma cigarettes’. Other principal uses are as a diuretic to treat urogenital diseases, such as kidney stones, menstrual problems, sterility, and venereal diseases. The plant is also used to treat affections of the skin and mucous membranes, including warts, scabies, taenia, thrush, aphthae, fungal afflictions, measles, Guinea-worm, and as an antiseptic to treat wounds, sores and conjunctivitis.

The plant has a reputation as an analgesic to treat severe headache, toothache, rheumatism, colic, and pains during pregnancy. It is used as an antidote and pain relief of scorpion stings and snakebites. It is antipyretic and anti-inflammatory. The use of the latex to facilitate removal of thorns from the skin is common. Its use in the treatment of jaundice, hypertension, oedema, anaemia, and malaria, as an aphrodisiac, and to facilitate childbirth has also been reported. In Central and Southern Africa, the plant is believed to relieve costal pains while in decoctions with other plants and is reported to make a soothing wash for infants with fever (Watt & Breyer-Brandwijk, 1962). In East Africa, the plant is reported to be useful in the treatment against *Oxyuris* threadworms while the leaf sap is used as a gargle for angina. The sap when squeezed in the eyes help against styes and

conjunctivitis. However, the latex is also reported of causing dermatitis.

In the Indian Ocean Islands, the leaf and stem decoction is used to treat hepatic colics, diarrhoea, dysentery, and rheumatism (Adjanohoun et al., 1983). To counteract asthma, around 20 leaves are boiled in one glass of water in which some coins had been added. Children usually take one spoonful, while adults drink a cup full. In Reunion Island, the plant is highly prized against asthma and intestinal disorders (diarrhoea, dysentery). A decoction made with 7 - 8 entire plants is used against coughs and is also given to encourage sleep. A decoction made with the entire plant, mixed with those of *Pissat de Chien* (*Cleome viscosa*) is drunk as an emmenagogue. In case of excessive period, a decoction made with a few leaves is drunk. The Jean Robert is also used in case of pains in the ovaries. The leaves can also be added to a bath so as to disinfect the body and act on vaginal discharges. The latex is applied directly on eczemas, warts, and other skin infections (Lavergne & Vera, 1989).

In Mauritius, a decoction is made from the plant by itself or mixed with the plants of *Cassytha filiformis* or the roots of *Psidium guajava* in order to treat diarrhoea. The plant decoction is also used to treat fever and allergies related to certain varieties of fish. The leaf and root decoction makes a refreshing drink and is also an emmenagogue. A root decoction treats dysmenorrhoea while the leaf decoction is used as an eye bath for sore eyes. The whole plant decoction also treats bronchitis and coughs. In the Seychelles, the plant treats impetigo, flatulence, diabetes, painful periods, and hypertension; while in the Comoros, a plant decoction treats urethral discharges. Mixed with the leaves of *Phyllanthus amarus*, it treats bloody diarrhea. It has also been reported that in Madagascar the plant is used as an astringent. It is also highly effective against anti-inflammatory and stimulates milk production in nursing mothers (Gurib-Fakim & Brendler, 2004). In Madagascar, the plant is used externally essentially as an anti-inflammatory agent. As a decoction, it is used to promote milk flow in nursing mothers and also used against dysentery (Boiteau & Allorge-Boiteau, 1993).

The plant is also reported to be useful against asthma and it has a special reputation for causing bronchial relaxation (Sofowora, 1982).

CHEMICAL CONSTITUENTS

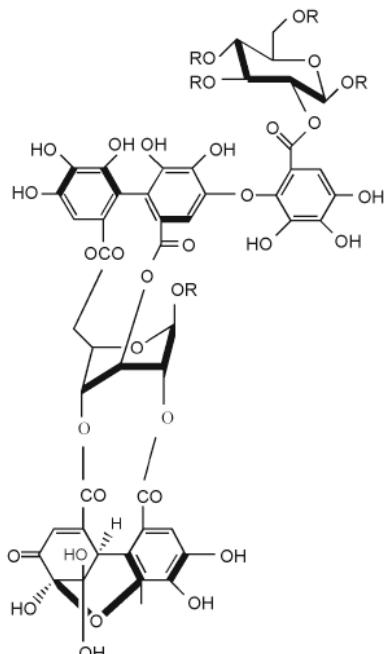
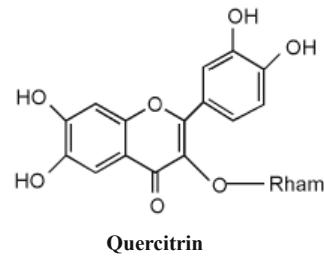
Compounds: The plant is rich in diterpenes (phorbol esters) triterpenes (including phytosterols), taraxerol, friedelin, α - and β -amyrins, cycloartenols, euphorbol hexacosonate, and friedelan. The dermatogenic latex contains euphorbon (Duke et al., 1985; Anon, 1992).

The diterpene tinyatoxin, 12-deoxy-4- β -hydroxyphorbol-13-dodecanoate-20-acetate, 12-deoxy-4- β -hydroxyphorbol-13-phenyl acetate-20-acetate, ingenol triacetate, the sterols β -sitosterol, campesterol, cholesterol, and a phytosterol glucoside are present in the plant (Rizk, 1986).

The latex is rich in (-)-inositol and pyrogallic and catechuic tannins and is reported to contain taxerol, friedelin, β -sitosterol, myricyl alcohol, ellagic, caffeic, chlorogenic, pyrogallic and gallic acids, hentriacontane, kaempferol, quercitol, quercitrin and other amino acids.

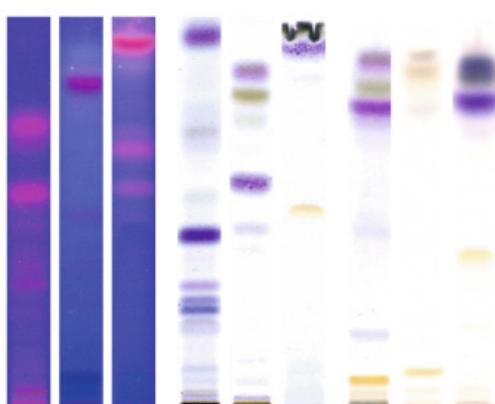
Equally present are leucocyanidol, camphol, quercitrin, quercitol derivatives containing rhamnose and chlorophenolic acid. The hydrocarbon triacontane as well as the alcohol 1-hexacosanol are equally present.

This species has also been reported to contain flavonoids namely leucocyanidol, camphol, quercetin, quercetol derivatives containing rhamnose and chlorophenolic acid, quercetin, cyaniding 3,5-diglucoside, and pelargonium 3,5-diglucoside. Shikimic, tannic and melissic acids have been isolated from this plant as well as inositol, jambulol, resins, cholines, alkaloids like xanthorhamnine, and various sugars (Oliver-Bever, 1986).



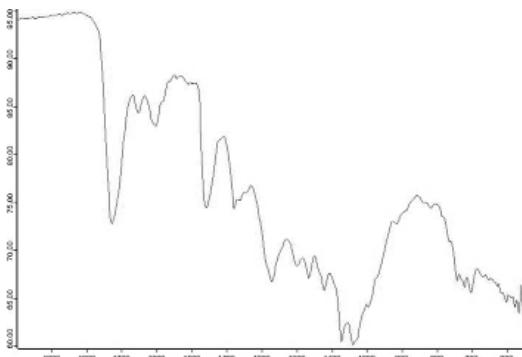
QUALITY CONTROL

TLC / HPLC / GC



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

NIR Spectroscopy



Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

Pharmacological studies have already been carried out on two of the active components of the plant, one exerting a spasmolytic or histamine potentiating activity and the other an antispasmodic property. The antispasmodic principle has been identified as shikimic acid, while the contracting principle as Choline. The latter also stimulates histamine activity.

The extracts are sedative and have anxiolytic properties in mice at a relatively high concentration ($>100\text{mg}$ of the extract of the plant/kg) (Lanher et al., 1990). The plant extract contains the flavonoid quercetin, which is used against diarrhoea and models have been used to validate this traditional use. The model were experimental models of diarrhoea induced by castor oil, arachidonic acid, and prostaglandins E2 but not in magnesium sulphate provoked diarrhoea. This activity may be attributed to the presence of hydrolysable tannins Euphorbin A, B, C, D, and E which have been isolated and characterised.

The hypoglycaemic activity of the plant and its antiprotozoal effect have been reported. The alcoholic extracts of the plant, besides being hypoglycaemic, manifests anti-cancerous properties against the leukaemia Friend Virus (Atta-Ur-Rahman et al., 1989). A maximum dose of 1g/kg per os, has been recorded as tolerated by mice. The drug appears to be well tolerated and was a proprietary product both in Europe and the United States.

The same extract is also antiprotozoal and acts against dysentery (Vijaya et al., 1995). It has been reported that the processed extract is 20x more active than the crude extract when tested against *Entamoeba histolytica*. The extract also has anti-inflammatory activities (Hiermann et al., 1994) and is antipyretic (Lanher et al., 1991). Anti-fungal activities against *Fusarium* species (Singh et al., 1994) as well as broad spectrum antibacterial activities have also been detected (Khan, 2001; Srivastava et al., 2001; Sudhakar et al., 2006). Nonetheless, it appears that the activity of *Euphorbia* is due to a wide range of compounds rather than a single isolate.

Lanher et al. (1991) have shown that the aqueous extract of the entire plant was endowed with central analgesic properties, apparently morphinomimetic, associated to anti-pyretic and anti-inflammatory effects on acute inflammatory processes. Lanher et al. (1996) have also demonstrated that the lyophilised extract of the entire plant did not possess neuroleptic properties but had a slight antidepressant activity against reserpine-induced ptosis or oxotremorine-induced hypothermia.

The plant extract has also been shown to interact with identified targets in allergic reactions and hence could be developed into a remedy for Type 1 allergic disorders (Singh et al., 2006).

The alcoholic extract of the aerial parts of this herb, containing shikimic acid, produces relaxation of the guinea pig ileum and has been used to treat asthma, hay fever, bronchitis, and other respiratory conditions. The extract showed an antispasmodic effect on the smooth muscles of mice and guinea pig.

Clinical Studies: Found to be effective in clinical trials for the treatment of amoebic dysentery. A single oral treatment appeared to be long lasting and produced remissions in 83% of the cases treated for up to 6 months.

SAFETY DATA

Preclinical Safety Data: The mineral content of a sample of the dried leaves was: Ca 1.1%, P 0.3%, Fe 0.03%, Mg 0.5%, Mn 0.01%, Zn 0.01%, and Cu 0.002%. Fresh leaves from *Euphorbia hirta*

plants of Nigerian origin were found to contain high levels of Mn (189 ppm), Cu (30.5 ppm), Zn (152 ppm), and NO (4600 ppm). Varying proportions of Fe, Mg, K, Ca, and Na were found. The levels of chemicals are high enough to constitute a source of toxicosis to animals consuming the plants and should also be a source of concern in medicinal use.

Few toxic effects have been documented for *Euphorbia hirta*. An ether extract was found to be toxic in a brine shrimp lethality test, whereas ethyl acetate and aqueous extracts were within safe limits. In another test, however, an aqueous crude extract was found to cause testicular degeneration in sexually mature male rats, as well as a reduction in the mean seminiferous tubular diameter. Several other extracts given orally to rats caused dullness and anorexia and induced a 20% mortality rate. Some fractions from the ethanolic extract showed potentially deleterious effects on the blood serum chemistry of rats. In feeding experiments with rats however, no difference in the blood serum was found after a prolonged period of adding *Euphorbia hirta* to the diet. It was also found that drying *Euphorbia hirta* prior to extraction considerably reduces the cytotoxic activity of certain of its extracts (Schmelzer & Gurib-Fakim Ed., 2008).

Experiments carried out on male rat reproductive organs have shown that the plant extract had a direct bearing on the seminiferous tubular volume as opposed to untreated rats. Testicular lesions have also been observed in treated rats (Adedapo et al., 2003).

Single Dose Toxicity cf. appendix 1

KEY (PROPOSED) USAGE

Therapeutic Indications: Analgesic, sedative, anxiolytic, hypoglycaemic.

Dosage, Method, and Duration of Administration: The recommended daily dose is three tablets containing 200 mg each.

Formulation and dosage poorly known and unrecorded. Tablets have been made from the dried leaf powder.

Pregnancy and Lactation: The product should not be used during pregnancy or while breast-feeding.

Evaluation of Efficacy: Efficacy pharmacologically proven, plant material with toxic potential.

TRADE INFORMATION

Nature of plant material: Flowering/harvesting time: Throughout the year.

Conservation status: Not threatened.

Nature of plant products: The plant preparations, under the name of 'Socambe' are available in France for dysentery (Sofowora 1982).

Processing and storage: Leaves are air-dried.

Stability unknown. The dried leaves are likely to be stable for considerable periods.

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Garcinia kola

GENERAL DESCRIPTION

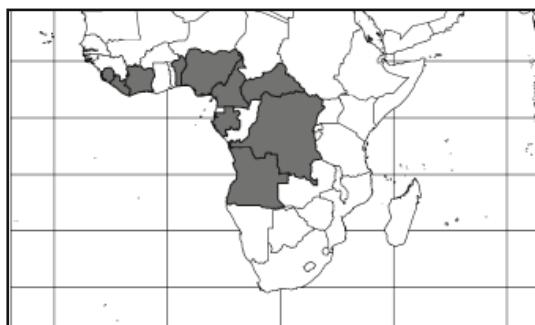
Scientific Name with Author: *Garcinia kola* Heckel

Family: Guttiferae

Vernacular Names: Adi, akara-inu oglu, akilu aki-inu, bitter kola, male cola, edum, efiari, efiat, false kola, mijin-gworo, okan.

Botanical Description: A medium sized tree, but sometimes growing up to 12 m tall and 1.5 m wide. It is a spreading tree with dense and heavy crown; the bole is straight, the bark is greenish-brown, thick, and smooth. It has broad leaves, 5 - 10 cm long, elongated elliptic, acute or shortly acuminate, cuneate, leathery, with very distinct resinous canals. It has ten pairs of lateral veins that run parallel to the margin but not forming a marginal nerve; the midrib is prominent at the underside; the stalk is stout, finely hairy in young leaves, about 8 mm long (Keay et al., 1964). It bares male and female flowers separately, usually December, March, and May-August. Female flowers are yellow and fleshy, globose, 1.5 cm wide; male flowers are smaller but with more prominent stamens (4 bundles), 4 sepals, and 4 greenish-white petals. It produces characteristic large fruits (6 cm in diameter), with the size and color of an orange, containing 2 - 4 brown seeds embedded in an orange colored pulp (Irvine, 1961).

Origin and Distribution: It is distributed throughout west and central Africa; it has been located in Sierra Leone, Ghana, Nigeria, Cameroon, and Congo.



Plant Part Used: Bitter kola consists of fresh or dried seed of *Garcinia kola* Heckel.

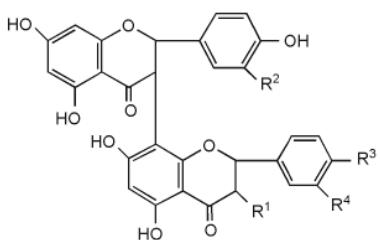


ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses: *Garcinia kola* is used extensively in traditional medicine for the treatment of various diseases. Medicinal uses include purgative, antiparasitic, antimicrobial. The seeds are used in the treatment of bronchitis and throat infections. They are also used to prevent and relieve colic, cure head or chest colds, and relieves cough. Also the plant is used for the treatment of liver disorders and as a chewing stick. Bitter kola is chewed in southern Nigeria and parts of West Africa as a masticatory, in spite of its very bitter taste. The stem bark is used as a purgative and the powdered bark for the treatment of malignant tumors. The sap is used for parasitic skin diseases. The latex (gum) is used internally for gonorrhea treatment and applied externally to fresh wounds. The twigs of *G. kola* can be used as tapers and the roots yield the favorite bitter chew-sticks sold in small bundles in local markets in West Africa. The seeds are chewed as an aphrodisiac and the dried nuts for dysentery treatment (Iwu, 1993).

CHEMICAL CONSTITUENTS

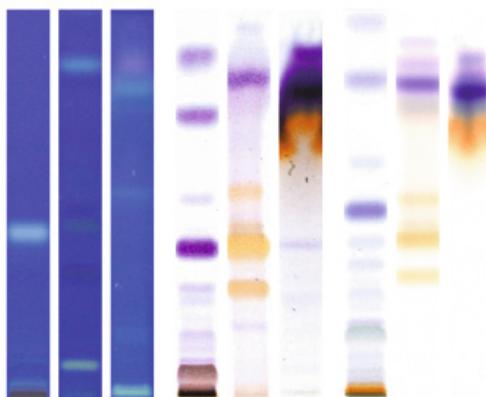
Compounds: *G. kola* and other members of the genus are known to contain a complex mixture of phenolic compounds including biflavonoids, xanthones, and benzophenones (Hussain et al., 1982; Iwu and Igboko, 1982; Iwu, 1985). The most important constituents of the plant include the antimicrobial benzophenone kolanone, and biflavonoids based on ericotryl/taxifolin moiety GB1, GB2, GB3, kolaflavanone, and garcininflavanone. Two arylbenzofurans, garcifuran-A and garcifuran-B, from the roots of *G. kola*, have been isolated and characterized (Kelly et al., 1997).



	R ¹	R ²	R ³	R ⁴
GB1	OH	H	OH	H
GB2	OH	H	OH	OH
Kolaflavanone	OH	H	OCH ₃	OH

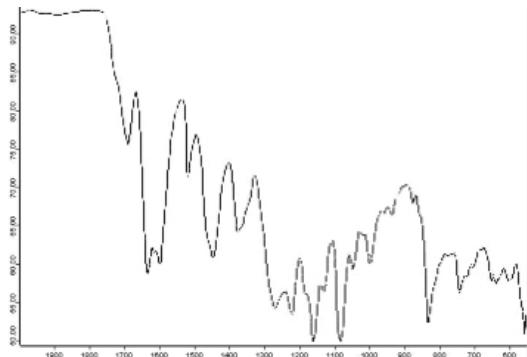
QUALITY CONTROL

TLC / HPLC / GC



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

NIR Spectroscopy



Standard Preparations: A number of products derived from *G. kola* have been manufactured and marketed as phytomedicines. Given the extent of use of *G. kola* and its dosage forms worldwide, a comprehensive quantitative analysis for monitoring the quality of these products is essential. The seeds of *G. kola* have been used in many herbal preparations either singularly or in combination with other plants. Among these are Heap-Vital Tea (a blend of *G. kola* and *Combretum micranthum*) and Hangover Tonic (a blend of kolaviron and *Cola nitida*). Most of Garcinia's pharmacological activities are believed to be related to its antioxidative activity and to its ability to increase immunity in general.

Like many dietary supplements and phytomedicines, *G. kola* products are susceptible to chemical variability due to growth. Plant collection through to production of finished phytomedicines is required to ensure safety and protect the consumers. Quality specifications for *G. kola* have been established based on classical pharmacopeia methods. These methods include: plant morphology, chromatography, and examination of pesticide residue and heavy metals, etc. In addition, for *Garcinia* formulations, capillary electrophoresis (CE) has been used in quantitative analysis of two proprietary dietary supplement products, Heap-Vital Tea and Hangover Tonic, containing *Garcinia kola* biflavonoids as the major biologically active compounds. (Okunji et al., 2002).

A CE method was used to estimate the pharmacological activity of biflavonoids in the Heap-Vital Tea and Hangover Tonic formulations. The method allows for quantitative

determination of independent biflavonoids in traditional formulations. This use of CE provides a sensitive, simple, and quantitative method that simultaneously quantifies the four major biologically active biflavonoids of *G. kola* (GB1, GB2, and GB1-glycoside and kolaflavanone). Optimal separation of four biflavonoids found in the formulations was achieved by systematically optimizing electrolytic and instrumental parameters. Baseline resolution of the biflavonoids was achieved in less than 10 minutes using a 100 mM borate buffer. The biflavonones completely resolve in less than 10 minutes using a 100 mM borate buffer, pH 9.5. Limits of quantization for the three flavanones are 2 mM. The assay successfully discriminate the four biflavonoids in proprietary products containing *G. kola*. The CE technique provides a powerful tool for the estimation and quality control of *G. kola* based products (Iwu et al., 2003).

Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

G. kola has been shown to possess remarkable antihepatotoxic activity against a variety of experimental hepatotoxins (Iwu et al., 1987). Chronic ingestion of *G. kola* seeds caused inhibition of gastrointestinal motility and weight reduction and prevented castor-oil induced diarrhea in rats. Other activities shown by the biflavonoid mixture include anti-inflammatory, antimicrobial, antidiabetic, and antiviral properties (Iwu et al. 1999, 2002). The biflavonoids also possess antidiabetic activity and inhibited the activity of rat lens aldose reductase. Examination of liver, kidney, and duodenum of rats fed a diet containing 10% (w/w) dry powdered seeds of *G. kola* for six weeks has been reported as revealing some histological alterations in these organs (Braide, et al. 1990). Most recently powerful antioxidative agents based on garcinoic acid from *G. kola* were reported by Terashima et al. (2002).

Some biflavonoids present in *G. kola* seed but isolated from *Rhus succedanea* have been reported to demonstrate significant antiviral activity against HIV-1 in phytohemagglutinin-stimulated primary human peripheral blood mononuclear cells at an EC₅₀ value of 6.9 µM and a selectivity index value of approximately 10 (Lin et al., 1997, 1999).

SAFETY DATA

Preclinical Safety Data

Single Dose Toxicity cf. appendix 1

KEY (PROPOSED) USAGE

Therapeutic Indications: Antioxidant, immunostimulant.

Interactions: No precautions have been reported concerning drug and laboratory test interaction.

Pregnancy and Lactation: Excretion of the components of *G. kola* into breast milk and its effect on the newborn has not been established. Not to be used during pregnancy. Not to be used while nursing.

TRADE INFORMATION

Nature of plant material: Found in moist forest, also cultivated in homesteads.

Nature of plant products: The seed extract and the dried powder have been formulated into various dosage forms including tablets, lozenges, creams, vial, and toothpaste.

Processing and storage: Seeds for decoction, tablets, capsules, extracts, tonic drink and lozenges store in airtight container protected from light and moisture.

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Griffonia simplicifolia

GENERAL DESCRIPTION

Scientific Name with Author: *Griffonia simplicifolia* Baill.

Synonyms: *Schotia simplicifolia* (Vahl ex DC.) Baill., *Bandeiraea simplicifolia* (M.Vahl ex DC.) Benth.

Family: Leguminosae

Vernacular Names: Atooto, gbogbotri, griffonia, kajya, kanya, kwakuo-aboto.

Botanical Description: Stout, woody, climbing shrub, woody vine, or liana growing to about 3 m with greenish flowers and inflated black seed pods. The seeds have a circular shape with a diameter of approximately 1.7 cm, weighing on average 0.5 g.

Origin and Distribution: Occurs mainly in West-Central Africa in thickets, usually associated with mounds of the termite Macrotermes on plains, in forests, in secondary vegetation and on old farms. It is evergreen, vigorous, and has wide adaptability.



Plant Part Used: The wildcrafted and dried seeds are the source of 5-hydroxy-L-tryptophan, 5-HTP (syn. Oxitriptan, L-5-HTP, 5HTP) and is commercially produced by extraction.

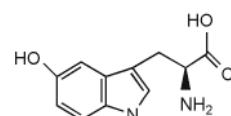


ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses: Traditional African uses for the plant include the use of the stem and roots as chewing sticks, leaves to aid in the healing of wounds, and leaf juice is used as an enema and for the treatment of kidney ailments. A decoction of stems and leaves is used to stop vomiting, to treat congestion of the pelvis and as an aphrodisiac. The bark-pulp is applied as a plaster to soft chancres.

CHEMICAL CONSTITUENTS

Compounds: Several indole derivatives including 5-Hydroxy-L-tryptophan (5-HTP), indole-3-acetyl-aspartic acid and 5-hydroxyl indole-3-acetic acid (5-HIAA). The seeds contain very high and varying levels of 5-HTP ranging from 14.3 to 17.1% (Kim et al., 2009).



5 - Hydroxytryptophan

QUALITY CONTROL

Identification: Seed color is black, brown. Endosperm color is yellow, yellow/green.

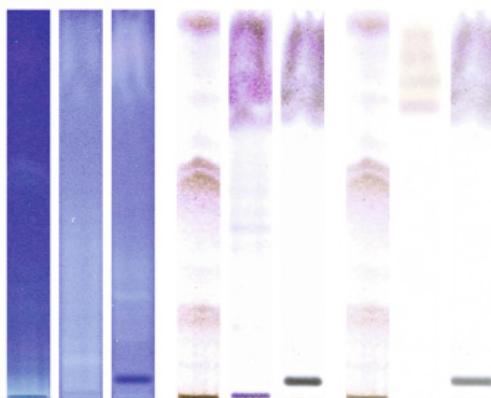
Organoleptic Properties: Aroma of seeds is a peppery odour.

Macroscopic Characteristics:

Damaged seeds - not more than 0.5 - 1%.
Seed weight - not less than 0.38 g.
Seed diameter - not less than 1.5 cm.

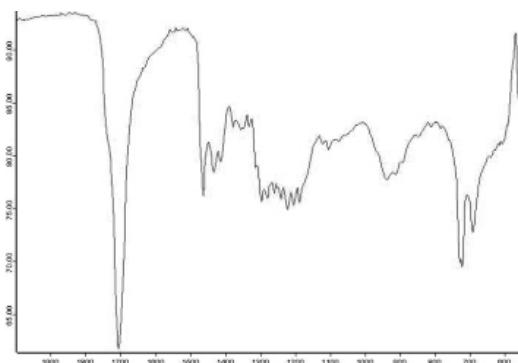
Moisture Content: not more than 10%

TLC / HPLC / GC



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

NIR Spectroscopy



Standard specifications cf. appendix 2

Extraneous/foreign matter content - not more than 0.5%.

PHARMACOLOGICAL PROPERTIES

5-Hydroxytryptophan (5-HTP) is the intermediate metabolite of the amino acid L-tryptophan (LT) in the serotonin pathway.

Therapeutic use of 5-HTP bypasses the conversion of LT into 5-HTP by the enzyme tryptophan hydrolase, which is the rate-limiting step in the synthesis of serotonin. Tryptophan hydrolase can be inhibited by numerous factors, including stress, insulin resistance, vitamin B6 deficiency, and insufficient magnesium. In addition, these same factors can increase the conversion of LT to kynurenone via tryptophan oxygenase, making LT unavailable for serotonin production.

5-HTP functions as an antioxidant (Simic et al., 1989).

5-HTP acts primarily by increasing levels of serotonin within the central nervous system (Chadwick et al., 1975). Other neurotransmitters and CNS chemicals, such as melatonin, dopamine, norepinephrine, and β -endorphin have also been shown to increase following oral administration of 5-HTP (Van Praag & Lemus, 1986; Den Boer & Westenberg, 1990; Guilleminault et al., 1973).

Clinical Studies: Most research on 5-HTP focuses on its use in the treatment of depression. Since the early 1970s, some 40 studies have evaluated the clinical effects of 5-HTP on depression. Significant clinical response of patients with unipolar or bipolar depression could be demonstrated over treatment periods of 2 - 4 weeks at doses of 150 - 900 mg daily (Loo et al., 1980; Van Hiele, 1980; Angst et al., 1977; Takahashi et al., 1975; Sano, 1972; Nakajima et al., 1978; Agren et al., 1991; Nardini et al., 1983; Poldinger et al., 1991).

These studies examined patients with different types of depression. The significant majority of subjects showed improvement while taking 5-HTP.

The current conventional therapies of choice for depression are the selective serotonin reuptake inhibitors (SSRIs). Thus, other

studies evaluated 5-HTP in comparison to SSRI drugs. Those studies found 5-HTP to be at least as effective as these drugs in treating severe depression, while displaying fewer side effects. In severe cases, 5-HTP dosages as high as 1200 mg daily were used.

Fibromyalgia patients have been found to have low serotonin levels. A number of clinical trials have demonstrated significant improvement in symptoms, including pain, morning stiffness, anxiety, and fatigue (Nicolodi & Sicuteri, 1996; Puttini & Caruso, 1992; Caruso et al., 1990).

Low serotonin levels in obese patients have been associated with carbohydrate cravings and resultant binge eating. Three studies with 5-HTP in obese patients resulted in decreased food intake and subsequent weight loss (Cangiano et al., 1991, 1992; Ceci et al., 1989).

5-HTP has been shown to be beneficial in treating insomnia, especially in improving sleep quality by increasing rapid eye movement (REM) sleep (Soulairac & Lambrinet, 1977; Guilleminault et al., 1973; Wyatt et al., 1971). According to anecdotal reports, higher doses may cause vivid dreams or nightmares.

Chronic headaches, especially migraines, are considered by to be the result of low serotonin levels, possibly as the result of increased breakdown of serotonin by the enzyme monoamine oxidase. 5-HTP has been used successfully in the prevention of chronic headaches of various types, including migraine, tension headaches, and juvenile headaches (Nicolodi & Sicuteri, 1996; Maissen & Ludin, 1991; De Giorgis et al., 1987; Titus et al., 1986; De Benedittis & Massei, 1985; Longo et al., 1984; Bono et al., 1982).

Pharmacokinetic Properties: 5-HTP is well absorbed from an oral dose, with about 70 percent ending up in the bloodstream (Magnussen & Nielsen-Kudsk, 1980; Magnussen et al., 1981). Absorption of 5-HTP is not affected by the presence of other amino acids; therefore it may be taken with meals without reducing its effectiveness.

Unlike LT, 5-HTP cannot be shunted into niacin or protein production. Serotonin levels in the brain

are highly dependent on levels of 5-HTP and LT in the central nervous system (CNS). 5-HTP easily crosses the blood-brain barrier, not requiring the presence of a transport molecule. LT, on the other hand, requires use of a transport molecule to gain access to the CNS. Since it shares this transport molecule with several other amino acids, the presence of these competing amino acids can inhibit LT transport into the brain.

SAFETY DATA

Preclinical Safety Data

Single Dose Toxicity cf. appendix 1

KEY (PROPOSED) USAGE

Therapeutic Indications: L-5-Hydroxytryptophan is of great benefit in psychiatric and neurological disorders connected to a deficiency of serotonin.

L-5-Hydroxytryptophan is used in alleviating the symptoms of anxiety and depression. It is a natural relaxant, helping alleviate insomnia by inducing normal sleep, for the treatment of migraine and headaches, and in the treatment of fibromyalgia.

It helps control cravings such as in eating disorders.

L-5-Hydroxytryptophan may strengthen the immune system and help reduce the risk of artery and heart spasms.

L-5-Hydroxytryptophan has proven to be useful in the management of Parkinson's disease (PD) and epilepsy.

Dosage, Method, and Duration of Administration:

Initial dosage for 5-HTP is usually 50 mg three times a day with meals. If clinical response is inadequate after two weeks, dosage may be increased to 100 mg three times a day. For insomnia, the dosage is usually 100 - 300 mg before bedtime. Because some patients may experience mild nausea when initiating treatment with 5-HTP, it is advisable to begin with 50 mg doses and titrate upward.

Contraindications: The primary concern regarding 5-HTP is the possibility of an

eosinophilia-myalgia syndrome (EMS) similar to the condition linked with contaminated LT.

Two cases of EMS-like symptoms have been described in patients taking 5-HTP (Sternberg et al., 1980; Michelson et al., 1994).

5-HTP should not be used in patients currently being treated or who have recently been treated with an SSRI antidepressant.

Interactions: When taken in combination with a selective serotonin reuptake inhibitor (SSRI) (antidepressants such as Prozac, Paxil, or Zoloft), 5-HTP may cause a condition known as serotonin syndrome (Martin, 1996). This syndrome is characterized by agitation, confusion, delirium, tachycardia, diaphoresis, and blood pressure fluctuations.

This syndrome has been reported in patients taking LT at doses above 1200 mg/day along with monoamine oxidase inhibitors (MAOIs), but was not identified in a 12-month study with 5-HTP (200 mg/day) taken in conjunction with an MAOI drug (Nicolodi & Sicuteli, 1996).

Pregnancy and Lactation: Clinical data is required to confirm safety for use in pregnant and lactating women. Therefore, it is not recommended for use during and post-pregnancy period.

Adverse Effects: Some patients may initially experience mild nausea when taking 5-HTP.

Evaluation of Efficacy: Efficacy clinically proven for the treatment of depression, headaches and migraines, fibromyalgia, insomnia, and for weight management.

TRADE INFORMATION

Nature of plant material: Conservation status: Not threatened. There are no readily available data on the current status, since exporters and agents have complained about declining stocks.

Nature of plant products: Most of the *Griffonia* entering into world trade has historically come from Ghana.

Processing and storage: The seeds are removed from the pods and dried under the sun.

Stability of the product: Unknown.

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Harpagophytum procumbens

GENERAL DESCRIPTION

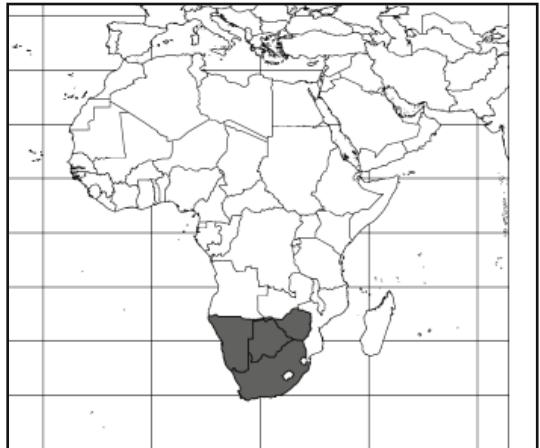
Scientific Name with Author: *Harpagophytum procumbens* DC.

Family: Pedaliaceae

Vernacular Names: Arpagofito, artiglio del diavolo, devil's claw, duiwelsklou, ekatata, elyata, grapple plant, griffe du diable, kloudoring, likakata, otjhangatene, Teufelskralle, Trampelklette, venustorn, Windhoek's root, burdock, wood spider, !ao!ao, //xsamsa-//oro, //xemta=’eisa, //khuripe//khams.

Botanical Description: The plant is a leafy, branched, prostrate perennial, with a branched root system, 1 - 1.5 m long shoots. The leaves are petiolate and lobed and may be opposite or alternate. The aerial parts die back during the dry season. The storage roots are formed from the main and lateral roots. The main roots have obtuse, quadrangular, 10 - 20 cm long and 30 - 60 cm thick erect collars which are covered in a fissured cork layer. The nodes of the lateral roots are up to 60 mm thick and 20 cm long. They are light-brown to red-brown on the outside and are found in an area of about 150 cm around the plant, growing to a depth of 30 - 60 cm. Flowers are solitary, large, resembling foxgloves or gloxinias, on short pedicels in the leaf axils. The petals are pale pink to crimson. The seed capsules are bivalvular, compressed at the sides, ovate, 7 - 20 cm long, approximately 6 cm in diameter, very woody with longitudinally-striped rind. They have a double row of elastic, arm-like, branched appendages with an anchor-like hook and contain about 30 oblong, dark seeds with a rough surface.

Origin and Distribution: Native to the red sand areas in South Africa, Botswana, and Namibia. It has spread throughout the Kalahari and Savannah desert regions.



Plant Part Used: Dried and powdered root slices

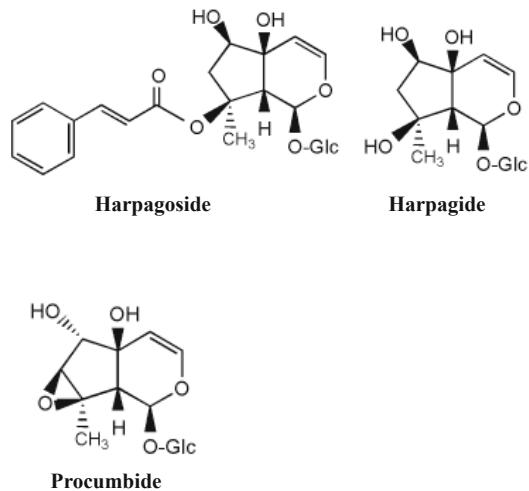


ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses: Ethnomedicinal uses of Devil's Claw include allergies, analgesia, arteriosclerosis, for boils, skin injuries, ulcers and sores (topical), in childbirth, dysmenorrhea, oedema, fever, gastrointestinal disorders, headache and migraine, malaria, myalgia, neuralgia, tendonitis, and urinary tract infections.

CHEMICAL CONSTITUENTS

Compounds: Constituents include iridoid glycosides (Kikuchi, 1983), harpagoside (0.5-1.6%) (Tunmann, 1962), 8-p-coumaroylharpagide, 8-feruloylharpagide, 8-cinnamoylmyporoside, pagoside, acteoside, isoacteoside, 6'-O-acetylacteoside, 2,6-diacylacteoside, cinnamic acid, caffeic acid (Ficarra et al., 1986; Boje et al., 2003; Munkombwe, 2003), procumbide and procumboside. Six-acetylacteoside, present in *H. procumbens* and absent in *H. zeyheri*, allows to distinguish between the two species (Chrubasik, 2004). Other compounds include flavonoids, fatty acids, aromatic acids, harpagoquinone, stigmasterol, β -sitosterol, triterpenes, sugars (over 50%), and gum resins (Tunmann, 1975).



QUALITY CONTROL

Identification: Hard, disc-shaped, medium brown slices of rhizome, with no odour.

Organoleptic Properties: Taste is moderately to extremely bitter and slightly sweet. Smell is almost neutral.

Macroscopic Characteristics: Cut drug: Irregular pieces, fan-shaped to wedge-shaped with curved exterior. Thin cortex, light grey-brown to dark brown. Cut area smooth, horn-like, light grey to whitish; raised lines radiate to the centre. In cross-section, the darker cambium layer shows up as a raised line between cortex and wood. The pieces are up to 2 cm in size, yellowish-grey to light rust colour on the outside, hard and difficult to break. Also present, isolated cylindrical, light brown pieces with wrinkled walls. Magnified appearance: 0.3 - 0.5 mm thick, torn, often multi-layered outer cortex, followed by a 4 - 8 mm thick inner cortex and a darker cambium layer. In the stele, thin vascular rays, in which further single or grouped vessels are present.

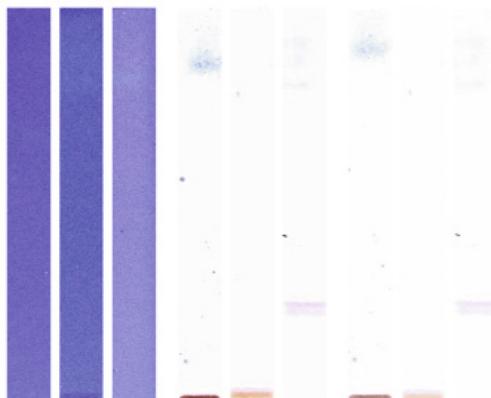
Powdered drug: Light brown powder.

Microscopic Characteristics: Cut drug: Outer cortex made up of thin-walled, regular cells followed by generally thin inner cortex, being made up of predominantly large, thin-walled, mottled cells. Isolated, angular sclerenchymal cells. Clusters of parenchymal cells filled with reddish brown inclusions, which stain dark brown in the presence of iron (III) chloride, and bright red with Schiff's reagent. In the cortex are longitudinally deformed sieve cells. In the stele, yellowish, in longitudinal section recognizable as mottled vascular cells; with intermittent tracheidal cells. The wood parenchyma resembles the cortex parenchyma and both contain isolated yellow or reddish brown resin droplets. Both tissue types contain isolated calcium oxalate crystals of up to 20 μ m in size, mostly small needles but also regular cuboid to isodiametrical crystals. No starch.

Powdered drug: Much woody parenchyma, with thickened vessels. Numerous, thin-walled, mottled parenchymal cells with resinous droplets, or yellow to reddish brown drops of resin, occasionally crystals or needles of calcium oxalate; long, thin-walled outer cortex cells with brown content. No starch. No sclerenchymal cells.

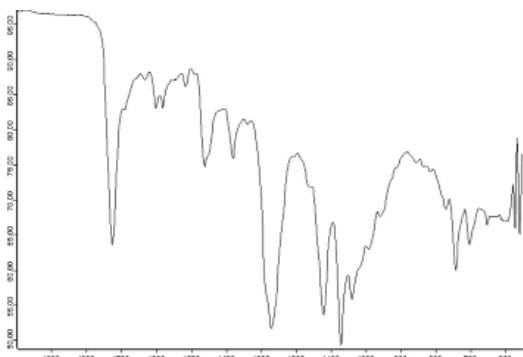
TLC / HPLC / GC: Using TLC for the ethanol drug extracts. Reference substance: Harpagoside; Sorption agent: Silica gel 60 F254; FM: water-methanol-ethylacetate (10+13+77); Detection: UV 254 nm, vanillin sulphuric acid reagent,

heated from 100 to 105°C; Evaluation: In UV 254 nm in the centre a fluorescent area which colours pink to red-violet after treatment with vanillin sulphuric acid reagent (Harpagoside). Further red tinted areas can be present. Colour spectrum of aqueous ethanolic drug extracts: 1. With vanillin sulphuric acid: dark red. 2. Extract concentrated by means of drying, water added and treated with potassium permanganate solution: A benzaldehyde-like odour results. Colour reaction with powdered drug: 1. Drug mixed with hydrochloric acid: colours red. 2. Drug mixed with sulphuric acid: colours red. 3. Drug mixed with vanillin sulphuric acid: drug colours red, colour slowly leaches, surrounding liquid yellowish green. 4. Drug mixed with phloroglucinol hydrochloric acid: colours green.



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

NIR Spectroscopy



Adulterants and Adulterations: Old, primary tubers of *Harpagophytum procumbens*; recognizable by black-brown colouring and

the lack of bitter taste. Adulterations with *Harpagophytum procumbens*, *Elephantorrhiza* spec. and *Acanthosicyos naudianus* have been found.

Standard Preparations: Capsules, film tablets, and compound preparations.

Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

A crude methanolic extract of Devil's Claw root showed significant, dose-dependent, protective action against arrhythmias in isolated rat hearts (Costa De Pasquale et al., 1985) and isolated rabbit hearts (Circosta et al., 1984). Investigation of the effects of *Harpagophytum* constituents on the stimulated release of inflammatory mediators from mouse peritoneal macrophages showed aucubin inhibited LCT4-release. None of the iridoid glycosides significantly affected the prostaglandin release or that of other products of the COX-2 pathway, while most of them (except harpagoside) showed significant inhibition of stimulated TXB₂ release (Benito et al., 2000), contrasting the findings of (Loew et al., 2001). Harpagoside inhibits both arachidonic metabolism pathways (Chrubasik et al., 1999). Kaszkin et al. (2004) demonstrated a downregulation of iNOS expression in rat mesangial cells by special *Harpagophytum* extracts which may indicate potential as an anti-inflammatory drug in the treatment of glomerular inflammatory diseases. Animal experiments show that *H. procumbens* extracts exert significant anti-oxidant activity, which may explain their role in relief of inflammatory arthritic conditions with pathophysiology involving oxidative free radicals (Bhattacharya & Bhattacharya, 1998). Bentancor-Fernandez et al. (2003) showed in the TEAC assay that harpagoside did not contribute significantly to the antioxidant activity of Devil's Claw. Antioxidant effects were also demonstrated by Langmead et al. (2002) by luminol-enhanced chemiluminescence in a xanthine/xanthine oxidase cell-free system. Although both an ethanolic extract (Fiebich et al., 2001) and an aqueous extract (Chrubasik et al., 2002a) prevented TNF-alpha synthesis, the latter had a greater inhibitory effect on COX-2 pathway products. This was confirmed by Jang

et al. (2003). The capability of *Harpagophytum* extract to suppress enhanced production of matrix metalloproteinases via the inhibition of the synthesis of inflammatory cytokines was shown by Schulze-Tanzil et al. (2004). Effects of the methanolic extract of *H. procumbens* on smooth muscle are due to a complex interaction of the different active principles of the drug with the cholinergic receptor. While harpagoside antagonizes smooth muscle contractile responses to acetylcholine and BaCl₂, harpagide increases the response at lower doses and antagonizes it at higher doses. This partial inhibition of BaCl₂ experimentally may explain the protective effect of the extract on arrhythmias by interference of the mechanisms regulating influx of calcium into cells. (Fontaine et al., 1981; Occhiuto et al., 1985; Occhiuto & De Pasquale, 1990; Circosta et al., 1984). Further studies are needed to confirm this hypothesis. A crude preparation of *H. procumbens* has shown antiplasmodial activity (Clarkson et al., 2003).

Devil's Claw extracts appeared to show anti-inflammatory effects in a number of studies (Eichler & Koch, 1970; Erdös et al., 1978), while others could not confirm these results (McLeod et al., 1979; Grahame & Robinson, 1981). In acute conditions (carrageenan-induced edema, etc.) no or only slight activity was found (Eichler & Koch, 1970; Erdös et al., 1978; McLeod et al., 1979; Grahame & Robinson, 1981; Whitehouse et al., 1983) while Jadot & Lecompte (1992) obtained a significant reduction of adriamycin-induced edema in rats with oral administration of powdered *Harpagophytum* root. Baghdikian et al. (1997) showed in the treatment of carrageenaan-induced hind paw edema in rats an efficacy of 1.200 mg/kg similar to that of indometacin 10 mg/kg. Significant, dose-dependent, anti-inflammatory effects were also shown by Lanfers et al. (1992). Anti-inflammatory effects, both in acute and chronic treatments of Freund's adjuvant-induced arthritis in rats, could be shown by Andersen et al., 2004. *Harpagophytum* extracts showed inhibitory effects on phorbol ester-induced COX-2 expression in mouse skin (Kundu et al., 2005) as well as inhibition of TPA-induced COX-2 expression in human breast epithelial cells and in mouse skin (Na et al., 2004).

Devil's Claw showed analgesic effects in several investigations. An aqueous root extract gave 53%

protection at an intraperitoneal administration of 200 mg/kg, which is comparable to 59% obtained with ASS at 68 mg/kg (Lanfers et al., 1992). Administration of 20 mg/kg harpagoside produced an analgesic effect similar to that of phenylbutazone at 50 mg/kg (Eichler & Koch, 1970). Writhings and stretchings induced in rats by 1.2% acetic acid were significantly reduced after administration of an aqueous Devil's Claw extract (2.2% harpagoside) (Baghdikian et al., 1997).

Antioxidant effects similar to selegiline were found in rats treated with a 53% ethanolic *Harpagophytum* dry extract (Bhattacharya & Bhattacharya, 1998). Protection against arrhythmias induced by calcium chloride was shown with a dry methanolic *Harpagophytum* extract (Circosta et al., 1984).

Clinical Studies: Clinical data on the treatment of degenerative joint disease and musculoskeletal disorders has been evaluated and compared in various reviews. In a systematic review, Ernst & Chrubasik (2000) evaluated randomized, placebo-controlled, double-blind trials of phyto-anti-inflammatories for the treatment of rheumatic symptoms. Chrubasik et al. (2003b) examined the effectiveness of treatment with *Harpagophytum* products in 20 studies. Long et al. (2001) evaluated the results of randomized controlled trials on the effectiveness of herbal products in the treatment of osteoarthritis. ESCOP (2003) and Chrubasik (2004) list and evaluate clinical data on the efficacy of Devil's Claw preparations. Finally, Gagnier et al. (2004) assessed the efficacy of *Harpagophytum* preparations dependent on extraction methods and harpagoside content.

SAFETY DATA

Preclinical Safety Data: Aqueous and methanolic extracts, as well as isolated compounds harpagoside and harpagide, have shown very slight toxicity in rodents (Van Haelen et al., 1983; Erdös et al., 1978; Whitehouse et al., 1983). The LD₅₀ in Swiss Webster mice is greater than 13.5 g/ kg of body weight (Whitehouse et al., 1983; Zimmermann, 1976). The acute LD₅₀ for pure constituents in mice was shown to be 1 g/ kg for harpagoside and greater than 3.2 g/kg for harpagide (Van Haelen et al., 1983). The acute

oral/intravenous LD₅₀ for aqueous, methanolic and butanolic extracts in mice was shown to be greater than 4.6 g/kg and 1.0 g/kg respectively (Erdös et al., 1978). Because there is no effect on the biosynthesis of prostanoids (Moussard et al., 1992; Loew, 1995), the adverse effects usually observed with non-steroidal anti-inflammatory and glucocorticoid medications are not expected with Devil's Claw.

Single Dose Toxicity cf. appendix 1

KEY (PROPOSED) USAGE

Therapeutic Indications: Major clinical uses of Devil's Claw are as an anti-inflammatory and analgesic in joint diseases, back pain, and headache.

Dosage, Method, and Duration of Administration: For treatment of painful osteoarthritis a duration of administration of 2 - 3 months is recommended (ESCOP, 2003). The clinical efficacy is dosage-dependent and has been documented not only for the powdered drug (daily dosage 2.6 g (Chantre et al., 2000)) but also for aqueous (2:1; daily dosage 2400 mg (Chrubasik et al., 1996)) and aqueous-ethanolic (4 to 5:1; daily dosage 960 mg (Laudahn & Walper, 2001)) dry extracts. Osteoarthritic/ Low Back Pain: 2 - 9 g/day of crude extract or equivalent amounts of extract (ESCOP, 2003; Chribasik, 2004). Daily dose for loss of appetite: 1.5 g of drug; other applications: 4.5 g of drug. Tea: 1 tsp. (= 4.5 g) cut drug to 300 ml boiling water, steeped for 8 hours, then strained, drink 3 times daily. Tincture (1:10, 25% ethanol) 3 ml / day (ESCOP, 2003).

Contraindications: Not recommended for children (ESCOP, 2003).

Special Warnings and Precautions for Use: Avoid use in patients with gastric or duodenal ulcers. Use with caution in patients taking anticoagulant or antiplatelet agents. Discontinue use at least two weeks prior to any surgery or dental procedure. Use with caution in patients with cholelithiasis due to the stimulant effect on the gall bladder. Use with caution in patients with diabetes, as Devil's Claw has been associated with cases of hypoglycemia (anecdotal). Use with caution in patients with heart disease,

especially in patients with arrhythmias or taking prescribed antiarrhythmic agents, due to potential negative chronotropic and inotropic effects of Devil's Claw. Use with caution when administered in the long-term (greater than 2-3 months) as its safety and efficacy have not been evaluated in long-term clinical trials.

Interactions: Use with other anticoagulant and antiplatelet agents may result in interaction. Use with antiarrhythmic agents may result in additive effects. Use with chronotropic agents may result in additive effects. Use with inotropic agents may diminish or potentiate the effects. Use with analgesics may result in additive effects. Use with anti-inflammatory agents may result in additive effects. Use with hyperlipidemic agents may reduce cholesterol concentrations.

Pregnancy and Lactation: Not recommended due to lack of sufficient data. Its use in pregnancy cannot be recommended because it may induce labour (may have traditionally been used for this).

Evaluation of Efficacy: Efficacy clinically proven.

TRADE INFORMATION

Nature of plant material: Conservation status: May be vulnerable, commercial planting has started.

Processing and storage: The parts used in medicine are the dried thick lateral tubular secondary roots. They are cut into slices or pieces or pulverized before they are sun dried.

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Harungana madagascariensis

GENERAL DESCRIPTION

Scientific Name with Author: *Harungana madagascariensis* Poir.

Synonyms: *Haronga madagascariensis* (Lam. ex Poiret) Choisy, *Haronga paniculata* (Pers.) Lodd. ex Steud., *Arungana paniculata* Pers.

Family: Clusiaceae

Vernacular Names: Bois harongue, fohatra, fohatse, haronga, harongana, miangaroka.

Botanical Description: Shrub or tree 2-12 m high, much branched, with scaly bark and orange latex becoming red on exposure to air. Branchlets pubescent-ferruginous, becoming glabrous. Leaves with 0.5-2.5 cm long petiole; leaf blade oblong or oblong-oval, or elliptic, 5-22 x 3-14 cm, acute or shortly acuminate, covered on the lower surface with ferruginous-stellate tomentum, sometimes glabrescent. Inflorescence corymbiform to pyramid-shaped, reaching 20 x 25 cm. Flowers numerous, fragrant, about 3 mm in diameter. Sepals oval to oblong, about 2 x 1 mm. Petals white, oval-elliptic, erect, 3-4 x 1-1.5 mm, with 2-4 glandular spots near the summit. Drupe yellow to orange, spherical, 3-4 x 3-4 mm, containing 5 pyrenes, each enclosing 1-4 curved cylindrical, 2 mm long seeds.

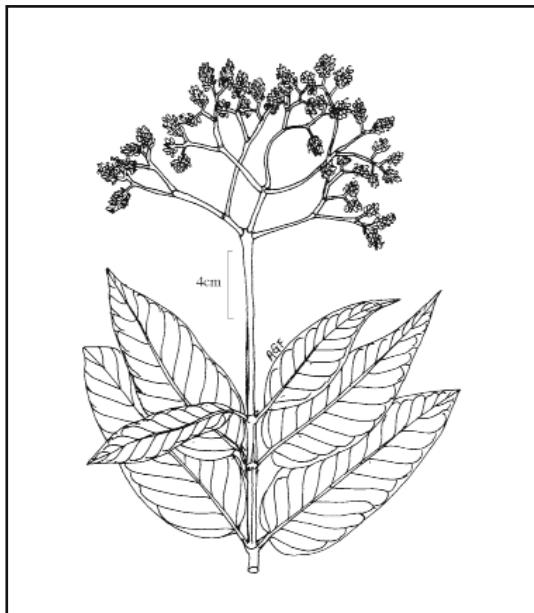
Origin and Distribution: The monotypic genus can be found in habitats from tropical Africa to Mauritius. It is native to continental Africa (Senegal, Sudan, Angola, Rhodesia, Mozambique) and Madagascar.

It is equally present in Mauritius and Reunion Island. It is found at the edge of the humid forests. In Mauritius it is more common in secondary forest formations at high altitudes.



Plant Part Used: Fruit, dried and powdered





ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses: Haronga bark and leaf are used mainly to treat dyspepsia and mild exocrine pancreatic insufficiency. It is considered effective in stimulating the production of gastric juices and increasing bile flow. In African countries, the reddish sap obtained from the bark or stems is used as a styptic and is applied topically to treat various skin diseases, wounds, leprosy, and itch. Bark extracts are also traditionally used to treat unspecified stomach ailments (Van Wyk & Wink, 2004). In Madagascar, the resin obtained after cutting the bark is mixed with animal fat and is then applied to parts of the body suffering from boils caused by the small arachnid responsible for transmitting the disease (Allorge & Boiteau, 1999).

CHEMICAL CONSTITUENTS

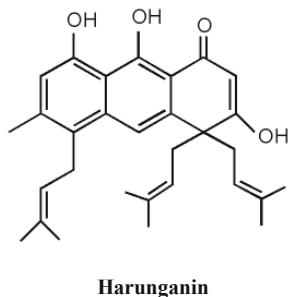
Compounds: Early investigations into the chemistry of *H. madagascariensis* were carried out by Fisel (1966). Gehrmann and Kraus (1989) prepared the essential oils of leaves and bark by steam distillation of the *n*-hexane extracts of the stored leaves and bark, and by direct steam distillation of 4-week-old shadow-dried leaves and bark. The different kinds of essential oil were compared by TLC and subsequently GC. Chrysophanic acid was derived from leaf, bark, and the alcoholic preparation of *H.*

madagascariensis. The essential oil of the dried and stored leaves showed a different composition from the 4-week-old drug.

Preliminary phytochemical screening of the aqueous stem bark extract showed the presence of glycosides, saponins, tannins, sterols, anthraquinones, fats, and oils (Akah, 1994). Haronga bark and leaf contain 1,8-dihydroxyanthracene derivatives. The bark contains 1,8-dihydroxyanthracene derivatives such as harunganin and madagascin. The leaves contain dimers of 1,8-dihydroxyanthracene derivatives such as hypericin, pseudohypericin and small amounts of quercetin. Also present are flavonol glycosides, tannins (especially condensed epicatechins and proanthocyanidins), and phytosterols. The leaves are reported to contain the acids gallic, protocatechic, paracoumaric, procyanidin B2 and B7, (-)-epicatechin, and derivatives of quercetin (Baldi et al., 1992).

Recently, two new prenylated anthronoids, harunmadagascarins A and B have been isolated from the bark. Six known compounds have been isolated along with the two anthronoids: harunganol B, harunganin anthrone; one benzophenone: methyl 3-formyl 2,4-dihydroxy-6-methyl benzoate; and three pentacyclic triterpenes: friedelin, lupeol and betulinic acid. Harunmadagascarins A and B have been characterised as being 8,9-dihydroxy-4,4-bis-(3,3-dimethylallyl)-6-methyl-2,3-(2,2-dimethylpyrano)anthrone and 8,9-dihydroxy-4,4,5-tris-(3,3-dimethylallyl)-6-methyl-2,3-(2,2-dimethylpyrano)anthrone. Methyl 3-formyl 2,4-dihydroxy-6-methylbenzoate has been isolated for the first time in this plant (Kouam et al., 2005).

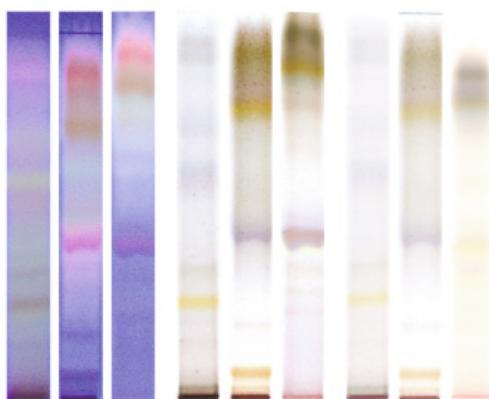
Three new prenylated anthronoids, harunmadagascarins C and D and kenganthranol D, were isolated from the leaves of *H. madagascariensis* (Kouam et al., 2007). Bazouanthrone, a new anthrone derivative, has been isolated from the root bark of *H. madagascariensis* (Ndjakou et al., 2007).



QUALITY CONTROL

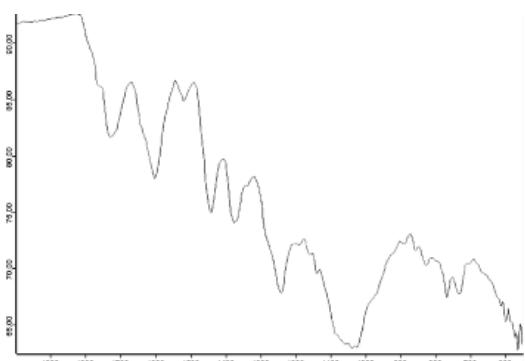
Identification: Crushed, dried, green-brown twigs and leaves.

TLC / HPLC / GC



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

NIR Spectroscopy



Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

The aqueous leaf extracts have been evaluated for their antibacterial activity against various strains of bacteria, namely *B. subtilis*, *Staphylococcus aureus*, *E. coli*, *Salmonella typhi*, and *Pseudomonas aeruginosa*. *B. subtilis*, *E. coli*, and *S. typhi*, but not *P. aeruginosa*, showed susceptibility at the following MICs, respectively: 2.0, 15.6 mg/ml; and MBCs of 2.0 - 3.9 mg/ml and 15.6 - 31.3 mg/ml, respectively, for the cold and hot extracts. *S. aureus* showed susceptibility only to the hot water extract. Concentrations of 2.5 - 10.0 mg/ml of the cold extract killed over 7 log₁₀ of the test bacterial population within 30 - 60 minutes of exposure. The hot extract needed higher concentrations and longer treatment to achieve similar levels of bacterial cell killing (Malcolm & Sofowora, 1969; Kambu et al., 1990; Okoli et al., 2002).

Harunmadagascarin A and B, harunganol B, and harunganin anthrone exhibited significant antioxidant activity (Kouam et al., 2005).

Moulari et al. (2006a) investigated the in vitro bactericidal activity of the ethyl acetate *H. madagascariensis* leaf extract (HLE) on the main oral bacterial strains largely implicated in dental caries and gingivitis infections, and the possibility of potentialization of HLE antibacterial effects using the poly (D,L-lactide-co-glycolide) nanoparticles (PLG-NP). HLE showed significant bactericidal effects against the bacterial strains tested, with minimal bactericidal concentration (MBC) to 5×10^2 mg/l or less, except for *Lactobacillus casei* with 7.5×10^2 mg/l. With the HLE incorporated into PLG nanoparticles (HLE-PLG-NP), diminution of the bactericidal concentration compared to HLE, the upper MBC being 1.875×10^2 mg/l, was observed.

Moulari et al. (2006b) evaluated the in vitro antibacterial activities of aqueous, ethanolic, and ethyl acetate crude extracts of *H. madagascariensis* leaves against bacterial strains representative of skin microflora. Only the ethyl acetate leaf extract presented important antibacterial activity. Concerning the antibacterial activity against the representative skin microflora of the armpit and feet, MIC and MBC ranged from 25 to 250 and 100 to 750 µg ml⁻¹, respectively.

The antiplasmodial activity of bazouanthrone, feruginin A, harunganin, harunganol A, harunganol B, friedelan-3-one, and betulinic acid was evaluated in culture against W2 strain of *P. falciparum*. All the compounds were found to be active against the *Plasmodium* parasites, with bazouanthrone showing particular potency ($IC_{50} = 1.80 \mu\text{M}$) (Ndjakou et al., 2007).

Harungana madagascariensis stem bark extract exhibited significant anti-protozoan effects against *Trichomonas* and *Plasmodium* both in vivo and in vitro (Iwalewa et al., 2008).

The leaf extract showed moderate antidiarrhoeal activity in mice (three of the five tested mice responded). Activity was exhibited against *Salmonella typhi*, *Salmonella D*, and *Shigella dysenteriae* (Malkere-Faniyo et al., 1989).

The butanol and saponin extracts of the stem bark have been tested for their ability to reduce pre-contracted guinea pig ileum using acetylcholine at a concentration of 5.1 - 7 M. The first fraction reduced the contraction by 25 + 1.7, whereas the second extract was effective at 100 + 0.0. When the depolarising solution was used, the relaxation was up to 40 + 3 for the first extract and 70.3 + 2.5 for the second (Kambu et al., 1990).

Nwodo (1989) showed that this plant contains pharmacologically active substances capable of stimulating smooth muscle of the guinea pig ileum. The extract also stimulated gravid and non-gravid rats and guinea pigs, acting to induce contraction in quiescent tissues and to increase the frequency and force of contraction in spontaneously active tissues. The contractions were found to be similar to those produced by oxytocin, and more pronounced in the gravid uterus (Akah, 1994). It can therefore be concluded that the extract possesses uterotonic activity and can hasten fetal delivery.

The concentrated methanol-water extract of the stem bark was tested for its anti-inflammatory properties in a concentration-dependent manner against carrageenan-induced rat paw oedema. The inhibition at the dose levels of 150 - 200 mg/kg were significant. Nonetheless, the extract was found to be less potent than phenylbutazone (Nwodo, 1989).

A herbal preparation made from *Parquetina nigrescens*, *Sorghum bicolor* and *Harungana madagascariensis* can increase the PCV and Hb concentrations in anaemic laboratory rabbits. The progressive recovery of *Brucella brucei*-infected rabbits is suggestive of progressive reduction of parasitaemia in the infected animals and restoration of PCV and Hb concentrations (Erah et al., 2003).

Clinical Studies: Jubi Formula® is a herbal preparation made from three medicinal herbs (*Parquetina nigrescens*, *Sorghum bicolor*, and *Harungana madagascariensis*). It has been reported to have been successfully used in the treatment of anaemia in humans.

SAFETY DATA

Preclinical Safety Data: Acute toxicity was tested in mice. Intraperitoneal LD₅₀ values were determined using the Dietrich (1983) method. The aqueous stem bark of *H. madagascariensis* was found to have a LD₅₀ (mg/kg) of 846.3 + 6.5 (Akah, 1994). Previous studies carried out by Nwodo (1989) showed no obvious toxicity effects in mice up to a dose of 300 mg/kg.

Isosaline leaf and stem-bark extracts of *H. madagascariensis* administered intraperitoneally into healthy Sprague-Dawley rats resulted in a significant elevation of liver, kidney, and serum alanine and aspartate aminotransferase activities. Serum urea, total proteins, total triglyceride, cholesterol, pyruvate, and lactate were also significantly elevated in the treated rats. These extracts alter metabolic activities in both the liver and kidney of treated rats and should therefore be used with caution in herbal preparations (Olagunju et al., 2004).

Single Dose Toxicity cf. appendix 1

KEY (PROPOSED) USAGE

Therapeutic Indications: Treatment of dyspepsia and mild exocrine pancreatic insufficiency, antioxidant.

Dosage, Method, and Duration of Administration: Dried alcoholic extracts can be taken three times daily, with a recommended daily dose of 7.5 - 15

mg. This corresponds to a daily dose of 25-50 mg of the crude herb. Commercial preparations made from Haronga are also available.

Special Warnings and Precautions for Use: Do not use in cases of acute pancreatitis and acute attacks of chronic recurring pancreatitis, severe liver function disorders, gallstones, obstruction of bile ducts, gallbladder empyema, ileus (Blumenthal Ed., 1998).

Pregnancy and Lactation: Not to be used during pregnancy or while nursing.

Evaluation of Efficacy: Efficacy pharmacologically proven.

TRADE INFORMATION

Nature of plant material: Conservation status: Not threatened.

Processing and storage: The leaves, stems, and resin of the plants are used (*Harunganae madagascariensis* cortex et folium, synon. Folium et cortex *Harongae*).

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Hibiscus sabdariffa

GENERAL DESCRIPTION

Scientific Name with Author: *Hibiscus sabdariffa* L.

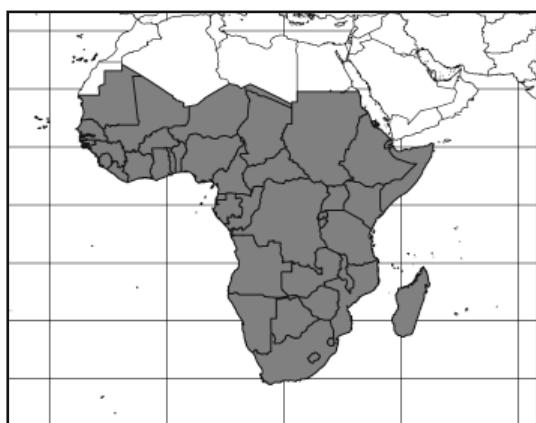
Family: Malvaceae

Vernacular Names: Bissap, Florida cranberry, hibiscus, Indian sorrel, Jamaica sorrel, karkade, oseille de Guinee, red-sorrel, roselle, sour sour, thé rose d'abyssinie.

Botanical Description: This shrub is an erect annual plant that can reach a height of 4 m. The leaves are lobed and the flowers are yellow in colour. The calyx and epicalyx are fleshy, bright red and persistent. They are collected at the fruiting stage. They are edible and have a sweet-sour taste and commonly referred to as the 'Hibiscus flowers'. The plant takes about 6 months to mature. The fleshy calyx is situated at the base of the flowers and is 1.5-2 cm wide, enlarging to 3-3.5 cm, fleshy, and bright red as the fruit matures.

Origin and Distribution: Originating from Africa, most probably Angola, this plant is now widely cultivated in the tropics for its edible calyx and epicalyx. The main producers across the globe are countries from North and West Africa (especially Sudan, Senegal, and Mali), Mexico, India, Thailand, and China. Different varieties produce different coloured calyces (deep red, red, or white).

Plant Part Used: Calyx and epicalyx, leaves and seeds.



ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses: The leaves are converted in a poultice and applied on the furuncles so as to accelerate the healing process (Raponda-Walker & Sillans, 1961).

The dried calyx is reported to have aphrodisiac properties (Adjanihoun et al., 1988). The leaf infusion is used as a diuretic, diaphoretic, and cholagogic. It is also hypotensive and helps to decrease blood viscosity, stimulate peristalsis, relieves cough, and biliousness. It is also given as a tonic in illness and during childbirth (Burkhill, 1997). The diuretic effect has also been reported in other parts of the world, namely in the Caribbean (Lans, 2006). The leaves are used externally on wounds, boils, and abscesses so as to accelerate the healing process. The leaf decoction is also used as a mouthwash against coughs and toothache. In some countries in Africa (Congo), the leaf sap is applied externally on the eyes. It is also drunk by women in confinement so as to hasten childbirth. The calyx also has laxative properties, and is reported to have hypotensive properties.

Fermented *Hibiscus* seeds (bi-kalga) are used as a source of protein in sauces and condiments in Burkina Faso. Protein values reportedly range from 25 - 30% (Bengaly, 2005).

Other Relevant Uses: Leaves are used as a nutritious vegetable, calyx as a herbal tea or infusion as a beverage, and a jam. Stems and leaves used as fodder and fibre.

In Jamaica and other Caribbean islands Hibiscus (Red Sorrel) is made into jams, jellies, and beverage products. In Europe the extract is used as a natural colouring. The seed oil has been reported to contain more than 25 volatile compounds and out of which many are unsaturated hydrocarbons and alcohols predominantly from C8 to C13. Among the fatty acids found: linolic acid (43.2%), oleic acid (24.7%), palmitic acid (17.3%), sterculic acid (2.1%), and linoleic acid (1.2%) (Jirovetz et al., 1992).

The stem yields a textile fibre known as Rosella Hemp and is grown as a crop for this purpose.

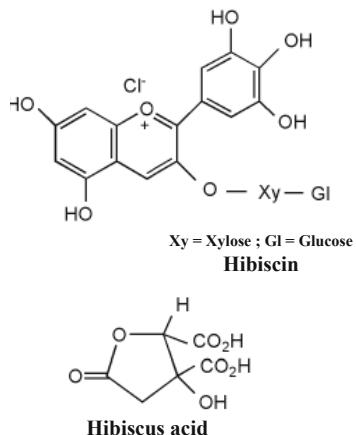
CHEMICAL CONSTITUENTS

Compounds: The water extracts of the plant contain up to 15% of mucilage polysaccharides and 2% pectin. The typical organic acids in the range of 15 - 30% contain hibiscus acid together with ascorbic, citric, malic, and tartaric acids. Among the polysaccharides present are the types arabinan and arabinogalactan; they occur with galacturonic acid, rhamnose, galactose, and arabinose.

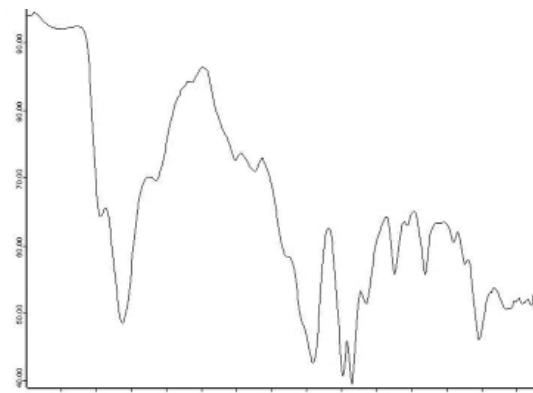
The seeds are also reported to be rich in proteins (25.2%). Among the most abundant amino acids found are leucine, lysine, and phenylalanine, which are also some of the essential amino-acids found (Al-Wandawi et al., 1984; El-Adawy et al., 1994).

The dried flowers contain hibiscic (hibiscus) acid ($C_6H_6O_7$), 13% citric and malic acids, the anthocyanins gossypetin (hydroxyflavone), hibiscin, and 40 - 50 mg/100gm of ascorbic acid. The petals are rich in the flavonol glucoside hibiscitin, which yields the crystalline aglycone hibiscetin ($C_{15}H_{10}O_9$). The latter has been synthesised and is a hepta-hydroxyflavone. The waxy substance isolated from the flower is a phytosterolin (Watt & Breyer-Brandwijk 1962).

The calyx has been found to be rich in delphinidin and cyanidine derivatives (delphinidin glucoxyloside, also known as Hibiscin and cyanidin-3-monoglucoside). Hibiscitin, discovered later to be 3-monoglucoside of hibiscetin, has also been isolated and identified. The flavonoids hibiscetin and their related glycosides have been identified in the plant. Gossypol, initially thought to be present in this plant has not been identified positively from the seeds or the aerial parts (Jaroszewski et al., 1992).



NIR Spectroscopy



QUALITY CONTROL

Identification: Raw material: A purple-red colour. Sold as dried whole or partially broken calyx or siftings.

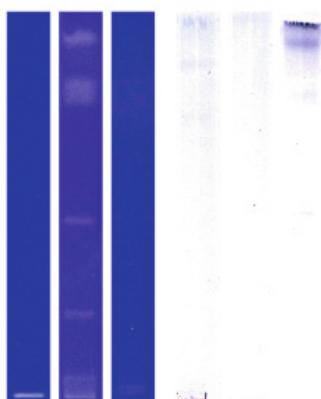
Extract: Clear, deep red solution with some purple hues. Blue hues are undesirable.

Organoleptic Properties: Raw material: Floral-like berry aroma, free from objectionable off odours.

Extract: Slight berry aroma with balanced tart and astringent flavour. Not excessively tart, acidic, or bitter. Free of off flavours.

Moisture Content: < 12%

TLC / HPLC / GC



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

Adulterants and Adulterations: Fibre may be confused with Kenaf (another *Hibiscus* species).

Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

The plant is rich in anthocyanins and these compounds have been shown to be very effective in providing protection against induced paracetamol hepatotoxicity (Ali et al., 2003). The plant has strong *in vitro* and *in vivo* antioxidant activity (Suboh et al., 2004).

In vitro testing of the oil extracted from the plant seeds has shown that it had some inhibitory properties on some bacteria and fungi (Ali et al., 2005).

While the consumption of *H. sabdariffa* was reported to protect against certain degenerative diseases, recent experiments carried out, *in vivo*, in mice have shown that the crude aqueous extracts of *H. sabdariffa* could be used as a dietary supplement to prevent clastogenic effects of arsenic exposure through drinking water (Adetutu et al., 2004).

In rats and rabbits, the extract showed strong anti-hypercholesterolaemic, antinociceptive, and antipyretic activity but no anti-inflammatory activity was detected (Chen et al., 2003). It has also been reported that a concentration of 1mg/ml of the dried calyx extracts of Roselle showed antioxidant activity in the inhibition of TBARs formation in a liposome model system induced by FeCl₃ and ascorbic acid (Hirunpanachi et al., 2005).

The extracts of the plant (40%) along with added nifedipin (51%) potentiated the diarrhoea inducing effect of Castor Oil ($IC_{50} = 350 \mu\text{m}$). It has been postulated that the effects are possibly generated by constituents such as quercetin and eugenol via a Ca^{2+} mode of action (Salah et al., 2002).

Clinical Studies: Patients treated with an aqueous extract of the calyx (10 g in 500 ml of water with the equivalent of 9.6 mg of the anthocyanin content) showed signs of reduced hypertension. The results were not very different to those treated with the positive control (Captopril). No other adverse effects were observed when the patients were subjected to the extract (Ali et al., 2005). Pharmacological studies have demonstrated that this effect was probably due to the diuretic activity and inhibition of the angiotensin-converting enzyme (ACE). Clinical trials along with positive controls with drugs like Lisinopril have confirmed the antihypertensive effects (Herrera-Arellano et al., 2007) The consumption of this plant by healthy men has resulted in the significant decrease of urinary creatinine, uric acid, citrate, tartrate, calcium, sodium, potassium, and phosphate.

SAFETY DATA

Preclinical Safety Data

Single Dose Toxicity cf. appendix 1

KEY (PROPOSED) USAGE

Therapeutic Indications: Antibacterial, diuretic, hypotensive.

Interactions: The consumption of the plant extract by human volunteers did not have any significant interaction with drugs (Ali et al., 2005). However, a recent study has shown impacts on the reduction of Diclofenac (a non-steroidal anti-inflammatory agent) excreted upon co-administration. Such interactions between drugs with beverages need to be documented as it may result in supra or sub therapeutic concentrations of certain drugs in clinical situations (Fakeye et al., 2007).

Pregnancy and Lactation: The product should not be used during pregnancy or while breast-feeding.

Evaluation of Efficacy: Antibacterial efficacy pharmacologically proven (Rabe & Van Staden 1997, 2000). Anticandida efficacy traditionally proven. Anti-ulcer: insufficient information for classification. General tonic: insufficient information for classification.

TRADE INFORMATION

Nature of plant material: Flowering/harvesting time: In the Southern Hemisphere: May - July. In Northern Hemisphere: June - October.

The plant is essentially cultivated at farm level. Roselle can be propagated from seeds or cuttings. The recommended spacing for seed propagation is 1m x 1m for the production of fleshy calyces. Closer spacing (30 - 46cm) is advocated for production of fibre or pulp. In some parts of Nigeria, the cultivation is under irrigation. The crop is often grown in association with cotton enjoying similar ecological conditions.

Conservation status: Not in danger.

Nature of plant products: A spray dried powder, a solid, and liquid extracts are sold on the international market.

Export of calyx is in lined boxes or multi-walled sacks.

Processing and storage: The calyx, as well as the leaves, are sold fresh or dried in local markets.

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Hoodia gordonii

GENERAL DESCRIPTION

Scientific Name with Author: *Hoodia gordonii* (Masson) Sweet ex Decne.

Synonyms: *Hoodia bainii* Thistleton-Dyer, *Stapelia gordonii* Masson

Family: Asclepiadaceae

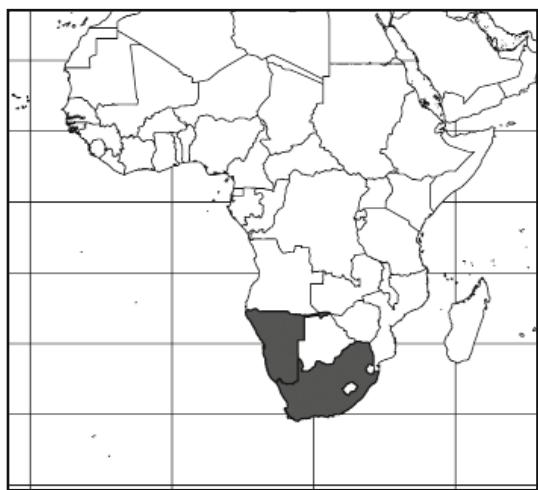
Vernacular Names: Bitterghaap, ghaap, hoodia.

Botanical Description: *Hoodia gordonii* is an erect, branched-stem succulent with greyish-green stems covered in short, single, bulbous-based spines. The flowers are large (50 - 100 mm in diameter) and flesh-coloured (Bruyns, 1993; Bruyns, 2005). *Hoodia gordonii* may be confused (in Namibia and Botswana) with *H. currorii* (especially when not in flower), but the former has erect, parallel stems forming a neat plant, whereas the latter has spreading stems that result in an untidy-looking plant.

Plant Parts Used: Sliced and dried stems



Origin and Distribution: South Africa: mainly the dry western parts, as far east as Kimberley and as far south as Oudtshoorn; Namibia: from the Brandberg (21° S) southwards (Bruyns, 2005). *Hoodia gordonii* does not occur in Botswana.

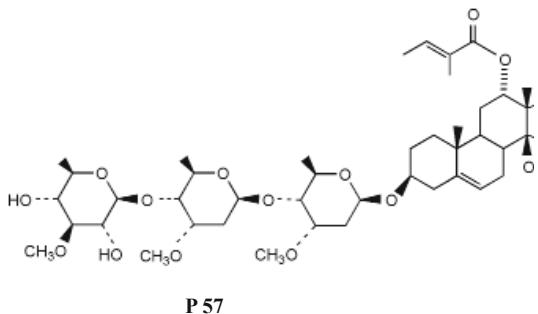


ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses: *Hoodia* is traditionally used as a functional food to suppress hunger and thirst (Van Wyk & Wink, 2004). Small pieces of the extremely bitter stem are chewed over long periods and have been recorded to have a marked appetite (food aversion?) effect (Marloth, 1932). Several species of *Hoodia* and related genera are used as wild food plants (Fox & Norwood Young, 1982). A number of other traditional medicinal uses have been recorded for *Hoodia* species, e.g. the treatment of haemorrhoids with brandy tinctures (Pappe 1857, 1862), use as a stomachic and for the treatment of tuberculosis (Watt & Breyer-Brandwijk, 1962; Bruyns, 1993), treatment of cancer with honey made from the flowers (Rood, 1994), diabetes (Von Koenen, 1996), indigestion, hypertension, diabetes, and stomach ache (Van Wyk & Gericke, 2000). *Hoodia pilifera* is known to contain P57 (Van Heerden et al., 2007) and this species has been used as appetite and thirst suppressant, to treat pulmonary tuberculosis, and as a brandy tincture (Pappe, 1857). It was once used as stomachic and for treating haemorrhoids. *Hoodia gordonii* has been used as appetite suppressant (and paradoxically also as an appetite stimulant) and to treat stomach pain (Van Wyk & Gericke, 2000).

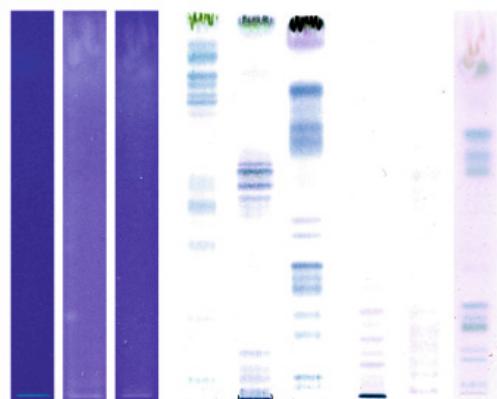
CHEMICAL CONSTITUENTS

Compounds: *Hoodia* species have numerous pregnane glycosides containing 6-deoxy- and 2,6-dideoxy sugars. *Hoodia gordonii* contains more than 20 different glycosides based on 12-hydroxypregnane (Abrahamse et al., 2007; Dall'Acqua & Innocenti, 2007; Pawar et al., 2007a, 2007b; Van Heerden et al., 2007). The appetite-suppressant compound in *H. gordonii* is an oxypregnane steroidal glycoside known as P57 [triglycoside of 12 β -trigloyloxy-14 β -hydroxypregn-5-en-20-one (commonly known as P57A53, or just P57)] (Van Heerden et al., 1998, 2007). The major steroid glycoside in *H. gordonii* is known as hoodigoside L (Pawar et al., 2007b). This compound does not have a 12-ester substituent.



QUALITY CONTROL

TLC / HPLC / GC

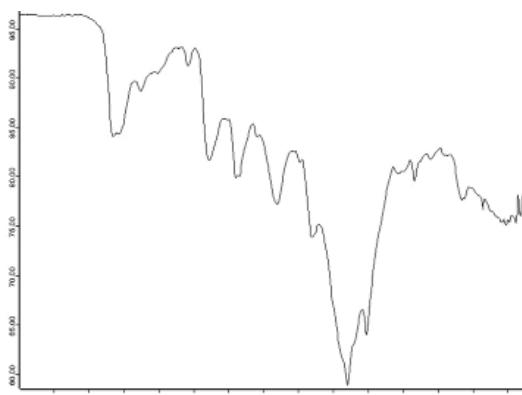


Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

Specialized TLC or HPLC if available. Standard TLC methods can be used, but the results are similar for different species of *Hoodia* (it may be difficult or impossible to detect species differences using TLC alone (Hulley, 2007). Adulteration with weedy species of Cactaceae can be detected by TLC (Hulley, 2007). The best method for quality control is to measure the presence and level of P57 by HPLC (Avula et al., 2006; Janssen et al., 2008).

Markers and Quantitative Methods: The active compound is a triglycoside of 12 β -trigloyloxy-14 β -hydroxypregn-5-en-20-one (commonly known as P57A53, or just P57). The level of P57 in two samples of *Hoodia gordonii* was determined to be 0.0051% or 0.0052% (sample 1) and 0.047% or 0.048% (sample 2) (Avula et al., 2006; Janssen et al., 2008).

NIR Spectroscopy



Adulterants and Adulterations: *Hoodia* species are very similar and it is extremely difficult to identify the source of raw material if its exact geographical origin is not known and if high-quality information (herbarium voucher specimens or good photographs of the plant, and especially of the flowers) are not available. Different *Hoodia* species (and especially *H. currori*) have been used as sources of raw material. Various species of Cactaceae have been used, including *Opuntia ficus-indica*, *Cereus jamacaru*, and *Echinopsis spachiana* (Henderson, 1995). Adulteration with *Aloe ferox* leaves has also been reported. Easy methods for the detection of adulterants (also in powdered material) have been developed by Hulley (2007), based on anatomical differences between Cactaceae (Metcalfe, 1983) and Apocynaceae. Fragments of epidermis can be studied to determine the type of stomatal complex (hexacytic in *Hoodia*, tetracytic in Cactaceae), the presence of hair-like spines (absent in *Hoodia*), and druse crystal (typical of *Opuntia*, absent in *Hoodia*).

Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

Feeding experiments in rats, using both crude extracts and the pure pregnane glycoside P57, have shown reductions in food consumption and body mass (Van Heerden et al., 2007). The anorectic effect is probably due to a central nervous system mechanism of action, as MacLean and Luo (2004) showed a 50 - 60% reduction in food intake plus an increase in ATP in the hypothalamus when P57 was injected directly into the brain of rats.

Anti-diabetic effects (Rubin et al., 2002) and the treatment of gastric acid secretion damage (Hakkinen et al., 2002) have been reported in patent applications.

Clinical Studies: None. A human study reached clinical phase II but was then discontinued for unknown reasons. It was speculated that there was a lack of efficacy owing to dosage problems. In view of the traditional use of *Hoodia* as a ‘kougoed’ – chewed for prolonged periods – it is possible that buccal absorption plays a major role and that oral dosage in tablet form is ineffective.

SAFETY DATA

Ethnic Use Safety Data: *Hoodia* species have a long tradition of use as wild food plants with no indications of toxicity.

Preclinical Safety Data

Single Dose Toxicity cf. appendix 1

Clinical Safety Data: A phase I clinical study by the company Pfizer has shown no signs of toxicity, but published details are not available.

KEY (PROPOSED) USAGE

Therapeutic Indications: Appetite suppressant.

Special Warnings and Precautions for Use: *Hoodia gordonii* can be safely consumed when used appropriately.

Pregnancy and Lactation: Owing to lack of data, the product is contraindicated during pregnancy and lactation.

Evaluation of Efficacy: Appetite suppressant efficacy pharmacologically proven (Van Heerden et al., 1998, 2007; MacLean & Luo, 2004). Antidiabetic efficacy pharmacologically proven (Rubin et al., 2002). Treatment of gastric acid secretion damage efficacy pharmacologically proven (Hakkinen et al., 2002). Abdominal cramps efficacy traditionally proven. Haemorrhoids efficacy traditionally proven. Hypertension efficacy traditionally proven. Gastric ulcer efficacy traditionally proven. Thirst suppressant efficacy traditionally proven.

Tuberculosis efficacy traditionally proven. Bitter tonic (amarum) efficacy traditionally proven.

TRADE INFORMATION

Nature of plant material: Flowering/harvesting time: Plants are harvested at any time of the year, but mainly in the growing season (spring in the western parts, summer in the central parts).

Cultivated/wild crafted: The plant has mainly been wild-harvested and there are serious conservation concerns (Oliver, 2006). Nature conservation authorities issue permits for wildcrafting under certain controlled circumstances. Methods for large-scale cultivation are being developed, and substantial quantities are said to originate from cultivated sources.

Conservation status: CITES Appendix II. Although *H. gordonii* is widely distributed and not considered endangered, several other *Hoodia* species are listed as Rare or Vulnerable in Red data lists (Golding, 2002). As harvesters are unlikely to distinguish between species (especially when plants are not in flower) the wild-harvesting of *Hoodia gordonii* is therefore considered a serious threat to other species.

Processing and storage: Stems are simply cut and dried. The product is likely to be highly stable when dried but relatively unstable in solution. No reliable data are available.

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Hypoxis hemerocallidea

GENERAL DESCRIPTION

Scientific Name with Author: *Hypoxis hemerocallidea* Fisch.Mey. & Avé-Lall.

Synonyms: *Hypoxis rooperi* L.

Family: Hypoxidaceae

Vernacular Names: African potato, hypoxis, hypoxis tuber, inkomfe, *Radix Hypoxidis*, star flower, *Tubera Hypoxidis*.

Botanical Description: The plant is a stemless, geophytic, perennial herb with large black corms (fibrous tubers) which are dark brown to black on the outside and bright yellow inside when freshly cut. The leaves are long, broad, strap-shaped, strongly keeled, slightly hairy, and typically arranged one above the other to form three distinct groups spreading outwards from the centre of the plant. The flowers are borne in simple racemes. They are bright yellow and star-shaped, with six broad perianth lobes and six anthers on short filaments. The species is sometimes confused with others, but the three-ranked leaves are distinct (Van Wyk et al., 1997).

Origin and Distribution: Southern Africa (widely distributed in the grassland region of South Africa, Swaziland, Mozambique and Zimbabwe).



Plant Parts Used: Fresh or dried mature corms (tubers) are used (extracts less often). Note: Some

commercial products with extracts directly or indirectly linked to *Hypoxis* actually contain pure sitosterols and sitosterol glycosides obtained from industrial sources (Schultz et al., 2001).



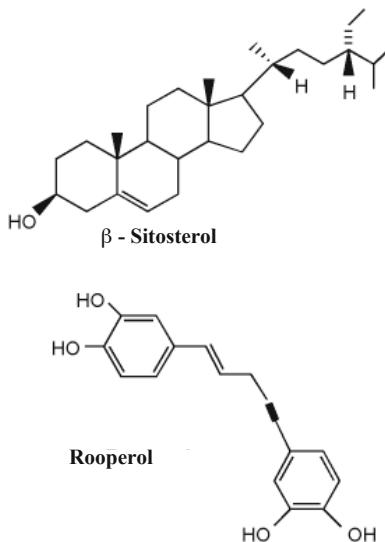
ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses: *Hypoxis* corms or extracts are used to a limited extent as general tonics in African traditional medicine (e.g., decoctions have been given to weak children) and the juice is applied to burns (Watt & Breyer-Brandwijk, 1962; Pujol, 1990, Hutchings et al., 1996; Van Wyk et al., 1997; Neuwinger, 2000; Van Wyk & Gericke, 2000). The stems and leaves are mixed with other ingredients to treat prostate problems and urinary infections (Pujol, 1990).

The main use of *Hypoxis* in modern times has been against urinary ailments (especially the treatment of benign prostate hypertrophy) (Schultz et al., 2001) and chronic polyarthritis (Burger & Wachter, 1998). It has also become well known as a panacea and general tonic.

CHEMICAL CONSTITUENTS

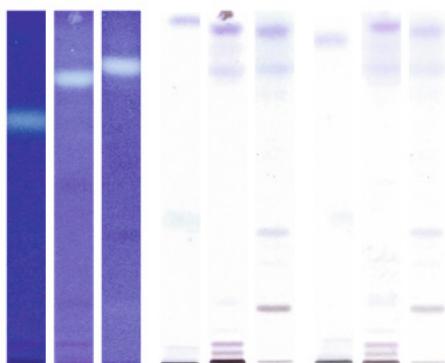
Compounds: The main compounds in the corms are phytosterols (mainly β -sitosterol and its glycoside, β -sitosterolin). The main phenolic compound is a norlignane 4,4'-digluicoside known as hypoxoside – its aglycone is known as rooperol (Marini Bettolo et al., 1982; Drewes et al., 1984; Drewes & Liebenberg, 1987a, 1987b).



QUALITY CONTROL

Identification: Dark-brown corm, approximately 6 cm in diameter, with a fringe of secondary roots at the base.

TLC / HPLC / GC

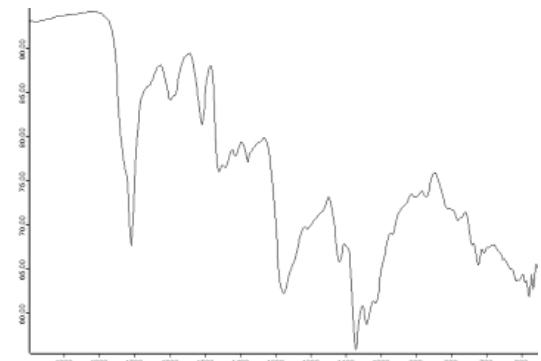


Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

β -Sitosterol, sitosterol glycosides, and hypoxoside are clearly visible on TLC. Corm extracts are easily analyzed by HPLC using standard methods.

Markers and Quantitative Methods: Phytosterol glycosides are present at a level of about 9 mg/100 g fresh tuber.

NIR Spectroscopy



Adulterants and Adulterations: *H. hemerocallidea* can easily be adulterated with other species of *Hypoxis*, such as *H. obtusa* (several have a similar chemical profile, with hypoxoside as main compound). One species, known as *ilabathetka* (*H. colchicifolia* = *H. oligothrica*) is known to be poisonous.

Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

Phytosterols and phytosterol glycosides resemble cholesterol and are of some use in the treatment of hypercholesterolemia (Schultz et al., 2001). They are also claimed to have beneficial effects in treating prostate hyperplasia (Pegel, 1984; Wagner & Wiesnauer, 1995). The activity of sitosterols is ascribed to enzymatic effects (inhibition of 5 α -reductase and aromatase) or to reduced binding of dihydrotestosterone within the prostate (Bruneton, 1999). It is speculated that an inhibition of prostaglandins may be responsible for the benefits seen in the treatment of chronic polyarthritis (Van Wyk & Wink, 2004).

Hypoxis or its sitosterols is considered to have immune-modulating properties (Bouic et al., 1996) and to be potentially useful as an adjuvant for the treatment of tuberculosis (Donald et al., 1997).

The main phenolic compound, hypoxoside, appears to be inactive as a prodrug but is broken down into a highly active aglycone, rooperol. Rooperol has been shown to have several biological activities. It is markedly antimutagenic, anti-inflammatory and cytotoxic to cancer cells (Theron et al., 1994; Albrecht, 1996). It is a potent inhibitor of lipoxygenase of leukotrienes (Albrecht, 1996). Hypoglycaemic effects have also been reported (Mahomed & Ojewole, 2003).

There is a difference in biological activity between corm and leaf extracts and it is not recommended that leaves be replaced by corms (Katerere & Eloff, 2008).

It is claimed to stabilize CD4 lymphocyte counts in patients with HIV, and may be useful in treating inflammatory conditions (Albrecht, 1996; Ojewole, 2002) and pain (Di Giannuario et al., 1993). See also Nicoletti et al. (1992).

Clinical Studies: Several clinical trials have been carried out on the effects of plant-derived sitosterols for ailments of the bladder and prostate (e.g., Ebbinghaus & Baur, 1977; Schultz, 2001). Sceptics point out that the beneficial effects seen in clinical trials are curious, as 150 - 300 mg of sitosterol are ingested daily in a normal diet (two to ten times more than the amounts recommended in commercial preparations).

A phase 2 clinical trial on anticancer effects showed little or no benefit (Albrecht, 1996).

SAFETY DATA

Preclinical Safety Data

Single Dose Toxicity cf. appendix 1

Clinical Safety Data: In a human clinical trial, oral ingestion of hypoxoside had no adverse effects (Smit et al., 1995; Coetzee et al., 1996).

KEY (PROPOSED) USAGE

Therapeutic Indications: Urinary ailments (especially benign prostate hypertrophy) and chronic polyarthritis.

Dosage, Method, and Duration of Administration:

Commercial and traditional formulations contain a daily dose of about 2 - 4 g of dry corm (or equivalent in extracts or decoctions). The effective oral dose of sterolin in treating prostate hypertrophy was reported to vary from 0.015 to 0.027 mg/day (Pegel & Liebenberg, 1972, 1973).

Special Warnings and Precautions for Use:

Safely consumed when used appropriately.

Tuber extracts are said to be purgative (Watt & Breyer-Brandwijk, 1962). In the absence of safety studies on the whole tuber, some caution is necessary.

Pregnancy and Lactation: Not to be used during pregnancy. Not to be used while nursing.

Evaluation of Efficacy: Prostate hyperplasia: efficacy pharmacologically proven (Pegel & Liebenberg, 1972; Ebbinghaus & Baur, 1977). Chronic polyarthritis: efficacy traditionally proven (Burger & Wachter, 1998; Van Wyk & Wink, 2004). Immune stimulant (tonic): efficacy traditionally proven (Bouic et al., 1996; Donald et al., 1997). HIV: efficacy traditionally proven (Albrecht, 1996)

TRADE INFORMATION

Nature of plant material: Flowering/harvesting time: Summer (September to May), when the plants are actively growing. They are dormant in winter.

Cultivated/wildcrafted: All material is wild harvested. In the interest of conservation, a cultivation protocol should be developed as a matter of urgency. Some crop development is currently under way.

Conservation status: Not threatened.

Processing and storage: Preparation/processing: The tubers are used fresh, or are peeled, sliced, and dried.

Stability of product: Unknown. Extracts are known to turn black when left in solution.

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Ipomoea pes-caprae ssp. brasiliensis

GENERAL DESCRIPTION

Scientific Name with Author: *Ipomoea pes-caprae* ssp. *brasiliensis* (L.) Ooststr.

Synonyms: *Ipomoea pes-caprae* Brown, *I. biloba* Forsk., *I. maritima* Roxb., *Convolvulus bilobatus* Roxb., *C. brasiliensis* Linne, *C. maritimus* Lamarck.

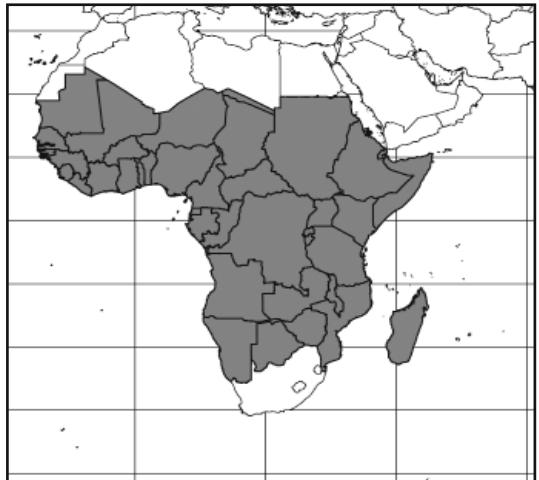
Family: Convolvulaceae

Vernacular Names: Batatran, goat's foot convolvulus, ipomée pied de chèvre, lalandia, liane batatran, liseron pied de chèvre, patate à durand, poumpou, vahindalalana.

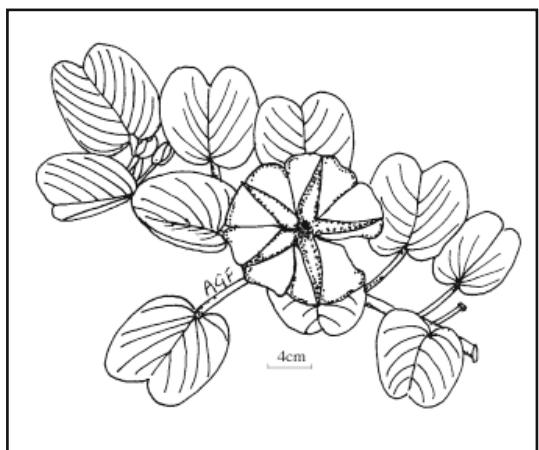
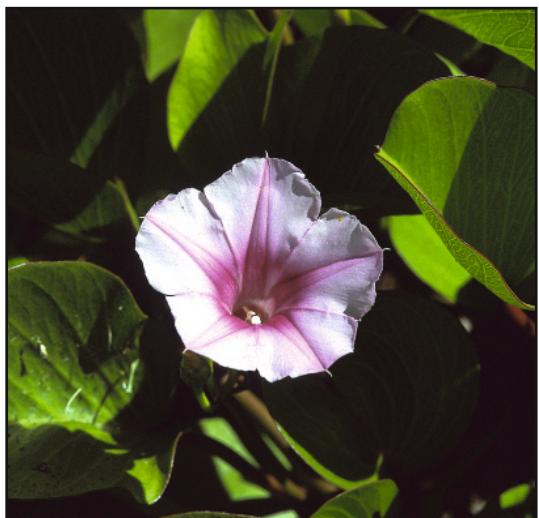
Botanical Description: Perennial plant, with stems trailing to 10-20 m long or more, rooting at the nodes. Leaves with a generally thick limb, margin entire, ovate, obovate, subcircular or subquadrangular, reaching 8 cm in length and 7 cm in width, generally emarginated or bilobed at the summit.

Inflorescence axillary, uni- or pauciflora and cymose; peduncle erect, 3 - 10 cm long; pedicels 1 - 3 cm, long; sepals ovate-oblong, 5 - 10 mm long, the external ones a little smaller. Corolla pinkish-mauve, with a darker centre, funnel-shaped, 3.5 - 5 cm long, limb spread out, 4 cm in diameter and with lobes less marked. Capsule bilocular, subglobose, 12 - 18 mm in diameter, containing four seeds, around 7 mm long, dark brown, pubescent.

Origin and Distribution: The genus comprises some 500 species in tropical and warm temperate habitats.



Plant Parts Used: Entire plant, leaves, roots.



ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses:

Different species contain different active compounds; relatively common, however, are resins derived from roots and seeds with laxative, emetic, and purgative effects. Several species have been reported to be poisonous on account of these activities. In addition, polyphenols and alkaloids are present, as well as saponins and tannins. Hallucinogenic ergolin derivatives have been isolated from the seeds. For *I. pes-caprae* ssp. *brasiliensis*, antihistamine action and jellyfish poisoning antagonism have been reported (Neuwinger, 1998).

A plant decoction is used to soak the feet in the event of swelling, rheumatism (Kokwaro, 1993), and cramps. In order to chase away evil spirits, the entire body is washed with the plant decoction and the remaining water is thrown into the sea. The root decoction is used against colic and fever. A poultice made of the crushed, warmed-up leaves, mixed with *Ricinus* oil is applied to furuncles, contusions, and parts of the body suffering from pain. Young and tender leaves are often crushed and applied to parts of the body that have been injured by sea urchins, facilitating the removal of the spine (Watt & Breyer-Brandwijk, 1962; Lavergne & Vera, 1989).

Leaf poultices are used to treat inflammation of the legs, and a hip bath is useful for a prolapsed anus. The leaf poultice is also used for 'panaris', colic, and rheumatic pain (Bouton, 1864; Daruty, 1886; Gurib-Fakim et al., 1993, 1994, 1995). In the Comoros, the warmed-up leaves are used as a haemostatic and analgesic, and to help the healing process. A hip bath is used to alleviate muscular pains (Adjano'houn et al., 1982, 1983). In Rodrigues, skin infections, eczemas, and dermatitis are bathed with a decoction made from a few pieces of *I. pes-caprae* and leaves of *Azadirachta indica*, *Jatropha curcas*, and *Vangueria madagascariensis*. Stings from the 'Laffe laboue' (venomous lagoon fish) are bathed with the juice extracted from the crushed plant (Gurib-Fakim et al., 1994, 1995).

Oliver-Bever (1986) reported that in India the root was used in the treatment of rheumatism, dropsy and colics. The juice is given in the event

of an oedema, and the bruised leaves are applied to swollen parts of the body. In most countries of the Pacific the leaves are used in preparations against rheumatism and also to extract the spines of sea urchins. In Asia, the plant poultice is applied to boils and other skin surface ailments (Burkhill, 1985).

Other Relevant Uses: In parts of Africa, e.g. Gabon, the long stoloniferous stem is converted into ropes which are used for hauling in fishing nets.

The plant is a pioneer colonizer of sand dunes and is a sand binder par excellence.

CHEMICAL CONSTITUENTS

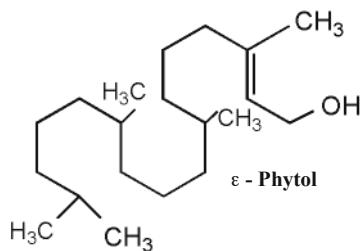
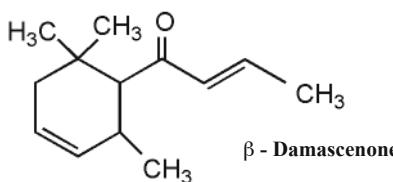
Compounds: The entire plant contains 7 - 8% resins, 0.05% of an essential oil, triacontane, pentatriacontane, sterol, and acids: behenic, butyric, melissic, and myristic. The presence of β -damascenone, triacontane, pentatriacontane, and a sterol ($C_{29}H_{49}OH$) has also been reported. Present in the roots are glycorrhettins, similar to the resins found in other *Ipomoea* spp. (Oliver-Bever, 1986).

For the plant growing in Reunion Island, Lavergne reported the following composition for the entire plant: steroids, terpenes, tannins. The seeds of the plant growing in the Seychelles are reported to contain alkaloids, flavonoids, sterols, and traces of flavones and anthocyanins. The leaves contain phenols and sterols, and the stems and leaves contain coumarins (Gurib-Fakim et al., 1999).

The seeds contain the alkaloids ergoline and clavine; the leaves are rich in malic, citric, maleic, tartaric, succinic and fumaric acids (Rastogi et al., 1991). The fatty acid glycosides present in the plant are pescaprosides A, B, and E. Pescaproside E is the major compound and has been characterised as a pentaglycoside of 11-hydroxyhexa-decanol acid (Gurib-Fakim & Brendler, 2004).

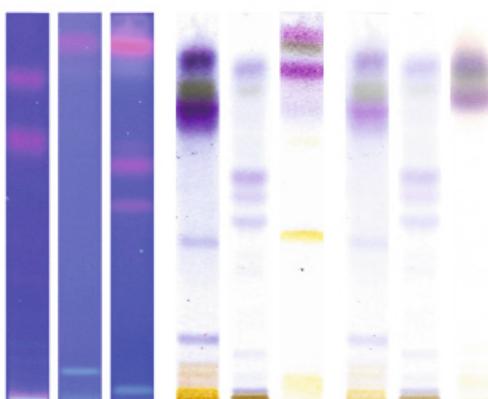
In Mexico, the plant-based herbal medicine is known under the registered name of 'Rinonina' and is sold in pharmacies. Further chemical studies on 'Rinonina' led to the isolation of the following compounds from the hexane extracts: pescaprosides A and B, pescapraeins (I-IX),

and stoloniferin III. Resin glycosides have also been isolated from the lipophilic hexane extracts (Escobedo-Martinez & Pereda-Miranda, 2007). The active compounds are the glycosidic esters (pescaprosides), as well as other molecules, such as β -damascenone, ϵ -phytol (for antispasmodic activity), β -glochidone, betulinic acid, α - and β -amyrin acetate, isoquercitrin, etc.



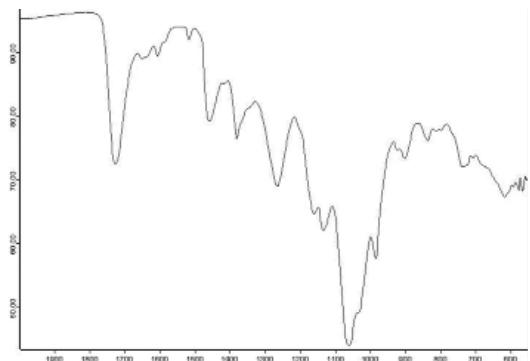
QUALITY CONTROL

TLC / HPLC / GC



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

NIR Spectroscopy



Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

It has been reported that the plant manifests antihistaminic (Oliver-Bever, 1986) and anti-inflammatory properties in vitro (Pongapyroon et al., 1991). β -Damascenone and ϵ -phytol, present in the plant, show antispasmodic properties equivalent to papaverine (Pongapyroon et al., 1992).

The whole plant extract is reported to neutralise certain toxins in poisonous fish. The effectiveness of the plant extract in the treatment of dermatitis, such as that caused by toxic jellyfish, is possibly due to the presence of β -damascenone and ϵ -phytol, which interfere with contraction of vascular smooth muscle. This would to some extent justify the use of this plant in traditional medicine against toxic fish (Pongapyroon et al., 1989, 1991).

Compounds such as glochidone, betulinic acid, α - and β -amyrin acetate and isoquercetrin have been shown to manifest antinociceptive properties in the writhing ($ID_{50} = 33.8 \text{ mg/kg/ip}$) and formalin tests ($ID_{50} = 37.7$ and 12.5 mg/kg ip) for the first and second phases in mice. The data again would to some extent justify the use of these plants in the treatment of pain (Krogh et al., 1999; de Souza et al., 2000).

Other tests carried out on *I. pes-caprae* extracts significantly inhibited ADP-induced platelet aggregation. These findings may justify the traditional use of this plant in the treatment of migraine in some countries (Rogers et al., 2000).

The leaves are reported to be inactive against *Staphylococcus aureus* but have been reported to have beneficial effects against bedsores. Extracts of the stem have been reported to have strong anti-tumour actions, and in the Philippines the leaves are said to extirpate fungoid growth of ulcers.

The quinic acid esters isolated from the plant have tested positively for their collagenase inhibitory activity. The compounds showed almost no cytotoxicity (Teramachi et al., 2005).

Clinical Studies: In Mexico, over-the-counter drugs known as 'Riñonina' are sold; the latter is a herbal preparation of *Ipomoea pes-caprae*.

SAFETY DATA

Preclinical Safety Data

Single Dose Toxicity cf. appendix 1

KEY (PROPOSED) USAGE

Therapeutic Indications: Antihistaminic, antibacterial, anti-inflammatory.

Dosage, Method, and Duration of Administration: Formulation and dosage: Poorly known and unrecorded. Nonetheless, some standardised plant-based herbal medicines involving this plant has been reported in Mexico.

Special Warnings and Precautions for Use: Unknown. Tablets and bark extracts have been used for many years with no reported side effects.

Ipomoea pes-caprae leaf: Can be safely consumed for appropriate usages. To be used only under the supervision of an expert qualified in the appropriate use of this substance.

Pregnancy and Lactation: The product should not be used during pregnancy or while breast-feeding.

Evaluation of Efficacy: Efficacy traditionally proven.

TRADE INFORMATION

Nature of plant material: Conservation status: Not threatened.

Collected from the wild. Grows along the beach. Flowering/harvesting time: Throughout the year.

Processing and storage: The leaves have been air dried and then dried further in the oven to constant weight.

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Kigelia africana

GENERAL DESCRIPTION

Scientific Name with Author: *Kigelia africana* (Lam.) Benth.

Synonyms: *Bignonia africana* Lam., *Crescentia pinnata* Jacq., *Tanaecium pinnatum* (Jacq.) Willd., *Kigelia pinnata* (Jacq.) DC., *Kigelia acutifolia* Engl., *Kigelia aethiopica* Decne

Family: Bignoniaceae

Vernacular Names: Arbre à saucisse, calebassier du Senegal, saucissier, sausage tree, worsboom.

Botanical Description: Tree reaching 15-20 m in height. Leaves opposite or grouped in 3-4, reaching 50 cm in length. Leaflets 7-11, opposite or sub-opposite, elliptic-oval, or oblong, rounded at the summit and at the base, reaching 20 x 6 cm. Inflorescence in panicles, narrow, elongated axis, reaching 2 m in length. Calyx greenish, 2-4 cm long, 5-dentate. Corolla yellow or brown, lined with green on the outside, dark brown to wine red in colour on the inside, 5-10 cm long; basal tube as long as the calyx or sometimes longer. Stamens 4, anthers sub-exsert, 7-9 mm long, staminodes 4-5 mm long. Disc large, annular. The fruit is cylindrical, indehiscent and rounded at the extremities, sausage-shaped, 25-50 cm long and 8-15 cm in diameter.

Origin and Distribution: Originating from the forested areas of subtropical savannas of Africa, this tree has been planted mainly as a curiosity because of its unique fruit. It has been introduced long ago in the Mascarene regions as an ornamental plant. In Africa, *K. africana* is a true multipurpose tree. It is widely cultivated in other tropical regions as an ornamental tree in parks and along roads.



Plant Part Used: The fruit and rarely the bark





ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses: The fruit decoction is administered as a galactagogue, internally, as well as externally, and is also used to treat cancer and oedema of the legs. The fruit is a purgative and toxic, and applied to treat syphilis, rheumatism, and a sexual stimulant. The powdered dried fruit is used as a dressing for ulcers, sores, syphilis and is also applied locally for rheumatism. The bark or fruit decoction is used orally or as enema for treating stomach ailments in children (Van Wyk & Wink, 2004). A decoction with peppers is used to treat constipation and piles, and powdered fruits are applied to ulcers and to treat rheumatism. The fruit sap is used as a wash against skin diseases. Slices of the unripe fruit are fermented in water and then used to wash the genital organs in the event of syphilis and venereal diseases.

The leaves and bark are sometimes applied to treat dysentery, stomach problems and kidney complaints. The slightly bitter bark is used in mixtures with other plants to treat epilepsy, snakebites, rheumatism, asthma, syphilis, and gonorrhoea and externally to treat wounds, sores, and ring worms. In parts of Africa, the ground fresh inner bark or dried pulverized inner bark is sprinkled on chronic wounds and sores. The

bark extract is also drunk against dysentery, rheumatism, and snake bites. A sweetened decoction of the wood and bark is drunk against cough and as an expectorant. An infusion made with the bark or root is applied to the parts of the body affected by tropical ulcers (Neuwinger, 2000).

The bitter root is administered as a remedy for boils, sore throat, constipation and tapeworm (Nasution & Lemmens, 2003). The root decoction is drunk against tapeworm and constipation.

Other Relevant Uses: It is highly esteemed for ritual purposes as well as for medicinal applications, as a shade and ornamental tree and for its wood. In traditional medicine, the fruits are most commonly used and sold on markets and their uses are often interwoven with ritual uses as they are considered a strong fetish. The fruit is also used to prepare beer, to make it stronger.

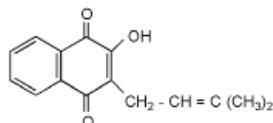
CHEMICAL CONSTITUENTS

Compounds: The root and the bark contain the naphthoquinone lapachol, as well as 2-(1-hydroxyethyl)-naphtho [2,3- β]furan-4,9-quinone, isopinnatal, kigelinol and isokigelinol, and the dihydroisocoumarine kigelin as major compounds. The naphthoquinones: kigelinone, dehydro- α -lapachone, and lapachol, and the phenylpropanoids: p-coumaric acid and ferulic acids have also been identified in the root extracts (Weiss et al., 2000).

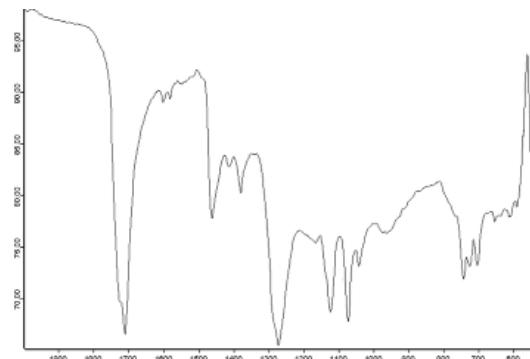
Among the other compounds present in the bark, roots, and fruits are: pinnatal, isopinnatal, stigmasterol, β -sitosterol, γ -sitosterol. The leaves and fruits contain the flavonoids luteolin and quercetin. The fruit extracts also contain kigelinone and caffeic acid (Binutu et al., 1996).

The fruit of *K. pinnata* has recently been reported to contain a new furanone derivative: 3-(2'-hydroxyethyl)-5-(2"-hydroxypropyl)-dihydrofuran-2(3H)-one and four new iridoids: 7-hydroxy viteoid II, 7-hydroxy eucommic acid, 7-hydroxy-10-deoxyeucommiol, and 10-deoxyeucommiol along with known iridoids: jiofuran and jioglitolide, 1-dehydro-3,4-dihydroxyaucubigenin, des-p-hydroxybenzoyl kisasagenol B, ajugol, verminoside, and 6-trans-

caffeoij ajugol (Gouda et al., 2003). Iridoids of the catalpol-type (specioside, verminoside, minecoside) and the breakdown product norviburtinal are also present.



NIR Spectroscopy



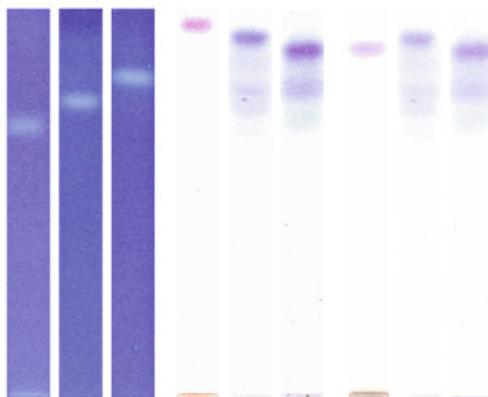
Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

QUALITY CONTROL

Identification: Hard fruit, medium brown in colour, with fibrous interior. Dimensions approximately 30 cm x 10 cm.

TLC / HPLC / GC



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

The naphthoquinoids isolated from the root bark have been assessed, in vitro, against chloroquine-sensitive (T9-96) and resistant (K1) *Plasmodium falciparum* strains and for cytotoxicity using KB cells. 2-(1-hydroxyethyl)naphtha[2,3,b]furan-4,9-dione possessed good activity against both strains [IC_{50} values 627 nM (K1), 718 nM (T9-96)]. Isopinnatal, kigelinol, and isokigelinol exhibited lower activity against both strains (Weiss et al., 2000). Previous testing of the methanolic extracts, evaporated crude water, and un-evaporated crude water extracts have been tested for molluscicidal activity on laboratory-reared *Lymnaea natalensis* (Kela et al., 1989).

The aqueous extracts of the stem bark are known to be rich in iridoids as the major components. In the light of traditional uses of this plant, the anti-microbial activities of the aqueous extracts and of the two major iridoids have been tested against *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans*. The crude aqueous extracts showed significant antimicrobial activity. This could be partially explained by the activity of the iridoids present (Akunyili et al., 1991). The naphthoquinones (kigelinone, isopinnatal, dehydro- α -lapachone, and lapachol) and the phenylpropanoids (p-coumaric acid and ferulic acids) have been identified as the compounds responsible for the observed antibacterial and antifungal activity of the root extracts. Kigelinone and caffeic acid are responsible for the activity from the fruits (Binutu et al., 1996). The activity

could have been enhanced by the presence of flavonoids such as luteolin and quercetin in the leaves and fruits.

The fruit extracts have been tested for molluscicidal activity and the alcoholic extract, evaporated to dryness and suspended in water to give a 100 ppm extract, was found to be able to kill 10% of the snails (Adewunmi & Sofowora, 1980).

The standardised water, ethanol, and dichloromethane extracts of the stem bark and fruits have been tested for their growth inhibitory effects against four melanoma cell lines and renal cell carcinoma line (Caki –2) using two different (MTT and SRB) assays. Lapachol, a possible constituent of these extracts, together with known therapeutic anti-neoplastic agents was also tested in the same way. The IC_{50} value of each extract (after dilution to 100 micrograms/ml) in 1% ethanol or water were noted. Significant inhibitory activity was shown by the dichloromethane extract of the stem bark and lapachol (continuous exposure). The activity was dose-dependent and the extract was found to be less active after 1h exposure. Chemosensitivity of the melanoma cell lines to the stem bark was greater than that seen for the renal adenocarcinoma line. In marked contrast, sensitivity to lapachol was similar amongst the 5 cell lines. Lapachol was not detected in the stem bark extract (Houghton et al., 1994).

Recently, the crude dichloromethane extracts of *K. pinnata* stem bark and fruit has also shown to manifest cytotoxic activity, *in vitro*, against cultured melanoma and other cancer cell lines using the sulphorhodamine B assay. The most active fractions have been found to be those of the stem bark and fruits. The active components in these fractions have been found to be norviburtinal and beta-sitosterol. Norviburtinal was found to be the most active compound but not very selective for melanoma cancer cell lines while isopinnatal also showed some cytotoxic activity. However, further analysis of the crude extract showed that Norviburtinal, present in the plant material, did not account for all the activity of the total extracts (Jackson et al., 2000).

SAFETY DATA

Ethnic Use Safety Data: The fresh fruit cannot be eaten. It is said to be strongly purgative and causes severe blisters in the mouth and on the skin. The green fruits are said to be poisonous (Watt, 1962).

Preclinical Safety Data

Single Dose Toxicity cf. appendix 1

KEY (PROPOSED) USAGE

Therapeutic Indications: Antimicrobial, antineoplastic.

Pregnancy and Lactation: Not to be used during pregnancy. Not to be used while nursing.

Evaluation of Efficacy: Efficacy traditionally proven, plant material with toxic potential.

TRADE INFORMATION

Nature of plant material: Conservation status: Not threatened.

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Mondia whitei

GENERAL DESCRIPTION

Scientific Name with Author: *Mondia whitei* Skeels

Synonyms: *Chlorocodon whitei* Hook. f., *Periploca latifolia* K.Schum, *Taccacea amplifolia* S.Moore, *T. viridis* A.Chev.

Family: Asclepiadaceae

Vernacular Names: Assase hwam, la racine, lufute lwa matwi, mbombongazi, umondi, White's ginger.

Botanical Description: *Mondia whitei* is a large creeper that scrambles over shrubs. It is a vigorous, robust, woody climber up to 6 m high, the twining lianas coming from a large tuberous rootstock. The yellow fleshy root is aromatic, with a pronounced characteristic vanilla-like odour. It tastes like liquorice or ginger but without the pungency. Its heart-shaped leaves are opposite and grow in distinctively frilly stipules, petiolate, ovate with a cordate base; acuminate, leathery, pale green and approximately 12 cm long. The panicles of attractive yellow and reddish-purple or maroon flowers with white star centres are in auxiliary clusters with five acute segments. The fruit is a paired follicle, grayish, 9 x 1.5 cm in dimension. It normally appears in bundles of ten and produces scarce seeds.

Origin and Distribution: *Mondia whitei* grows all over Africa, from Guinea Bissau to the Congo (Adjanohoun et al., 1988; Burkhill, 1997), in East Africa (Kokwaro, 1976; Morris, 1996) and from the Congo Basin to Southern Africa (Msonthi et al., 1989; Van Wyk & Gericke, 2000).



Plant Part Used: Although the root is the part mostly used for medicinal purposes, there are reports of the entire plant and other parts being used for treatment.



ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses: *Mondia whitei* is used in African traditional medicine for the treatment of a wide range of diseases and conditions, the most common being as an aphrodisiac (Kokwaro, 1976). The use of the entire plant as an aphrodisiac has been reported in Senegal. There is also a report of the use of a hot water extract of the entire plant in South Africa for impotence in men (Watt & Breyer-Brandwijk, 1962).

In Kenya, a preparation from it known as 'mkombelo' is used to enhance milk production in lactating mothers, to manage diabetes mellitus, to flavour foods, and to induce appetite (Karimi, 2002). *M. whitei* is also known to be oxytocic and is therefore used as uterine stimulant during parturition (Burkhill, 1997; Watt & Breyer-Brandwijk, 1962; Kerharo, 1971). In Kenya, the root is taken as a purgative and to relieve body pains. An extract of *M. whitei* roots is used in Tanzania as a cure for gonorrhoea, a treatment that tends to cause profuse urination. The use of such a preparation for the treatment of gonorrhoea has also been reported in Malawi (Morris, 1997) and in other parts of Africa (Msomthi, 1989).

In South Africa, *M. whitei* is used as an expectorant in treating bronchitis. It is also used by the Zulus to flavour soft drinks. It is said to have antibiotic properties and helps in the management of sexually transmitted diseases (McCartan & Crouch, 1998). In the same country, McGaw et al. (2000) have reported on the use of the plant to treat stomachaches. Sparg et al. (2000) have also reported on its use against schistosomiasis, and the seeds are used as arrow poison (Burkhill, 1997). In Zimbabwe, the root infusion is used to treat constipation, anorexia, schistosomiasis, and as an aphrodisiac. The plant is reported to be used for the treatment of these same diseases and conditions in other parts of Eastern, Central, and Southern Africa (Msomthi, 1989). It is also used for fits in children and to treat stress and tension in adults (Van Wyk & Gericke, 2000).

Its most common use is to manage erectile dysfunction (Msomthi, 1989; Kokwaro, 1976; Watt & Breyer-Brandwijk, 1962). The Luo and Luhya tribes in Western Kenya use a preparation

of *M. whitei*, called respectively ogombera and mukombeera, to increase male libido (Karimi, 2002; Watcho et al., 2001). In Cameroon, the bark of the dried root is used to achieve the same effect (Watcho et al., 2001), and in Malawi the fresh root tuber is eaten as an aphrodisiac (Msomthi, 1991). In Ghana, anecdotal evidence suggests that both aqueous and alcoholic extracts of the roots of *M. whitei* are effective in managing erectile dysfunction (Ofosuhene, 2005). Adjanohoun et al. (1988) also mention the use of the root of *M. whitei* as an aphrodisiac from Guinea to the Congo.

Other Relevant Uses: The stem is fibrous and the fibre is said to be suitable for use as a cord. It has a latex which is white and sticky and appears to come from the central pith. There is no known special property for the latex.

CHEMICAL CONSTITUENTS

Compounds: 2-Hydroxy-4-methoxybenzaldehyde is a simple benzaldehyde derivative isolated from the roots of *M. whitei* (Kubo & Kinst-Hori, 1999; Mukonyi & Ndiego, 2001; Patnam et al., 2005). The same compound had been referred to as an isomer of vanillin (Burkhill, 1997). It is a tyrosinase inhibitor, a mixed-type inhibitor, probably forming a complex with both the enzyme and the substrate, as shown by Lineweaver-Burk plots, inhibiting the oxidation of L-3,4-dihydroxyphenylalanine (L-dopa) by mushroom tyrosinase with an LD₅₀ of 4.3 µg/ml (0.03 mM). Preincubation did not affect enzyme action, indicating that the inhibitor found in *M. whitei* does not inactivate the enzyme. This inhibition is specific to this compound, as it is not shown by closely related congeners such as vanillin (4-hydroxy-3-methoxybenzaldehyde), isovanillin (3-hydroxy-4-methoxy-benzaldehyde), and o-vanillin (2-hydroxy-3-methoxybenzaldehyde). Isovanillin has been shown to be present in *M. whitei* (Koorbanally et al., 2000; Mukonyi & Ndiego, 2001; Patnam et al., 2005).

The dried roots of *M. whitei* collected from Malawi were shown to contain a phenolic glycoside with the sugar 2-O- β-D-glucopyranosyl-(1-6)-O- β-D-xylopyranoside (Msomthi, 1989, 1991).

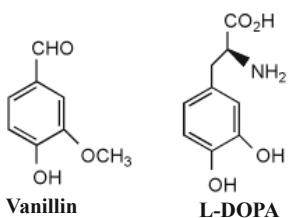
The glycoside was isolated from the methanolic extract of the roots using a combination of techniques: direct countercurrent chromatography

(DCCC), Sephadex LH-20 column chromatography, and reverse-phase medium-pressure liquid chromatography. The structure was determined by spectroscopy and synthesis of the aglycone.

Burkhill (1997) reported the presence of a volatile oil said to contain coumarin in the roots of *M. whitei* at a concentration of 1 - 1.2%. He also reported 2.8% fixed oil, 20% glucose, 0.7% resin, and 0.045% glycoside.

Putnam et al. (2005) isolated and characterized the unusual chlorinated coumarinolignan 5-chloropropacin from the dried roots of *M. whitei* collected from Togo.

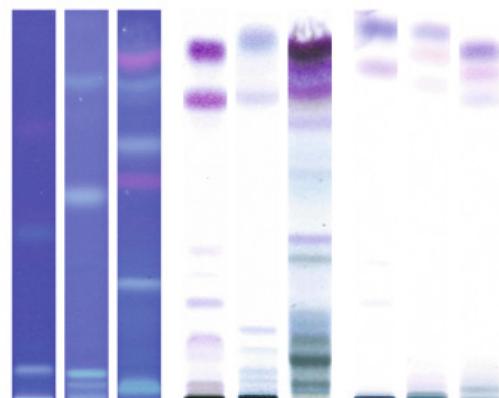
The structure of the chlorinated coumarinolignan was determined by NMR and mass spectroscopic analysis. Other coumarins were also isolated and characterised, including propacin, 7,8-dihydroxy-6-methoxy, and the 7-hydroxy-4,6-dihydroxy derivatives.



QUALITY CONTROL

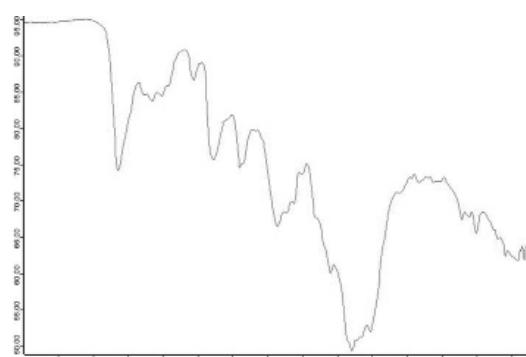
Organoleptic Properties: Tuberous fleshy root with sweet-tasting vanilla-like flavour.

TLC / HPLC / GC



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

NIR Spectroscopy



Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

Spargs et al. (2000), using aqueous extract of *M. whitei* at a concentration of 50 mg/ml, have shown the extract to be active against *Schistosoma haematobium*. In a study involving screening of East African plants for antimicrobial, anti-yeast, and antifungal (plant pathogen) activities using broth culture, a 70% ethanolic extract of *M. whitei* at a concentration of 100 µg/ml, did not show any activity against *Bacillus subtilis*, *Escherichia coli*, *Penicillium crustosum*, and *Saccharomyces cerevisiae* (Taniguchi et al., 1978).

Ofosuhene (2005) carried out studies using male rabbits to investigate the effect of ethanolic extract of *M. whitei* on some biochemical processes

leading to penile erection. In these investigations, blood and corpus cavernosal tissue levels of nitric oxide (NO), cyclic guanosine monophosphate (cGMP), and malondialdehyde (MDA) were measured in response to a suspension of freeze-dried extract of *M. whitei* in water, administered by oral gavage for a period of 8 weeks. Three different concentrations of the freeze-dried powder (2, 20, 200 mg/kg body weight) were used with Viagra (sildenafil citrate) as a positive control (50 mg/kg) and the vehicle as the negative control. There were elevations of NO and cGMP in response to the oral administration of *M. whitei*. There was a statistically significant increase in plasma NO, especially in response to the medium dose, with no such increase for the single dose of Viagra used. The increase in plasma cGMP was statistically significant at the high dose used. Again, the increase in response to Viagra was not statistically significant. Levels of NO and cGMP in corpus cavernosal tissue increased significantly in response to *M. whitei*. Compared to Viagra, the increase in NO was lower for the animals on *M. whitei*, even at the highest dose used, whereas the increase in cGMP, which was dose dependent, was the same as that for Viagra at the highest dose used. Levels of MDA in erythrocytes decreased for both *M. whitei*-treated and Viagra-treated animals, and the percentage decrease was the same at the highest dose of *M. whitei* used. In the corpus cavernosal tissues, however, the percentage decrease was the same for the two treatments and at all three doses. The author concluded that *M. whitei* has multiple biochemical/pharmacological effects and acts through elevation of NO and cGMP levels in corpus cavernosal tissues. There were additional effects on free-radical scavenging, from which antioxidative activity was inferred.

Watcho et al. (2001) studied the anti-spermatogenic and anti-fertility effects of *M. whitei* by chronic administration of a root bark extract of the plant (400 mg/kg/day) for 55 days. The treatment resulted in cessation of spermatogenesis and degenerative changes in the seminiferous tubules and the epididymides. A recovery period resulted in normal spermatogenesis and fertility, suggesting reversible antispermatic and antifertility effects of the plant.

In a study to determine the effect of an aqueous extract of *M. whitei* roots on testosterone

production and fertility in male rats, Watcho et al. (2004) used four groups of adult male Wistar rats, treated with a single dose (400 mg/kg) of *M. whitei* for different lengths of time, varying between 1 and 6 hours, in an acute and a chronic study, during which the plant material was administered for 2, 4, and 8 days. In the acute treatment groups, the serum and testicular concentrations of testosterone, determined by radioimmunoassay, remained unchanged. Chronic treatment for 8 days induced a significant increase in the testicular weight, the serum and testicular testosterone, the testicular protein content, and the sperm density ($P < 0.05 - 0.01$), but did not affect the accessory gland weights, the serum protein contents, the testicular concentration of 17 β -estradiol, and the fertility compared to the controls. From these studies, the authors concluded that *M. whitei* root extract possesses androgenic property.

M. whitei has traditionally been used to relieve headache. In a study by Nielson and co-workers to screen plants used for anxiety and depression, *M. whitei* showed affinity to the serotonin transport protein in rat brain (Nielsen et al., 2003).

SAFETY DATA

Preclinical Safety Data

Single Dose Toxicity cf. appendix 1

KEY (PROPOSED) USAGE

Therapeutic Indications: Aphrodisiac, erectile dysfunction.

TRADE INFORMATION

Nature of plant material: In the Congo it is specially cultivated, whereas in some parts in South Africa, the plant is almost extinct because people simply uproot the plant and chew the roots (Burkhill, 1997).

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Moringa oleifera

GENERAL DESCRIPTION

Scientific Name with Author: *Moringa oleifera* Lam.

Synonyms: *Moringa pterygosperma* Gaertn., *M. moringa* Millsp., *Guilandina moringa* L.

Family: Moringaceae

Vernacular Names: Behen tree, ben and ben-oil-tree, benzoline-tree, bred mouroung, brede mouroungue, drumstick tree, horseradish tree, mouroungue, tree of life.

Botanical Description: Tree reaching between 5 - 6 m in height, with an open umbrella-shaped crown. Depending on the climate, the foliage can be either evergreen or deciduous. The leaves are 1 - 2 cm large and alternate, bi-, or tripinnate. Leaflets obovate, 4 - 24 x 3 - 12 mm, with margins entire. Flowers scented, with sepals and petals white, greenish at the base, sometimes tinted red. Sepals 10 - 13 mm long. Fruit is an elongated capsule, 24 - 30 cm long. Seeds globose, 6 - 7 mm in diameter, with three large, papery wings.

Origin and Distribution: Indigenous to sub-Himalayan regions of northern India and Pakistan, but now has pan-tropical distribution in Africa, Asia, the Pacific and Caribbean Islands, and South America.



Plant Parts Used: Virtually every part of the tree is beneficial. Leaves, flowers, seeds, and pods are eaten as highly nutritious vegetables. The bark, roots, leaves, and pods are used for their medicinal properties.



ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses: All parts of *Moringa oleifera* have numerous medicinal applications, including wound healing, anthelmintic, anti-inflammatory, antibiotic, and antihypertensive properties, among others.

The leaves are reported to aid in wound healing and to have anthelmintic properties (Adjanohoun et al., 1990). A soup made from the leaves is used

to treat hypertension. In Madagascar, the leaves are used against nervous ailments (Gurib-Fakim et al., 2004).

The juice extracted from the crushed bark, flowers, roots, and leaves, mixed with honey, is used for nervous disorders (Pernet, 1957; Adjanooun et al., 1982, 1992). Bark or leaf, mixed with the leaves of *Catharanthus roseus* in a decoction form, has antispasmodic properties. A bark decoction, with the leaves of *Persea americana*, is a well-known abortifacient (Gurib-Fakim et al., 1994).

The root is chewed against gingival ulcers and also as an aid to digestion. The root pulp is applied as a chest poultice for 2 minutes against pulmonary diseases such as coughs and bronchitis. The root decoction is drunk against epilepsy, hysteria, fever, and dyspepsia (larger doses prove to be dangerous). Lightly boiled leaves, bark or root pulp, or pulverized root alone is applied to painful joints to relieve symptoms of rheumatism and arthritis. Extracts of bark or the root are drunk against scurvy. The root and pounded flower are applied as a dressing on wounds. An infusion made from four root pieces, along with the seeds of mustard (*Brassica juncea*), is used as a gargle. The root poultice is a stimulant, used for some forms of paralysis and fever (Pernet, 1957). The juice extracted from the crushed roots is used as an ear drop for ear infections.

A leaf poultice mixed with coconut oil acts against tetanic infections, and a leaf decoction mixed with cooking oil is used for constipation. Among some farmers in Niger, dried *Moringa* leaves are an important cash crop. The leaf infusion is oxytocic (Gurib-Fakim et al., 1999). The leaf pulp is used as a dressing against inflammation. The leaf sap is used as an ear drop to treat ear infection (Gurib-Fakim et al., 2004).

A whole plant decoction is drunk daily against viral hepatitis. Jaundice is treated with a decoction of the leaves along with those of *Senna occidentalis* and the leaves of *Citrus aurantifolia*. Oedemas are treated by rubbing with the root bark mixed with lemon juice (Neuwinger, 2000). In Mauritius and Seychelles, the plant is traditionally used against sore throats, hoarseness and throat-related infections. In Rodrigues, the plant is mainly

known for its action on arterial pressure (Gurib-Fakim et al., 1994).

The seed oil is rubbed on affected joints for the treatment of rheumatism, gout and arthritis.

Other Relevant Uses: *Moringa* leaves may be pounded, cooked, and eaten like spinach, or added to rice, stews and soups. In every country of West Africa the leaves of *Moringa oleifera* are used to make a sauce. These leaves can be found for sale in both rural and urban markets. In Senegal this sauce, eaten with rice or millet, is called Mboum. Collected during dry seasons, the leaves are frequently used to combat malnutrition. The dried leaf powder is used traditionally as a nutritional additive to sauces and infant formulas, whereby one or more spoonfuls of powder are stirred into the sauce or formula. One rounded soup-spoon (tablespoon) contains about 8 g of powder; 100 g of powder is a little less than one and a half cups, American measure. Thus, listings of leaf powder content are expressed as one heaped soup-spoonful: 100 g of fresh leaves contain 6.7 g protein, and one heaped soup-spoon of leaf powder contains 2.2 g protein.

Moringa pods are also eaten as a vegetable in a manner similar to green beans. The pods are highly nutritious, containing all the essential amino acids. In Asia, fresh and canned pods are sold commercially; 100 g of the edible portion of the pods contain 2.5 g protein.

During pregnancy, or when a woman is breastfeeding, the recommended daily intake of protein is 65 g. Hence a meal of 100 g *Moringa* pod will satisfy 3.8% of the daily protein requirement, and a meal of 100 g of fresh *Moringa* leaves will satisfy 10.3% of the protein requirement. Each rounded soup-spoonful of leaf powder added to the diet will satisfy 3.3% of the daily protein requirement during pregnancy or breastfeeding.

The quantities of nutrients found in the various parts of the *Moringa* plant are variable, depending on the source, genetic background, environmental conditions and cultivation methods. Nevertheless, the following tabulation provides a list of typical nutrients found in the leaves, pods, and leaf powder. *Moringa* leaves are a rich source of vitamin E (90.0 mg/kg) (Ching & Mohamed,

2001), a potential source of vitamin A (Nambiar & Seshadri, 2001), and a rich source of essential fatty acids, such as linoleic and alpha-linoleic acids, as well as significant levels of selenium and phosphorus (Freiberger et al., 1998). Thus, the dehydrated leaves have been used to supplement the diet of children deficient in vitamin A and other nutrients (Nambiar et al., 2003). The fruit extracts have also been shown to possess hypolipidaemic effects (Mehta et al., 2003).

In some Asian countries the seeds are also consumed as food, either as immature and fresh or as fried mature seeds.

Moringa seeds have been reported to be rich in glucosinolates. These molecules are anionic, hydrophilic plant secondary metabolites which are of particular interest in the prevention of cancer and other chronic and degenerative diseases (Fahey et al., 2003).

During the 19th century, *Moringa oleifera* plantations in the West Indies were exporting the oil (known as Ben oil) to Europe for use in making perfumes and as a lubricant for fine machinery. The pleasant-tasting edible oil, which can be extracted from the seeds, was highly valued by the ancient Roman, Greek and Egyptian civilizations for use in making perfume and in protecting the skin. As the seed oil does not become rancid, it makes an excellent salad oil and a good soap (Neuwinger, 2000).

In the search for alternatives to currently used fungicides, the potential of aqueous *Moringa* seeds extract was evaluated. The results showed that the extracts had potential as a biofungicide on groundnut seeds, as most concentrations tested significantly reduced the incidence of fungi on the seeds; this effect correlated with the concentration of the *Moringa* extracts. There was no significant difference, however, between *Moringa* extract and that of the commercial fungicide Apron Plus at the manufacturer's recommended dosage (Donli & Dauda, 2003).

CHEMICAL CONSTITUENTS

Compounds: Leaves: Quercetin-3-O-glucoside; quercetin-3-O-(6'-malonyl-glucoside), kaemferol-3-

O-glucoside, kaemferol-3-O-(6'-malonyl-glucoside), 3-caffeoylequinic acid, 5-caffeoylequinic acid. Proanthocyanidins and anthocyanins have not been detected (Bennett et al., 2003).

Bark: Several glucosinolate isomers have been reported; 4-(α -L-rhamnopyranosyloxy)-benzylglucosinolate (Bennett et al., 2003).

Seeds: Pterygospermine.

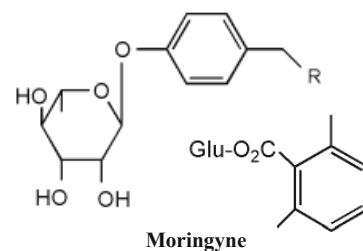
Seed Oil: Seed oil from plants grown in Pakistan accounts for 38 - 42% of the seed weight and contains 26.5 - 32% proteins, 5.8 - 9.3% fibre, 5.6 - 7.5% ash.

Seeds and Roots: 4-(α -L-rhamnosyloxy)benzylisothiocyanate.

Roots: High concentrations of 4-(α -L-rhamnopyranosyloxy)-benzylglucosinolate; benzyl glucosinolate (Bennett et al., 2003).

Root Bark: Afomine; spiraquine; thiol amine bases: moringine, spirochine; benzylic amines (moringine) and glucotropaeoline.

Chemical and physical analyses of the extracted oil: Iodine value: 68.0-71.8; refractive index (40°C): 1.4590 - 1.4625; density (24°C): 0.9036 - 0.9080 mg/ml; saponification value: 180.60 - 190.50; unsaponifiable matter: 0.70 - 1.10%; tocopherols (α , γ and δ): up to 123.5 - 161.3, 84.1 - 104.0, 41.0 - 56.0 mg/kg, respectively. The oil was also found to contain high levels of oleic acid, up to 78.6%; palmitic, stearic, behenic, and arachidic acids: up to 7.0, 7.5, 6.0, and 4.2%, respectively (Anwar & Bhanger, 2003).



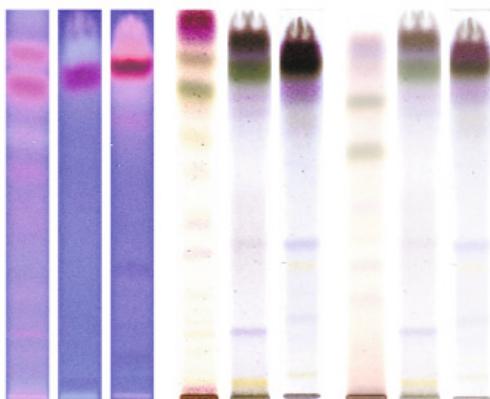
Niazinin A: R = (E)NHC(=S)OMe

Niazinin B: R = (Z)NHC(=S)OMe

Moringyne: R = (E)NHC(=S)OEt

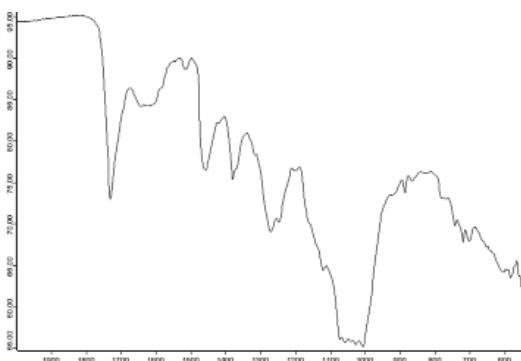
QUALITY CONTROL

TLC / HPLC / GC



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

NIR Spectroscopy



Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

Pterygospermine is a compound known to exhibit antibiotic properties. Pterygospermine is also known to possess antimicrobial, antifungal and antiviral properties (Berdy et al., 1992).

The antimicrobial and antimyotic properties of pterygospermine have been demonstrated in vitro (Caceres et al., 1990). The seed extract of *Moringa* shows antimicrobial activity against *Staphylococcus aureus*, in vitro, in a manner similar to that of neomycin (Caceres et al., 1991). Spirochicine is a prophylactic and an antiseptic, antibiotic against Gram-positive bacteria. *Moringa* has been

shown to be very active against *Cholera vibrio* (Oliver-Bever, 1986).

In one study the seeds of *Moringa* were used as a water purifier, by removing up to 90% of microbes in drinking water (Gilani et al., 1994).

The seed flour from *Moringa oleifera* is widely used as a natural coagulant for water treatment in developing countries. The extracts obtained by soaking intact *M. oleifera* seeds in water showed the presence of water-soluble lectins, trypsin inhibitor, tannin and antioxidant activity (Mandlo et al., 2004; Santos et al., 2005).

Pterygospermine isolated from the roots has been reported to have anti-fertility properties (Sangeeta et al., 1987) as well as significant antibiotic properties against a wide range of Gram-positive and Gram-negative bacteria (Oliver-Bever, 1986). The aqueous and ethanolic leaf extracts are powerful abortifacients in mice at a dose of 175 mg/kg (Nath et al., 1991). Anti-inflammatory and antispasmodic properties of the seeds and roots have also been reported (Caceres et al., 1990; Ezeamuzie et al., 1996).

As the root extract has been used traditionally in Western Africa against rheumatism, the anti-inflammatory action of the aqueous root extract was tested in rats using indomethacin (10 mg/kg) as a positive control. At a dose of 750 mg/kg, the *Moringa oleifera* treatment significantly inhibited the development of oedema at 1, 3, and 5 hours (reduction of 53.5, 44.6, and 51.1%, respectively). Treatment with the positive control under the same conditions gave the following results: 49.1, 82.1, and 46.9%, respectively. Increasing the dose of *Moringa* extract did not increase the inhibitory effect on oedema development. It was concluded that the root may contain anti-inflammatory effects useful in the treatment of acute inflammatory conditions (Ndiaye et al., 2002).

It was also observed that an extract of *M. oleifera* exerts hepatic protection by reducing lipid peroxides and enhancing antioxidants (Ashok Kumar & Pari, 2003). These extracts also possess antioxidant activity, attributable primarily to phenolics of the flavonoid group, namely quercetin and kaemferol (Siddhuraju & Becker, 2003).

The standardized aqueous root extract of *M. oleifera* has been shown to inhibit penicillin-induced seizures and to markedly reduce locomotor activity (Ray et al., 2004a). It has also been reported to potentiate pentobarbitone-induced sleeping time and to increase alpha wave activity through 5-hydroxytryptamine (Ray et al., 2004b).

Moringa extracts have also been shown to be effective against thymidine kinase-deficient HSV-1 and phosphonate acetate-resistant strains of HSV-1. These therapeutic effects were characterised using a cutaneous HSV-1 infection in mice. The extracts of *M. oleifera* at a dose of 750 mg/kg/day significantly delayed the development of skin lesions, prolonged the mean survival time, and reduced the mortality of HSV-1-infected mice compared to controls (Lipipun et al., 2003).

SAFETY DATA

Preclinical Safety Data

Single Dose Toxicity cf. appendix 1

In mice, a single peroral dose of 5 g of powdered seed per kg body weight resulted in hyperkeratosis in the stomach and liver cell steatosis. Parenteral doses of 22 - 50 mg/kg body weight of the glucosinolate present in seeds was fatal to mice (Gruenwald et al., 2000).

KEY (PROPOSED) USAGE

Therapeutic Indications: Antibiotic, anti-inflammatory, antioxidant.

Special Warnings and Precautions for Use: No health hazards are known with therapeutic doses of the root. Ingestion of large quantities of the root can produce nausea, dizziness, and vomiting.

There are no health hazards known in conjunction with the proper administration of the seeds.

Pregnancy and Lactation: The root should not be used by pregnant or nursing women (Gruenwald et al., 2000).

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Nauclea latifolia

GENERAL DESCRIPTION

Scientific Name with Author: *Nauclea latifolia* Sm.

Synonyms: *Sarcocephalus esculentus* Afzel. ex Sabine, *Sarcocephalus russeggeri* Kotschy ex Schweinf., *Sarcocephalus sambucinus* K.Schum, *Nauclea esculentus* (Afzel. ex Sabine) Merrill, *Sarcocephalus sassandrae* A. Chev., *Sarcocephalus latifolius* (J.E. Smith, E.A. Bruce).

Family: Rubiaceae



Vernacular Names: African peach, bari, baro, bati country fig, Guinea peach, negro peach, Pêcher africain, peyae biasa, Sierra Leone peach, tafashia.

Botanical Description: *Nauclea latifolia* is a straggly, multi-stemmed tree or shrub, rarely over 6 metres high. It has an open canopy. Inflorescence with terminal, spherical, head-like cymes of small, whitish, fragrant flowers.

In *Nauclea* spp, the flowers are joined by their calyces. The fruit is reddish and syncarp. Leaves are elliptic or rounded-ovate, acuminate tip, cuneate, rounded or subcordate base, 10-20 cm long, 6-12 cm broad; pedicel is 1-2 cm long. The tribe Naucleae to which *Nauclea latifolia* belongs shows similarities to the family Combretaceae (Abbiw, 1990).

Origin and Distribution: *Nauclea latifolia* is a savanna shrub sometimes found in undisturbed fringing forest and closed savanna woodland. *Nauclea latifolia* is commonly found in Republic of Benin, Burkina Faso, Cameroun, Democratic Republic of Congo, Gabon, Gambia, Ghana, Mali, and Nigeria.

Plant Parts Used: Roots, stem bark, leaves, and fruits.



ETHNOBOTANICAL INFORMATION

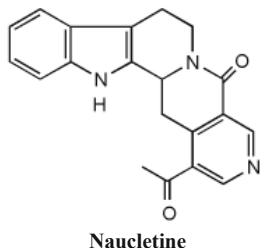
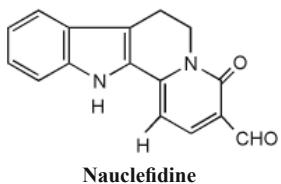
Major Ethnopharmacological Uses: In most parts of West Africa, the trunk bark is used against fever and malaria, hence the description “African quinine” (Oliver-Beaver, 1986). It can be used alone or in association with *Khaya senegalensis*.

The decoctions of the leaves or trunk bark are used as antienteralgesic, vermifuge and diuretic. The trunk bark is also used as haemostatic. Leaves and roots are used against amenorrhea and sterility (Kerharo & Adam, 1974; Malgras, 1992). The fruit is eaten as a cough remedy. In Kinshasa, *N. latifolia* is used by traditional health practitioners to treat diabetes. In Nigeria, *Nauclea latifolia* is used as a cure for malaria fever, and treatment for piles and dysentery.

Treatment for abdominal pain, arthritis, diarrhoea, wounds, infective hepatitis, septic mouth, halitosis, toothache, and dental caries (Mshana et al., 2000).

CHEMICAL CONSTITUENTS

Compounds: Strictosidine (isovincoside) lactam was isolated from *Nauclea latifolia* (Brown et al., 1977). Furthermore, several indoloquinolizidine alkaloids were isolated from the root of *Nauclea latifolia*. Hotellier et al. (1975) identified two known compounds (angustine and angustoline) and two new chemical entities (nauclefidine and naucleline). In addition, the alkaloid, strictosamine, is obtained from the roots, leaves and stem bark (Duez et al. 1994).



QUALITY CONTROL

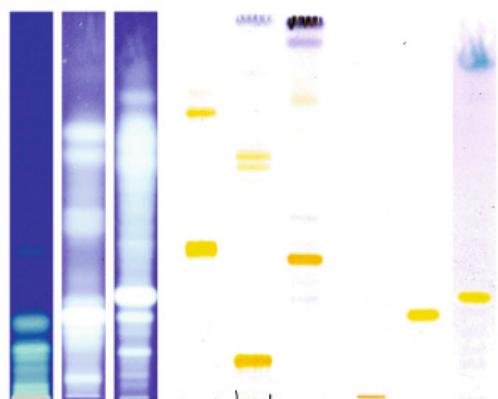
Organoleptic Properties: Dried stem bark and root are yellow. Powdered plant material is yellowish-brown; odour pleasant; taste bitter.

Macroscopic Characteristics: Root cylindrical or broken; light to medium material; lenticellate

bark, grayish-brown outer surface; yellow wood (pinkish when cut fresh) (GHP, 2007).

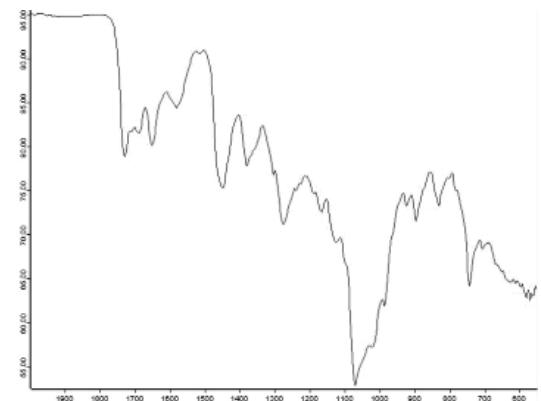
Microscopic Characteristics: The transverse section shows a bark region comprising several layers of stratified, lignified, cork cells. Cortical region is made up of parenchymatous cells interspersed with lignified sclereids. Phloem tissue consists of phloem parenchyma interspersed with aggregates of fibres; medullary rays separate the fibres; rosette crystals occur in the phloem parenchyma; starch grains abound throughout the bark tissue except in the cork cells. The wood is lignified and made up of large xylem vessels, tracheids, wood fibres, and parenchyma cells which contain abundant starch grains (GHP, 2007).

TLC / HPLC / GC



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

NIR Spectroscopy



Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

The toxicity and the genotoxicity of antimalarial alkaloid rich extracts derived from two plants used in Mali (*Mitragyna inermis* and *Nauclea latifolia*) were evaluated on in vitro and in vivo systems. The results demonstrated that an alkaloid rich extract derived from *N. latifolia* could interact in vitro with DNA of bacteria and mammalian cells, leading to G2-M cell cycle arrest and heritable DNA-damage, as well as inducing in vivo single-strand breaks in liver, kidney and blood cells (Traore et al., 2000).

The aqueous extract of *N. latifolia* leaves (0.25 - 2.0 mg/ml) paralysed *T. columbriformis* larvae in a concentration-dependent manner (ED₅₀ value of 0.52 mg/ml at 24 h). The paralysing effects of the extract increased with period of exposure. Other studies on *Nauclea latifolia* extract include the molluscicidal activity and antimicrobial activity of the plant (Kela et al., 1989). *Nauclea latifolia* exhibited activity against *Escherichia coli*, *Shigella flexneri*, *Salmonella typhi*, and *Staphylococcus aureus* (Deeni & Hussain, 1991; Sourabie et al., 1994). The extracts of three plants including *Nauclea latifolia* were tested, by agar diffusion and macrobroth dilution methods, for activity against five strains of *Staphylococcus aureus* and two of *Escherichia coli* isolated from cases of STD and/or urethritis. Four typed bacterial strains, *S. aureus* ATCC 12600, *Bacillus subtilis* ATCC 6051, *Pseudomonas aeruginosa* ATCC 10145, and *Escherichia coli* ATCC 117755 were included as reference organisms. *Nauclea latifolia* extracts were bacteriostatic to both Gram positive and Gram negative strains (Okoli & Iroegbu, 2004).

Aqueous extracts of *Nauclea latifolia* manifested antiplasmodial activity in vitro against chloroquine-resistant FcB1-Columbian strain of *Plasmodium falciparum*. Cytotoxicity was determined on human MRC-5 and rat L-6 cell lines. (Zirihi, et al., 2005). A similar study which included a Nigerian chloroquine-sensitive strain was undertaken by other workers (Benoit-Vical et al., 1998; Traoré-Kéita et al., 2000). Extracts used were obtained from stems and roots of the plant in two forms, infusion and decoction, both methods

used by most traditional health practitioners. The in vitro activity of *N. latifolia* extracts on *P. falciparum* was assessed visually under the microscope and also by a radioactive method. Similar results were obtained applying fresh, frozen, or lyophilized extracts. The IC₅₀ values determined were within the range already reported for other antimalarial plants such as *Azadirachta indica* A. Juss (Meliaceae) and *Artemisia annua* L. (Asteraceae). Aqueous extracts of *N. latifolia* inhibited *P. falciparum* (FcB1 strain) mainly at the end of the erythrocytic cycle (32nd to 48th hour).

Nauclea latifolia is used in Nigerian traditional medicines against gastrointestinal helminths of animals and man. Proanthocyanidins were detected in *Nauclea*. Extract of the plant killed 50% of brine shrimp nauplii at < 10 ppm (*Nauclea*), the *Nauclea* LD₅₀ being similar to the anthelmintic drug piperazine. Extracts were also toxic to the parasitic nematode *Haemonchus* infective L3 stage. Nematode glutathione-S-transferases (GSTs) are potential drug targets. It contained heat-stable inhibitory activities against recombinant *Ascaris* and *Onchocerca* GSTs in vitro (Fakae et al., 2000). The leaf and root parts or their combination of *Nauclea latifolia* were separately extracted and their pharmacological effects on purinergic transmission in the bladder investigated. The leaf extract was very potent in potentiating purinergic neurotransmission by potentiating ATP induced contractions. These actions were more pronounced than the depression of potassium chloride-evoked contraction of the bladder. The root extract depressed purinergic contraction of the bladder by a direct depressant action on the bladder smooth muscle, since it did not modify ATP induced contractions. The leaf and root extracts antagonized each other when combined together. It is concluded that the leaf and root extracts of *N. latifolia* possess interesting pharmacological actions on the bladder (Udoh, 1995).

Extract of *Nauclea latifolia* root bark (50 - 200 mg/kg p.o.) significantly (P < 0.05) decreased the spontaneous motor activity and exploratory behaviour in mice, and prolonged pentobarbital sleeping time in rats, dose-dependently. The extract also remarkably attenuated the intensity of apomorphine-induced stereotypy dose-dependently in mice, but had no effect on motor

coordination as determined by the performance on rota-rod. These results indicate the presence of psychoactive substances in the aqueous extract of the root bark of *Nauclea latifolia* (Amos et al. 2005).

The anthelmintic efficacy of *Nauclea latifolia* stem bark aqueous extract was studied in sheep with natural acute/sub-acute parasitic gastro-enteritis, due primarily to mixed nematode species. Graded doses of the extract (400, 800, and 1600 mg/kg, p.o for 5 consecutive days) significantly reduced faecal egg counts in infected animals. The percentage reduction (93.8%) by 1600 mg/kg of the extract was comparable to that of 5 mg/kg of albendazole (94.1%). The administration of the extract resulted in improved haemoglobin and leucocytosis values in worm-infected sheep (Onyeyili et al., 2001).

Ethanolic extracts of *N. latifolia* leaves demonstrated hepatoprotective properties in rats (Akapanabiati et al., 2005), while the root extract showed antihepatotoxic effect and inhibited the multiplication of *Trypanosoma brucei* infection in mice in a dose-dependent manner (Madubunyi, 1995). Aqueous extracts of the leaves possess hypoglycaemic activity (Gidado et al., 2005).

Clinical Studies: Gamaniel et al. (unpublished data) conducted a comparative randomized clinical trial using standardized extracts of the root of *Nauclea latifolia* against symptomatic, but uncomplicated malaria in human volunteers at two district hospitals located in Abuja, Nigeria. The study had three arms, with 30 study participants per arm; the test arm received 300 mg of standardized extract orally two times daily for five days, the second arm was given chloroquine 600 mg base orally once daily for three days, and the last arm received sulphadoxine/pyrimethamine (Fansidar). The results indicate that the standardized extract was efficacious against uncomplicated malaria. The parasite clearance was better than chloroquine and there were no threats of serious side effects affecting the organs or tissues.

SAFETY DATA

Preclinical Safety Data: Abbah et al. (Unpublished data) studied the non-clinical efficacy and safety profiles of the extract of the

root of *Nauclea latifolia* and found the product to be safe and efficacious against *P. berghei* in mice. The extract had an LD₅₀ > 2000 mg/kg per oral in rats and mice, and did not show any significant toxic activity on the organs in the 28 day study. There was no abnormality in the liver enzymes AST, ALP, and ALT in rats. There was no effect on both conjugated and total bilirubin which often results from jaundice or liver disease. The extract did not alter the levels of creatinine or urea, which are sensitive indicators of kidney function. Furthermore, the extract had no effect on triglycerides, cholesterol, and glucose (products of carbohydrate and fatty acid metabolism). These agents: increase in liver disease, renal damage, hypertension, and diabetes mellitus are risk factors in coronary heart disease.

Single Dose Toxicity cf. appendix 1

KEY (PROPOSED) USAGE

Therapeutic Indications: Antimalarial effects (Benoit-Vical, F. et al., 1998, Traoré-Kéita et al., 2000, Zirihi, G.N et al., 2005, Abbah et al. (Unpublished data), Gamaniel et al. (unpublished data)). Antineurological effects (Udoh, 1995, Amos et al. 2005). Antibacterial effects (Deeni and Hussain, 1991; Sourabie et al.. 1994, Okoli, A.S. and Iroegbu, C.U. 2004). Antihelmintic/ Antitrypanosoma (Fakae et al., 2000, P. A. Onyeyili et al., 2001, Madubunyi II. 1995). Diuretic effect (Kerharo et Adams, 1974, Malgras, 1992).

Evaluation of Efficacy: Antimalarial effects efficacy pharmacologically proven (Benoit-Vical, F. et al., 1998, Traoré-Kéita et al., 2000, Zirihi, G.N et al., 2005, Abbah et al. (Unpublished data), Gamaniel et al. (unpublished data)). Antineurological effects efficacy pharmacologically proven (Udoh, 1995; Amos et al., 2005). Antibacterial effects efficacy pharmacologically proven (Deeni & Hussain, 1991; Sourabie et al., 1994; Okoli & Iroegbu, 2004). Antihelmintic/Antitrypanosoma efficacy pharmacologically proven (Fakae et al., 2000; Onyeyili et al., 2001; Madubunyi II, 1995). Diuretic effect efficacy traditionally proven (Kerharo & Adams, 1974; Malgras, 1992).

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Traore F, Gasquet M, Laget H, Guiraud C, Di Giorgio N, Azas N, Doumbo P, Timon-David P (2000) Toxicity and genotoxicity of antimalarial alkaloid rich extracts derived from *Mitragyna inermis* O. Kuntze and *Nauclea latifolia*. Phytother. Res. 14(8): 608-611.

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Zirihi GN, Mambu L, Guédé-Guina F, Bodo B, Grellier P (2005) In vitro antiplasmodial activity and cytotoxicity of 33 West African plants used for treatment of malaria. J. Ethnopharmacol. 98(3): 281-285.

Pelargonium sidoides

GENERAL DESCRIPTION

Scientific Name with Author: *Pelargonium sidoides* DC.

Family: Geraniaceae

Vernacular Name: Umckaloabo

Botanical Description: A small perennial herb with tuberous rhizomes, rounded to heart-shaped and slightly silvery leaves on long petioles, and small tubular flowers that are dark maroon red to almost black. The closely related *P. reniforme* is morphologically very similar but has pink flowers (Van der Walt, 1977; Van Wyk & Wink, 2004).

Origin and Distribution: Eastern parts of southern Africa (Eastern Cape Province, Kwazulu-Natal, and Lesotho).



Plant Part Used: Dried tuberous rhizomes.

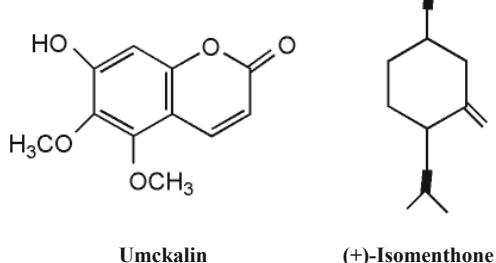


ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses: The traditional medicinal uses of *Pelargonium sidoides* are poorly recorded. The plant is traditionally used by Zulu people to treat gonorrhoea, diarrhoea, and dysentery. A large number of *Pelargonium* species with tuberous rhizomes are used in traditional medicine against diarrhoea and dysentery, and only this use is well documented (Watt & Breyer-Brandwijk, 1962; Forbes, 1986; Hutchings et al., 1996; Van Wyk et al., 1997).

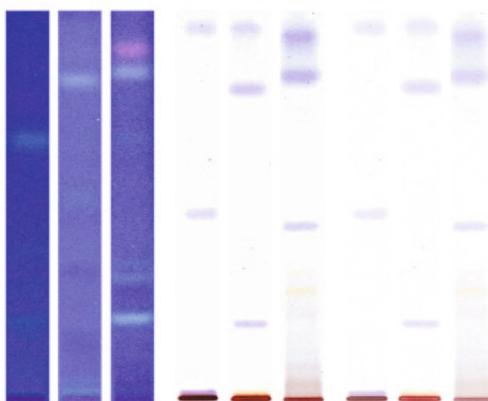
CHEMICAL CONSTITUENTS

Compounds: The dried rhizomes contain at least eight different coumarins, of which umckalin and 5,6,7-methoxycoumarin are considered to be useful marker compounds (Kayser & Kolodziej, 1994, 1995, 1997; Kolodziej & Kayser, 1998; Kayser et al., 2001). The herb also contains gallic acids and methyl esters of gallic acids, as well as flavonoids (quercetin), flavan-3-ols (catechin, gallocatechin), and phytosterols (sitosterol-3-gluoside). Above-ground parts contain a wider diversity of phenolic compounds but no coumarins. Mono-terpenoids, such as isomenthone (common in *Pelargonium* species), appear to be absent. A comprehensive study of all the chemical constituents of both underground and aerial parts of *P. sidoides* and *P. reniforme* has been carried out by Kolodziej (2007).



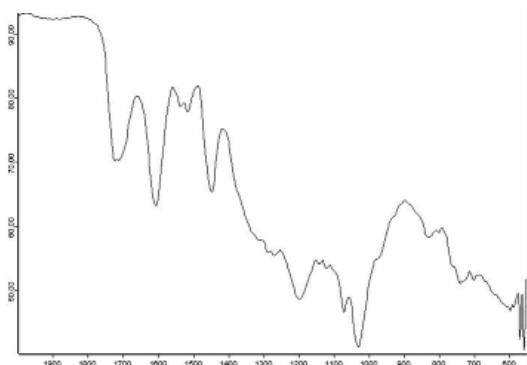
QUALITY CONTROL

TLC / HPLC / GC: General TLC systems for coumarins can be used. No specialized systems for HPLC analysis appear to have been published.



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

NIR Spectroscopy



Adulterants and Adulterations: The dried product may be adulterated with the very similar-looking *P. reniforme*. Morphological distinction of the dried product is extremely difficult, so that

chemical analysis is the only reliable method. Whereas *P. sidoides* contains umckalin and its 7-O-methylether (= 5,6,7-trimethoxycoumarin) as major constituents, these are characteristically low or absent in *P. reniforme*.

Standard Preparations: Various products in solid and liquid forms.

Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

Evidence for immune stimulation (Kayser et al., 2001), NO induction, and antibiotic effects of the proprietary substance Umckaloabo have been published (Kayser & Kolodziej, 1997; Kolodziej et al., 2003). Recent studies showed significant effects on nasal epithelial cells (Neugebauer et al., 2005) and against mycobacteria (Seidel & Taylor, 2004). The antibacterial and antiviral effects are attributed to gallic acids and other phenolic compounds, whereas the immunomodulatory activity is considered to be due to a combination of phenolic compounds and the numerous coumarins (umckalin and derivatives). For a comprehensive review of the known biological activities of *P. sidoides* see Brendler and Van Wyk (2008).

Clinical Studies: A total of 18 clinical trials have thus far been conducted, several of which were randomized, double-blind and placebo-controlled. EPs® 7630, an extract of *P. sidoides*, has been shown to effectively shorten the severity and duration of acute bronchitis and tonsillopharyngitis, most notably in children. Several other randomized, double-blind, placebo-controlled studies of special extracts on children and adults have followed (Bereznoy et al., 2003; Haidvogl et al., 1996; Matthys et al., 2003). For a review of the clinical evidence see Agbabiaka et al. (2008) and Brendler and Van Wyk (2008).

SAFETY DATA

Preclinical Safety Data

Single Dose Toxicity cf. appendix 1

Overall safety and a very low incidence of side effects have been confirmed (Conrad et al., 2007). An unpublished brine shrimp test indicated complete safety.

Clinical Safety Data: The observational study carried out by Haidvogl et al. (1996) as well as other clinical trials indicate safety (very low incidence of side effects).

KEY (PROPOSED) USAGE

Therapeutic Indications: Acute bronchitis in children and adults.

Dosage, Method, and Duration of Administration:

Ethanol extracts are used in a proprietary herbal tincture known as Umkaloabo. The recommended dose of EPs® 7630, a root extract from *P. sidoides*, for adults and children over the age of 12 years, is 30 drops (1.5 ml) three times per day for 7 days. Children aged 6 - 12 years may take 20 drops (1.0 ml) three times per day. Infusions or decoctions are traditionally used, but dosage information on the crude herb is not available.

Contraindications: A theoretical risk of interactions with anticoagulants and anti-platelet drugs could not be confirmed.

Special Warnings and Precautions for Use: Can be safely consumed when used appropriately. A total of 34 case reports of allergic (hypersensitivity) reactions have been recorded through the WHO's pharmacovigilance programme, which may be associated with the use of *Pelargonium* extract, all originating from Germany (De Boer et al., 2007).

Pregnancy and Lactation: The extract of *P. sidoides* root (EPs® 7630) is contraindicated during pregnancy and lactation, as no specific data on its effect on pregnant or lactating women are available.

Evaluation of Efficacy: Acute bronchitis in children and adults: efficacy clinically proven (special extract) (Brendler & Van Wyk, 2008).

TRADE INFORMATION

Nature of plant material:

Conservation status: Not listed.

Origin: Eastern Cape Province.

Most of the material is still wildcrafted, but crop

development has progressed to a point where significant quantities of raw material will soon be produced from cultivated plants.

The plant flowers over a long period during the summer months. Harvesting usually takes place after the end of the growing season.

Processing and storage: The tuberous rhizomes are simply sliced and dried. Rapid kiln drying yields a better-quality product.

Stability of product: Unknown.

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Prunus africana

GENERAL DESCRIPTION

Scientific Name with Author: *Prunus africana* (Hook. f.) Kalkman

Synonyms: *Pygeum africanum* Hook. f.

Family: Rosaceae

Vernacular Names: African plum tree, African prune tree, alumty, armaatet, chati, iluo, inkhokhokho, inyangazoma-elimnyama, gwaami, kiburabura, kirah, kondekonde, lemalan, ligambo, mdundulu, mfila, migambo, mconde-konde, mpembaati, mueri, mufubia, muiru, murugutu, mutimailu, mweria, mwiluti, mwiritsa, Natal tree, nuwehout, ol-koijuk, olkonjuku, oromoti, prunier d'afrique, red stinkwood, rooistinkhout, tenduet, tendwet, twendet, umdumizulu, umkakase, umkhakhazi, umlalume, vla, wotangue.

Botanical Description: An evergreen tree, usually 10 - 25 m high, with straight, cylindrical trunk and dense, rounded crown. Leaves alternate, 8 - 12 cm long, long-stalked, simple, elliptic, bluntly pointed at apex, with shallow crenate margins; leathery, deep green, and glossy, with midrib sharply impressed or channeled on upper surface and strongly prominent on underside; smell of almonds when bruised. Leafstalks and young branchlets often reddish. Flowers small, white or cream, fragrant, in axillary racemes 3 - 8 cm long; corolla lobes up to 2 mm long. Fruits cherry-shaped, red to purplish-brown, 8 - 12 mm in diameter; very bitter flesh and bony stone. Wood pale red, with strong cyanide smell when freshly cut, darkening to rich dark red or mahogany-brown on exposure to air; straight grained and even textured, strong and elastic, very hard, and very heavy.

Origin and Distribution: The tree occurs in tropical and subtropical parts of Africa including Angola, Burundi, Cameroon, Ethiopia, Ghana, Guinea, Kenya, Madagascar, Malawi, Mozambique, Principe, Republic of Congo, Rwanda, Sao Tome, South Africa, Sudan, Swaziland, Uganda, United Republic of Tanzania, Zambia, and Zimbabwe.



Plant Part Used: The bark is used to produce medicinal extracts commonly known as pygeum extract.



ETHNOBOTANICAL INFORMATION

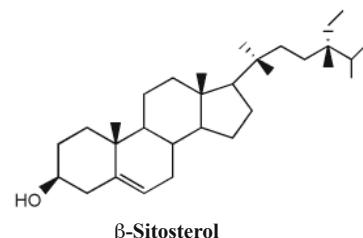
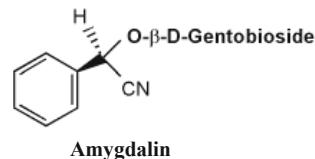
Major Ethnopharmacological Uses: Aphrodisiac, bladder disorders, impotence, inflammation, prostate cancer, prostatic adenoma, prostatitis, sexual performance.

Prunus africana is a traditional medicine in Africa used to treat chest pain, malaria, and fevers (Cunningham & Mbenkum, 1993). The bark was traditionally powdered and drunk as tea for genitourinary complaints, allergies, inflammation, and kidney disease. The bark extracts are used to make capsules for benign prostatic hyperplasia and propecia for male pattern baldness.

Other Relevant Uses: The wood is used for construction poles and firewood.

CHEMICAL CONSTITUENTS

Compounds: Alkanes and fatty acids: N-hentriaccontane, N-nonacosane, tetracosan-1-ol, N-triacontane, arachidic acid. Other major constituents include alkanols: tetracosanol (0.5%) and trans-ferulic acid esters of docosanol and tetracosanol; fatty acids (62.3%): comprising myristic, palmitic, linoleic, oleic, stearic, arachidic, behenic, and lignoceric acids); sterols: docosanol (0.6%), β -sitosterol (15.7%), campesterol, daucosterol, β -sitosterone (2.0%), 3 β -sitosteryl glucoside, stigmast-4-en-3-one, and daucosterol; triterpenes: colosolic acid, corsolic acid, malinic acid, ursolic acid (2.9%), friedelin (1.4%), 2 α -hydroxyursolic acid (0.5%), epimaslinic acid (0.8%), maslinic acid and oleanolic acid, oleanolic-3-acetate, 2 α ,3 α -dihydroxyurs-12-en-28-oic acid, 2 α ,3 β -dihydroxyurs-12-en-28-oic acid, 2 α ,3 β -dihydroxyolean-12-en-28-oic acid, 3 β ,24-dihydroxyurs-12-en-28-oic acid, 2 α ,3 α ,23-trihydroxyurs-12-en-28-oic acid, 2 α ,3 α ,24-trihydroxyurs-12-en-28-oic acid, 24-O-trans-ferulyl-3 α -hydroxy-urs-12-en-28-oic acid, 24-O-cis-ferulyl-hydroxy-urs-12-en-28-oic acid; andphenolics:catechin,epicatechin,5-procyanidin, docosyl ferulate, N-trans-tetracosyl ferulate, and atraric acid.



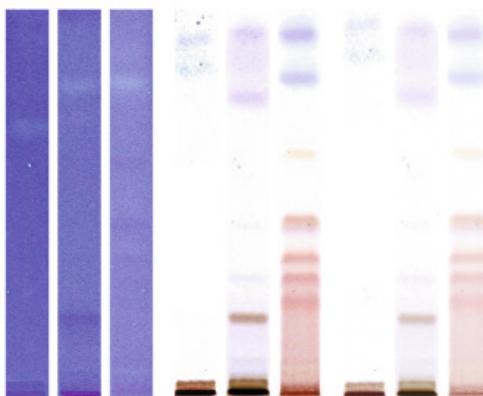
QUALITY CONTROL

Identification: Reddish powdered bark.

Organoleptic Properties: Strong, characteristic almond-smell.

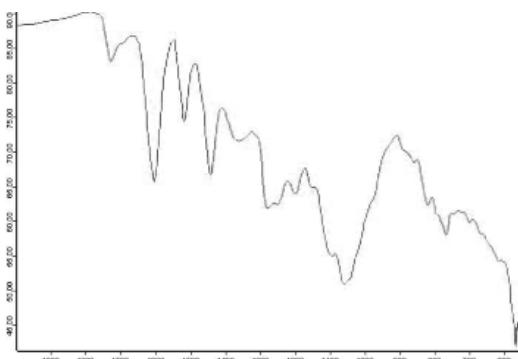
Macroscopic Characteristics: The bark is red to blackish-brown, deeply square-fissured or corrugated. Bark pieces consist of long fragments of variable dimensions, from only a few cm to 1 m long, with a thickness varying from a few mm to 1-2 cm. The color is brown, more or less dark on the external surface; light brown to red-brown on the internal surface. The transverse section of the bark consists of multiple layers of small, square cells with the walls of moderate thickness. It presents a cortical parenchyma of more or less round cells, with a few apparent formations of very thin-walled sclereids. Often, in the parenchyma, there are groups of cells containing oxalate crystal druses; a few bigger cells with highly thickened walls can also be observed. It shows a liber with phloem zones and presenting medullary rays. The phloem portions contain groups of fibrous cells with highly thickened walls as well as phloem and parenchymal elements, sometimes containing druses of oxalate. The medullary rays are of conical shape; larger on the external surface and thinner on the internal side, they can also contain druses of oxalate.

TLC / HPLC / GC



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

NIR Spectroscopy



Standard Preparations: There are a number of herbal products in the European and North American market based on *Pygeum* either alone or in combination with Saw Palmetto and Pumpkin seed, which are also used as a treatment of BPH.

Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

Multiple mechanisms have been proposed for the genitourinary effects of Prunus, including 5α -reductase inhibition, estrogenic effects, and anti-inflammatory properties.

Genito-urinary effects: *P. africana* extract appears to inhibit prostatic 5α -reductase in rats and aromatase from human placenta cells (Hartmann et al., 1996), but only slightly inhibits

5α -reductase from human prostate compared to finasteride (Rhodes et al., 1993). Reduction of urethral obstruction and improvement of bladder function have been observed. Most studies of *P. africana* extract have addressed outcomes related to the obstructive component (Levin et al., 1996, 2000, 2002; Gomes et al., 2000; Choo et al., 2000; Yoshimura et al., 2003). In rats and men, lipophilic extracts have been found to stimulate secretory activity of the prostate and seminal vesicles (Thieblot et al., 1971, 1977; Clavert et al., 1986; Doremieux et al., 1973).

Anti-inflammatory effects: In vitro studies report *P. africana* extract to inhibit production of 5-lipoxygenase metabolites (Paubert-Braquet et al., 1994a, 1994b).

Clinical Studies: Efficacy and safety of *Prunus* extracts in the treatment of mild to moderate BPH has been confirmed in numerous clinical trials (Barlet et al., 1990; Bassi et al., 1987; Bongi, 1972; Chatelain et al., 1999; Doremieux et al., 1973; Dufour et al., 1984; Dutkiewicz, 1996; Mathe et al., 1995; Rizzo et al., 1985) and systematic reviews (Ishani et al., 2000; Bombardelli & Morazzoni, 1997; Andro & Riffaud, 1995; McQueen & Bryant, 2001).

SAFETY DATA

Preclinical Safety Data

Single Dose Toxicity cf. appendix 1

Single doses up to 8 g/kg BW were well tolerated in rats and mice (Andro & Riffaud, 1995).

Repeated Dose Toxicity: Short and long-term administration (1 - 6 months) in dogs, mice and rats caused no adverse effects or pathological parameters (Andro & Riffaud, 1995). Only at doses of 3.3 g/kg BW in rats the extract showed signs of marked toxicity after 6 days of administration (Gathumbi et al., 2000, 2002).

KEY (PROPOSED) USAGE

Therapeutic Indications: Treatment of lower urinary tract symptoms of benign prostatic hyperplasia (BPH) stages I and II, as defined by Aiken (e.g., nocturia, polyuria, and urinary

retention), where diagnosis of prostate cancer is negative.

Dosage, Method, and Duration of Administration: Oral administration, 75 - 200mg lipidostericolic extract of the crude drug, in divided doses (Andro & Riffaud, 1995; Bombardelli & Morazzoni, 1997; Ishani et al., 2000; McQueen & Bryant, 2001). Long-term administration may be advisable.

Contraindications: None known.

Interactions: None reported.

Pregnancy and Lactation: Not applicable.

Overdose: No toxic effects reported in human use.

Evaluation of Efficacy: Clinically proven efficacy.

TRADE INFORMATION

Volume of production in the country: The estimated world trade in *Prunus africana* bark either in dried bark or extract form is between 3,500 - 5,000 tonnes per year. Seventy-two percent comes from Cameroon, 18% from Madagascar and the remainder from Kenya, Tanzania and Uganda (Pomatto 2001).

Nature of plant material: *Prunus africana* has been listed in Appendix II of CITES as of March 1995.

Nature of plant products: The drug consists of the dried bark of the trunk of *Prunus africana*. Wild-harvesting of bark is very destructive and some attempts have been made to establish plantations for sustainable bark production.

Processing and storage: Fresh bark is harvested, dried, crushed, and extracted so as to obtain the drug. Bark harvesting is done throughout the year and approximately 5 kgs of extract are produced from one tonne of air-dried bark. The best quality bark extract can contain as much as 25% phytosterols. Most finished products are usually standardised to around 12% - 13% phytosterol content.

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Rauvolfia vomitoria

GENERAL DESCRIPTION

Scientific Name with Author: *Rauvolfia vomitoria* Afzel.

Synonyms: *Rauvolfia congolana* De Wild & T. Durand, *Rauvolfia senegambiae* A.DC., *Rauvolfia stuhlmannii* K.Schum.

Family: Apocynaceae

Vernacular Names: African *Rauwolfia*, African serpent-wood, akakapenpen, apoto, apototso, asofeyeje, bakapembene; blonyatsho, dodemakpowoe, kakapenpen, nsusua-dua, swizzle-stick tree, wada.

Botanical Description: Small tree up to 10 m in height. The bark is grey-brown and exudes a white, bitter latex. The branches are whorled, nodes enlarged and lumpy, long and thin, and covered in white lenticles. The leaves have 1 - 2 cm petioles and are approximately 7 - 24 x 3 - 10 cm, in whorls of three to five, elliptic to slightly oblanceolate, acuminate, with narrow, triangular tips; 8 to 16 paired lateral veins. The central peduncle is up to 7.5 cm long. The flowers have five very small cream-white sepals up to 10 mm long, 6 - 8 mm of which make up the tube. The drupe-like fruit is a 7 - 10 mm thick roundish berry, solitary or in pairs, and reddish in colour.

Origin and Distribution: Tropical West Africa from Senegal to northern Nigeria; also central Africa, Mozambique and East Africa. Occurs naturally in gallery forests but is mostly found in forest regrowth where fallow periods are prolonged; widely cultivated.



Plant Parts Used: The drug is extracted mainly from the roots, but also from the leaves, bark, and branches of *R. vomitoria*.



ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses: This tree is used medicinally in many African countries. As indicated by the name, the plant is an emetic. On the Ivory Coast, the juice of the crushed root is used together with other plants to treat epilepsy. Women drink the macerate of the root as a remedy for blennorrhoea. The fresh pounded root together with lime juice and kaolin is said to be a remedy for scaly eczema.

Other uses in tropical Africa are haemorrhoids, enlargement of the liver, as a sedative and sleeping agent, and for fever; in the form of a bark decoction for body parasites. In Ghana, Cameroon, and Senegal the root bark is used as a strong laxative,

emetic, and diuretic. A Ghanaian anti-arthritis herbal preparation contains *R. vomitoria*.

In West Africa, the bark is a remedy for fever, digestive problems, and scabies; the root is used as a tonic and purgative; the leaves as an emetic; the milky latex for colic and diarrhoea. In Nigeria, an enema of the root is administered for gonorrhoea. Nigerian traditional healers also use it to treat psychiatric patients. The dried root of *R. vomitoria* showed antipsychotic effects.

A widely used arrowhead poison is prepared from the plant. *R. vomitoria* is used to treat leprosy in the Democratic Republic of Congo.

Other Relevant Uses: *R. vomitoria* bark yields a good bast fibre. It also yields a white and finely grained wood, which reddens with time. The bark yields a yellow dye. In Gabon, the bark and root powder is used as an insecticide and vermicide. The root bark is reported to be poisonous. The seeds are used in making decorative necklaces.

CHEMICAL CONSTITUENTS

Compounds: The main biologically active compounds are considered to be alkaloids. The best known are reserpine and ajmaline, which have been known since the 1950s and are used in western medicine. Root, leaf, branch tips, and stem bark all contain alkaloids; the composition of the alkaloids in the aerial parts differs from that of roots. The total alkaloid content of the leaves is 0.03% - 0.8%, that of the trunk bark is approximately 0.6%, that of the roots 0.15% - 0.20%, and that of the root bark 1.5% - 2.0 % (roots from Zaire contain 7 - 18 times as many alkaloids as the root wood).

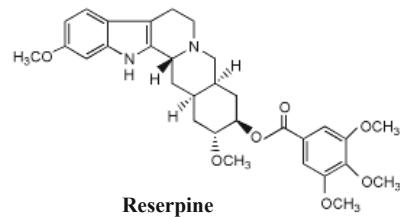
To date, 43 alkaloids have been identified in the trunk bark, 44 in the root, 19 in the leaves and 4 in the fruit. The main alkaloids in the leaves are rauvoxin and rauvoxinin. In root and trunk bark, alkaloids of the heteroyohimbin type (especially reserpine) and the dihydroindol type are dominant, while the leaves mainly contain oxindole alkaloids (carapanaubine) and heteroyohimbine alkaloids (aricin and isoreserpilin). Dihydroxyindole type alkaloids are absent from the leaves. The leaves contain geissoschizol,

geissoschicine, desacetyldesformoakuammilin, desacetyldesformopicralin (= picrinin), and peraksin. A leaf sample from Ghana contained carapanaubine (0.012%), aricin (0.0063%), isoreserpine (0.0031%), rauvoxin (0.0025%), tetrahydroalstonin (0.0017%), and reserpilin (0.0015%).

The following alkaloids have been identified from the root bark: reserpilin (1.09%), reserpin (0.218%), rescinnamin (0.105%), ajmalin (0.09%), serpentin (0.018%), tetraphyllin (0.012%), norajmaline, ajmalidin and neoajmaline (0.010%), neonorreserpine, ajmalinol, serpenticin, reserpinic acid, aricine, carapanaubine, carapanaubine-Nb-oxide, rauvoxin, suaveoline, vellosimine, and 3-epi-rescinnamin and 3,4-dimethoxybenzoylreserpinic acid methyl ester.

The trunk of the bark contains: reserpilin (0.04%); isoreserpilin (0.006%); and 18-hydroxyyohimbin, yohimbin, purpelin, norpurpelin, norseradamine, nortetraphyllicine, carapanaubine, indolenine-RG (all between 0.001% and 0.002%).

Non-alkaloid compounds have been reported, including kaempferol glycosides, the 3-rhamnoglucoside of nicotiflorin, 3-glucoside (astragalin), free ursolic acid, monocyclic terpene alcohol (vomifoliol), serposterol (= 28-hydroxystigmasterol), gallic acid, and flavonoids (kaempferol and quercetin). Tetrahydroalstonin, rauvominin, yohimbin, and the ester ajmaline-17-O-trimethoxybenzoic acid (= willicourtin) have been isolated from unripe fruit. No alkaloids were found in the ripe fruit.



QUALITY CONTROL

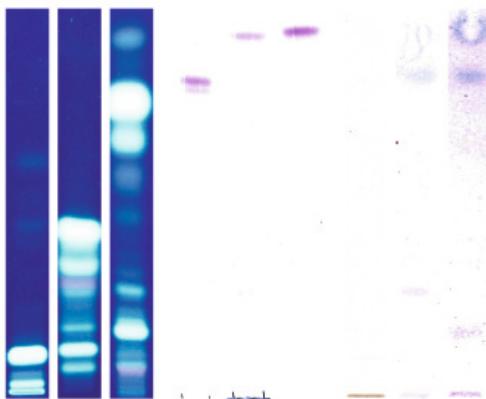
Organoleptic Properties: Bitter taste, slight odour.

Macroscopic Characteristics: Cylindrical root, occasionally branched, light yellow outer surface, short and splintery fracture, cream-coloured wood, and thin bark (Ghana Herbal Pharmacopoeia, 1992).

Microscopic Characteristics: Root: Transverse section with thin bark of stratified cork, compressed and alternate with larger lignified cells, cork cambium; secondary cortex with parenchyma cells with starch grains and interrupted with lignified sclereids; phloem with sieve elements interspersed with medullary ray cells; xylem made up of vessels, tracheids, and parenchyma, lignified.

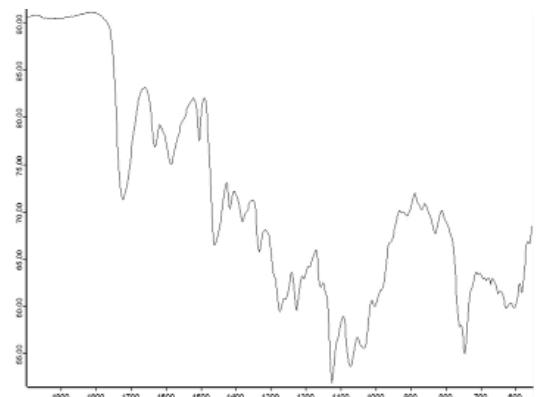
Powder: Starch grains, abundant lignified fibres, tracheids, parenchymatous cells with prismatic calcium oxalate crystals, and lignified cork (Ghana Herbal Pharmacopoeia, 1992).

TLC / HPLC / GC



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

NIR Spectroscopy



Purity Tests / Requirements: Loss on drying not less than 12% at 100 °C, reserpine-like alkaloids not less than 0.2% (Ghana Herbal Pharmacopoeia, 1992).

Adulterants and Adulterations: Other *Rauvolfia* spp.

Standard Preparations: Aqueous decoctions, alcoholic extracts.

Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

Along with several central and peripheral effects, reserpine is extremely sedative and hypertensive. The central sedative effect of reserpine comes through the blocking of the Mg²⁺-dependent, ATP-ase controlled absorption mechanism of the catecholamine, in the central and peripheral neuronal storage vesicles. This is due to a depletion of the neurotransmitters noradrenaline, serotonin, and dopamine, or the emptying of the storage vesicles, leads to sedation in higher doses to neuroleptic effects and in toxic doses to severe mental depression.

Its efficacy for schizophrenia is based on the breakdown of excess dopamine in the synapses. The peripheral main effect of reserpine is to decrease blood pressure, which is particularly severe in hypertensive people. This effect is caused by a lowering of the sympathetic tone and of the peripheral vessel resistance, in combination with a depletion of catecholamine, in turn leading to vasal dilation, sinus bradycardia, and a lowering of cardiac output (1 per min).

As a hypertonic agent it is also only used in lower doses or in combination with other antihypertonic agents (e.g. ajmalicine). The rescinnamine isolated from the plant acts qualitatively, like reserpine, where the degree of effect and the side-effects are milder, and toxicity is markedly less. Ajmaline differs significantly in its main mechanism of action from reserpine. It is neither sedative nor does it inhibit catecholamine content in the CNS and atrium. The substance is a classic anti-arrhythmic agent with effects similar to quinidine: negatively bathmotropic, dromotropic, inotropic, and weakly negatively chronotropic, whereby the lowering of the stimulation conduction is twice as strong as for quinidine. The negative bathmotropic effect is also seen in the peripheral and central nerve plexi and on smooth muscle. Ajmaline is a potent sodium antagonist, while at the same time having an inhibitory effect on potassium outflow, which leads to a consecutive lengthening of the action potential. Therefore, the substance is used mainly for tachycardic arrhythmia, extra systolia, and cardiac functional disturbances caused by conduction defects (e.g. WPW syndrome).

The reserpilin isolated from the plant is markedly sympatholytic and hypotensive, but without having the centrally depressive or sedative effects. In contrast to reserpin and rescinnamin it causes no other side-effects such as gastric ulcers, diarrhoea, etc. Ajmalicin (raubasin) is a short-acting hypotensive and adrenolytic agent which does not have a tranquilizing effect in therapeutic doses. It increases the peripheral circulation above cardiac volume and is mainly used today for cerebral and peripheral circulation, usually in combination with dihydroergocristin. It increases the effect of reserpine when used in combination.

Sarpagin is somewhat less hypotensive than reserpine, mainly adrenolytic, barely sympatholytic, and only sedative in high doses. Rauvanin is much less toxic than reserpine, mildly sedative and weakly hypotensive. Raumitorin is hypotensive and is similar in its toxicity to rauvanin; its sedative effect is similar to that of reserpine. Serpentine and serpentinin display hypotensive, but not sedative, adrenolytic, or ganglia-blocking properties. Serpentin is more negatively chronotropic than ajmaline. Aricine is negatively chronotropic and renoxidine is hypotensive and sedative (half the efficacy of reserpine). Yohimbin

and α -yohimbin (rauwolscine) are briefly adrenolytic and hypotensive. The substances are α -adrenoreceptor antagonists selective for presynaptic α -adrenoreceptors in vivo and in vitro. Methylreserpat displays similarities in effect to reserpine, but on the contrary, it in fact increases blood pressures. Alstonin and its C20-epimer serpentine are strong cholinesterase inhibitors, and in rats, in vivo, showed approximately one-third of the effect of physostigmin. Normacusine B (tombozin) is sedative for mice at a dose of 20 - 50 mg per kg. A dose of 0.05 to 5 mg per kg lowers blood pressure in dogs and cats (is not blocked by 1 mg per kg atropine), where as a dose of 20 mg per kg caused ganglia-blocking and cardiodepression similar to papaverine. Carapanaubine causes a severe and lasting drop in blood pressure.

Clinical Studies: Efficacy has not yet been proven using the valid criteria for clinical trials. Prostabel® is a herbal supplement comprised of *Rauvolfia vomitoria* and *Geissospermum velosii* extracts (Natural Source, International, Ltd). A clinical trial is under way to assess the safety and tolerability of Prostabel® when administered to men with elevated PSA and a negative prostate biopsy.

SAFETY DATA

Preclinical Safety Data: Experiments with dogs using a water-soluble root bark extract in low doses produced tachypnoea; in medium doses tachypnoea followed by bradypnoea; in high doses increased bradypnoea with death from respiratory and cardiac arrest. An LD₅₀ of 0.5 mg/kg dog IV was determined. The LD₅₀ of a reserpine-free extract was 14 mg/kg mouse ip; the LD₁₀₀ for guinea-pigs was calculated as 17 mg/kg IV.

In therapeutic doses, ajmaline displayed barely any adverse effects, whereas toxic or lethal doses caused extreme reductions in blood pressure and convulsions. At a dose of 8.1 mg/kg cat IV, 75% of the animals died within 1 - 5 minutes, following a severe drop in blood pressure and pulse. Like quinidine, ajmaline acts as an Na⁺ antagonist in cardiac-cell membranes, and has a very strong inhibitory effect on the rapid Na⁺ inflow at rest and in action. After simultaneous administration of quabain and reserpine, reserpine increased

the quabain-induced cardiac arrhythmia, as well as markedly increasing toxicity: whereas 50% of dogs died after a single dose of 0.1 mg/kg IV, when this was combined with reserpine, 90% died.

Single Dose Toxicity cf. appendix 1

Clinical Safety Data: In humans, long-term administration of high doses of reserpine causes extreme neuroleptic effects and severe mental depression. The dopamine deficiency caused in particular by long-term use leads to anxiety states and danger of suicide. In cases of overdose, the dopamine deficiency at the dopaminergic neurons of the cerebrum is so extreme that it causes parkinsonism. On the other hand, the cholinergic system remains unaffected by reserpine, and because the sympathetic tone is lowered by reserpine, there are, as well as central effects, dose-dependent, strong peripheral side effects (saliva flow, increased intestinal motility, stomach cramps, diarrhoea, ulcer formation, bradycardia, miosis, ptosis, swelling of the nasal mucous membrane (rhinitis serpentina), reduction in kidney performance, and orthostatic complaints up to and including collapse and loss of consciousness as a result of extreme drops in blood pressure). In lethal doses, initial ataxia is followed by tremor, convulsions, and death from respiratory failure. At the beginning of the 1970s, fears were expressed that *Rauvolfia* alkaloids and reserpine increased the risk of breast cancer in women over 50. These fears have, however, not been confirmed in long-term studies with over 2000 at-risk subjects.

KEY (PROPOSED) USAGE

Therapeutic Indications: *Rauvolfia* is currently used as a treatment for mild essential hypertension, usually in association with a diuretic. It is also recommended for nervous and mental disorders. Experimental pharmacology has shown it to be an effective treatment for high blood pressure and anxiety. The root has a pronounced sedative and depressant effect on the sympathetic nervous system. It may also be used to treat anxiety and insomnia, as well as more serious mental health problems such as psychosis.

Internal Use: Sedative and tranquilliser, hypotensive and antiarrhythmic. External Use: Parasitic skin diseases.

Effects on Ability to Drive and Use Machines:
May impair reactions.

Interactions: Combination with alcohol may increase the impairment of reactions; combination with neuroleptics and/or barbiturates may increase their effect; bradycardia may occur when combined with digitalis glycosides.

Pregnancy and Lactation: Not to be used during pregnancy and lactation.

Adverse Effects: Nasal congestion, drowsiness, depression, erectile dysfunction.

TRADE INFORMATION

Nature of plant material: Most of world demand is presently met from wild sources. Its demand in allopathic medicine, however, has receded due to increased use of ajmalicine alkaloid from *Catharanthus roseus*. *Rauvolfia* has become an endangered species and was included in CITES Appendix 2 in January 1990. Attempts are being made to preserve the existing germplasm and develop agroforestry plantation of *Rauvolfia*. There is a considerable danger that *Rauvolfia vomitoria* will become extinct. There has been indiscriminate exploitation in countries such as Congo (Zaire), Rwanda and Mozambique from where the root bark is exported to Europe.

Nature of plant products: Mostly crude, cylindrical root pieces up to 14 cm long.

Processing and storage: Roots for medicinal use may be harvested non-destructively, annually, by cutting them 10 cm from the taproot.

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Ravenala madagascariensis

GENERAL DESCRIPTION

Scientific Name with Author: *Ravenala madagascariensis* J.F.Gmel.

Family: Strelitziaceae

Vernacular Names: Akondrohazo, antandro, antrandra, antsandra, arbre du voyageur, bakabia, barabia, bemavo, falafa, fitroka, fontsimavo, fontsy, fony, hazobaka, hirina, hisatr'atandro, hodokembo, honkona, honkondambo, hovitra, iripafa, kasaka, konko, lamaka, lovia-ravimpotsy, mahabevola, mandravalana, ontsy, rapaka, ravenale, ravimafy, ravimboafotsy, ravimpotsy, ravina, ravinala, ravinamafy, voadora, voafontsy.

Botanical Description: Plant reaching between 15-20 m in height and having a fibrous trunk. The leaves are large and distichal, arranged in the shape of a fan. The inflorescence is axillary with imbricate bracts. The flowers are white, 20 - 30 cm long. The capsules are dehiscent with 3 valves, bearing numerous seeds, and covered with a blue aril.

Origin and Distribution: Originating from Madagascar, this plant has been introduced and naturalized in the Mascarenes. It is commonly found in Reunion Island and on Mauritius where it has become an invasive plant. It is occasionally found on Rodrigues.



Plant Part Used: Young leaves



ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses: In African countries where this plant grows, the plant is particularly known for the potable water found stored in the leaves (Raponda-Walker & Sillans, 1961).

In Mauritius, a decoction of the leaves is taken orally at night against diabetes (Sussman 1980; Adjanohoun et al., 1983; Gurib-Fakim et al., 1997; Gurib-Fakim & Brendler 2004). In Madagascar, the blue aril surrounding the seeds contains a pigment rich in fatty acids with antiseptic properties (Pernet, 1957). In Rodrigues, a decoction of the young leaves is administered orally against diabetes and diarrhoea. A tea made from the young leaves or heart yields a refreshing drink (Gurib-Fakim et al., 1994).

In Madagascar, Boiteau (1999) reported that the pulp surrounding the seeds is used to prepare

'Odihozona'. The latter is prepared by crushing a fresh water mollusc with the leaves of *Ravenala*, which is then used to strengthen the gum and hence prevent the premature loss of teeth. Descheemaeker (1990) reported that an infusion is made with a handful of the leaves. This infusion drunk at the rate of a cup 3 times daily is useful against diabetes and hypertension. The leaf decoction is also a good diuretic (Rabesa, 1986).

Rakotobe (1993) reported on the use of the leaf decoction as a means of treating loss of albumin in the urine and also to halt the yellowing of the eyes. The dried leaves can also be smoked in the same manner as a cigarette. Pere Rimbault used the leaf petiole to treat localised oedemas; 60-70 g of the leaf petiole (otherwise known as 'Cotton') is boiled in 1 L of water. This decoction is then used during the day, as well as subsequent days. After a week or two, the oedema was reported to have disappeared, especially if the diet has been poor in salt and alcohol.

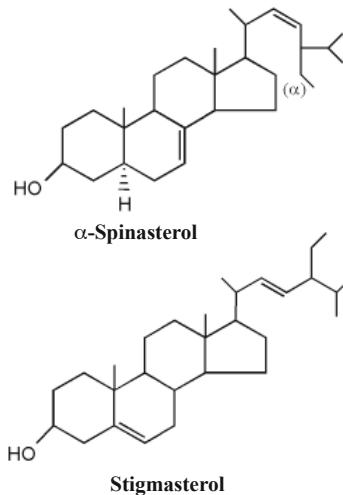
CHEMICAL CONSTITUENTS

Compounds: Preliminary phytochemical screening of the young leaves has yielded phenols, tannins, saponins, leucoanthocyanins, flavonoids, sterols, terpenes, and traces of alkaloids (Gurib-Fakim et al., 1997, 1999).

Oils extracted from various parts (seed and aril) of *R. madagascariensis* were studied for their fatty acid and sterol contents. Oil contents of seed and aril are 4.1% and 68.7%, respectively.

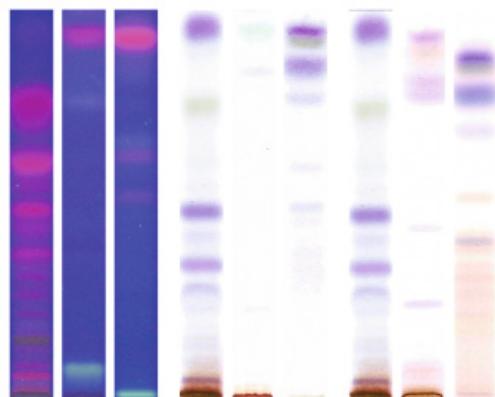
These oils, partially solidified, may be used as edible food products. These oils contain rather high levels of oleic (39%) and palmitic (34 - 42%) acids. Sterol fraction analysis of seed oil reveals 7 sterols, mainly β -sitosterol [83-46-5] (65%). Sterol fraction of aril oil reveals 12 sterols, mainly stigmasterol [83-48-7] (18%), 24-methyl-5- α -cholest-7-en-3- β -ol [4198-70-3] (16%), α -spinasterol [481-18-5] (28%), and δ 7-avenasterol [23290-26-8] (19%).

R. madagascariensis oils have a fatty acid composition that is intermediary between the palm oil and cocoa butter. They form a possible new source of vegetable butter (Rabarisoa et al., 1981).



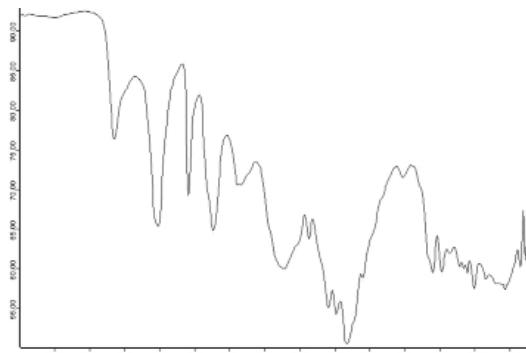
QUALITY CONTROL

TLC / HPLC / GC



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

NIR Spectroscopy



Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

The antiseptic properties may be attributed to the presence of the tannins and flavonoids found in the plant.

SAFETY DATA

Ethnic Use Safety Data: Tablets and bark extracts have been used for many years with no reported side effects.

Preclinical Safety Data

Single Dose Toxicity cf. appendix 1

KEY (PROPOSED) USAGE

Therapeutic Indications: Antiseptic, antibacterial.

Pregnancy and Lactation: The product should not be used during pregnancy or while breast-feeding.

Evaluation of Efficacy: Antibacterial efficacy pharmacologically proven. Anticandida efficacy traditionally proven. Anti-ulcer: insufficient information for classification. General tonic: insufficient information for classification.

TRADE INFORMATION

Nature of plant material: Cultivated/wildcrafted: Wildcrafted

Conservation status: Not endangered.

Processing and storage: The leaves are air dried, then dried to constant weight in an oven at a temperature not exceeding 30C.

The dried leaves are likely to be stable for considerable periods.

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Sceletium tortuosum

GENERAL DESCRIPTION

Scientific Name with Author: *Sceletium tortuosum* (L.) N.E.Br.

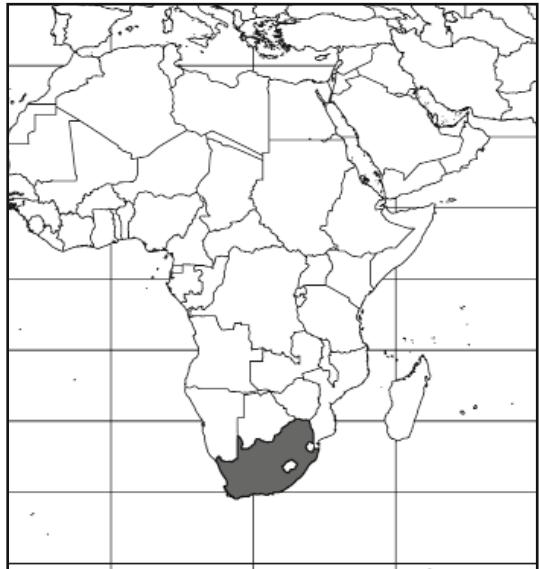
Synonyms: *S. boreale* L.Bol., *S. compactum* L.Bol., *S. concavum* (Haw.) Schwantes, *S. framesii* L.Bol., *S. gracile* L.Bol., *S. joubertii* L.Bol., *S. namaquense* L.Bol. (both varieties), *S. ovatum* L.Bol., *S. tugwelliae* L.Bol.

Family: Aizoaceae

Vernacular Names: Kanna, kougoed, sceletium, sceletium herb.

Botanical Description: *Sceletium tortuosum* is a short-lived, perennial, succulent plant with creeping stems and overlapping pairs of leaves that have glistening water cells (bladder cell idioblasts) on their surfaces. The leaves become “skeletonised” when they dry out – the persistent leaf veins remain on the plant – hence the generic name *Sceletium*. Pale to bright yellow or orange-yellow flowers are borne along the branch tips, followed by pale brown, papery capsules containing numerous small, reddish-brown kidney-shaped seeds (Van Wyk & Gericke, 2000; Van Wyk & Wink, 2004). There are eight species of *Sceletium*, but only *S. tortuosum* is well known and used in commercial products. It differs from other species in having straight secondary veins, prominent idioblasts, incurved leaf tips and imbricate leaves (Gerbaulet, 1996).

Origin and Distribution: Western and northern Cape Provinces of South Africa (western parts, from the Little Karoo northwards to Namaqualand) (Gerbaulet, 1996).



Plant Part Used: Dried whole plant

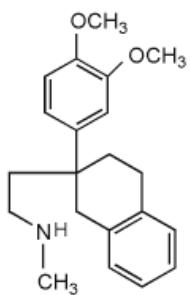
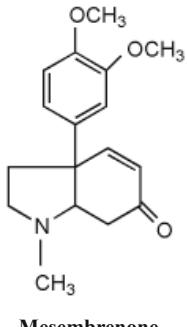
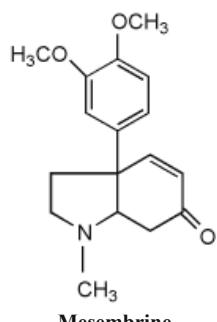


ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses: *Sceletium* elevates mood and reduces anxiety, stress, and tension. The traditionally prepared dried plant material is chewed, or smoked, or powdered and inhaled as a snuff (Watt & Breyer-Brandwijk, 1962; Watt, 1967; Rood, 1994; Forbes, 1986; Smith et al., 1996; Van Wyk & Gericke, 2000; Van Wyk & Wink, 2004). It is also taken as a tea and as a tincture (Pappe, 1868).

CHEMICAL CONSTITUENTS

Compounds: *Sceletium tortuosum* contains mesembrenine as the major alkaloid, together with mesembrenone, mesembrenol, and tortuosamine (Smith et al., 1996; Gericke & Van Wyk, 1997).

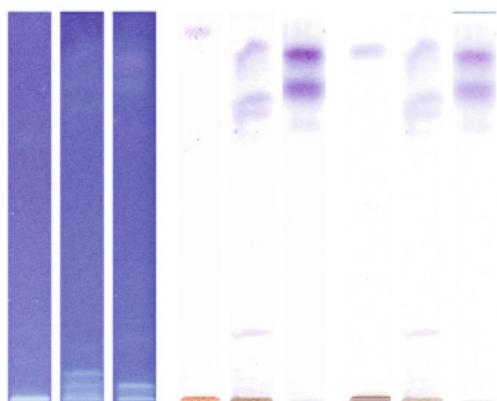


QUALITY CONTROL

Identification: Whole dried plant fibrous, with small greenish-brown leaves, having no smell.

TLC / HPLC / GC: The alkaloids are easily studied

on TLC using published methods. They are also easily studied by GC (using standard methods for alkaloids) and HPLC.



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

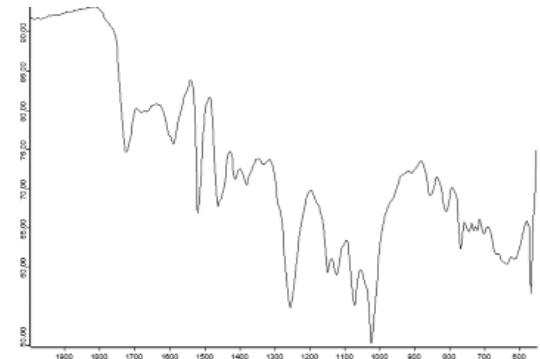
Markers and Quantitative Methods: Total alkaloid content is variable and ranges from 0.05% to 2.3%.

Commercial plant material should have a total alkaloid content not less than 0.5%. Of the alkaloids, the mesembrenine-alkaloids (mesembrenine, mesembrenone, and mesembrenol) are important active compounds.

Mesembrenine itself is present in highly variable yields of 0 - 2.4% of dry weight, depending on the source and processing of the plant material.

With high-yielding clones, the mesembrenine content of unprocessed material should be at least 0.5 - 1% dry weight (Gericke & Van Wyk, 1997).

NIR Spectroscopy



Adulterants and Adulterations: *Sceletium* may be adulterated with *Aptenia* species, or with low-yielding material of unknown provenance. TLC and GC identification and quantification is essential.

Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

Published case reports and observations by doctors, psychiatrists, and psychologists indicate clinical efficacy in treating depression, including major depressive disorder, anxiety, social phobia, and low libido. It is also used to facilitate the psychotherapeutic process and for support in alcoholism rehabilitation and smoking cessation (Gericke, 2001).

Pharmacokinetic Properties: Mesembrane is a potent serotonin-uptake inhibitor, with a novel mechanism of action for this known molecule, that suggests therapeutic applications for anxiety, depression and other serious mental health conditions (Gericke & Van Wyk, 1997).

SAFETY DATA

Ethnic Use Safety Data: The plant is not hallucinogenic, and no adverse effects have been documented. It is remarkable that there appears to be no physical or psychological dependency, even after many years of habitual use (Van Wyk & Gericke, 2000).

Preclinical Safety Data

Single Dose Toxicity cf. appendix 1

KEY (PROPOSED) USAGE

Therapeutic Indications: Anxiolytic

Dosage, Method, and Duration of Administration: In tablet form, a dose of 50 - 200 mg of the dried, powdered herb is included in tablets and capsules (about 1 - 4 mg of alkaloid) and taken two or three times a day. Traditionally, the dried product is regularly chewed throughout the day (the frequency is controlled by the slight hypnotic effect, similar to the practice of smoking tobacco). Teas, decoctions, and tinctures are also

reported to be used, but details of dosage levels are unknown and/or unpublished.

Contraindications: Contraindicated when taking any psychiatric medication, including antipsychotic drugs, monoamine oxidase inhibitors, serotonin-uptake inhibitors, and benzodiazepines.

Special Warnings and Precautions for Use: None. Can be safely consumed when used appropriately.

Interactions: None known.

Adverse Effects: Occasional side-effects include self-limiting headache, mild loose stools, mild abdominal cramping, sedation, and stimulation.

Evaluation of Efficacy: Sedative (anxiety, stress) efficacy pharmacologically proven. Antidepressant efficacy pharmacologically proven.

TRADE INFORMATION

Nature of plant material: Flowering/harvesting time: The whole plants are harvested at the end of the flowering period (spring or summer, depending on the region of cultivation; winter or summer in rainfall regions).

Cultivated/wildcrafted: Substantial quantities of raw material from cultivated sources are available. Wildcrafting is unacceptable.

Conservation status: Not threatened (but rare in nature).

Nature of plant products: Origin: South Africa.

Processing and storage: Preparation/processing: The whole plants are simply dried. A traditional method is used to prepare the plant (Smith et al., 1996), but this is not considered necessary for commercial purposes.

Stability of product: Limited data and experience. The alkaloids are fairly stable in the dry product but unstable in solution.

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Siphonochilus aethiopicus

GENERAL DESCRIPTION

Scientific Name with Author: *Siphonochilus aethiopicus* (Schweinf.) B.L.Burtt

Synonyms: *Siphonochilus natalensis* (Schltr. & K.Schum.) J.M.Wood & Franks

Family: Zingiberaceae

Vernacular Names: African ginger root, African ginger, Afrikanischer INGWER, gingembre Africaine, isiphephetho, indungulo, wild ginger, zenzero Africano.

Botanical Description: African ginger is an aromatic, deciduous plant with large, hairless leaves developing annually from a small, distinctive, cone-shaped rhizome. The spectacular pink flowers appear at ground level; they are broadly funnel-shaped, pink and white in colour, with a small, yellow blotch in the middle. Most plants are bisexual, and they have much larger flowers than female plants. The small, berry-like fruits are borne below or above the ground (Wood & Franks, 1911; Kam, 1980; Burtt, 1982; Gordon-Gray et al., 1989; Smith, 1998; Van Wyk et al., 1997; Crouch et al., 2000; Van Wyk & Gericke, 2000; Van Wyk & Wink, 2004). The species is morphologically uniform but genetically very polymorphic (Makhuvha et al., 1997).

Origin and Distribution: Widely distributed in tropical Africa, from South Africa (Limpopo Province) northwards to Zambia, Malawi, Ethiopia, West Africa, and Gambia.



Plant Parts Used: Rhizomes and fleshy roots.



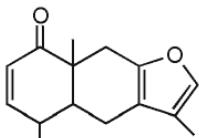
ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses: Fresh roots or rhizomes are chewed for coughs, colds, and asthma. Tablets made from dried rhizomes or extracts are highly effective against tension headache, and also against influenza, sore throat, mild asthma, sinusitis, PMS, and menstrual cramps. The main traditional uses in the Zulu

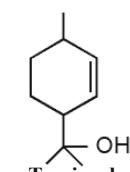
culture were against malaria and candida (Watt & Breyer-Brandwijk, 1962; Cunningham, 1988; Hutchings et al., 1996; Van Wyk et al., 1997; Van Wyk & Gericke, 2000; Crouch et al., 2000; Van Wyk & Wink, 2004).

CHEMICAL CONSTITUENTS

Compounds: The product is rich in essential oil (0.1 - 0.3% of fresh weight) and contains more than 70 monoterpenoids and sesquiterpenoids, including 1,8-cineole, cis-alloocimene, α -terpineol, and germacrene B (to name only a few) (Viljoen et al., 2002). The main furanoterpenoid, siphonochilone, was reported to represent at least 20% of total composition of the oil (Van Wyk et al., 1997; Holzapfel et al., 2002; Viljoen et al., 2002).



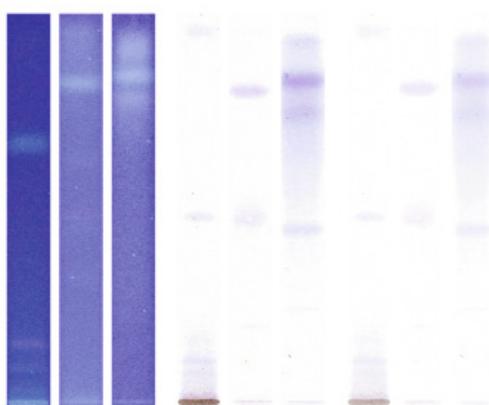
Siphonochilus sesquiterpenoid



α -Terpineol

QUALITY CONTROL

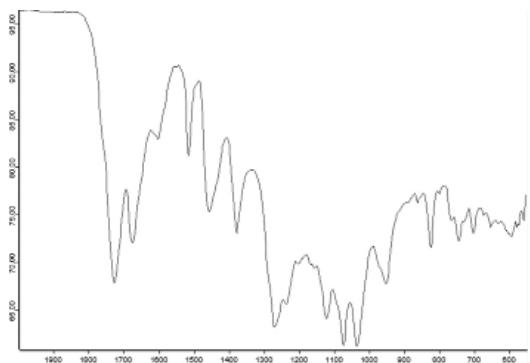
TLC / HPLC / GC: Standards methods of TLC can be used (similar to those for commercial ginger, *Zingiber officinalis* – see Van Beeck, 1991). The essential oil fraction, containing the main furanoterpenoid, can be studied by GC using a published method (Viljoen et al., 2002).



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

Markers and Quantitative Methods: The essential oil contains very high concentrations (up to 0.2% of dry weight) of a single compound loosely referred to as *Siphonochilus sesquiterpenoid* or siphonochilone (Van Wyk et al., 1997; Holtzapfel et al., 2002; Viljoen et al., 2002). It is not clear whether this compound is the active principle, but it is a useful and unique marker substance for quality control.

NIR Spectroscopy



Adulterants and Adulterations: Rare (unlikely). It may possibly be confused or adulterated with other members of the Zingiberaceae, such as Zingiber or Hedychium, but the difference is easily detected by TLC. Quality can easily be controlled by TLC or GC (characteristic furanoterpenoids are present in *Siphonochilus aethiopicus*).

Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

Extracts showed antibacterial activity, especially against Gram-positive bacteria, but no anti-viral effects (Light et al., 2002).

Antimycobacterial compounds have been isolated (Lategan et al., 2008).

In vitro anti-inflammatory and uterine relaxing activities have been reported (McGaw et al., 1997; Lindsay et al., 1999; Light et al., 2002).

SAFETY DATA

Preclinical Safety Data

Single Dose Toxicity cf. appendix 1

KEY (PROPOSED) USAGE

Therapeutic Indications: Antibacterial, anti-inflammatory.

Dosage, Method, and Duration of Administration: About 200 mg of dry rhizome is taken three or more times per day. In traditional medicine, a small piece of the fresh rhizome is chewed.

Special Warnings and Precautions for Use: None known. As a precaution, pregnancy and lactation are contraindicated, but no published information exists.

Can be safely consumed when used appropriately.

Pregnancy and Lactation: Not to be used during pregnancy. Not to be used while nursing.

Evaluation of Efficacy: Anti-inflammatory efficacy pharmacologically proven (McGaw et al., 1997; Lindsay et al., 1999; Light et al., 2002). Antibacterial efficacy pharmacologically proven (Light et al., 2002). Uterine relaxing effects efficacy pharmacologically proven (Lindsay et al., 1999). Coughs, colds efficacy traditionally proven. Tension headaches efficacy traditionally proven. Influenza efficacy traditionally proven. Sore throat efficacy traditionally proven. Mild asthma efficacy traditionally proven. Sinusitis efficacy traditionally proven. PMS, menstrual cramps efficacy traditionally proven. Malaria efficacy traditionally proven. Candida efficacy traditionally proven.

TRADE INFORMATION

Nature of plant material: Flowering/harvesting time: Rhizomes and roots are harvested at the end of the growing season when the plants start to become dormant.

Cultivated/wildcrafted: Product is available from commercial plantations and rapid scale-up is possible. No wildcrafting should be allowed.

Conservation status: Rare or extinct in KwaZulu-Natal and other parts of South Africa. Conservation status in other countries is not clear.

Origin: South Africa.

Processing and storage: Preparation/processing: The rhizomes and roots are simply sliced and dried.

Stability of product: Unstable in solution. Possibly stable in dry form. Stafford et al. (2005) reported that the antibacterial activity remained more or less unchanged, whereas anti-inflammatory activity decreased over time.

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Strophanthus gratus

GENERAL DESCRIPTION

Scientific Name with Author: *Strophanthus gratus* Franch.

Synonyms: *Roupellia grata* (Wallich & Hooker) Baillon

Family: Apocynaceae

Vernacular Names: Bego-esé, climbing oleander, cream fruit, eguro-eguro, ejoku ja mfa, gohodo, gondo isa, isagere, isa-gidi, isa-ogbubu, kalanmeni, konen, matwa, m-moropo, o-maatwa, oroko, ota, oubain, rose allamanda, sagere, salo, siniabie, smooth strophanthus, wabayó.

Botanical Description: Liana, 2 - 25 m high, with a stem up to 10 cm in diameter, or less often a shrub, 2 - 3 m high, latex clear or white. Branches are dark brown or purplish-brown, rather densely lenticellate, lenticels becoming corky with age, glabrous. Leaves opposite, glossy and medium to dark green above, 1 - 15 mm long, rounded or cuneate at the base, thinly coriaceous, 5 - 18 x 2 - 9 cm, 5 - 11 pairs nearly straight. Petiole 5 - 17 (-32) mm long. Flowers are fragrant with unequal calyx lobes and a corolla tube four times as long as the calyx. Corolla white, turning yellow near the base outside, reddish near the mouth outside, white and red-streaked inside; corolla lobes white with stripes of purple, turning red or purple all over. The fruit is a follicle tapering towards the apex, 23 - 41 cm long; fruit wall dark brown to purplish-brown and enclosing glabrous, pale brownish seeds, 11 - 20 x 2.5 - 5.0 mm, flat.

Origin and Distribution: The plant is commonly found in deciduous forests of tropical West and Central Africa, from Senegal (Casamance) to Guinea, Sierra Leone, Liberia, Ivory Coast, Ghana, Nigeria, Cameroon, Gabon, Congo, CAR, and Zaire. It is seen growing between 0 and 650 m altitude in primary and secondary forest, forest margins, and on river banks. In Nigeria, Cameroon, and Gabon the plant is under cultivation.



Plant Part Used: Seeds



ETHNOBOTANICAL INFORMATION

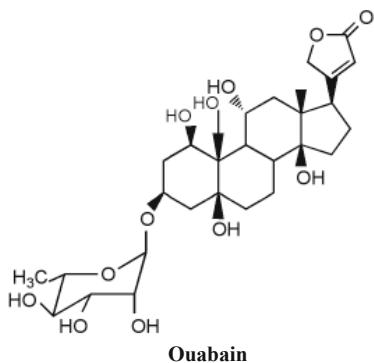
Major Ethnopharmacological Uses: Traditional health practitioners use the crushed leaves to rub on the body when the patient is suffering from fever. The leaves are also crushed and applied as a poultice to treat skin ulcers, wounds, and parasites; a decoction is taken orally against gonorrhoea. In Nigeria, *S. gratus* has been used to treat snake bite. The seeds have been shown to delay blood clotting.

Other Relevant Uses: *S. gratus* is the ideal arrow poison plant. Its predominant principle is ouabain. It fulfills all conditions for a perfect hunting poison: extremely high toxicity, fast and sure effect, unusually high concentration in the seeds, highly soluble in water, and therefore easy to extract from the seed.

CHEMICAL CONSTITUENTS

Compounds: After the exhibition of the seeds at the 1865 World Fair, in Paris, *S. gratus* became the object of chemical and pharmacological studies. In 1904, the presence of ouabain (3.6%) was reported in the seeds of *S. gratus* and was named g-strophanthin (*gratus*-strophanthin). The presence of several cardiac glycosides have also been reported (strogoside and gratoside). Ouabain is the most abundant, followed by acolongifloroside K and strogoside. The leaves have yielded three lignans, pinoresinol, 8-hydroxypinoresinol, and olivil.

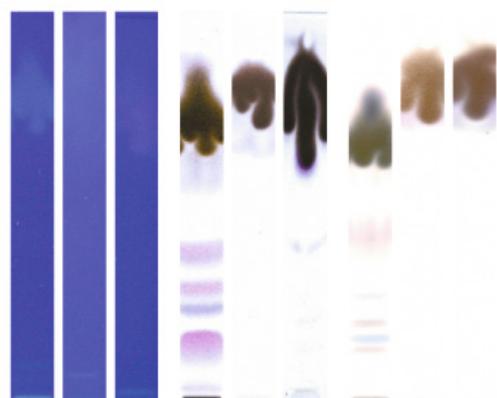
Ouabagenin is the aglycone richest in oxygen (contains six –OH groups), and its water solubility is correspondingly high.



QUALITY CONTROL

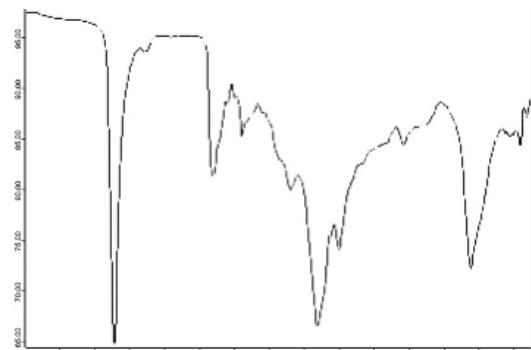
Organoleptic Properties: Yellowish-brown in colour, aromatic, pungent, and bitter.

TLC / HPLC / GC



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

NIR Spectroscopy



Adulterants and Adulterations: The two most mixed with the official drug before exportation are those of *S. gratus* from Senegal and Congo, where *S. hispidus* is found, and which are recommended by some authorities because they are easily recognised and yield strophanthin readily in crystalline form; and *S. thallone*.

Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

When applied externally, preparations containing *S. gratus* appear to exert no special effects unless they are applied with hydrous wool fat. The seeds, however, applied to the cornea, produce prolonged anaesthesia. Three or four drops of a solution of strophanthin (1:1000) applied to the cornea also produce total anaesthesia, including sensitivity to heat and cold (main differences from cocaine).

Strophanthus is a muscle poison. When taken internally its action is exerted primarily upon the voluntary muscles, increasing their contractility, and if the dose is near the toxic level a tetanic paralysis will occur, with the net result that the muscles are unable to regain their former normal flexibility. It is this effect that renders *S. gratus* an efficient arrow poison. When the muscles are in extreme paralysis, lactic acid has been observed to replace the normal alkaline condition. *S. gratus* muscular paralysis consists chiefly of the diminishing ability of the muscles to relax, and then in destroying this capability, producing a condition difficult to distinguish from rigor mortis. The muscular paralysis produced renders the animal an easy prey to its pursuer.

Strophanthus does not appear either to affect the spinal cord or to act upon its nerve trunks. Its specific action upon the heart is due to direct contact (through the blood) with the muscular fibres of that organ, and not to any effect upon the cardiac nerves. A large dose increases contractility, leading to a more perfect, energetic and prolonged systole while the capability of the muscle to relax is lost, or so diminished that diastole cannot take place. Examination of the heart after death shows the ventricle to be so completely contracted as to almost efface the cavity - the heart passing from life directly into rigor mortis. According to some, it may cease either in systole or in diastole. The calibre of the blood vessels is only slightly influenced by *Strophanthus*. It is strongly diuretic in so far as lack of secretion depends on low blood pressure, i.e. it increases diuresis, resulting in increased blood pressure, which consequently produces an increased urinary product. It is also thought by some to act especially upon the renal secreting structures. When one is in good physiological condition it is said to have little or no diuretic action; but in diseased conditions,

with low blood pressure, it is claimed to exceed digitalis in diuretic power.

Clinical Studies: If an *S. gratus* preparation is given in large doses it produces gastrointestinal irritation with vomiting and diarrhoea. Small doses, however, act as a bitter tonic, improve the appetite, augment gastric action, and promote digestion. In proper doses, it strengthens the heart muscle, slows cardiac action, increases the interval between beats, reduces the pulse rate, and powerfully increases arterial tension, not by any effect (to any extent at least) upon the vessels, but by strengthening the heart muscle. Whether or not the drug is cumulative is still an unsettled question, though it is probably not cumulative unless given too freely in overlapping doses. The action of *Strophanthus* on the heart is probably greater than that of any other drug, and its active principle is far more potent than the digitalis derivatives.

Strophanthus is a remedy for weak heart caused by debility of the cardiac muscle, with lack of proper contractile power, as shown by a rapid, weak pulse and very low blood pressure. The disordered action of the heart is due to lack of tonicity and not to walls weakened by deposition of fat, in which case the drug must be used with extreme circumspection; although in small doses it has been recommended by some as a remedy for cardiac fatty degeneration, as well as for atheroma of the arteries in the aged. It is a remedy for precordial pain and for cardiac dyspnoea. It has been strongly endorsed in heart afflictions with disorders of compensation. *Strophanthus* is a remedy for valvular heart disease only in so far as there is muscular insufficiency, where the compensatory increase of muscular action is not sufficient to offset the valvular insufficiency. It has been reported to be useful in cases of mitral regurgitation with dilatation; mitral stenosis with regurgitation; regurgitation with oedema, anasarca, dyspnoea, etc.; mitral insufficiency with palpitation, praecordial pain, cyanosis, etc. When the balance in the circulation has become impaired, as a result of insufficiency of the valves of the heart from organic disease with a general dropsical condition, *Strophanthus*, although affording temporary relief in some cases, has failed in every case to re-establish the compensation. The result was the same whether

the mitral, the tricuspid, or the semilunar valves were most involved. These are the cases of heart disease in which digitalis is the remedy. However, evidence is strong to show that when the muscular insufficiency can be corrected in these cases, then the remedy is beneficial. Acute endocarditis and the reflex palpitation of neurasthenic, hysterical, and chlorotic subjects have been signally benefited by *Strophanthus*, and it appears to give better cardiac power during or after typhoid and other adynamic fevers, when heart failure threatens. It may be used as a remedy for threatened cardiac failure in any disease. Full doses should be given for the relief of angina pectoris, and the remedy should be continued for a period after the attack. It is less efficient, because slower in action, than amyl nitrite or nitroglycerine, but may be given for more permanent effects after the evanescent action of these agents has worn off. In pulmonary congestion and in acute bronchitis or acute pneumonia it may be used when there is defective heart action.

Strophanthus has been praised for prompt results in cardiac and bronchial asthma, with oedema; in whooping-cough it has many advocates; it assists the action of iron salts in pernicious anaemia; and is credited with the cure of traumatic tetanus. Goitre is claimed to have been cured with it, and large doses (8 - 25 drops, several times a day) have been said to cure a large proportion of cases of exophthalmic goitre, with irregular cardiac action.

Great relief of the heart symptoms was seen in two cases of exophthalmic goitre, with disappearance of the bronchocoele in one case. *Strophanthus* has also been used as a remedy for chronic nephritis, with albuminuria, in anasarca, and in ascites due to hepatic cirrhosis. It is of little value in oedema and other forms of dropsy or kidney disorders unless dependent on cardiac disorders. A case report involving the use of 5-drop doses, every 2 hours, produced an enormous flow of urine and thin alvine discharges, completely draining the tissues in less than 12 hours. Finally, *Strophanthus* is credited with the cure of a large number of cases of Asiatic cholera, and it has been asserted useful in urticaria and psoriasis.

This agent does not take the place of digitalis, each having its own field of action. It may, however, follow the use of other heart tonics, and

particularly those that are evanescent in action, such as amyl nitrite and nitroglycerine. As it does not affect the calibre of the vessels it may be used in preference to digitalis when it is not desirable to add extra work to the heart. It is tolerated well by the aged and by children. The advantages of *Strophanthus* over digitalis are greater rapidity, modifying pulse-rate within an hour; absence of vasoconstrictor effects; greater diuretic power; no disturbance of digestion; absence of cumulation.

SAFETY DATA

Preclinical Safety Data

Single Dose Toxicity cf. appendix 1

KEY (PROPOSED) USAGE

Therapeutic Indications: Ouabain is one of the strongest cardiac tonics known. It is used internally by injection for heart failure, angina, hypertension, pulmonary oedema, and during anaesthesia and surgery. It acts rapidly when given intravenously, and therefore used for cardiac arrest.

Dosage, Method, and Duration of Administration: There is great variation in the strength of various batches of tincture of *Strophanthus*, owing to lack of uniformity in the crude drug employed. The dose of tincture of *Strophanthus* is from 1 to 10 drops; of specific strophanthus, 1/2 to 10 minims; of strophanthin, 1/500 to 1/60 grains, all of which should be cautiously administered.

Contraindications: *Strophanthus* should be avoided or used very cautiously in advanced muscular degeneration, in pronounced mechanical defects of the heart, and in fully and overcompensated hearts.

Interactions: There is a reported interaction between ouabain and reserpine. Pretreatment with this alkaloid reduces the toxicity of ouabain, whereas simultaneous treatment increases it. Whereas 5 out of 10 dogs died after the intravenous administration of 100 g/kg of ouabain, with the simultaneous administration of 1 mg/kg reserpine, nine out of 10 died. This may play an important role in arrow poisons which are prepared from *Rauvolfia vomitoria* and *Strophanthus* with other

cardenolide-containing plants. Such combinations may often be observed in Zaire and the Central African Republic.

Evaluation of Efficacy: Efficacy proven, but too few results to be conclusive.

TRADE INFORMATION

Nature of plant material: Prefers a protected, partly shaded, warm position in fertile, humus-rich, moist but well-drained soil. Propagated by seed or cuttings.

Nature of plant products: Dried, ripe seeds deprived of their awns.

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Sutherlandia frutescens

GENERAL DESCRIPTION

Scientific Name with Author: *Sutherlandia frutescens* R.Br.

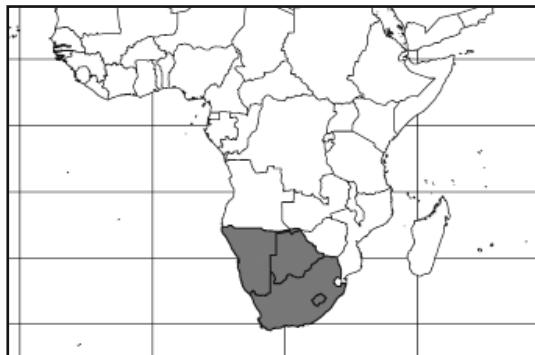
Synonyms: *Lessertia frutescens* (L.) P.Goldblatt & J.C.Manning

Family: Fabaceae

Vernacular Names: Cancer bush, kankerbos, musaphelo, sutherlandia, unwele.

Botanical Description: Cancer bush is a perennial shrub, up to 2 m, with pinnately compound leaves comprising pubescent to white-tomentose, entire leaflets. The flowers are large, bright red with a long, recurved standard petal, a slightly longer keels, and very short wing petals (adapted for bird-pollinated). The fruits are distinctive, inflated, bladdery, and papery pods (Phillips & Dyer, 1934). The various inland species of *Sutherlandia* (including a form with narrow pods, known as *S. microphylla*) are regarded by some botanists as a single, variable species, *S. frutescens* (Moshe, 1998; Moshe et al., 1998). *Sutherlandia* is very closely related to the genus *Lessertia* and is sometimes considered to be part of the latter (Goldblatt & Manning, 2000).

Origin and Distribution: South Africa (mainly the dry western parts), Namibia, and Botswana.



Plant Parts Used: Dried leaves and twigs



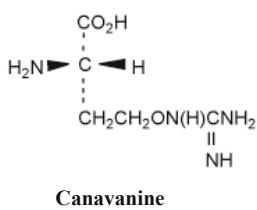
ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses: Mainly a bitter tonic (*amarum*) and adaptogen. It has a long history of traditional use against self-limiting conditions, as well as an adjuvant in the treatment of serious ailments. *Sutherlandia* has been used to treat colds and influenza, cough, asthma,

bronchitis, fever, indigestion, poor appetite, gastritis, peptic ulcers, dysentery, diabetes, unspecified liver conditions, rheumatism, urinary tract infections, tuberculosis, stress, and anxiety. It has been used in the treatment and prevention of cancer, hence the common name. Recent anecdotes and observations indicate immune modulatory properties (similar to those of *Astragalus*), and significant benefits in the treatment of wasting in cancer and AIDS. Extracts are used topically in the treatment of burns, wounds, and inflammatory skin conditions (Dykman, 1891; Smith, 1895; Watt & Breyer-Brandwijk, 1962; Rood, 1994; Van Wyk, 1997; Van Wyk & Gericke 2000; Van Wyk & Wink 2004).

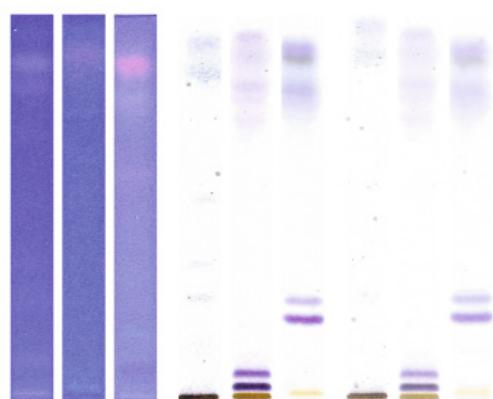
CHEMICAL CONSTITUENTS

Compounds: The herb is exceptionally variable but contains a large number of triterpenoid saponins as main bitter compounds. The main saponin in commercial *Sutherlandia* is known as SU1 (unpublished). Also present are large quantities of free amino acids, (including L-canavanine, a non-protein amino acid) at variable levels (usually 2 mg per g in commercial material but up to 20 mg per g (or more) in some provenances. A number of flavonoid glycosides are present, as well as pinitol (a cyclitol).



QUALITY CONTROL

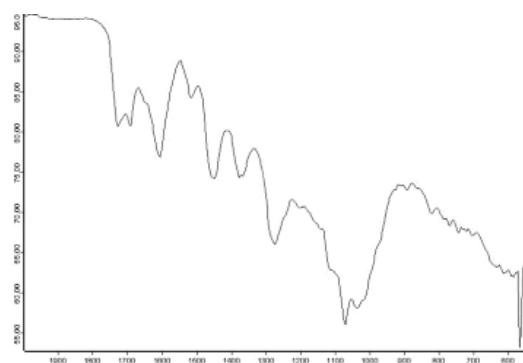
TLC / HPLC / GC: Standard TLC methods for triterpenoid saponins can be used. The flavonoids can be studied using standard methods (reverse phase C18, methanol-water gradient). The free amino acids, including canavanine, are analysed by HPLC using specialised amino acid analysers.



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

Markers and Quantitative Methods: Active/unknown. Canavanine occurs in leaves at a level of 2 - 7 mg per g dry weight.

NIR Spectroscopy



Adulterants and Adulterations: Very unlikely, as *Sutherlandia* has very unique flowers and fruits. However, the choice of provenance (geographical origin) is important, as the plant is morphologically, genetically, and chemically exceptionally variable.

Possible with low quality material (e.g., thick branches or unsuitable chemotypes) or lucern (*Medicago sativa*).

Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

Recent in vitro studies indicate anticarcinogenic (Tai et al., 2004; Chinkwo, 2005), antioxidant (Ojewole, 2004; Fernandes et al., 2004), anti-inflammatory (Ojewole, 2004; Na et al., 2004; Kundu et al., 2005), anti-HIV (Harnett, 2005), antidiabetic (Ojewole, 2004; Sia, 2004) and anti-stress effects (Prevo, 2004; Smith & Myburg, 2004).

The various chemical compounds identified in *Sutherlandia* provide a possible rationale for its varied traditional uses. L canavanine is a potent L arginine antagonist that has documented anticancer (Swaffar et al., 1995; Crooks & Rosenthal 1994) and antiviral activity, including activity against the influenza virus and retroviruses (Green, 1988). Canavanine is an inhibitor of nitric oxide synthase and has potential for the treatment of septic shock and chronic inflammation (Anfossi, et al., 1999; Levy et al., 1999). A USA Patent (Ostlund, 1996) suggests clinical application in treating the wasting in cancer and AIDS patients. The amino acid GABA is an inhibitory neurotransmitter that could account for the use of *Sutherlandia* for anxiety and stress. Triterpenoids related to astragalosides may have significant immune modulatory and anti-inflammatory activities besides their obvious bitter tonic effects. Pinitol is a known anti diabetic agent (Narayanan et al., 1987) that also may have an application in the treatment of wasting in cancer and AIDS (Ostlund & Sherman, 1996).

A comprehensive animal study (vervet monkeys) of the SU1 type of *Sutherlandia* indicated no adverse effects in more than 50 parameters evaluated. The results are unpublished but available from the website of the Medical Research Council of South Africa (who conducted the study).

Johnson et al. (2007) report results from a randomized, double-blind, placebo-controlled trial exploring the safety of *Sutherlandia frutescens* leaf powder in healthy adults. They report no significant differences in general adverse events or physical, vital, blood, and biomarker indices between the treatment and placebo groups. However, participants consuming *Sutherlandia* reported improved appetite compared to those in the placebo group. Although the treatment group

exhibited a lower respiration rate and higher platelet count, MCH, MCHC, total protein, and albumin than the placebo group, these differences remained within the normal physiological range, and were not clinically relevant. The *Sutherlandia* biomarker canavanine was undetectable in participant plasma.

Clinical Studies: Convergent clinical observations by health professionals and community workers suggest that *Sutherlandia* taken on a daily basis can improve appetite, facilitate weight gain, and improve the CD4 counts of HIV positive people (Gericke, 2001). These observations need to be verified by a controlled clinical study.

SAFETY DATA

Preclinical Safety Data

Single Dose Toxicity cf. appendix 1

KEY (PROPOSED) USAGE

Therapeutic Indications: Adaptogen.

Dosage, Method, and Duration of Administration: A daily dose of about 1 - 2 g of the dry herb is taken by infusion or decoction. Tablets are also used (one to two tablets containing 300 mg of the dry herb, taken two to three times per day).

Special Warnings and Precautions for Use: *Sutherlandia frutescens* (commercial type) can be safely consumed when used appropriately.

Interactions: L-canavanine reduces the uptake of essential amino acids from the intestines and disturbs protein biosynthesis. Massive amounts may cause reversible pancytopenia and/or lupus erythematosus. (Alfalfa seeds and sprouts are sold as a food for humans and have GRAS status in the USA, despite their high levels of L-canavanine). No serious side effects have been reported despite large numbers of people taking *Sutherlandia* tablets daily on a long-term basis.

A preliminary in vitro study demonstrated that *Sutherlandia* could interfere with blood levels of some anti-retroviral drugs (Mills et al., 2005.). Products containing *Sutherlandia* should thus not be taken at the same time as antiretroviral drugs

without formal monitoring of blood levels by a suitably qualified healthcare professional.

Pregnancy and Lactation: The product is contraindicated during pregnancy and lactation.

Evaluation of Efficacy: Anticarcinogenic effects efficacy pharmacologically proven (Tai et al., 2004; Chinkwo, 2005). Antioxidant efficacy pharmacologically proven (Ojewole, 2004; Fernandes et al., 2004). Anti-inflammatory efficacy pharmacologically proven (Ojewole, 2004; Na et al., 2004; Kundu et al., 2005). Antidiabetic efficacy pharmacologically proven (Ojewole, 2004). Adaptogen (stress, anxiety) efficacy pharmacologically proven (Prevoo, 2004; Smith & Myburg, 2004). Anti-HIV efficacy traditionally proven (+) (Harnett, 2005). Bitter tonic (amarum) efficacy traditionally proven. Tuberculosis efficacy traditionally proven. Cancer (prevention) efficacy traditionally proven. Cachexia (wasting, in cancer and AIDS) efficacy traditionally proven. Topical use (burns, wounds, and inflammatory skin conditions) efficacy traditionally proven.

TRADE INFORMATION

Nature of plant material: Cultivated/wildcrafted: The plant is wildcrafted to some extent but large quantities of cultivated material from a selected chemotype with a long history of human use (the so-called “Phyto Nova Sutherlandia” or “SU1 Sutherlandia”) is available. Rapid scale-up is possible.

Flowering/harvesting time: Plants are harvested after the growing season (spring in the western parts, late summer in the eastern parts).

Conservation status: Not listed.

Processing and storage: Preparation/processing: Small twigs, leaves, and leaflets are simply cut and air dried.

The product is highly stable when dried but relatively unstable in solution. No reliable data is available.

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Terminalia sericea

GENERAL DESCRIPTION

Scientific Name with Author: *Terminalia sericea* Burch. ex DC.

Synonyms: *Terminalis angolensis* O.Hoffm., *T. fischerii* Engl., *T. nyassensis* Engl., *T. brosigiana* Engl. & Diels, *T. velutina* Rolfe, *T. bubu* De Wild.

Family: Combretaceae

Vernacular Names: Konono, mangwe, mhungweluwala, mogunono, mokonona, mpululu, m konono, musunu, mususu, namatipo, omuolo, si ver cluster leaf, silver terminalia, vaalboom, umHonono, umKhonono.

Botanical Description: A deciduous tree that can rarely grow as high as 12 m. It frequently occurs as shrubs or saplings, 2 - 4 m high. The leaves are blue-silvery, which makes it very easy to identify (Carr, 1998).

Origin and Distribution: Western parts of southern and eastern Africa, with its northern limits in Zaire and Tanzania. It is invariably restricted to sandy soil, and also occurs in *Combretum-Terminalia*, *Brachystegia*, and *Colophospermum* woodland (Miombo woodland). It is frequently dominant or co-dominant forming dense stands.



Plant Parts Used: Mainly roots, but also leaves.



ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses: *T. sericea* has a wide range of uses in traditional medicine, including having anticarcinogenic effects (Fyhrquist, 2007).

Roots are prepared in various ways to treat many indications in African traditional medicine. Hot water decoctions of the roots are used for diarrhoea, and to relieve colic pains (Neuwinger, 1996; Tshikalange et al., 2005; Moshi & Mbwambo, 2005); also for certain eye diseases (Drummond & Coates-Palgrave, 1973). Decoctions of the roots in treatment of bilharzia (Kokwaro, 1976) and gonorrhoea (Hedberg et al., 1982), whereas venereal diseases are treated with root decoctions of *T. sericea* mixed with *Carica papaya*, *Citrus limon*, and bark of *Parinari excelsa*, or infusions of the roots of *T. sericea* with *Peltophorum africanum*, *Pterocarpus angolensis*, and *Xymania caffra* (Neuwinger, 2000). Enemas of *T. sericea* root decoctions for menstrual

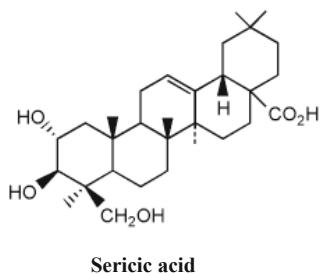
cramps, and roots steeped in water for 1 hour for trachoma (eye disease). A tea is made from roots for skin diseases and coughs; for headaches and general body weakness, root is powdered and swallowed with food. For pneumonia roots are boiled in water for a hot fomentation (Drummond & Coates-Palgrave, 1973), or the chest is exposed to the vapours of this liquid (Neuwinger, 1994). The powdered root bark is taken with corn meal for diabetes (Watt & Breyer-Brandwijk, 1962). Roots of *T. sericea* are taken as an emetic (Watt & Breyer-Brandwijk, 1962).

Use of other plant parts are rarely used in traditional medicine, but there are some reports of the uses of leaf infusions for chest complaints (pneumonia) and diarrhoea. Wounds are dressed with stem bark or leaves (Neuwinger, 2000). Use of dried fruit for tuberculosis is also recorded (Eldeen et al., 2005).

CHEMICAL CONSTITUENTS

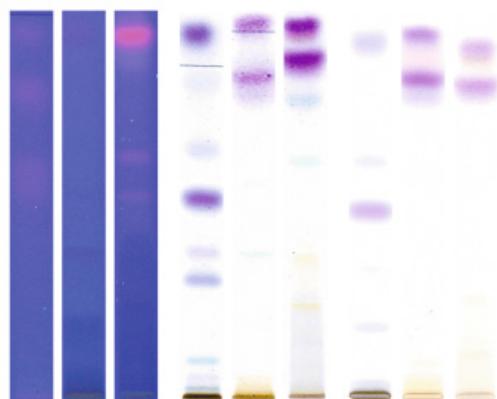
Compounds: The triterpenoids sericoside and arjunglucoside have been isolated from the roots and stem bark of *T. sericea* (Bombardelli et al., 1974, 1986), without any biological assays. Terminoic acid was isolated as one of the antibacterial compounds from leaves of *T. sericea* (Kruger, 2004), and anolignan B from the roots (Eldeen et al., 2005).

Joseph et al. (2007) isolated a stilbene glycoside, resveratrol-3-O- β -rutinoside, and resveratrol from an ethanol extract of the root bark.



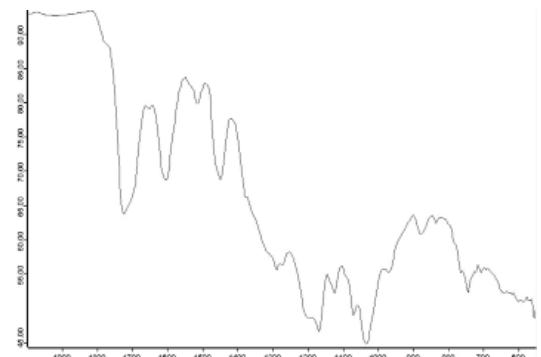
QUALITY CONTROL

TLC / HPLC / GC



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

NIR Spectroscopy



Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

Antibacterial activity of root extracts: Aqueous extract has limited activity (MIC 1 - 20 mg/ml) against Gram-positive bacteria (Tshikalange et al., 2005;), but no activity against Gram-negatives (MIC >50 mg/ml). Chloroform extracts were inactive against all bacterial species (Tshikalange et al., 2005; Steenkamp et al., 2004). Moshi and Mbwambo (2005) found that in addition to being active against Gram-positive bacteria, extracts of the roots of *T. sericea* showed activity also against the Gram-negative bacterium *Escherichia coli*. An ethyl acetate root extract of *T. sericea* yielded termilignan B and arjunic acid with activity against

Gram-positive and Gram-negative bacteria, with MIC values ranging between 1.9 and 15.6 µg/ml (Eldeen et al., 2008).

Antiviral activity of root extracts: *T. sericea* root extract showed alpha-glucosidase inhibitory activity ($IC_{50} = 92$ mg/ml) which was better than that of acarbose ($IC_{50} = 131$ mg/ml). In a reverse transcriptase assay, *T. sericea* exhibited inhibitory activity ($IC_{50} = 43$ mg/ml) higher than that of adriamycin ($IC_{50} = 100$ mg/ml) (Tshikalange et al., 2008).

Antibacterial activity of bark extracts: Water and methanol extracts of bark had no antibacterial activity against five Gram-positive or Gram-negative bacteria (Rabe & van Staden, 1997).

Antibacterial activity of leaf extracts: Acetone extracts of leaves had some activity against both Gram-positive (MICs 0.4 and 0.6 mg/ml) and Gram-negative bacteria (MICs 12 and 6 mg/ml; the activity increased to 3 and 0.8 mg/ml after storage at 7 °C for 6 weeks) (Eloff, 1999). Kruger (2004) used 10 different extractants and found good activity against Gram-negative and Gram-positive organisms, with average MICs ranging from 0.14 to 1.96 mg/ml. Non-polar extractants gave the best results.

Antifungal activity of leaf extracts: Different leaf extracts had excellent activity against five animal fungal pathogens, with MICs varying between 0.02 mg/ml and 0.32 mg/ml. With the exception of *Aspergillus fumigans*, the inhibition appeared to be fungicidal and not fungistatic (Masoko et al., 2005). Based on bioautography, at least seven different antifungal compounds, against one or more of the five fungal pathogens evaluated, were present. Most of the compounds were in the dichloromethane extracts, and the fewest in the methanol extract.

Intermediate and polar extracts of the roots of *T. sericea* have in general been found to possess the highest antimicrobial activity compared to more non-polar extracts (Moshi & Mbwambo, 2005; Tshikalange et al., 2005), and have also been found to be antifungal against *Candida albicans* and *Aspergillus niger*. In addition to the roots, the leaves of *T. sericea* have also been found to be antimicrobial, and activity was demonstrated

both against Gram-positive and Gram-negative bacteria (Eloff, 1999). It is possible that the leaves contain different antimicrobial compounds from the roots.

Bioactivity-guided fractionation led to the isolation of terminoic acid from the leaves (Kruger, 2004) and anolignan B from the roots of *T. sericea* (Eldeen et al., 2005); isolated against Gram-negative (MIC value of 3.8 µg/ml against *Bacillus subtilis*) and Gram-positive bacteria. A 20% acetone crude leaf extract and a 1% terminoic acid cream were more effective than gentamicin in controlling *Staphylococcus aureus* infections on skin lesions in rats (Kruger, 2002).

The triterpene saponin sericoside from leaf and root of *T. sericea* has a strong lipolytic activity (Mochizuki & Hasegawa, 2006).

SAFETY DATA

Preclinical Safety Data

Single Dose Toxicity cf. appendix 1

KEY (PROPOSED) USAGE

Therapeutic Indications: Antibacterial, antioxidant, anti-inflammatory, adaptogen.

Evaluation of Efficacy: Anticarcinogenic efficacy pharmacologically proven (Fyrquist, 2007). Antioxidant efficacy pharmacologically proven. Anti-inflammatory efficacy pharmacologically proven. Antidiabetic efficacy pharmacologically proven. Adaptogen (stress, anxiety): efficacy pharmacologically proven. Anti-HIV efficacy traditionally proven; efficacy pharmacologically proven; insufficient information for classification. Bitter tonic (amarum) efficacy traditionally proven. Tuberculosis efficacy traditionally proven. Cancer (prevention) efficacy traditionally proven. Cachexia (wasting, in cancer and AIDS) efficacy traditionally proven. Topical use (burns, wounds and inflammatory skin conditions) efficacy traditionally proven.

TRADE INFORMATION

Nature of plant material: Cultivated/wildcrafted: Because *T. sericea* occurs so widely frequently as the dominant species, there has been no incentive to cultivate it. The stem bark of *T. sericea* is exported to Europe and possibly to Germany from Mozambique, according to some reports (Rukangira, 2000).

Conservation status: Not listed.

Nature of plant products: Origin: Tropical and subtropical areas of southern and eastern Africa.

Processing and storage: Preparation/processing: Plant parts are dried and ground.

Stability of product: The product is highly stable when dried, but relatively unstable in solution. No reliable data are available.

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Toddalia asiatica

GENERAL DESCRIPTION

Scientific Name with Author: *Toddalia asiatica* Lam.

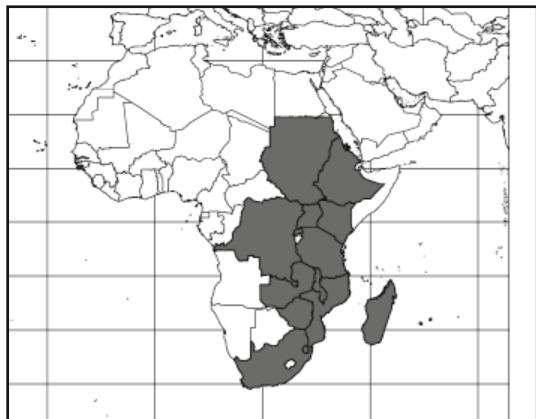
Synonyms: *Toddalia nitida* Lam., *Toddalia angustifolia* Lam., *Toddalia aculeata* Pers.

Family: Rutaceae

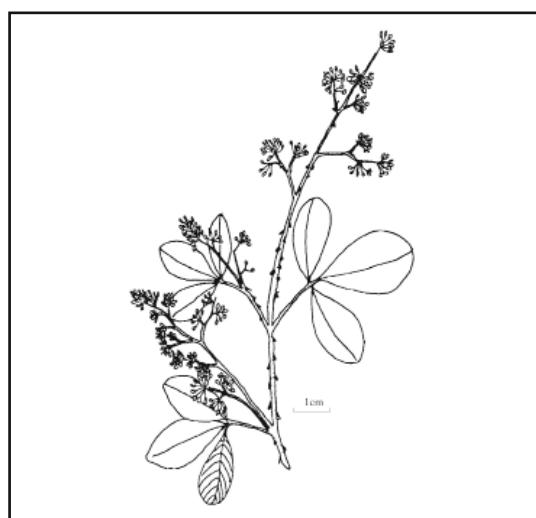
Vernacular Names: Ajua, bambara, chikombe za chui, liane de patte poulet, katemwe, mdakakomba, mkuro, mwikunya, olebarmonyo, patte poule piquant, patte poule, petit patte poule, ronce.

Botanical Description: Scandent shrub or woody liana, bearing short, retrorse, dark brown prickles, usually pubescent to shortly hispid on younger parts, sometimes glabrous. Leaves trifoliolate-palpate; petiole 2-4 cm long; leaflets dark, shiny green, oboval to elliptic, 2.5-5.5 x 1-2 cm, the terminal one a little larger, often acuminate; margins slightly crenate to more or less sinuous in the upper half, often revolute. Inflorescences usually axillary, sometimes terminal on lateral branchlets, 2-6 cm long, longer than broad, pubescent; male inflorescences bearing 50-100 flowers borne in fascicles or umbels; female inflorescences with 10-20 flowers loosely arranged. Petals yellowish-white, spreading or reflexed, oblong to narrowly triangular, 2.5-0.5 mm, more or less valvate at the base and imbricated at the summit. Male flowers with stamens having filaments 1.5-2 mm long; ovary rudimentary, style simple with discoid stigma. Female flowers with staminodes, more or less globular-ellipsoid, ovary with sessile thick, peltate, lobed stigma. Fruit orange when ripe, more or less glandular, 5-8 mm in diameter, gland-dotted. Seeds 1-4, incurved, brownish-black, laterally compressed.

Origin and Distribution: This species is indigenous to the Mascarene region and is also found in Malawi (Morris, 1996) and Mozambique. It is fairly common and is found in numerous locations from sea level to high-altitude forests.



Plant Part Used: Leaves



ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses: Two teaspoons of the root decoction, along with the leaves and root bark of *Rhus vulgaris*, the root bark of *Sympodia globulifera*, are drunk once daily against colds (Neuwinger, 1996). A bark maceration is used against blenorragia (gonorrhoea). The leaves and twigs are boiled and the vapours inhaled against bronchial diseases and malaria. The fruit or fruit decoction is used against cough, colds, and stomach pain. A decoction of the root sap is taken as an emetic and against paralysis resulting from snake bites. The root sap is also applied to scarification resulting from snake bites. The root decoction is also drunk against cardiac and back pain, and also when there are digestive troubles resulting from overconsumption of meat. The root decoction is also taken in the event of rheumatism and sterility. A poultice made from the root is applied to swollen limbs.

The leaves are boiled with butter and drunk against pneumonia and rheumatism. A glassful of the leaf decoction is drunk twice daily against rheumatic pains. This decoction is also gargled against toothache. The leaves are also chewed against toothache, and a decoction of the aerial parts of the plant is drunk against malaria. A poultice made from the ground leaves is applied on scabies. Boiled in water, the decoction made from the ground leaves is drunk against snake bite. The plant resin is drunk by pregnant women as an oxytocic agent (Kokwaro, 1976; Neuwinger, 1996).

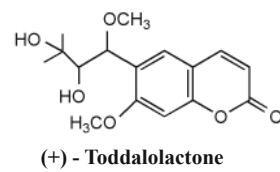
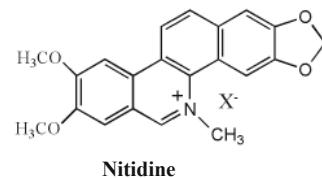
In Reunion Island, the plant is macerated in rum and the resulting solution is used to rub on limbs suffering from cramps. An infusion made with four to five leaves in one cup of water is used against cough, fever, colds, fatigue, rheumatism, and gout. Alternatively, the grated plant is macerated in rum and the solution taken at the rate of 1 soupspoon twice daily, morning and night. A handful of the plant is boiled in 2 litres of water and this decoction is drunk several times per day as a purgative (Lavergne & Vera, 1989).

In Mauritius and Rodrigues, the leaves are burnt and the fumes used against nasal catarrh. A decoction of the leaves is used against colds and cough (Adjanohoun et al., 1983).

In Madagascar, the plant is used as a tonic. The root bark is used as a stimulant, against intermittent fever, cholera, diarrhoea, rheumatism, and venereal diseases (Watt & Breyer-Brandwijk, 1962).

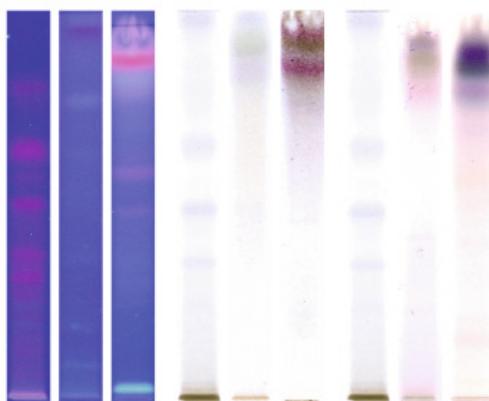
CHEMICAL CONSTITUENTS

Compounds: Coumarins (toddaculin, coumurrayin, toddanone, 8-(3,3-dimethylallyl)-6,7-dimethoxycoumarin, isopimpinellin, 6-(3-chloro-2-hydroxy-3-methylbutyl)-5,7-dimethoxycoumarin, 6-formyllimettin, 5,7,8-trimethoxycoumarin, toddasin, (+)-toddanol, 6-(2-hydroxy-3-methoxy-3-methylbutyl)-5,7-dimethoxycoumarin, and toddalolactone); five new coumarins (toddalenol, toddalosin, 5-methoxysuberon, toddalenone, and 8-formyllimettin); alkaloids (benzo[c]phenanthridine, alkaloids (des-N-methylchelerythrine, oxychelerythrine, arnotianamide, oxyavicine, avicine, chelerythrine and chelerythrine-psi-cyanide); four known quinoline alkaloids (*N*-methylflindersine, 4-methoxy-1-methyl-2-quinolone, skimmianine, integriquinolone); and one known triterpenoid (β -amyrin).



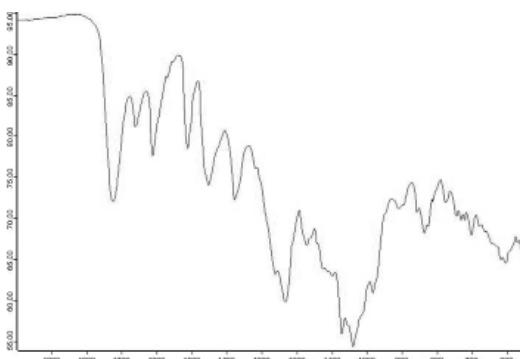
QUALITY CONTROL

TLC / HPLC / GC



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

NIR Spectroscopy



Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

In vitro assay of the root bark of *T. asiatica* has shown significant activity against the malarial vector *Plasmodium falciparum*. Bioguided fractionations of the bioactive extracts has revealed that the alkaloid nitidine, as well as other coumarins present in the plant (Gakunju et al., 1995; Oketch-Rabah et al., 2000), is largely responsible for this activity. Nitidine has previously received considerable attention as it is a well-known cytotoxic agent, particularly against leukaemia (Gakunju et al., 1995). Dihydronitidine, on the other hand, has also been reported to exhibit tumour-specific cytotoxicity in vitro (Iwasaki et al., 2006).

The aerial parts of *T. asiatica* var. *floribunda* have been tested for spasmolytic activity in vitro on guinea-pig ileum. Although the activity was significant, it was not attributed to the presence of the coumarins toddalolactone and toddanone, as previously reported. Attempts have been made to assign this activity to the presence of chlorocoumarin in vitro (Rastogi & Mehrotra, 1998). However, these observations would justify the use of this plant against colics (Lakshmi et al., 2002).

Chinese scientists have tested the plant extracts on the cardiovascular system and have isolated an active constituent, which has been identified to be isopimpinellin (Guo et al., 1998). The wood extract yielded seven compounds having strong antiplatelet aggregation activity in vitro (Tsai et al., 1998).

The aqueous leaf extracts have also been tested for their antifeedant activity, and a 1% concentration showed 86.1% mortality (Sundararajan & Kumuthakalavalli, 2001).

The essential oil extracted from the leaves (Veeramuthu et al., 2006) and the leaf extracts (Duraiapandian et al., 2006) inhibited the growth of several bacteria in vitro, namely *Bacillus subtilis*, *Staphylococcus aureus*, and *S. epidermidis*, among others.

T. asiatica has also been shown to have anti-inflammatory and analgesic effects in rats, and it appears that long-term administration does not cause injury to the liver (Hao et al., 2004).

Recent *in vivo* tests on mice using extracts of the root bark of *T. asiatica* has shown that the latter shows remarkable suppression of parasitaemia (38 - 66%) either alone or in combination with chloroquine. The survival rate of the mice was found to be longer than that of controls (Muregi et al., 2007a, b).

SAFETY DATA

Preclinical Safety Data

Single Dose Toxicity cf. appendix 1

KEY (PROPOSED) USAGE

Therapeutic Indications: Antimalarial, anti-inflammatory, spasmolytic.

Dosage, Method, and Duration of Administration:

Long-term consumption of *T. asiatica* may be deleterious to health, given the cytotoxicity of some of the components found within, nitidine, for example. However, short-term use to treat a life-threatening disease may be supportable on a risk-benefit analysis, and in view of the availability of the plant and its low cost.

Special Warnings and Precautions for Use:

The roots are reported to be oxytocic and hence can promote very strong uterine contractions. It is thus a very potent abortifacient. In Madagascar, its use is regulated. The author also mentions the presence of toddaline, a powerful neuromuscular toxin which is dangerous to the heart, and recommends that this plant be used only externally (Rivière, 2007).

Pregnancy and Lactation: The product should not be used during pregnancy or while breastfeeding.

Evaluation of Efficacy: Antibacterial efficacy pharmacologically proven. Anticandida efficacy traditionally proven. Anti-ulcer: insufficient information for classification. General tonic: insufficient information for classification.

TRADE INFORMATION

Nature of plant material: Conservation status: Not threatened. Generally the plant is wildcrafted.

Processing and storage: Leaves are air dried.

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Muregi FW, Ishih A, Suzuki T, Kino H, Amano T, Mkoji GM, Miyase T, Terada M (2007a) In vivo

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Trichilia emetica

GENERAL DESCRIPTION

Scientific Name with Author: *Trichilia emetica* Vahl.

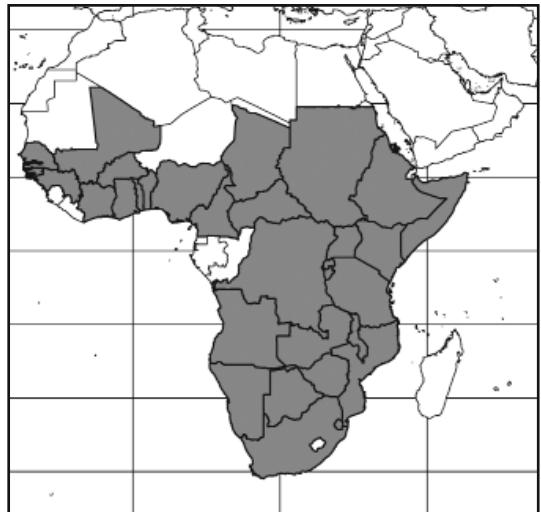
Synonyms: *Trichilia roka* Chiov.

Family: Meliaceae

Vernacular Names: Apolgum, asabrabise, ashapa, bouriète, chele, goro-mas, gumeh, ixolo, jan saiwa, kisiga, korillon, mhisí, misikiri, miti-mai, mmaba, mnwamai, mnwamají, mosikiri, msikitsi, msikizi, msukidzi, muchichiri, mukeka, mukeko, mururi, mushikiri, mushikishi, musikili, musunui, mutshikili, mutuhu, mwavi, muzaramanya, Natal mahogany, nkhuhlu, omenyakige, queco, roka, rooinessenhout, roqah, rugah, safsafa, sok, sorget, *Trichilia emetica* cortex, tsandi, umkhuhlu, umkhulu, vungu, yofunosi.

Botanical Description: A medium-sized to large evergreen tree, up to 20 m tall, with a smooth to rough bark (subsp. *emetica*), or a shrub or small tree with a corky bark (subsp. *suberosa*). The leaves are pinnate, with four or five pairs of leaflets, plus a terminal leaflet. They are dark green and glossy above, pale and densely hairy below. The small, fragrant flowers are pale yellowish-green, and are followed by rounded pale brown seed capsules up to 30 mm in diameter. Characteristic for this species is the presence of a distinct neck (stipe) at the base of the fruit. Each of the shiny black seeds is surrounded by a bright red fleshy aril that almost completely surrounds it, leaving only a characteristic black “eye” (White & Styles, 1963, 1986; Palmer & Pitman, 1972; Coates Palgrave, 2002).

Origin and Distribution: Widespread in the tropical parts of Africa, extending to Yemen. It occurs from Senegal to the Red Sea, and southwards throughout East and Central Africa to northern Namibia, northern Botswana, and South Africa.



Plant Parts Used: Bark. The root, root bark, leaves, and seed oil are also used.





ETHNOBOTANICAL INFORMATION

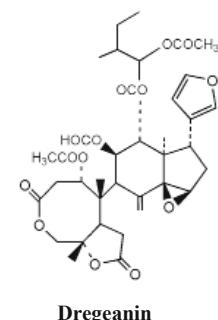
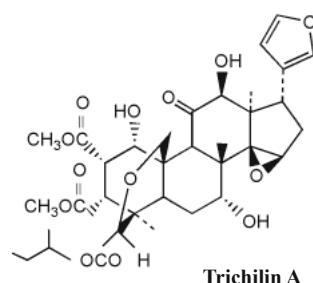
Major Ethnopharmacological Uses: The bark is traditionally used as an emetic, hence the specific epithet. The most common medicinal use throughout Africa is that of a purgative to treat constipation. The traditional method of administration is as an enema (Watt & Breyer-Brandwijk, 1962; Vercourt & Trump, 1969; Kerharo & Adams, 1974; Arnold & Gulumian, 1984; Iwu, 1993; Van Wyk et al., 1997; Coates Palgrave, 2002; Neuwinger, 2000; Diallo et al., 2003). Small doses of bark macerations or decoctions are taken orally to treat constipation, stomach ailments, intestinal parasites (mainly as an anthelmintic), fever, cough, bronchial ailments, gastritis, internal tumours, ulcers, gonorrhoea, syphilis, and several other conditions (Neuwinger, 2000; Diallo et al., 2003).

Root decoctions are used as a purgative, for ascaris, cirrhosis, dysmenorrhoea, fever, hepatitis, ochocerca, and other ailments (Kokwaro, 1976; Burkill, 1997; Neuwinger, 2000; Diallo et al., 2003).

The leaves are important for healing chronic wounds (Diallo et al., 2003). Numerous external uses of the bark, leaves, and seed oil have also been recorded (Burkill, 1997; Neuwinger, 2000, and references cited therein).

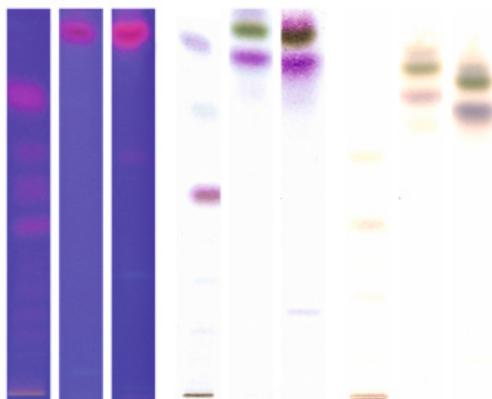
CHEMICAL CONSTITUENTS

Compounds: A large number of limonoids (also called trichilins) have been isolated from the roots, root bark, and seed arils of *T. emetica* (Taylor, 1980; Nakatani et al., 1981). Trichilin A is usually the major compound, but possible variation over the large distribution range of the species is unknown. Seeds of the closely related *T. dregeana* yielded dregeanin and several other typical trichilins (Mulholland & Taylor, 1980). Bioactivity-guided fractionation of an extract from the stem bark of *T. emetica* led to the isolation of the limonoids nymania 1, drageana 4, trichilin A, rohituka 3, and Tr-B. Also present was seco-A protolimonoid (Gunatilaka et al., 1998). Roots are rich in free and bound phenolic acids, including caffeic, ferulic, p-coumaric, syringic, vanillinic, protocatheeucic, gallic and chlorogenic acids (Germanò et al., 2006). Leaves are rich in polysaccharides, including a pectin of the rhamnogalacturonan type I with side chains of the arabinogalactan type II (Diallo et al., 2003).



QUALITY CONTROL

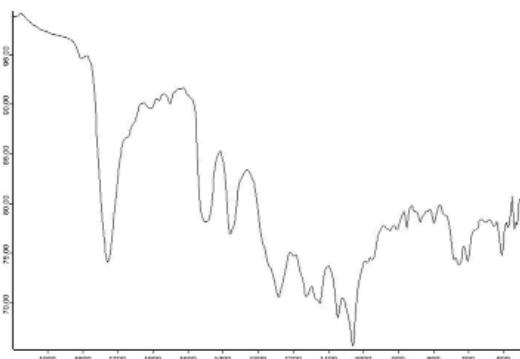
TLC / HPLC / GC: Standard TLC methods for limonoids. The phenolic fraction can be studied using standard methods (reverse-phase C18, methanol-water gradient).



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

Markers and Quantitative Methods: The root bark, stem bark and seed aril contain complex mixtures of limonoids (trichilins), of which trichilin A is usually a major constituent.

NIR Spectroscopy



Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

The trichilins are well-known insect antifeedants and have insecticidal and larvicidal activity (Nakatani et al., 1981, Xie et al., 1994) but they also have antibacterial and anti-inflammatory activity (Bruneton, 1995; Jäger et al., 1996).

Antibacterial and antifungal activity has been documented (Shai et al., 2008). Stem bark extracts have antiplasmodial (El Tahir et al., 1999; Kamanzi Atindehou et al., 2004), as well as antitrypanosomal activity (Kamanzi Atindehou et al., 2004). Antitrypanosomal effects were also found in leaf extracts (Hoet et al., 2004). Antischistostomal properties have been reported (Sparg et al., 2000). The root has hepatoprotective and antibacterial activities (Germanò et al., 2005). In addition, the leaf has shown GABA- benzodiazepine receptor-binding activity (Nah et al., 2006) and the root was found to have good antioxidant effects (Germanò et al., 2006). Significant antipyretic effects were found (Diallo et al., 2003).

SAFETY DATA

Ethnic Use Safety Data: The toxicity of *Trichilia emetica* is well known, and some human fatalities have been reported (Verdcourt & Trump, 1969; Burkhill, 1997; Neuwinger, 2000). The seed oil is edible (provided the poisonous aril is removed) and has been used on a large scale for human consumption (Burkhill, 1997).

Preclinical Safety Data

Single Dose Toxicity cf. appendix 1

KEY (PROPOSED) USAGE

Therapeutic Indications: Emetic, antibacterial, anti-inflammatory, antioxidant.

Dosage, Method, and Duration of Administration: Precise details are not available. When (commonly) used as an enema (Hutchings et al., 1996), a small piece of bark ("about the length and breadth of two fingers") is powdered and mixed with two cups of warm water. In the case of *Trichilia dregeana*, one teaspoon of powdered bark in one cup of milk.

Special Warnings and Precautions for Use: Toxic effects and human fatalities have been recorded, so that *Trichilia* bark extracts and other products should only be used for medicinal purposes under the supervision of an experienced health-care practitioner.

Pregnancy and Lactation: The product is contraindicated during pregnancy and lactation.

Evaluation of Efficacy: Anti-inflammatory efficacy pharmacologically proven; plant material with toxic potential (Bruneton, 1995; Jäger et al., 1996). Antibacterial and antifungal efficacy pharmacologically proven; plant material with toxic potential (Shai et al., 2008). Antiplasmodial efficacy pharmacologically proven; plant material with toxic potential (El Tahir et al., 1999; Kamanzi Atindehou et al., 2004). Antitrypanosomal efficacy pharmacologically proven; plant material with toxic potential (Kamanzi Atindehou et al., 2004). Antischistosomal efficacy pharmacologically proven; plant material with toxic potential (Sparg et al., 2000).

TRADE INFORMATION

Nature of plant material: Flowering/harvesting time: Any time of the year.

Cultivated/wildcrafted: The plant is wildcrafted, as it is exceptionally common. The sustainability and conservation implications of large-scale bark harvesting need to be considered.

Conservation status: Not threatened.

Nature of plant products: Origin: Various parts of tropical South, Central, East, and West Africa.

Processing and storage: Preparation/processing: The bark is harvested and dried. It is often pounded or powdered before use.

Stability of product: The product is stable when dried, but relatively unstable in solution. No reliable data are available.

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Vernonia amygdalina

GENERAL DESCRIPTION

Scientific Name with Author: *Vernonia amygdalina* Delile

Synonyms: *Vernonia eritreana* Klatt, *V. giorgii* De Wild., *V. randii* S. Moore, *V. vogeliana* Benth., *V. weisseana* Muschl., *V. adenosticta* Fenzl, *Gymnanthemum amygdalinum* (Delile) Walp., *Gymnanthemum abyssinicum* Sch.Bip. ex Walp (Beentje, 2000).

Family: Asteraceae

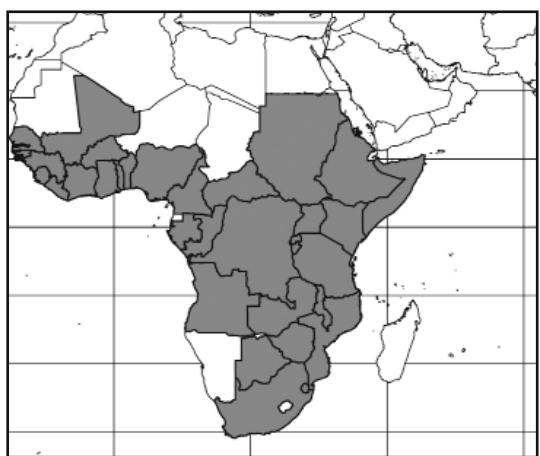
Vernacular Names: Alomagbo, amanvivè, awonwone, bantara bururé, bitter leaf, chichilusa, ekibirizi, ewuro, kayakaya, kibirizi, ko safoune, kossa fina, labori, labwore, lubirizi, lugon, luluza, matoulou, mubirizi, muburizi, mululuza, munkankali, musikavakadzi, muuluza, okelo okelo, omubiriri, omubirizi, omululisi, omululusa, shiwaka.

Botanical Description: *Vernonia* is a shrub or small tree of 2-5 m high. The leaves are green, with a characteristic odour and a bitter taste. Petiolate leaves oblong-lanceolate to narrowly elliptic-lanceolate in shape, 7-15 cm long, 3-7 cm broad, gradually cuneate at the base, gradually acuminate at the apex, pubescent; inflorescence corymbiform with numerous capitula; floret white or bluish; pappus white or russet (Mshana et al., 2000). Similar with *V. colorata* in appearance, it shares many of the same vernacular names and uses. The only reliable way to separate these two species is to examine the achenes with a lens (this also works with the ovaries when the plants are in flower). If only papillae are present on the body of the achene, it is *V. colorata*; if both papillae and hairs are present, then it is *V. amygdalina*.

Consequently, it is highly probable that traditional healers use these two species interchangeably and usually do not differentiate between them.

Origin and Distribution: Savanna and forest margins, often forming thickets, from Guinea to W Cameroon. It is widely distributed throughout

tropical Africa, drought tolerant, growing in a range of ecological zones, and producing a large mass of forage (Hutchison & Dalziel, 1963, cited by Bonsi et al., 1995a). In Africa, some 200 species of *Vernonia* are known. In contrast to *V. colorata* (which appears to be a plant of generally drier areas at lower altitudes), *V. amygdalina* occurs in higher rainfall areas at higher altitudes.



Plant Parts Used: Leaves, twigs, and roots.



Possible Alternative Source Species: *Vernonia colorata*, a plant which shares close resemblance to *V. amygdalina* and is often used ethnobotanically for similar purposes.

ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses: *V. amygdalina* is well known as a medicinal plant with several

uses attributed to it. It is used in Nigerian folk medicine as a tonic, and as a remedy against constipation, fever, diabetes, high blood pressure, and many infectious diseases. The roots are chew sticks against gastro-intestinal diseases, enteritis, and anthelmintic. The twigs, as chew sticks, are haemostatic and cicatrizing. Infusion of the bark, stem, and root is used for the treatment of fever, diarrhea, and rheumatism. The dried flowers are used to treat stomach disorders (Burkhill, 1985). In the Guruve District of Zimbabwe, the chopped roots of *Vernonia amygdalina* are used for the treatment of sexually transmitted diseases (njovhera) (Kambizi & Afolayan, 2001). In South Africa, the root of the plant is used for its antifertility effect and for treatment of amenorrhoea and dysmenorrhoea. In Ethiopia, it is one of the traditionally used antifertility plants. Studies to confirm the antifertility effect of the plant have been carried (Destá, 1994).

V. amygdalina is one of the most commonly used plants against fevers and malaria throughout Africa. In a survey in eastern Uganda, it was by far the most widely quoted plant for the treatment of malaria (Tabuti, 2008). It is also used as a treatment for fever and malaria in other parts of Uganda (Adjanohoun et al., 1993; Adriaens, 2005; Bitahwa et al., 1997), Kenya (Kokwaro, 1993), Angola (Watt & Breyer-Brandwijk, 1962), Democratic Republic of Congo (Tona et al., 1999), Burundi (Baerts & Lehmann, 1993), Rwanda (Hakizamungu & Weri, 1998), Ghana (Asase et al., 2005), Nigeria (Ainslie, 1937), Ethiopia (Gedif & Heinz-Jurgen, 2002), and Zimbabwe (Gelfand et al., 1985). Anecdotally, evidence is available to support its effectiveness. In Ghana, 15% of respondents knew about its use for malaria, and rated it as very effective (Asase et al., 2005).

The bark of stem and roots is particularly bitter and is more or less astringent. The leaves of *V. amygdalina* may be consumed either as a vegetable (macerated leaves in soups) or aqueous extracts as tonics for the treatment of various illnesses. In Ethiopia, the leaves of the plant are used to treat skin wounds by Zay people (Giday et al., 2003).

A survey in Uganda of 492 health workers, mothers, and people with AIDS revealed that 84% said they had used the tree *Vernonia amygdalina* as a medicinal plant (Challand, 2005). The roots of *V. amygdalina* have been used for gingivitis and toothache, and consequently antimicrobial activity was established (Elujoba et al., 2005).

Other Relevant Uses: The leaves are said to be used as an appetizer and digestive tonic. They have a sweet and bitter taste, sold fresh or dried, and are a typical ingredient in egusi soup. Also used as a vegetable, especially in Nigeria (fatefate or maye maye) and Uganda (Katende et al., 1999). Several species of *Vernonia*, including *V. calvoana*, *V. amygdalina*, and *V. colorata*, are eaten as leaf vegetables. They are one of the most widely consumed leaf vegetables of Cameroon, where they are a key ingredient of ndole stew.

Vernonia amygdalina is used, instead of hops, to make beer in Nigeria and Uganda, and is found in homes and villages as pot-herb and fence post.

During the dry period, dairy farmers from southern Ethiopia feed boiled *Vernonia* to cattle. Boiling decreases the content of secondary plant compounds and makes the feed more palatable. It is also used as a supplement to teff straw (*Eragrostis tef*), fed to Ethiopian Menz sheep (Bonsi et al., 1995).

The crushed leaves of *V. amygdalina* are made into paste and rubbed on the body of livestock against ectoparasites (Chifundera, 1998). In the wild, chimpanzees have been observed to ingest the leaves when suffering from parasitic infections. (Huffman & Seifu, 1989, Huffman et al., 1993, 1996). It has also been suggested to be a possible insect antifeedant (Ganjian et al., 1983).

CHEMICAL CONSTITUENTS

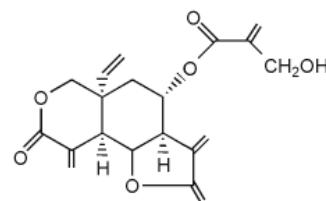
Compounds: Chemical studies have shown the presence of different compounds including flavonoids, sesquiterpene lactones, cardiotonic glycosides, steroidal glycosides, and similar natural product compounds.

From the root, a glycoside cardiotonic (vernonin) has been isolated with an action comparable to that of digitalin. Two sesquiterpene lactones

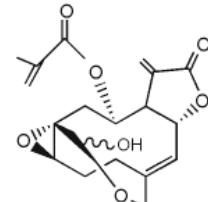
(vernolide and vernodalol), isolated from an ethanolic extract of the leaves, have been shown to exhibit antioxidative effect, which might be responsible for the health-promoting ability of this plant species (Erasto et al., 2006, 2007). Several sesqui-terpene lactones from the plant have demonstrated interesting biological activities. Examples include vernolepin, vernodalol, vernomygdin, and vernodalin as antitumour agents (Oketch-Rabah, 1996). Vernodalin and vernodalol have significant antimalarial activity in vitro (Ohigashi et al., 1994).

Other compounds include stigmastane-type steroid glucosides, vernonioside A1, A2, A3, A4, and B1, B2, B3; they have been found to be antiparasitic (Oketch-Rabah, 1996). Some of these compounds and their aglycones appear to be responsible for the bitter taste in the plant (Jisaka et al., 1993). Other compounds identified are 7,24(28)-Stigmastadien-3 β -ol (Arene, 1972), elemanolide, and 11,13-dihydrovernodalin (Ganian et al., 1983).

Flavonoids and cynamoyl derivatives occurring in *Vernonia amygdalina* leaves have also been identified and quantified. The identified flavonoids were luteolin, luteolin 7-O-glucuronoside, luteolin 7-O-glucoside, and two rutinosides of luteolin (with the sugar moiety linked in 4' and 7'). Three isomers belonging to the same class (dicaffeoyl derivatives) were identified as the cynamoyl derivative (dicaffeoyl quinic acid and 1,5 di-caffeooyl quinic acid), in addition to chlorogenic acid and caffeooyl quinic acid. The quantitative estimation of the compounds identified in *Vernonia amygdalina* (mg/g dried weight) revealed that luteolin-7-O-glucuronoside (0.47) and luteolin-7-O-glucoside (0.36) were the dominant flavonoids, while caffeooyl quinic acid derivatives were shown to be the dominant cinnamoyl derivative. Dicaffeoyl quinic acid (0.78) and 1,5 dicaffeoyl quinic acid (0.41) were also present. These phenolic compounds may be responsible in part for the antioxidant activity (Salawo et al., 2007).



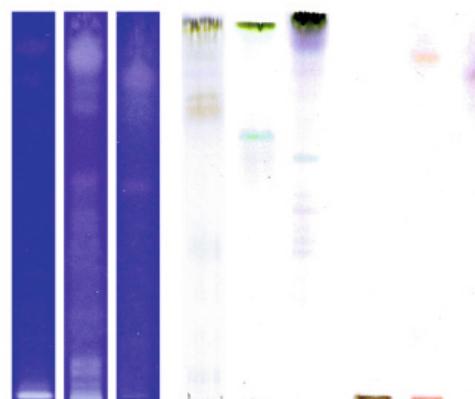
Vernodalin



Vernolide

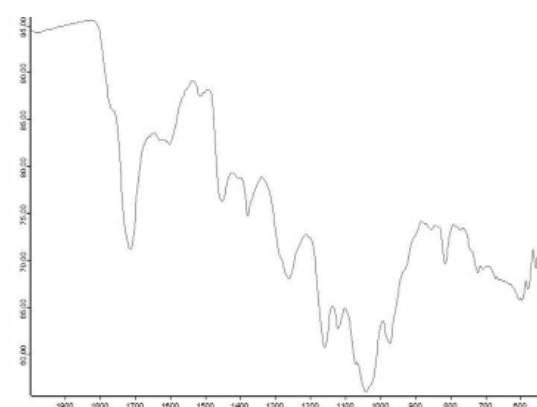
QUALITY CONTROL

TLC / HPLC / GC



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

NIR Spectroscopy



Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

Vernonia amygdalina leaf (60% methanol extract) was found to be active at 25 mg/ml against six of eight bacterial isolates (Akinpelu, 1999). Two known sesquiterpene lactones (vernolide and vernodalol), obtained from the leaves of *Vernonia amygdalina*, were tested by agar dilution method against 10 bacteria strains and 5 fungi species (Erasto et al., 2006) Both compounds exhibited a significant bactericidal activity against five Gram-positive bacteria, while lacking efficacy against the Gram-negative strains. In the antifungal test, vernolide exhibited high activity against *Penicillium notatum*, *Aspergillus flavus*, *Aspergillus niger*, and *Mucor hiemalis*. Vernodalol showed moderate inhibitions against *Aspergillus flavus*, *Penicillium notatum*, and *Aspergillus niger*. Both compounds were ineffective against *Fusarium oxysporum*, a microbe known to be highly resistant to chemical agents.

The aqueous leaf extract of *Vernonia amygdalina*, given intra-peritoneally, produced a dose-related fall in blood sugar. A dose of 80 mg/kg body weight of adult rabbit produced a maximum lowering of blood sugar in both fasted (normal) and alloxanized rabbits. The fasting blood sugar in normoglycaemic rabbits was reduced from 96 mg% to 48 mg% in 4 h. In alloxanized rabbits, the blood sugar was reduced from the mean value of 520 mg% to 300 mg% in 8 h. (Akah & Okafor, 1992). Studies conducted using streptozotocin-induced diabetic laboratory animals showed that *V. amygdalina* administration decreased blood glucose by 50% compared to untreated diabetic animals (Nwanjo, 2005).

Extracts of *Vernonia amygdalina* have been evaluated for antithrombotic activity in mice. It offered protection against thrombosis produced by an intravenous injection of a mixture of ADP and adrenaline (Awe et al., 1998).

Aqueous extract of *Vernonia amygdalina*, infused intragastrically at a dose of 10 mg/ml in rats, induced a significant increase in acid output which was reduced by ranitidine (5 mg/kg b.w.) or atropine (1.2 µMol/kg b.w.). Moreover, the extract evoked a dose-dependent contraction of

the guinea pig ileum, at a dose range of 0.6 mg/ml to 66 mg/ml, which was inhibited by atropine (2.4×10^{-8} - 2.4×10^{-6} M) (Owu et al., 2007). A methanol extract of *Vernonia amygdalina* leaves was evaluated for cathartic effect. The extract exhibited a significant promotion of intestinal motility on charcoal meal test in mice. Frequency of defecation and amount of faeces were markedly increased following administration of the extract, which also promoted gastric emptying in rats.

The lipid-lowering effects of methanolic extract of *Vernonia amygdalina* leaves, in rats fed an high cholesterol diet, was compared with a standard hypolipidemic drug Questran (Adaramoye et al., 2008). The results suggest that *Vernonia amygdalina* could serve as a new potential natural product for the treatment of hyperlipidemia.

In vitro activity against *Plasmodium falciparum* has been tested by many authors (Tona et al., 1999, 2004; Bogale & Petros, 1996; Masaba, 2000; Madureira et al., 2002). *V. amygdalina* leaf extracts also have antimalarial activity in vivo (Abosi & Raseroka, 2003; Hakizamungu & Weri, 2008; Iwalokun, 2008). One study has even shown that *Vernonia amygdalina* aqueous extracts given together with chloroquine can help to overcome chloroquine resistance (Iwalokun, 2008).

The hepatoprotective and antioxidant effects of an aqueous extract of *V. amygdalina* leaves were evaluated against acetaminophen-induced hepatotoxicity and oxidative stress in mice (Iwalokun et al., 2006). Pre-administration of *V. amygdalina* resulted in a dose-dependent (50 - 100 mg/kg) reversal of acetaminophen-induced alterations of all the liver function parameters by 51.9 - 84.9%. Suppression of acetaminophen-induced lipid peroxidation and oxidative stress by the extract was also dose-dependent (50 - 100 mg/kg). The results of this study suggest that *V. amygdalina* elicits hepatoprotectivity through antioxidant activity on acetaminophen-induced hepatic damage in mice. In another study, an aqueous leaf extract of *V. amygdalina* administered to rats for 3 weeks, after establishment of CCl₄ induced liver damage, resulted in significantly less ($P < 0.05$) hepatotoxicity than CCl₄ (Arhogho et al., 2009). Histopathological study also showed significant reduction and even reversal of liver damage in the

rats. The results of this study show that aqueous leaf extract of *V. amygdalina* has a potent anti-hepatotoxic action against CCl₄ induced liver damage in rats.

Clinical Studies: A clinical study to investigate the effect of *Vernonia amygdalina* on postprandial blood glucose concentration of healthy human subjects was undertaken by a group of researchers in Nigeria (Okolie et al., 2008). Proximate analysis indicated that this vegetable contained fiber, which is a non-soluble polysaccharide (NSP). The effect of the vegetable was compared with that of another indigenous vegetable, also identified to be taken by diabetic patients, *Gongronema latifolium*. The vegetables (50 g each) were processed and administered by squeeze-wash-drink and chew-raw to the subjects, who also served as their own controls, on separate days, in randomized order. Blood glucose levels were checked at fasting (0 min), and postprandially, at 30 min intervals for 2 h. Compared with the other vegetable, *Vernonia amygdalina* elicited significant reductions ($P<0.05$) in blood glucose levels at most postprandial time points, and for area-under-curve (AUC) values. The researchers postulated that the bioactive antioxidant substances which occur naturally in stems, roots, and leaves of the two vegetables may possess insulin-like effect.

A clinical trial of the antimalarial effect of *V. amygdalina* leaf infusion in Ugandan patients with uncomplicated malaria found that the remedy was associated with an adequate clinical response (ACR) at d 14 in 67% of cases. However, complete parasite clearance occurred in only 32% of those with ACR; of these, recrudescence occurred in 71% (Challand & Willcox, 2009). Further studies are needed to determine whether the efficacy can be improved by increasing the dose, changing the preparation, or adding other antimalarial plants.

SAFETY DATA

Ethnic Use Safety Data: *Vernonia amygdalina* is very widely used in human diet as well as a medicine (see above) without any reported cases of serious toxicity.

Preclinical Safety Data

Single Dose Toxicity cf. appendix 1

The LD₅₀ was established to be 560 +/- 1.21 mg/kg i.p. in mice and 3.32 +/- 0.15 g/kg p.o. in rats (Njan et al., 2008).

Repeated Dose Toxicity: The leaves did not cause any toxicity when fed to rats as part of their diet (making up to 75% of their food) over a period of 65 days (Ibrahim et al., 2001). Chronic treatment (six weeks) of male rats with aqueous leaf extract of *V. amygdalina* produced no significant changes in packed cell volume (PCV), white blood cell (WBC), and platelet counts. There were no significant changes in the morphology of the heart, liver, kidney, and intestinal tissues of the treated and un-treated animals. Body weights of both the treated and untreated animals rose progressively as the period increased. This study suggests that the plant extract is non-hazardous, even when taken for some time (Amole et al., 2006).

In another study involving toxicity in rats, results show no clinical signs of toxicity or adverse toxicological effects in the treated groups, except for a significant decrease in red blood cell count, and a dose-dependent increase in serum bilirubin. These changes were within control values, based on historical reference ranges, at doses of 500 - 2,000 mg/kg/day for 14 consecutive days, as compared to the control (Njan et al., 2008).

Clinical Safety Data: In the clinical trial of *V. amygdalina* leaf infusion for malaria, patients were closely monitored for adverse events, both with a screening questionnaire and with blood tests (Challand & Willcox, 2009). There were no serious or severe adverse effects or toxicity from the medication, and no patient requested to withdraw from the study because of assumed side effects. Sixty-five percent reported at least one minor adverse event. The most common adverse events were nocturia, insomnia, and cough. *Vernonia amygdalina* is generally reported to have an unpleasant taste, and drinking 250 ml at night may be the cause of the nocturia.

There was a trend towards a reduction in haemoglobin between day 0 and day 28, although this did not reach statistical significance. However two patients were withdrawn at days 9 and 10 because their Hb had fallen from 9 to

7.4, and from 8.6 to 7.6 respectively (Challand & Willcox, 2009). The apparent trend towards a fall in haemoglobin may have several explanations. Haemoglobin (Hb) measurement was only repeated at d 14 and 28 in those who had an abnormality at d 7 (N=15), so patients with normal Hb were not re-tested. Persisting parasitaemia in the majority of patients is the most likely cause of anaemia. Oboh (2006) showed that *V. amygdalina* leaf infusion causes haemolysis in vitro, especially of erythrocytes from sickle-cell patients (Hb-SS) or carriers (Hb-AS). It causes less haemolysis in normal (Hb-AA) erythrocytes. It is thought that the haemolysis was principally caused by saponins, which are poorly absorbed in vivo. However, rats treated with comparable doses (0.5 g/kg orally) did show a reduction in red cell count and increase in bilirubin, suggesting that some saponins may be absorbed and cause haemolysis (Njan et al., 2008).

KEY (PROPOSED) USAGE

Therapeutic Indications: Antidiabetic (Akah & Okafor, 1992; Okolie et al., 2008; Nwanjo, 2005). Antithrombotic (Awe et al., 1998). Antibacterial (Akinpelu, 1999; Taiwo et al., 1999, Erasto et al., 2006). Insect antifeedant (Ganjian et al., 1983). Ulcerogenic activity (Owu et al., 2007). Cathartic effect (Awe et al., 1998). Antimalarial (Tona et al., 2004; Abosi & Raseroka, 2003; Challand & Willcox, 2009).

Dosage: Many different dosages for different conditions are reported by Adriaens (2005). The Watongwe in Eastern Congo use 2 - 3 fresh leaves pounded and drunk in 300 - 400 ml of water (Huffman et al., 1996). In the clinical trial in malaria patients, the medicinal infusion was made using a standardised procedure of 25 g of fresh, chopped leaves steeped in 1 litre of boiled water for 15 minutes (Challand & Willcox, 2009).

Method and Duration of Administration:

The patients were given 1 litre of freshly made infusion daily to be taken as 250 ml, four times daily, for 7 days. This equates to a daily dose of 0.5 g/kg for a patient weighing 50 kg (Challand & Willcox, 2009).

Pregnancy and Lactation: One source states that the plant is not recommended for consumption by pregnant women but the reason for this is not given (Adriaens, 2005).

Adverse Effects: Adverse events reported in a clinical trial (Challand & Willcox, 2009) included: nocturia, insomnia, cough, numbness, unpleasant taste, irregular heartbeat, diarrhoea, constipation, dysuria, dry mouth, chest pain, stuffy nose, heartburn, and abdominal pain. However, none of these symptoms were reported by more than three patients (of a total of 40) and some reported events may have had causes other than the medicinal infusion. No patient reporting irregular heart beat or chest.

Evaluation of Efficacy: Appetizer and digestive tonic efficacy traditionally proven. Laxative efficacy traditionally proven. Chewsticks against gastro-intestinal diseases efficacy traditionally proven. Anthelmintic, enteritis, antischistosomiasis efficacy traditionally proven. Haemostatic and cicatrizing efficacy traditionally proven. Against malaria, *Vernonia amygdalina* leaf infusion is not as efficacious as quinine or artemisinin derivatives (Mueller et al, 2004).

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Vernonia colorata

GENERAL DESCRIPTION

Scientific Name with Author: *Vernonia colorata* Drake

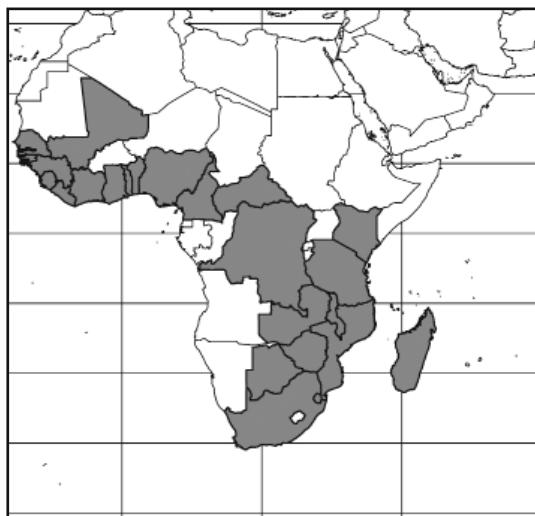
Synonyms: *Vernonia amygdalina* Delile

Family: Asteraceae

Vernacular Names: Abovi, abowi, aovi, bitter leaf, ewuro, ko-safandé, ko-safina, ndumberghat, todzo, shiwaka, zidor.

Botanical Description: *Vernonia colorata* is a highly branched shrub, up to 3-5 m high. The leaves are bitter, pubescent, ovale, 8-15 cm long, 5-10 cm broad; lateral nerves 7-9, prominent below; petiole 15-30 mm long, pubescent. Inflorescences flattened panicles composed of small capitulum, 5 to 15 cm long. Flower white or bluish, tubular, 8-10 mm. Achenes glabrous, with reddish-brown pappus, 3 mm long (Ake Assi & Guinko, 1986).

Origin and Distribution: *Vernonia colorata* grows as well in savanna as in rainforests, especially in wet places and secondary growth. The plant is common in most of West Africa (from Guinea to Cameroon), central Africa, and the tropical south.



Plant Part Used: Leaves



ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses: Several species of *Vernonia*, including *V. colorata*, are eaten as leaf vegetables. Common names for these species include bitterleaf. They are one of the most widely consumed leaf vegetables of Cameroon, where they are a key ingredient of ndole stew. The leaves have a sweet and bitter taste. They are sold fresh or dried, and are a typical ingredient in egusi soup in Nigeria.

Vernonia colorata has long been used in traditional medicine by the indigenous people for the treatment of cough, fever, hepatitis, gastritis, diarrhoea, stomach pain, gastrointestinal disorders, diabetes, venereal diseases, skin eruptions, boils, rheumatism, dysentery, diabetes, ulcerative colitis, and as a general tonic, for which decoctions of the leaves are generally used (Hutchings et al., 1996).

Commonly, the leaves are used as water infusions or decoctions and employed to rinse the mouth, for tonsillitis, to cure earache, and as a febrifuge. The extract of fresh leaves, softened by heating, is applied to wounds for cicatrisation (Kerharo & Adam, 1974; Ake Assi & Guinko, 1986; Oliver-Bever, 1996; Adjano'houn, et al., 1985).

CHEMICAL CONSTITUENTS

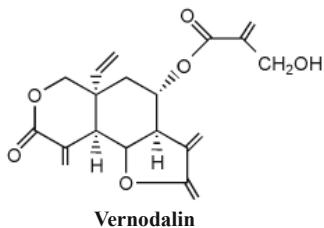
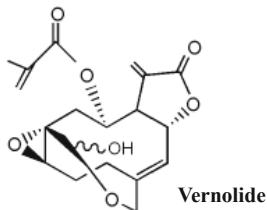
Compounds: *Vernonia* contains mainly saponins and sesquiterpene lactones. The investigation of

two East African and a Madagascanian *Vernonia* species afforded several new sesquiterpene lactones. *Vernonia colorata* subsp. *grandis* gave 19-hydroxyglaucolide A and three vernodalin derivatives. The structures were elucidated by high field NMR techniques.

Sesquiterpene lactones were isolated from *V. colorata* leaves and identified as vernolide (I) and hydroxyvernolide (II) by IR, NMR, and mass spectrophotometry. Three compounds were obtained by the hydrogenation of hydroxyvernolide from *Vernonia colorata* (Toubiana, & Gaudemer, 1967; Toubiana, 1969).

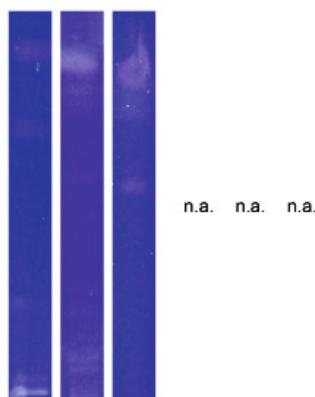
A fraction enriched in amino acids from the powder of *V. colorata* leaves contained 2 free and 12 bound amino acids (Bamba et al., 1984). A number of sesquiterpene lactones have been isolated from other African *Vernonia* species (Toubiana & Gaudemer, 1967; Kupchan et al., 1969; Asaka et al., 1977; Zdero et al., 1991).

The essential oils from the aerial parts of *Vernonia colorata* and *Vernonia nigritiana*, grown wild in Mali, were obtained by hydrodistillation. 115 Components have been identified (Senatore et al., 2004).



QUALITY CONTROL

TLC / HPLC / GC



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

NIR Spectroscopy



Standard specifications cf. appendix 2

The total ash was evaluated at $12.615 \pm 0.06\%$.

PHARMACOLOGICAL PROPERTIES

Vernonia possess significant in vitro antischistosomal, plasmodicidal, and leishmanicidal activities (Ohigashi et al., 1994). These lactones have anthelmintic and amebicidal properties.

Bioassay-directed fractionation of an anti-inflammatory $\text{CHCl}_3\text{-MeOH}$ (9:1) extract of leaves of *Vernonia colorata*, using a carrageenan-induced rat paw model, led to the isolation of six

new compounds. Compounds were tested for the anti-inflammatory activity, but all were inactive or weakly active as anti-inflammatory agents (Cioffi et al., 2004).

Extracts of *Vernonia colorata* were active against *Pseudomonas aeruginosa* (Jonathan et al., 2000). Vernodalin isolated from *Vernonia colorata* leaves demonstrated antibacterial activity, with major minimal inhibitory concentration (100 µg/ml) against *S. aureus* (Reid et al., 2001). The leaves of *V. colorata* have shown high antibacterial activity in the disc-diffusion bioassay (Kelmanson et al., 2000).

Leaf extracts of *Vernonia colorata* had antimalarial activities with IC₅₀ in vitro 4.7 ± 2.2, 7.82 ± 6.6, 9.38 ± 4.4, 12.5 ± 0, 0.4 ± 0, 2.35 ± 1.1, 9.38 ± 4.4, and 12.5 ± 0 on two strains of *P. falciparum*, the first one was chloroquine-resistant, and the other was chloroquinesensitive (Benoit-Vical et al., 1996, 2000; Menan et al., 2006). Extracts from *V. colorata* were inhibitory at concentrations as low as 1 mg/l, with IC₅₀ of 1.7, 2.6, and 2.9 mg/l for dichloromethane, acetone, and ethanol extracts, respectively. These results indicate a promising source of new anti-toxoplasma drugs from *V. colorata* and African medicinal plants (Benoit-Vical, et al., 2000). The lipophilic extracts from the aerial parts of *Vernonia colorata* proved to be the most active against the chloroquine-sensitive strain PoW, and against the chloroquine-resistant clone Dd2, of *Plasmodium falciparum*. Evaluation of *V. colorata* revealed four sesquiterpenes, 11β,13-dihydrovernodalin, vernodalol, 11β,13-dihydrovernolide and 11β,13,17,18-tetrahydrovernolide, of which the first two constituents exhibited the strongest antiplasmodial activity (IC₅₀ values: 1.1 - 4.8 µg/ml) (Kraft et al., 2003).

As well as glibenclamide, the aqueous extract of *V. colorata*, at a dose of 300 mg/kg per os, decreased the blood glucose in alloxanic rats from 2.80 ± 0.10 to 1.00 ± 0.20 g/l (p < 0.05, n = 5).

Vernolide and hydroxyvernolide, two lactones isolated from *V. colorata* leaves, have anthelmintic and amoebicidal properties. The compound vernolide is more active than hydroxyvernolide in vitro, and has a level of action comparable to metronidazole. However, only hydroxyvernolide

has significant in vivo action, especially as an amoebicide (Gasquet et al., 1985). With this, the antiplasmodial effects of the two sesquiterpenes lactones (vernolide and hydroxyvernolide) were supported.

Vernonia spp. are used in Latin America in menstrual and stomach disorders, suggesting smooth muscle relaxing properties of some of their chemical constituents. Sesquiterpene lactones, glaucolides D and E, were assayed on isolated rat smooth muscle. Glaucole E proved more potent than glaucolide D to relax high KCl- or noradrenaline-induced contractions in aorta, and to relax the high KCl-contraction in uterus. Hirsutinolide-type sesquiterpene lactone also was tested but displayed no effect. Relaxation of smooth muscle, by structurally related sesquiterpene lactone parthenolide, has been attributed mainly to the α-methylene γ-lactone moiety; because glaucolides D and E lack this functional group, their relaxant properties may rely on other alkylating sites, such as C10 of the germacra-1(10),4-diene-4-epoxide skeleton (Campos et al., 2003). Different extracts of the leaves of *V. colorata* possess both hypoglycaemic and antidiabetic effect in normoglycaemic and alloxan-induced diabetic rats (Sy et al., 2004, 2005, 2006).

SAFETY DATA

Preclinical Safety Data

Single Dose Toxicity cf. appendix 1

Vernonia colorata leaves are not toxic (Taylor et al., 2003). The LD₅₀ of *Vernonia colorata* in mouse is about 10g/kg.

KEY (PROPOSED) USAGE

Therapeutic Indications: Antibacterial, antimalarial.

Evaluation of Efficacy: Antimalarial activities efficacy traditionally proven (Benoit-Vical et al., 1996; Kraft et al., 2003). Active against *Pseudomonas aeruginosa* efficacy traditionally proven (Jonathan et al., 2000). Antibacterial activity efficacy traditionally proven (Kelmanson et al. 2000).

Appetizer and digestive tonic efficacy traditionally proven. Fever and substitute for quinine efficacy traditionally proven. Laxative efficacy traditionally proven. Chewsticks against gastrointestinal diseases efficacy traditionally proven. Anthelmintic, enteritis, antischistosomiasis efficacy traditionally proven. Haemostatic and cicatrizing efficacy traditionally proven.

TRADE INFORMATION

Nature of plant material: Leaves of *Vernonia colorata* are harvested at any period of the year. They are used fresh or dried and powdered into a greenish powder.

Processing and storage: The preparation differs depending on the use. For soup, because of their bitterness, fresh leaves are boiled into water, washed, and then used in the soup. For the treatment of malaria, fresh leaves are abundantly washed into water and pressed to collect the bitter water. The product is highly stable when dried but relatively unstable in solution.

Processing and preservation methods used are thought to influence the nutrient content of these vegetables. A study was aimed at determining the effects of processing and preservation methods on vitamin C and total carotenoid levels of some species of *Vernonia* (*V. amygdalina*, *V. calvoana* var. *bitter*, *V. colorata*, and *V. calvoana* var. *non bitter*) consumed in Cameroon. Results show these leafy vegetables were good sources of vitamin C and total carotenoid. Drying caused significant losses, especially in Vitamin C. The best temperature for drying the vegetables to preserve carotenoids and vitamin C was at 45°C, whereas sun drying and oven drying at 75°C caused the highest losses. Freezing for up to ten days had no significant influence on total carotenoids and vitamin C levels. Comparatively, squeezewashing proved to be the best processing method that ensured minimum loss of both vitamins (Ejoh et al., 2005a). The leafy vegetables from *Vernonia* species are good sources of iron and total carotenoids. In *Vernonia colorata*, iron value is 15.2 to 0.4 mg/100 g (Ejoh et al., 2005b).

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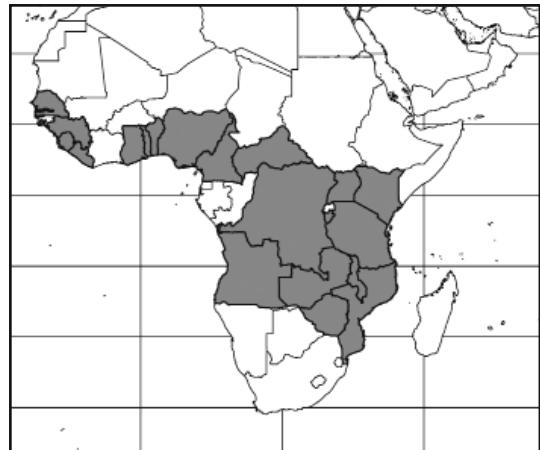
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Voacanga africana

GENERAL DESCRIPTION

Scientific Name with Author: *Voacanga africana* Stapf ex Scott-Elliott

Synonyms: *V. africana* var. *glabra* (Schum.) Pichon, *V. angolensis* Stapf ex Hiern., *V. eke-tensis* Wernham, *V. glabra* K.Schum, *V. glaberrima* Wernham, *V. magnifolia* Wernham, *V. obtusa* Schum., *V. schweinfurthii* var. *parviflora* Schum., *V. talbotti* Wernham, *V. thouarsii* Roem. and Schult., *Annularia natalensis* Hochst., *Tabernaemontana thouarsii* Pal.



Family: Apocynaceae

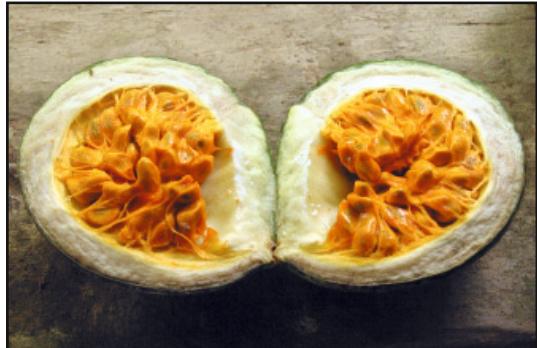
Vernacular Names: Afaf, akete, aka dodo, bunbo, degbe degbe, deguedegue, dodo, foba, gboni, ibalak, igi dodo, kagil, kaka pempe paapaku, ksiso, kwatie kwatie, ninge-ekunyi, o-banawa, ovien-ibu, pete pete, piegba, small-fruit wild frangipani, sulaberekilo, voacanga, voacanga tree.

Botanical Description: *Voacanga africana* is a small, evergreen, tree or shrub, reaching up to 6 meters in height, with a low widely spreading crown. The leaves are opposite, obovate, and acuminate. They are dark green and glossy above, green below, usually stalkless. The flowers are white, borne in loosely branched, glabrous, axillary or terminal inflorescences. The fruits occur mainly in pairs, spherical, mottled green with seeds wrapped in yellow pulp.

Parts Used: The seeds are the most economically important part of the plant, though the bark and roots are also widely used in traditional medicines.



Origin and Distribution: *V. africana* is native to West Africa, specifically Ghana and Ivory Coast. It is mostly found in the wild as a shade plant around communities but occasionally cultivated on commercial basis. *V. africana* is also widespread in mainland tropical Africa, from West Africa (e.g., Senegal) to East Africa (e.g., Kenya) and Southern Africa (e.g., Angola, Zimbabwe, and Mozambique).



ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses: Traditional African uses include the root bark as a cerebral stimulant, and the seeds for visionary purposes. A root-decoction prepared from boiled root is used for painful hernia, dysmenorrhoea, heart troubles (spasm, angina), and blennorrhoea. A paste of the roots is applied to the head to kill lice. The dried and powdered roots without the outer bark are mixed with porridge, and taken against kidney troubles and menstruation problems in women. Decoctions, infusions, or latex of the stem bark, leaves, or roots are put on wounds, boils, and sores, and used to treat gonorrhoea, eczema, fungal infections and scabies. They are also taken to treat heart problems, hypertension, and rheumatic afflictions. The bark sap is used for treating sores, furuncles, abscesses, fungal infections, filarial, and eczema. An infusion of the twigs is applied in bronchitis.

A decoction of the leaves is taken by enema for diarrhoea, in baths for general oedema, by frictions and draughts for leprosy, and in lotions for convulsions in infants. The sap of the leaves is given as nose-drops to control the level of aggression common in patients. It is also used for the treatment of fatigue due to shortness of breath, and against infectious diseases. It has been reported to have been used to treat mental disorders, and as an analgesic. The latex is used as a rubber adulterant, and also put in teeth to treat caries, or dripped in the eye to cure ophthalmia.

The fruit and seeds are extracted with cold water, and the extract taken against peptic ulcer. The seeds are also used to treat high blood pressure. Pharmaceutical companies in Europe extract tabersonine from the seeds, which is readily converted into vincamine, a compound widely used in medicines for geriatric patients. Seed extracts are also used in medicines to treat heart diseases, to lower blood pressure, and to treat cancer.

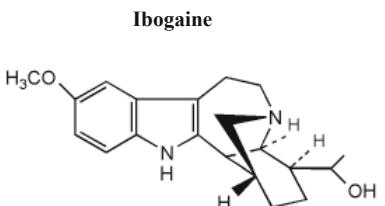
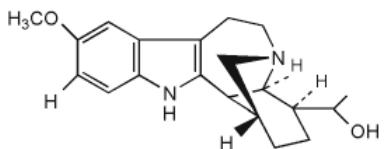
CHEMICAL CONSTITUENTS

Compounds: *Voacanga* contains several alkaloids as well as edible oil. Alkaloids are present in root bark (5 - 10%), trunk bark (4 - 5%), leaves (0.3 - 0.45%) and seeds (1.5 - 3.5%). The main alkaloids

of the root bark are corynanthean-ibogan class dimers (mostly voacamine, voacamidine, and voacorine); vobtusine (a rare plumeran-plumeran class dimer) has also been isolated. Among the monomers found, the ibogan class voacangine and voacristine are the most important constituents; the plumeran class tabersonine has also been found. In the stem bark, voacamine and congeners predominate; vobtusine has also been identified. The leaves contain mainly dimeric alkaloids of both the voacamine and vobtusine groups; but the monomeric plumeran class voaphylline is the main alkaloid.

The alkaloid composition of the seeds is very different, and consists almost exclusively of tabersonine. The seeds of *V. africana* contain up to 10% indole alkaloids, including voacamine, voacangine, and many related compounds. The same alkaloids are found in the bark, but in much lower levels (2%). This group of indole alkaloids when ingested causes a mild to strong stimulation lasting several hours. Higher doses have a strong hallucinogenic effect. The total alkaloidal fraction may be slightly toxic, acting as CNS depressants and hypotensives. Extracts of the seeds are used in commercial phytopharmaceutical production of several interesting cognitive-enhancing compounds.

Some of the important alkaloids fall into three categories: a) vincamine and vinburnine, which are alkaloids used in the cerebro-vascular and geriatric markets (mainly in Japan). In the U.S., they are known as memory enhancers and considerable research is being done with these alkaloids in the treatment of Alzheimer's diseases and Parkinson diseases; b) voacamine, voacangine, voacangerine, and vobtusine, which are hypotensive and as such have ventricular cardio-stimulant action, and a slight action on the sympathetic and parasympathetic nervous system; c) ibogamine, ibogaine, ibolute and iboxygaine which are certified compounds used to treat drug addicts.



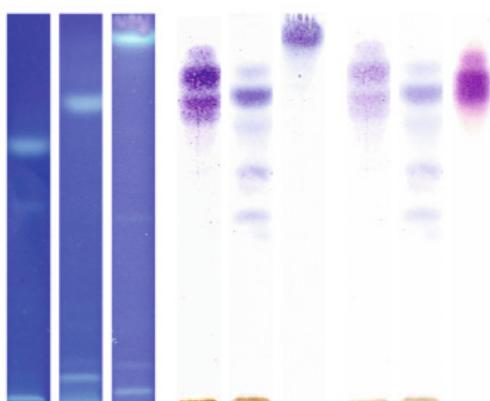
QUALITY CONTROL

Identification: Dark brown or brown.

Macroscopic Characteristics: Damaged seeds: not more than 0.5%. Percent of seed clusters (particles > 4.75 mm): not more than 30%. Seed density: not less than 0.4 (g/ml).

Moisture Content: Not more than 12%.

TLC / HPLC / GC



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

NIR Spectroscopy



Adulterants and Adulterations: Adulteration from other alkaloid-producing plants is possible, since the ibogan and bisindole type occur in several other Apocynaceae genera (e.g., *Catharanthus*, *Hunteria*, *Picralima*, *Rauvolfia*, *Tabernaemontana*, and *Taber-nanthe*). Vincamine is commercially extracted from *Vinca major*.

Standard specifications cf. appendix 2

Extraneous/foreign matter content: not more than 0.5%.

PHARMACOLOGICAL PROPERTIES

The alkaloids show a vast range of pharmacological activities. High doses of voacangine induce convulsions and asphyxia, but in lower doses voacangine exhibits, mostly, rather moderate central nervous system-stimulant activity. It exhibits some cataleptic activity. In regular doses, it has anticonvulsant activity, increases hexobarbital sleeping time in mice, and decreases body temperature. In addition, it has local analgesic activity, hypotensive properties, and causes bradycardia. The hydrochloride salt of voacangine has significant diuretic activity.

Most of the pharmacological work on voacamine and voacorine has focused on their cardiotonic properties. Voacamine shows little tendency to accumulate, and is less toxic than cardiac glycosides, such as digitoxin. Voacamine sulphate resembles cardiac glycosides. When tried clinically on patients with chronic cardiac insufficiencies of various origins, it caused considerable improvement in their clinical status and in haemodynamic parameters, while there was little

effect on the heart rate. The alkaloid was effective both orally and intravenously. In high doses, both voacamine and voacorine are hypertensive, due largely to peripheral vasoconstriction. These compounds also have parasympatholytic and sympatholytic properties, bringing about contraction of smooth muscle fibres, and they are also central nervous system depressants. Voacamine, voacorine, and voacamidine are all cytotoxic in the P-388 cell culture assay. In experiments with rats and mice, parenteral and oral administration of these compounds slowed the growth of transplanted and primary induced neoplasms. Vobtusine causes hypotension as a result of peripheral vasodilatation and a direct depressant action on the heart. In moderate doses initial agitation is followed by a sedative effect; high doses may bring about convulsions and death. Presently, there is no evidence for clinical use of vobtusine.

Tabersonine, the major alkaloid from the seeds, is readily converted to vincamine and vincamine derivatives. Tabersonine is only slightly toxic. It has about a quarter of the hypotensive activity of reserpine (which is commonly used to treat high blood pressure), and a spasmolytic effect on the smooth muscle of the intestine. It has no tumour-inhibiting activity. Vincamine shows protective activity, and improves performance in animal models of cognitive dysfunction produced experimentally by cerebral ischaemia, and by amnesia producing agents. Subsequently, vincamine was shown to increase cerebral blood flow as a result of cerebral vasodilatation, and may also enhance cellular respiration. It has become very popular in Europe especially for geriatric patients and for patients suffering from cerebral arteriosclerosis; there is improvement in the EEG and clinical status, it ameliorates disturbances of attention, memory and mood.

The aqueous extract of the root bark showed antibacterial activity, anti-amoebic activity against *Entamoeba histolytica* and antispasmodic activity on the guinea-pig ileum. This triple action may well explain its traditional use as an antidiarrhoeal agent. The fruit extract contains a compound with cytoprotective and ulcer-healing properties. Finally, the stem bark was tested for molluscicidal activity on the freshwater snail, *Bulinus globulus*, but was found to be only moderately effective.

Leaf-cell suspension cultures of *Voacanga*, grown for 20 days under standard conditions, yielded 6 alkaloids. Tabersonine, lochnericine, and minovincinine were the major ones. Voafrine A and B, dimers of the plumeran-plumeran class, not previously detected from nature, were also produced. These compounds are of pharmacological interest because they are related to vincaleucoblastine (vinblastine). The seed oil is a by-product of the commercial extraction of tabersonine for vincamine synthesis. The main fatty acids are palmitic (15 - 20%), stearic (7 - 16%), oleic (49 - 60%), and linoleic (15 - 20%) acid. The oil has cosmetic and nutritional value.

Voacamine, voacangine and voacorine also have a hypotensive action, and are simultaneously mildly parasympatholytic and sympatholytic. Voacangine manifests analgesic and local anaesthetic effects, while vobtusine increases the hypnotic effect of barbiturates. Voacorine is also cardiotonic through direct action on the heart muscle and on the coronary perfusion, and seems to contribute considerably to the action of the total alkaloids in *Voacanga*. Ibogaine is a natural alkaloid of *V. africana* that is effective in the treatment of withdrawal symptoms and craving in drug addicts. As the synaptic and cellular basis of ibogaine's actions are not well understood, the study tested the hypothesis that ibogaine and *Voacanga* extract modulate neuronal excitability and synaptic transmission in the parabrachial nucleus using the nystatin perforated patch-recording technique. Ibogaine and *Voacanga* extract dose-dependently, reversibly, and consistently attenuate evoked excitatory synaptic currents recorded in parabrachial neurons. The ED₅₀ of ibogaine's effect is 5 µM, while that of *Voacanga* extract is 170 µgrams/ml. At higher concentrations, ibogaine and *Voacanga* extract induce inward currents or depolarization that are accompanied by increases in evoked and spontaneous firing rate. The depolarization or inward current is also accompanied by an increase in input resistance, and reverses polarity around 0 mV. The depolarization and synaptic depression were blocked by the dopamine receptor antagonist, haloperidol. These results indicate that ibogaine and *Voacanga* extract: 1) Depolarize parabrachial neurons with increased excitability and firing rate; 2) Depress non-NMDA receptor-mediated fast synaptic transmission; 3) Involve dopamine

receptor activation in their actions. These results further reveal that the *Voacanga* extract has one-hundredth the activity of ibogaine in depressing synaptic responses. Thus, ibogaine and *Voacanga* extract may produce their central effects by altering dopaminergic and glutamatergic processes. Extracts of *Voacanga* also have cytoprotective, ulcer healing properties through the promotion of mucus secretion.

Clinical Studies: Clinical studies have demonstrated the efficacy of ethyl apovincamine, a derivative of vincamine, in a wide range of conditions including dementia, memory and attention, brain blood supply insufficiency, vascular diseases, and CNS degenerative disorders.

Cerebrovascular dysfunction: There is a growing demand for the use of ethyl apovincamine in the management of cerebro-vascular dysfunction and has been found to be especially effective in improving memory, maintaining cognitive function, and enhancing overall attention, and concentration. It exhibits an activity on neural metabolism by favouring the aerobic glycosis and promoting the redistribution of blood flow toward ischemic areas.

Utilization of oxygen: It is also reported to act to increase cerebral circulation and the utilization of oxygen, and thus may help in other conditions related to insufficient blood flow to the brain including vertigo, Meniere's syndrome, difficulty in sleeping, mood changes, and depression and headaches.

Cerebral circulation: Ethyl apovincamine is believed to increase cerebral blood flow without significantly affecting systemic blood pressure. Furthermore, it decreases cerebrovascular resistance without producing general vasodilatation, or any serious side effects. In addition to aiding memory and promoting healthy brain function, it has also shown promise in the treatment of tinnitus (ringing of the ears), strokes, Alzheimer's disease, macular degeneration, and glaucoma.

Enhancement of the cerebral circulation: It produced a greater increase in cerebral than in peripheral blood flow, which has in some cases

been interpreted as indicating selective cerebral vasodilatation. However, in vitro studies show insignificant selectivity for cerebral, as opposed to peripheral blood vessels, is observable indicating that the selective increase in cerebral blood flow produced may be due to an additional indirect effect of the molecule such as increasing cerebral metabolic rate.

Cerebral metabolic rate: Studies have also shown that this compound can act to increase cerebral metabolic rate. This stimulation can act to increase cerebral blood flow, and may explain the observed enhanced cerebral circulation. The important aspect of this phenomenon is that, despite some vasodilator activity, it does not appear to produce any stealing of blood from the under perfused areas of the brain. This could be due to the fact that the molecule normalises the flexibility of human erythrocytes exposed to lactic-acidosis and hyper osmolality.

Anti-ischemic and neural protector: It has been shown to have an anticonvulsant activity in mice and rats, and neuronal protective activity, by protecting the brain from the deleterious effects of ischemia. The mechanism of action of the compound as a neuronal protector is not completely known, but it is thought that it inhibits the cellular re-uptake of adenosine, which is considered the brain's endogenous anticonvulsant and a neuronal protector.

Memory formation: It inhibits cyclic nucleotide phosphodiesterase, with consequent effects on noradrenalin release and neuronal excitability, thus may also play a relevant role on memory formation.

SAFETY DATA

Preclinical Safety Data

Single Dose Toxicity cf. appendix 1

The acute toxicity LD₅₀ was estimated at 14.5 and 11 g/kg body weight (bw) for the bark methanol extract of *V. africana*. The LD₅₀ of ibogaine has been determined in guinea pig (82 mg/kg i.p.) and rat (327 mg/kg intragastrically, and 145 mg/kg i.p.). Voacamine does not bind to the cardiac proteins and has no cumulative action, but it

has a direct myotonic effect on the cardiac fibre. Lethal doses for guinea pigs (by instillation in the jugular vein) are 313mg/kg for voacamine sulphate and 348 mg/kg for voacandine sulphate compared to 2.5mg/kg for digitalin and 0.9 mg/kg for strophanthin. In mice, the LD₅₀ of voacangine, given intravenously, is 41 - 42 mg/kg. Therapeutic doses (1 - 3 mg daily) are well tolerated, act rapidly, and are quickly eliminated without any cumulative effect. Its minimum LD₅₀ in guinea pigs (by slow intravenous injection) is 228mg/kg. In the acute toxicity tests performed with oral, intravenous, and intraperitoneal routes of administration of Ethyl apo-vincamine, LD₅₀ ranged from 58.7 to 534 mg/kg in mice and from 42.6 to 503.3 mg/kg in rats. Ataxia and clonic convulsions were induced by lethal doses.

Repeated Dose Toxicity: Sub-chronic toxicity was tested with oral administration in rats (25 - 100 mg/kg, for 4 weeks) and with parenteral administration, in rats (5 - 25 mg/kg ip for 3 months) and dogs (2 - 5 mg/kg iv for 3 - 4 months). Chronic toxicity was tested in the course of oral, 6-month administration in rats (5 - 100 mg/kg) and dogs (5 - 25 mg/kg). Results of both sub-chronic and chronic tests indicated that human oral and iv doses of could be considered safe, since multiple doses of 25-80-125 fold were well tolerated by animals without any toxic damage. In reproduction and teratogenicity studies conducted in rats and rabbits, there was no evidence of toxic effects.

KEY (PROPOSED) USAGE

Therapeutic Indications: Stimulant, cardio-tonic

Pregnancy and Lactation: Not recommended for administration in pregnancy and lactation.

TRADE INFORMATION

Volume of production in the country: 800-1,000 Mt (Ghana)

Volume of domestic consumption: All production exported.

Average price: USD \$5 to \$11 per kilogram (Ghana).

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Warburgia salutaris

GENERAL DESCRIPTION

Scientific Name with Author: *Warburgia salutaris* (G.Bertol.) Chiov.

Family: Canellaceae

Vernacular Names: Isibhaha, peperbasboom, pepperbark tree, Pfefferrindebaum, warburgia.

Botanical Description: A medium-sized tree of about 10 m in height, with a rough, mottled bark which is reddish to pinkish on the inner side. The leaves are oblong, about 60 mm long, glossy green above and paler below. Small, greenish-yellow flowers are produced between the leaves on the stem, followed by round, green fruits with several flat seeds inside (Codd, 1976).

Origin and Distribution: Eastern and southern Africa.



Plant Parts Used: Dried leaves. Bark harvesting is not sustainable and plant part substitution (the use of leaves instead of bark) has been practiced on a small scale since 1996 (Rabe & Van Staden, 2000).



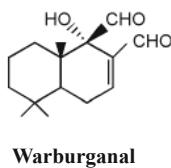
ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses: Traditional knowledge about this popular traditional medicine is surprisingly poorly recorded considering its obvious importance and popularity. For example, it has been estimated that about 17 tons of *Warburgia* bark are traded a year in the Durban medicinal plants trade alone (Mander, 1998). It is one of the great tonics and panaceas of southern Africa; its specific name *salutaris* means salutary to health. The bark is generally used, and it is usually taken orally in a powdered form, or as infusions and decoctions. *Warburgia* is used as a tonic for all health conditions, including fever, malaria, colds, influenza, venereal diseases, abdominal pain, toothache, constipation, cancer, rheumatism, stomach ulcers, headache, as an expectorant in coughs, and as a natural antibiotic to treat chest infections. Cold water infusions of the powdered bark are

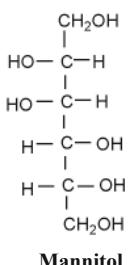
traditionally taken orally as expectorants, or the bark is smoked as a cough and cold remedy (Watt & Breyer-Brandwijk, 1962; Gelfand et al., 1985; Hutchings et al., 1996; Van Wyk et al., 1997; Van Wyk & Gericke, 2000; Neuwinger, 2000; Van Wyk & Wink, 2004).

CHEMICAL CONSTITUENTS

Compounds: Key active compounds are drimane sesquiterpenoids (Mashimbye et al., 1999) such as warburganal, mukaadial, salutarisolide, polygodial, and isopolygodial, as well as muzigadial (Rabe & Van Staden, 2000). The levels of these compounds appear to be unreported. Bark is said to contain mannitol (Watt & Breyer-Brandwijk, 1962).



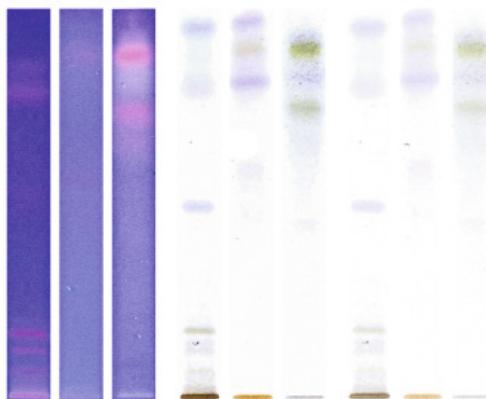
Warburganal



Mannitol

QUALITY CONTROL

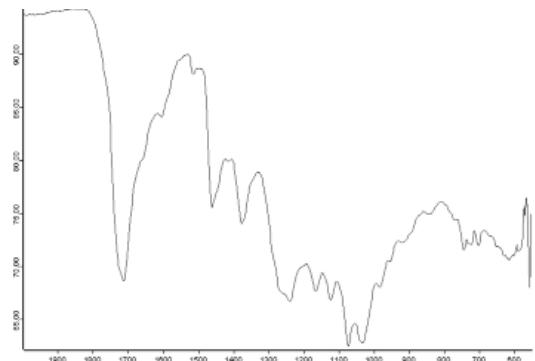
TLC / HPLC / GC



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

Markers and Quantitative Methods: The active compounds are drimane sesquiterpenoids (Mashimbye et al. 1999) but their levels in bark or leaves have not been accurately recorded.

NIR Spectroscopy



Adulterants and Adulterations: The bright green colour and sharp, peppery taste is a useful way to distinguish the product. The bark has a pinkish underbark, and is also extremely peppery.

Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

The drimane sesquiterpenoids, such as warburganal and polygodial, are known to be highly active in low concentrations against candida (Kubo & Taniguchi, 1988). Polygodial is potentially useful in clinical medicine as an adjunct to treatment with antibiotics and antifungals that have poor membrane permeability (Iwu, 1993). Polygodial has a protective effect against the development of gastric mucosal lesions (Pongpiriyadacha Y, et al. 2003). *Warburgia* should never be taken during pregnancy as warburganal is a potent cytotoxic. Recent studies have confirmed antibacterial activity (Rabe & Van Staden, 1997, 2000) and also showed molluscicidal activity (Clark & Appleton, 1997). Mannitol is used for dyspepsia, as a diuretic, and as a sweetener for diabetics (Bruneton, 1999).

Clinical Studies: There are no controlled clinical studies of efficacy. Based on limited clinical observations of medical doctors, there is preliminary evidence for efficacy of freeze-dried *Warburgia* leaf tablets for treating vaginal candida and confirmed acute bacterial cystitis (Gericke, 2001).

SAFETY DATA

Ethnic Use Safety Data: Tablets and large volumes of the more potent bark powder and extracts have been used for many years with no recorded toxicity.

Preclinical Safety Data

Single Dose Toxicity cf. appendix 1

KEY (PROPOSED) USAGE

Therapeutic Indications: Antibiotic, antibacterial, antifungal.

Dosage, Method, and Duration of Administration: Unrecorded and poorly known. Tablets have been made from the dried leaf powder, and the recommended daily dose is one to two tablets, each containing 100 mg freeze-dried leaf material, taken three times a day for up to 7 days.

Special Warnings and Precautions for Use: *Warburgia salutaris* leaf can be safely consumed when used appropriately. Should not be taken continuously for longer than two weeks.

Occasional incidents of mild loose stool has been reported from use of *Warburgia* leaf tablets, that resolves spontaneously. The inner bark can cause self-limiting vomiting if taken in excess, or on an empty stomach, probably on the basis of gastric irritation.

Pregnancy and Lactation: The product should not be used during pregnancy or while breast-feeding.

Evaluation of Efficacy: Antibacterial efficacy pharmacologically proven (Rabe & Van Staden, 1997, 2000). Anticandida efficacy pharmacologically proven (Kubo & Taniguchi 1988; Gericke, 2001). Anti-ulcer: Insufficient information for classification. General tonic efficacy traditionally proven for short term use only (two weeks).

TRADE INFORMATION

Nature of plant material: Flowering/harvesting time: Throughout the year.

Cultivated/wildcrafted: Cultivated on a small scale. Only the leaves are used, as bark harvesting is destructive and unacceptable in view of the rarity of the species.

Conservation status: Endangered.

Processing and storage: Preparation/processing: Leaves are rapidly air-dried in the shade, or preferably freeze-dried within 12 hours of harvesting. Preliminary observations suggest that if air-drying is prolonged, or if drying conditions are too hot, the phytochemical quality of the leaf material may be poor.

Stability of product: Unknown. The dried leaves are likely to be stable for considerable periods.

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Xylophia aethiopica

GENERAL DESCRIPTION

Scientific Name with Author: *Xylophia aethiopica* A.Rich.

Synonyms: *Annona aethiopica* Dunal, *Xylophia emini* Engl.

Family: Annonaceae

Vernacular Names: African grains of Selim, atta, eru, erunje, Ethiopian pepper, *Fructus Xylopiae*, grains of Selim, granen van Selim, hwentia, kani pepper, Kanipfeffer, kili, kimba, kyimba, Mohrenpfeffer, moor pepper, Negerpfeffer, negro pepper, piment noir de Guinée, poivre de Sénégal, Selimskörner, Senegal pepper, Senegalpfeffer, spice tree seeds, tsunfyanyi, unien, uda.

Botanical Description: *Xylophia* is an evergreen, aromatic tree, growing up to 20 m high, 50 cm in diameter, with a straight bole. The bark is grayish-brown to reddish, fairly smooth but with a network of fissures; the slash is reddish-brown and fibrous beneath; the crown is much branched, the branches and branchlets having numerous whitish lenticels. The leaves are broad (5 - 15 cm long and 2.5 - 6 cm wide), acuminate, elliptic, simple, with stipules absent; the margins are entire, coriaceous, midrib very broad at base, slightly impressed above. The flowers are greenish-white, solitary or small clusters of 3 - 5. There are 6 petals, in 2 whorls, cream, with the outer petals linear, 2.5 - 5 mm long, thick, gradually tapering to the apex, covered with rust-colored hairs, and the inner petals shorter and narrower. The fruits are peppery and occur as monocarp, cylindrical, up to 9 cm long, reddish at first, eventually blackish, with 1 - 8 orange-red to black, cylindrical seeds (Irvine, 1961).

Origin and Distribution: *Xylophia aethiopica* is native to the lowland rainforest and moist fringe forests in the savanna zones of Africa. It is distributed from Senegal to Zaire, and has been located in Gambia, Angola, Guinea, Sierra Leone, Liberia, Ivory Coast, Togo, Dahomey, Nigeria, Gabon, Congo, Sudan, Uganda, Tanzania, Zambia, and Mozambique.



Plant Part Used: Fruits



Possible Alternative Source Species: In Tropical Africa (Ethiopia to Ghana) both the species, *X. aethiopica* and *X. striata*, are used for local cooking. In South America, *X. aromatica* (burro pepper), has found similar applications among Brazilian Indios.

ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses: The fruit of *Xylophia aethiopica* is used as a soup condiment, and is valued for its carminative effect as a cough remedy. The Igbos give the soup to women after child birth as a general tonic and to promote healing and lactation, or to promote fertility in women. It is an ingredient for the preparation of a Yoruba herbal remedy, Agbo. A decoction of the fruit is drunk as a remedy for stomach ache, and a treatment for bronchitis, biliousness, and dysentery.

The seed extract is used to eliminate roundworms (Oliver-Bever, 1986). A decoction of the fruits of *Xylopia aethiopica*, leaves of *Alstonia boonei* and *Wissadula amplissima* is used to bathe children as an anticonvulsant. A decoction of the fruits and stem bark of *Newbouldia* leaves is drunk as a remedy for amenorrhea.

In Ghana, the fruits are also used to treat malaria, uterine fibroid, fungal infections, boils, hemorrhoids and flatulence, while the seeds are used to treat numbness, epilepsy and anaemia. The leaves are used to treat dyspepsia, laryngitis, splenomegaly, and headache. The stem bark is used to treat dysentery, female infertility, wounds, and rheumatism (Mshana et al., 2000).

External uses of the plant include a poultice for headache and neuralgia, and with lemon grass as douching solution for female hygiene. A soup prepared with the ground fruits, those of *piper guineense*, and leaves of *Leptaspis cochleata*, is taken as a remedy for dizziness.

Xylopia aethiopica has a wide variety of applications; the very odorous roots of the plant are employed in West Africa in tinctures, administered orally to expel worms and other parasitic animals from the intestines, or in teeth-rinsing and mouth-wash extracts against toothaches. The fruits are also used in various forms and exhibit revulsive properties, especially when mashed with grains. These properties are used advantageously in the external treatment of rheumatism.

Other Relevant Uses: Crushed powdered fruits can also be mixed with shea butter fat and coconut oil and used as creams, cosmetic products, and perfumes (Burkill, 1985) The dried fruits are also used as spices in the preparation of pepper-soup and two special local dishes named “obe ata” and “isi-ewu,” taken widely in the southwest and southeastern parts of Nigeria.

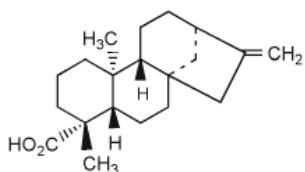
CHEMICAL CONSTITUENTS

Compounds: The fruits contain the diterpenic acid xylopia acid (15β -acetoxy(-)kaur-16ene-19-oic acid), and its analogs kaurenoic, 15-oxo-kaurenoic acid, and kauran- 16 α -ol (Ekong et al., 1968; Ogan, 1971). The plant also yields cuminal,

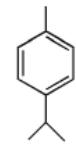
acyclic compounds, glycosides, alkaloids, fats, oils, and a pleasant smelling volatile oil (Odebisi et al., 1978; Puri et al., 1964). The volatile oil contains α - and β -pinenes, carene, cymene, α -phellandrene, limonene and terpinolene, cineole, bisabolene, linalool, terpinen-4-ol, terpineal, cuminal alcohol, and cumminic aldehyde. Smith et al. (1996) have reported the presence of monoterpenoids: cineole, cuminic aldehyde, terpinone, β -pinene; diterpenoids: xylopic acid, other kaurane, trachylobane diterpenes; oleoresins, and minerals (copper, manganese, and zinc) in the volatile oils.

Analyses of the essential oil from dried fruit and powdered dry fruits of *Xylopia aethiopica* using combined GC and GC/MS isolated 33 compounds from the fruit, and 42 compounds from the powdered fruit (Keita et al., 2003). The predominant compounds in the oil extracted from the fruit were β -pinene (19.1%), γ -terpinene (14.7%), trans-pinocarveol (8.6%), and p-cymene (7.3%). In the oil isolated from powder, the main components were β -pinene (9.9%), α -cadinol (6.9%), trans-pinocarveol (4.6%), α -pinene (4.1%), and 1,8-cineole (4.0%).

The analysis results showed that, in oil of fruit of *X. aethiopica* from Mali, monoterpenes were the main constituents (81.8%), 30.7% being oxygenated monoterpenes. This proportion was much lower in the powder; only 48.8%, whereas the amount of sesquiterpenes increased, 22.3% against 9.0% in the fruit. While levels of the various constituents were much lower in the powder, more constituents were found in the powder than in the fruit (42 against 33). This difference is probably due to partial breakdown of the various constituents when the fruit was crushed, or after it was dried.



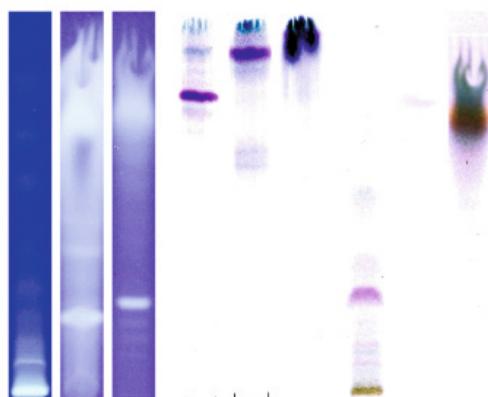
Kaurenoic acid

 α -Pinene

p-Cymene

QUALITY CONTROL

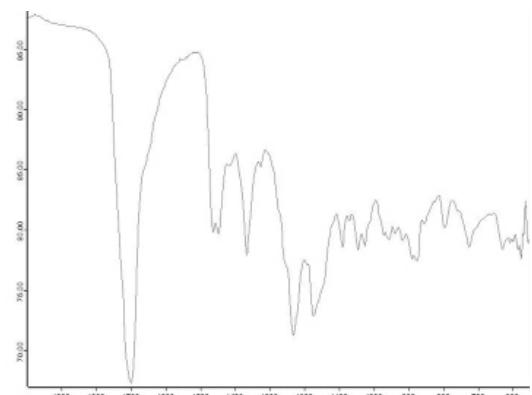
TLC / HPLC / GC



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

Identification: In order to rank their flavor contribution, volatiles isolated from *Xylophia aethiopica* from Nigeria have been analyzed by High Resolution Gas Chromatography (HRGC) and eluate sniffing (Tairu et al., 1999). The volatiles were isolated by sublimation in vacuo using the equipment described by Guth and Grosch (1989). The volatiles were separated into acidic and neutral/basic fractions, and the flavor dilution (FD) factors of the odour-active compounds evaluated by aroma extract dilution analysis (AEDA).

NIR Spectroscopy



Standard specifications cf. appendix 2

Organoleptic Properties: Colour dark brown; odour aromatic; taste spicy (Ghana Herbal Pharmacopoeia, 2007).

Macroscopic Characteristics: Pod is unilocular, multiseeded (4 - 10 seeds), length up to 6.5 cm and 0.5 cm diameter, wrinkled surface; fracture fibrous (Ghana Herbal Pharmacopoeia, 2007).

Microscopic Characteristics: Transverse section of the fruit shows distinct multi-layered epicarp; isolated sclereids and groups of fibres occur in the broad mesocarp and may be lignified, starch grains and oil cells present in mesocarp; the seed endosperm shows numerous globoid resinous bodies (Ghana Herbal Pharmacopoeia, 2007).

Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

The fruit extract has been shown to be antimicrobial against both Gram-positive and Gram-negative bacteria, but inactive against *Escherichia coli*; the activity being mainly due to the diterpene xylopic acid (Boakye-Yiadom et al., 1977; Malcom, 1978). Extracts of *Alepidea amatymbica* and *Xylophia aethiopica* demonstrated diuretic activity and natriuretic effects comparable to the effects of chlorothiazide, suggesting inhibition of Na⁺ and K⁺ reabsorption in the distal tubule (Somova et al., 2001). The essential oils of *Xylophia aethiopica*, *Monodora myristica*, *Zanthoxylum xanthoxyloides*, and *Z. leprieurii*, showed antibacterial and antifungal activity (Tatsadjieu et al., 2003).

Clinical Studies: Aqueous extract of *Xylopia aethiopica* was neither a miotic nor a mydriatic, but lowered the intraocular pressure amplitude of accommodation in visually active volunteers (Igwe et al., 2003).

SAFETY DATA

Preclinical Safety Data

Single Dose Toxicity cf. appendix 1

KEY (PROPOSED) USAGE

Therapeutic Indications: Antimicrobial, diuretic.

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Xysmalobium undulatum

GENERAL DESCRIPTION

Scientific Name with Author: *Xysmalobium undulatum* R.Br.

Family: Asclepiadaceae

Vernacular Names: Ishongwe, milk bush, uzara.

Botanical Description: A perennial herb of about 1 m in height, with thick, erect, flowering stems developing from a branched, fleshy root system. The characteristic large leaves are arranged in opposite pairs and exude a milky latex when broken. Rounded clusters of small, yellowish-brown, bell-shaped flowers are borne along the stems, followed by large, hairy capsules which contain numerous hairy seeds (Van Wyk et al., 1997, Van Wyk & Wink, 2004).

Origin and Distribution: Grassland regions of southern Africa.



Plant Part Used: The fleshy roots



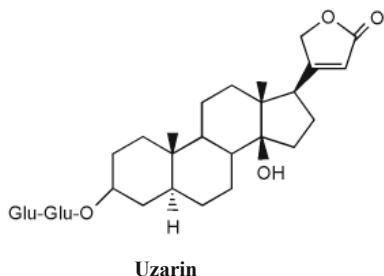
ETHNOBOTANICAL INFORMATION

Major Ethnopharmacological Uses: The bitter root is traditionally used mainly to treat diarrhoea and afterbirth cramps. Traditional uses also include the treatment of headache, indigestion, and dysmenorrhoea, as well as external application for sores and wounds (Watt & Breyer-Brandwijk, 1962; Forbes, 1986; Von Koenen, 2001; Hutchings & Van Staden, 1994; Hutchings et al., 1996; Neuwinger, 2000; Pahlöw, 1993; Pujol, 1990; Schmitz et al., 1992; Van Wyk et al., 1997; Van Wyk & Gericke, 2000; Van Wyk & Wink, 2004).

The uses in modern phytotherapy are as antispasmodic and antidiarrhoeal to treat non-specific acute diarrhoea (Blumenthal et al., 1998), and to a lesser extent afterbirth cramps and menstrual cramps.

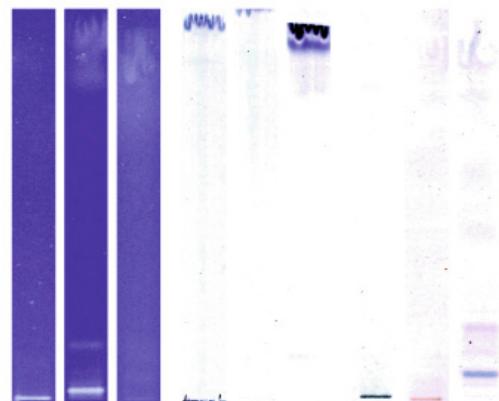
CHEMICAL CONSTITUENTS

Compounds: Uzara root contains several cardiac glycosides, of which uzarin is the major and best known compound. It co-occurs with substantial quantities of xysmalorin (differing from uzarin only in the presence of a double bond in ring B). Also present are the two corresponding H-17 β isomers, allouazarin and alloxyssmalorin (Ghorbani et al., 1997; Wagner, 1999).



QUALITY CONTROL

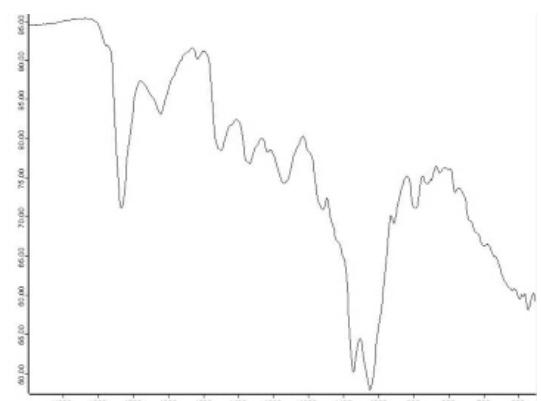
TLC / HPLC / GC: The dried product is easily studied by TLC (Wagner & Bladt, 1995) and HPLC, but the uzarin may not separate well from xysmalorin.



Chromatograms of acetone extract separated by BEA, CEF and EMW from left to right and visualized under UV 235 nm and by using the vanillin and anisaldehyde spray reagents.

Markers and Quantitative Methods: The dried root should not contain less than 6% glycosides (Schultz et al., 2001). An average level of about 30 mg per g dry weight has been recorded. The level of uzarin is about 5 mg per g dry weight (Huber et al., 1951).

NIR Spectroscopy



Adulterants and Adulterations: Not recorded but wildharvested material may be of doubtful identity (several plants of the Asclepiadaceae are known by the generic name ishongwe). Wildharvested material may be mixed with other species of the family.

Standard specifications cf. appendix 2

PHARMACOLOGICAL PROPERTIES

The biological activity of uzara is relatively well known. The cardiac glycosides have proven antidiarrhoeal and antispasmodic activity. They work on the visceral smooth muscles (probably by local stimulation of sympathetic nerves), thereby inhibiting intestinal motility (Schultz et al., 2001). Uzarin inhibits Na⁺, K⁺, and ATPase, like other cardenolides.

Clinical Studies: An observational study has shown that treatment with about 60 mg total glycosides per day was effective against diarrhoea in 80% of patients (Anonymous, 1994).

SAFETY DATA

Preclinical Safety Data

Single Dose Toxicity cf. appendix 1

The relatively low toxicity of the Asclepiadaceae glycosides compared with other cardiac glycosides may be linked to their unusual trans-trans-cis configuration (Burger & Wachter, 1998). Because of its low bioavailability after oral application, digitalis-like symptoms are only found at very high doses. The lethal dose of dry extract in dogs was determined to be about 100 to 200 mg per kg body weight (Schmitz et al., 1992).

KEY (PROPOSED) USAGE

Therapeutic Indications: Antidiarrhoeal and antispasmodic.

Dosage, Method, and Duration of Administration: In adults, a daily dose of 45 - 90 mg of total glycosides is recommended, after an initial dose of 1 g of the herb (75 mg of total glycosides). The recommended dose for children is 15 - 30 mg total glycosides. Standardized tinctures and dried extracts have been used in Germany since 1911 (Schultz et al., 2001).

Special Warnings and Precautions for Use: Uzara root can be safely consumed when used appropriately.

Interactions: Concomitant treatment with heart glycosides is contraindicated (Blumenthal et al., 1998). Mild side effects (pruritus) was observed in one patient (0.18% of participants) during an observational study (Anonymous, 1994), indicating that the product is relatively safe.

Pregnancy and Lactation: Not to be used during pregnancy. Not to be used while nursing.

Evaluation of Efficacy: Nonspecific acute diarrhoea efficacy clinically proven (Anonymous, 2004; Blumenthal et al., 1998). Antispasmodic efficacy pharmacologically proven (Burger & Wachter, 1998). Dysmenorrhoea efficacy traditionally proven. Wound healing efficacy traditionally proven.

TRADE INFORMATION

Nature of plant material:

Flowering/harvesting time: The herb flowers in summer (November to February) and are harvested at the end of the growing season (March to May).

Cultivated/wildcrafted: Commercial product is cultivated; small scale in South Africa.

Conservation status: Not listed.

Processing and storage: Preparation/processing: The roots are sliced and rapidly air-dried. Water-alcohol extracts were traditionally used for commercial medicinal products.

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Methods and procedures

JN ELOFF AND LJ MCGAW

1. INTRODUCTION

It is widely accepted that in the order of 80% of people from developing countries use medicinal plants for their primary health care. In addition to the use of plants in developing countries there has been a substantial growth in the use of herbal medicines in developed countries. According to a survey done in the United States (McCaleb and Langner, 2000) people use herbal medicines because they prefer natural products (47%), there are fewer side effects (17%), it is more efficient (17%), it is less expensive (10%) and it is milder (8%). Within his group of consumers price was not a major factor.

Robbers and Tyler (1999) distinguish between paraherbalism, which is based on pseudoscience, and rational herbalism where herbal medicine is used based on scientifically verified evidence. In paraherbalism, which includes homeopathy as “a particularly pernicious form of paraherbalism”, the effects achieved could be a placebo effect or are at least not reproducible and scientifically verifiable at this stage. On the other hand rational herbalism is based on plants containing relatively low concentrations of pharmacologically active compounds and the efficacy can be proven in clinical trials. Herbal medicines are therefore in effect dilute drugs.

The main factors that limit the rational use of herbal medicine are (a) the efficacy of the herbal medicine has to be proven, (b) the safety of the herbal medicine has to be proven and (c) there is a large variation in the quality of products marketed as herbal medicines.

The fact that people continue using herbal medicines for long periods may be an indication of efficacy of herbal medicine despite the absence of well designed clinical trials in most cases. Because the use of these herbal medicines cannot easily be patented, adequate clinical trials are very expensive and funding restriction limits clinical trials.

As far as safety is concerned, many herbal medicines have been used for centuries by rural people and for decades by urban people and are frequently considered to be safe. Caution is

required when a different extractant or formulation is used. For many herbal medicines approved in the German Commission E⁺ Monographs (Blumenthal et al 1998) as prescription medicines, no clinical trials or long term toxicity studies have been carried out.

Quality control is therefore one of the major problems in the rational use of herbal medicines. This is related to two aspects: in the first place, is the product the plant species presented on the label? Secondly is it free of dangerous contaminants and does it contain the required concentration of the active principle? In many herbal medicines, the active component is not known. Different compounds may be responsible for different therapeutic properties and there is a growing body of evidence that there are synergistic interactions between different components of a plant extract. Many factors influence the concentration of plant secondary compounds. Frequently a marker compound is selected and this is used to determine the quality of the herbal medicine, although it may not be related to the biological activity.

There are many examples where herbal medicines sold in developed countries are of poor quality. In a study in North America no sample of feverfew examined contained the 0.2% parthenolide required for activity (Groenewegen and Heptinstall, 1986). With more people collecting and distributing medicinal plants, the wrong plant is frequently offered either as a genuine mistake or in a fraudulent effort to increase profits. In one study of 54 ginseng products, 60% were worthless and 25% contained no ginseng at all (Liberty and Marderosian, 1978).

One of the important components of quality control is therefore to validate the identity of the plant. High performance liquid chromatography is valuable to quantify chemical compounds in plant extracts, but planar chromatography, widely known as thin layer chromatography (TLC), is generally better, cheaper and easier than HPLC to identify plants by analyzing the chemical components of extracts.

Many of these methods are specified in publications such as the British Herbal

Pharmacopoeia, surprisingly without including images of the chromatograms. With the growth in number of medicinal plants used and many role players with doubtful ethics it is becoming increasingly important to have a technique that could verify the identity of the plant species in question with reasonable certainty. One should keep in mind that identifying plant species frequently requires such a high level of expertise that only specialists are able to accomplish it with certainty in some taxa.

Wagner and Bladt (1996) did pioneering work in providing a TLC atlas of many herbal medicines with colour photographs of the chromatograms of plant extracts. Many different scientists developed the methods for specific medicinal plants over many years. This led to the use of many different extractants, a large number of TLC solvent systems, several types of TLC plates and 44 different spray reagents. It is difficult for a relatively small company preparing a variety of herbal medicines from different suppliers of plant material to handle so many different systems.

The question arose whether it would be possible to have a simplified system that could be used to characterize plant extracts by TLC. This has been addressed in an MSc thesis by Theresa Ntloedibe (2002) and the results will be published in another scientific journal.

The following aspects will be discussed in different sections: preparation of the plant material, extraction of the plant material, dissolving and making up extracts to a known concentration, separation by TLC using fewer solvent systems, evaluation of the chromatogram and storing the information. In a separate section, safety of extracts will also be discussed.

2. PREPARATION OF PLANT MATERIAL

If the plant material to be used is in a finely ground dry form (particle size less than 1 mm) it can be used directly. Material obtained from bulk suppliers will usually be in this form. If not, the material has to be dried first before grinding it to a fine powder.

The excess moisture must be removed prior to storage or further treatment. The main reason is to limit possible contamination by bacteria or fungi leading to changes in the chemical composition of the plant material and even the possible production of toxic fungal metabolites. Another reason is that there may be changes in the chemical composition within the plant material due to enzymatic changes if the plant material remains moist. Once plant material is dry compounds present in it may remain biologically active for very long periods. Leaves of *Combretum erythrophyllum* collected from the same area lost no antibacterial activity when stored in an herbarium over a period of more than 90 years (Eloff, 1999).

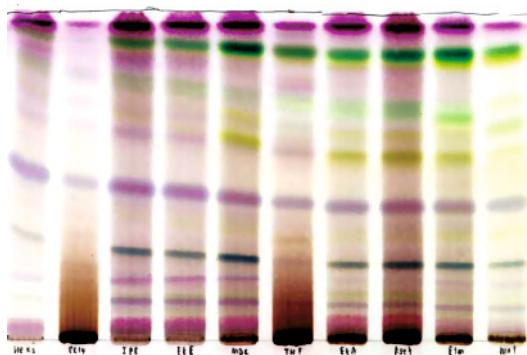
Drying should be in the shade to limit photo-oxidative change. Because every 10°C increase in temperature increases the chemical reactions, for practical reasons drying should be undertaken at room temperature to limit chemical changes. It should be rapid by generating a flow of air across the plant material. Because spreading plant material on paper to dry takes up so much space we fill open-woven mesh bags used for the sale of oranges loosely with a limited quantity of the plant material and hang these in a room with a stream of air flowing across it. With non-fleshy leaf material the procedure is simple: if the leaves break easily when bent, it should be sufficiently dry. With fleshy leaves, corms and bulbs it should be cut into thin sections to facilitate drying to constant weight. Root or bark should be cut into thinner pieces for the same reason.

To promote efficient extraction the plant material should be ground to a particle size of 1 mm or less.

3. WHICH EXTRACTANT SHOULD BE USED?

Compounds usually have to be in solution before they can take part in chemical reactions. The insoluble portion consists mainly of structural material in the plant such as cellulose and lignin. The soluble compounds consist of metabolites that participate in the life processes of the plant. These life processes involve making food from light energy by photosynthesis and forming all the compounds that are required to ensure the propagation of the plant cells and protecting the

plant against other organisms. Different solvents extract different compounds from the same plant material. In many cases compounds with biological activity are relatively non-polar. Cold water used under the same conditions as the other extractants frequently extracts no relatively non-polar compounds that can be separated with the non-polar eluent BEA (benzene:ethanol:ammonia 9:1:0.1) (Figure 1).



*Figure 1. Separation of components present in 100 µg of 10 different extracts of *Combretum microphyllum* using BEA as eluent and the vanillin-sulphuric acid spray reagent. Lanes from left to right extracted by hexane, carbon tetrachloride, isopropyl ether, diethyl ether, methylene dichloride, tetrahydrofuran, ethyl acetate, ethanol, and methanol. No components separated in the water extract (results not shown). (From Kotze and Eloff, 2002).*

Earlier results have shown that acetone is probably one of the best solvents to extract compounds of a wide range of polarity from dried plant material (Eloff, 1998). In a previous study in our group (Ntloedibe, 2002) we compared acetone extraction with methanol under reflux extraction used in the British Herbal Pharmacopoeia with five currently used selected Western herbal medicines. The acetone extraction yielded much better results than the methanol extraction as far as number of different compounds isolated with all the plant species. The procedure was also much easier and quicker than the methanol extraction. We also tested existing solvent systems and developed additional TLC solvent systems to separate compounds with a large variation in polarity.

Furthermore we also investigated using different spray reagents for the TLC chromatograms.

Initially we extracted samples in centrifuge tubes on a Vortex mixer. This is tedious as one sample has to be handled at a time. We compared extracting the finely powdered material using the Vortex mixer with extraction using an orbital shaker containing a test tube rack for the centrifuge tubes. There was hardly any difference in the results obtained with the two procedures. In most cases the first extraction with acetone removed close to 80% and two extractions removed c 90–95% of material extracted after repetition on the marc (residue left after extraction). We found that with a very fine powder within one minute on the vigorously shaking machine there was no increase in the yield. The equilibrium therefore establishes very quickly if the plant material is finely powdered and a good extractant is used under vigorous shaking.

4. WHICH TLC SOLVENT SYSTEM SHOULD BE USED?

We tested several existing solvent systems and selected benzene:ethanol:ammonia (9:1:0.1) which is excellent for non-polar compounds, chloroform:ethylacetate: formic acid (5:4:1) which is good for intermediate polarity compounds and ethyl acetate: methanol:water (10:1.35:1) which is best for polar compounds (Kotze and Eloff, 2002). With these three systems compounds with a very wide range of polarity can be separated. In addition to this, one system is basic, one neutral and one acidic which also helps in separating different compounds in the extracts.

5. WHICH SPRAY REAGENT SHOULD BE USED?

A large number of spray reagents are used for visualizing chromatograms by Wagner and Bladt (1997) and in the British Herbal Pharmacopoeia. We found few differences between plastic and aluminium coated TLC plates. The aluminium covered plates tended to be destroyed by harsh chemicals and the plastic backed plates were deformed by heating for long periods. There were large differences using the different spray reagents, but in general using vanillin-sulphuric acid on aluminium backed plates gave good results, were not as sensitive to overheating as the widely used *p*-anisaldehyde spray reagent and this was selected as standard treatment (Ntloedibe, 2002).

As an example of the separation obtained (Kotze and Elof, 2002), chromatograms developed with BEA and sprayed with vanillin-sulphuric acid for the different herbal medicines examined is presented in Figure 1. When these chromatograms were investigated in 230 nm UV light and photographed with a digital camera before spraying many significant differences were observed (results not shown here).

Even with only one of the three solvent systems described above, most of the Western herbal medicines investigated could already be distinguished from each other. It is also clear that analyzing the same product from different origins e.g. three Valerian samples led to virtually identical chromatograms. The BEA and CEF chromatograms indicated that with even one solvent system and detection system, extracts of most of the herbal medicines tested to date give unique patterns. By using the different solvent systems and uv fluorescence and vanillin sulphuric acid on chromatograms any uncertainty should be resolved (Ntloedibe, 2002).

It remains to be seen how wide the difference between different populations of the same species is. By co-chromatography of standards occurring in most plants such as β -sitosterol, rutin or a standard known to occur in the plant investigated, the identification can be more definite.

6. PRACTICAL EXTRACTION METHODS AND PROCEDURES

6.1 Preparation of plant material before extraction

1. Grind the dried plant material to a fine powder with a diameter of less than 1 mm. Figure 2 shows a commercial grinding machine that can be used with different sieves to determine the particle size after grinding. The larger the particle size the more inefficient the extraction is. Efficiency of extraction should not have a major effect on the fingerprint obtained.
2. Weigh the required quantity of plant material off on an analytical balance in a c. 15 ml centrifuge tube supplied with a cap.
3. Ensure that the opposite centrifuge tubes are balanced and centrifuge the tubes for c. 5 minutes at c. 1200 x g.

4. Decant the extract into a small (c. 50 ml) beaker.
5. Add the same volume of acetone used for the first extraction to the centrifuge tube containing the marc (remaining insoluble plant material) and repeat the extraction and centrifugation process two times more to give a total of about 30 ml of extract.

Ensure that only clear extract and no particulate matter is decanted. If this is not possible the time and speed of centrifugation can be increased or the extract can be filtered to ensure that no particulate matter is included with the extract.



Figure 2 A commercial grinder for plant material

6.3 Quantifying the sample concentration

If the quantity of material separated by TLC is too low, some compounds present in the extracts may not be present in a high enough concentration to react visibly with the spray reagent. On the other hand if some compounds are present in too high a concentration, it may lead to smearing and poor separation on a chromatogram. We found that loading a quantity of 100 μ g of the plant extract usually leads to a good separation. In practical terms this means that the extract is made up to 20 mg/ml and then 5 μ l is chromatographed or that it is made up to 10 mg/ml and 10 μ l is chromatographed.

6.4 Storage of extracts

One of the advantages of using acetone as solvent of the sample before TLC is that due to its volatility it is easy to apply the 5-10 µl on a TLC plate in a thin line. Another advantage is that there is little chance for microbial growth on the sample, especially if samples are stored at low temperatures. One has to be careful with the stopper that is used if the samples are to be stored because acetone will pass through some stoppers, especially polyethylene and lead to an increase in the concentration of the extract (Eloff, 2003).

7. PRACTICAL METHODS AND PROCEDURES FOR IDENTIFICATION OF PLANT EXTRACTS

7.1 Preparation of TLC plates

Commercially available TLC plates 20 X 20 cm without a fluorescence indicator are cut in half and with a soft pencil a line is lightly drawn 1 cm from the longest side. At least 2 cm from the edge marks are made 1 cm long where the sample will be placed in a thin even line. A distance of at least 0.5 cm should be kept between the different lines for different sample applications (See Figure 1).

7.2 Applying the samples

A 5 µl micropipette is used to load about 2.5 µl in a thin line on the marked area of the TLC plate and waiting until the first part has dried before the second part is applied across the first. With some experience it is possible to get similar results to that of a commercial sample applicator (Figure 3). If different samples are to be applied it goes much quicker by hand. It is important to rinse the micropipette between applying samples.

7.3 Preparing solvent systems and chromatography tank

Solvent systems should be prepared fresh just prior to use and placed in the TLC tanks about an hour ahead of time to ensure proper equilibration with the tank which must have a well fitting lid. With a volatile eluent such as BEA it helps the equilibration if the sides of the TLC tank are covered with filter paper and moistened with the solvent. The solvent should be added to the tank to a depth of less than 1 cm so that the solvent level is below the plant extract sample applied.

7.4 Development of chromatograms

The TLC plates should not be touched with fingers on the silica layer and should rather be put into the chromatography tank using tweezers or a pair of tongs. When the solvent front has nearly reached the top of the plate (i.e. has moved about 9 cm) it should be taken out and the level where the solvent reached marked with a pencil. Subsequent plates can be run in the same solvent system within a period of one or two hours without preparing new solvent.

7.5 Drying of plates

Without touching the silica gel covered area the plates should be placed in a draft of cold air until the solvent has evaporated. The plates should not be left too long before the next step to limit breakdown of separated compounds by photo-oxidation or the evaporation of volatile compounds. If no perceptible smell of the solvents remain, the next step can be taken.

7.6 Visualizing of separated compounds on chromatograms under UV

Chromatograms should be placed under an ultraviolet light source at a wavelength of 350 nm. By experimenting with different settings and a tripod, the fluorescence on the chromatogram can be photographed if a permanent record is needed.

7.7 Visualizing the chromatograms with the vanillin sulphuric acid spray reagent

The spray reagent should be made up, using analytical quality reagents in a ratio of 0.1 gram of vanillin to 1 ml sulphuric acid and 28 ml methanol. The sulphuric acid should be added carefully with constant stirring to the methanol before the vanillin is added. The mixture may be kept closed at a low temperature for later use, but should be discarded if any pink colour has developed in it. The chromatograms should be sprayed with a fine mist using a commercial spray gun in a fume cupboard until the whole chromatogram is just moist. The chromatogram is then placed in an oven at c. 100°C and observed carefully until optimal colour development. Be careful not to overheat or it will spoil the quality of the final product. Any development of colour in the area between the lanes containing extracts is a sign of incipient overheating. It is better to scan the chromatogram (see next section), then heat it more to see if any additional colour develops.

7.8 Scanning of chromatograms

To obtain a permanent record of the chromatogram it can be scanned using a commercial scanner from a company such as Hewlett Packard. A computer program such as Microsoft Office Picture Manager can be used to optimize the contrast and the intensity to result in a better representation of the chromatogram.



Figure 3 Commercial sample applicator for TLC

8. NEAR INFRARED SPECTROSCOPY METHODS AND PROCEDURES

Near infrared spectroscopy is a method that has been used widely in the quality control of agricultural products.

Near infrared light in the wavelength range of 700-100 nm can go through outer surfaces of cells and react with different chromophores to yield reflectance spectra that depend on the type and concentration of the different chromophores present in the cells. This non-destructive technique has found very wide application in the fields of medicine and agriculture. It has also been used to detect counterfeit drugs (Puchert et al 2010) and for identification of herbal medicines (Woo et al., 1999).

We have evaluated near infrared reflectance spectroscopy as a parameter for identifying the African Herbal medicines in this volume. We found that using an acetone extract and drying it on the stage of the Bruker Tensor 27 apparatus

(Figure 4) gave much better results than using the powdered plant material. An acetone plant extract was therefore dried, the dried material was scraped off and placed on the stage of the apparatus measuring the reflectance spectra. This method is quick and the results can be useful in identifying herbal medicines.



Figure 4 Bruker Tensor 27 apparatus used to determine the near infrared reflectance spectra of samples.

9. BACKGROUND TO TOXICITY ASSAYS AND PRACTICAL APPLICATION

9.1 Introduction

Toxicity evaluations of plant extracts and natural products isolated from plants are required to be certain that biological activity is not caused by general toxic effects against microorganisms in anti-infective tests. *In vitro* toxicity screening is also a necessary aspect of the preliminary safety assessment of plant-based preparations. Naturally, *in vivo* toxicity experiments are crucial, because laboratory-based assays detecting cytotoxic effects are not sufficient to indicate toxicity of ingested or topically applied medications.

9.1.1 The brine shrimp larval toxicity assay

The brine shrimp assay involves incubating test substances with freshly hatched brine shrimp larvae and observing the larvae for mortality after incubation. This simple, inexpensive assay has been used by various researchers to detect *in vitro* cytotoxic or pharmacological effects (Meyer et al., 1982; Solís et al., 1993; McLaughlin et al., 1998; McGaw and Eloff, 2005). The assay shows a good correlation with cytotoxicity in cell lines

such as 9KB, P388, L5178Y and L1210 (Meyer et al., 1982; McLaughlin, 1991; De Rosa et al., 1994; McLaughlin et al., 1998). A drawback of the brine shrimp assay is the lack of data correlating lethal effects of test substances against brine shrimp larvae to mammalian *in vivo* toxicity. The assay also does not detect activity in those compounds requiring metabolic activation.

9.1.2 Cytotoxicity assays using cell lines

Cytotoxicity test methods generally involve culturing cells in microtitre plates until they are actively growing, and measuring the inhibition of growth of the cells after addition of the test substance and incubation for a predetermined period. This measurement of growth inhibition (or proliferation) is often performed indirectly by detecting colour formation in certain assays by actively dividing cells, as the intensity of colour formation is directly proportional to the cell number (Houghton et al., 2007). A variety of colour formation assays are available. The LC₅₀ values are calculated as the concentration of test compound resulting in a 50% reduction of absorbance compared to untreated cells. A number of different cell lines are suitable for use in the assay. Radioactive methods are also available but require specialized equipment, and radiolabeled chemicals. When carrying out a cytotoxicity assay, important considerations are the seeding density or concentration of cells to be used, and the time of incubation with test compounds. These criteria differ according to the cell line used. Further information is provided in Houghton et al. (2007).

Among the assays available, we have been using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay as an indicator of cell proliferation following incubation with plant extracts. The MTT tetrazolium salt is metabolized by mitochondria in living cells to a deep purple formazan product. The intensity of this colour change can be measured spectrophotometrically using a microtitre plate reader. The viability of treated cells can be compared with the viability of untreated control cells. It is important to note that some plant extracts can reduce MTT in the absence of living cells (Shoemaker et al., 2004), so the extract should be removed from the cells adhering to the bottom of the microtitre plate wells prior to addition of MTT.

MTT formazan is shown in Figure 5.

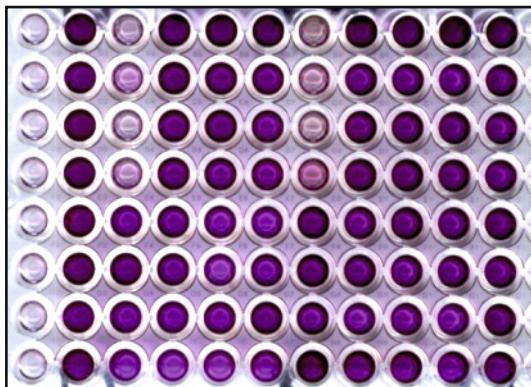


Figure 5. Microtitre plate showing layout of cytotoxicity assay, with blank in column 1, and control untreated cells in column 2. The darker the colour of the wells, the more coloured formazan is produced by actively growing cells.

9.2 Materials and Methods

9.2.1 Brine shrimp assay

Artemia salina Lech. (brine shrimp) larvae are used in this assay. Brine shrimp eggs can be obtained from local pet shops, and are hatched in artificial sea water (3.8% NaCl in distilled water). After 48 hours, the phototropic nauplii are collected using a Pasteur pipette prior to conducting the brine shrimp assay (Solís et al., 1993; Desmarchelier et al., 1995). Plant extracts are tested in a range of concentrations, initially 0.1, 0.2, 0.5, 1 and 2 mg/ml, and podophyllotoxin (Sigma) is used as a positive control. After incubating the extract with approximately 10-15 nauplii per microtitre plate well for 24 hours in the dark at room temperature, the number of dead and live larvae in each well is counted using a stereomicroscope. If control deaths occur, the percent death values are corrected using Abbott's formula as expressed by Rasoanaivo and Ratsimamanga-Urverg (1993). Tests are carried out in triplicate and the experiments are repeated three times.

METHOD

1. Hatch brine shrimp eggs in artificial sea water (3.8 g sea salt/NaCl + 100 ml dH₂O).
2. After 48 h, collect phototropic nauplii using a Pasteur pipette and transfer to a fresh beaker to separate from egg shells and unhatched eggs.

3. Dissolve organic extracts in as small an amount possible of DMSO (maximum final concentration = 0.5%) and make up to required volume with water (include solvent blank control to test effect of DMSO concentration used on larvae).
4. Test plant extracts in a range of concentrations to obtain an LD⁵⁰ value.
5. Place 100 µl plant extract solution in replicate wells of a 96-well microtitre plate.
6. Reference compound = podophyllotoxin.
7. Place 100 µl nauplii suspension (10-15 nauplii) in each well.
8. Cover microplate and incubate for 24 h at room temperature.
9. Count number of dead and live nauplii in each well using a stereomicroscope. If control deaths occur, correct percent death values using Abbott's formula:

Corrected mortality percentage = $(m - M)/S \times 100$
 m = mean % of dead larvae in treated tubes

M = mean % of dead larvae in controls

S = mean % of living larvae in controls

9.2.2. Cytotoxicity assay

Plant extracts are tested for cytotoxicity against the Vero African green monkey kidney cell line. The cells are maintained in minimal essential medium (MEM, Highveld Biological, Johannesburg, South Africa) supplemented with 0.1% gentamicin (Virbac) and 5% foetal calf serum (Adcock-Ingram). To prepare the cells for the assay, cell suspensions are prepared from confluent monolayer cultures and plated at a density of 2.5×10^3 cells into each well of a 96-well microtitre plate. After overnight incubation at 37°C in a 5% CO₂ incubator, the cells in the microtitre plate are used in the cytotoxicity assay. Stock solutions of the plant extracts are prepared at a concentration of 100 mg/ml in an appropriate solvent. Serial 10-fold dilutions of each extract are prepared in growth medium (1–1000 µg/ml). Viable cell growth after 120 hours incubation with plant extracts is determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma) tetrazolium based colorimetric assay described by Mosmann (1983) and modified slightly by McGaw et al. (2007). The absorbance is measured at a wavelength of 570 nm on a Versamax microplate reader (Molecular Devices). Berberine chloride

(Sigma) is used as a positive control. Tests are carried out in quadruplicate and each experiment is repeated three times.

The cells are rinsed with PBS and fresh medium is added to the wells. Then, MTT is added to each well, and the plates are incubated for 4 hours at 37°C. The supernatant is removed and the MTT formazan crystals are dissolved in DMSO prior to reading the plate in a microplate reader.

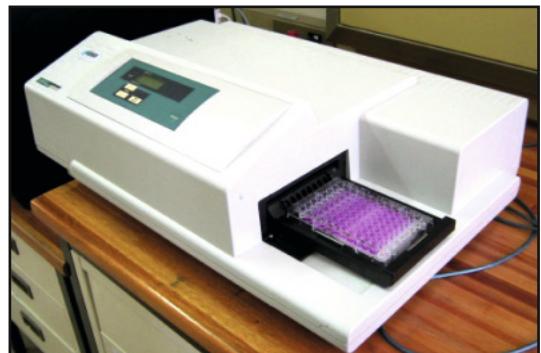
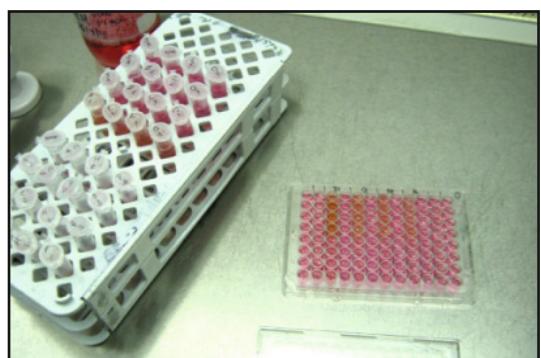


Figure 6. Preparing for dilution of plant extracts. Plant extracts added to cells. Absorbance read in microplate reader.

METHOD

10. Prepare 96-well microtitre plates with adherent cells in wells of columns 2 – 11 (cell density needs to be optimized for different cell lines). Leave columns 1 and 12 containing the minimal essential growth medium (MEM) only.
11. Prepare a serial dilution of the test sample in MEM in Eppendorf tubes (e.g. 1, 0.1, 0.01, 0.001 mg/ml). (Figure 6 a)
12. Prepare a serial dilution of berberine or other positive control drug in MEM (test control at 100, 10, 1, 0.1 µg/ml).
13. Prepare serial dilutions of solvent blanks (e.g. acetone, ethanol).
14. Remove the medium from the wells in columns 2 and 11 (use a syringe with a needle attached to a thin tube to aspirate the medium). Feed the cells in the eight wells in columns 2 and 11 with 200 µl of fresh MEM; these cells are the controls.
15. Replace growth medium with the test sample dilutions (200 µl) in wells of columns 3 to 10 (four wells are used for each drug concentration) (Figure 6b)
16. Incubate the plates for a defined exposure period e.g. 5 days (this needs to be optimized if necessary for different cell lines).
17. Remove the MEM from the wells, wash wells with 200 µl PBS and replace with 200 µl fresh MEM.
18. Add 30 µl of a 5 mg/ml MTT in PBS solution to each well.
19. Incubate the plate for a further 4 h at 37°C.
20. After incubation with MTT, centrifuge the plate for 10 min at 850 g (optional).
21. Carefully remove the medium in each well without disturbing the MTT concentrate in the wells.
22. Add 150 µl PBS to each well and centrifuge again for 10 min at 850 g (optional).
23. Repeat wash step (optional).
24. After the washing steps, aspirate liquid from the cells and dissolve the MTT formazan crystals by adding 50 µl DMSO to each well.
25. Shake the plate gently until the solution is dissolved.

Measure the amount of MTT reduction by immediately measuring absorbance at 570 nm (reference wavelength 620 nm). Use the wells in column 1, which contain medium and MTT but no cells, to blank the plate reader. The results are described as a percentage of the control cells (Figure 6c).

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APPENDIX 1 - Extractability and toxicity assays

Species	Plant part tested	Extractability (mg/g)			Cytotoxicity (LC50 in mg/ml)	
		water	ethanol	acetone	Brine shrimp	Vero cell line
<i>Acacia senegal</i>	gum exudate	472,6	1,6	0,6	0,7262	>1
<i>Adansonia digitata</i>	leaves	17,9	99,3	31,2	>2	0,1155
<i>Aframomum melegueta</i>	seeds	110	124	126	n.a.	0,0144
<i>Agathosma betulina</i>	leaves	22	45	30	>2	0,5035
<i>Aloe ferox</i>	exudate	35	487	250	>2	>1
<i>Antidesma madagascariensis</i>	leaves	91	35	26	0,876	0,338
<i>Aphloia theiformis</i>	leaves	248	124	124	>2	0,951
<i>Artemisia afra</i>	leaves	5,5	13,0	32,1	0,0697	0,2346
<i>Aspalathus linearis</i>	leaves	39	2	3	>2	>1
<i>Balanites aegyptica</i>	bark	35	33	12	>2	0,6446
<i>Boswellia sacra</i>	resin	93	370	470	0,5435	0,2825
<i>Bulbine frutescens</i>	leaves	58,3	77,9	28,7	0,1702	0,2113
<i>Cajanus cajan</i>	seeds	51,5	20,3	11,9	0,0889	0,1025
<i>Catharanthus roseus</i>	leaves	65,0	82,2	36,4	0,0160	0,0539
<i>Centella asiatica</i>	leaves	43,0	73,4	15,7	0,0540	0,1298
<i>Carissa edulis</i>	leaves	309	183	124	n.a.	>1
<i>Combretum micranthum</i>	leaves	124,3	26,5	13,8	0,0092	0,1490
<i>Commiphora myrrha</i>	resin	414,5	145,5	163,9	0,2008	0,1489
<i>Cryptolepis sanguinolenta</i>	leaves	56,6	16,5	14,5	0,0493	0,0119
<i>Cyclopia genistoides</i>	leaves, twigs	41	60	15	>2	0,401
<i>Danais fragrans</i>	leaves	175	36	46	>2	0,0614
<i>Euphorbia hirta</i>	leaves	33,8	32,1	20,2	0,3253	0,2208
<i>Garcinia kola</i>	leaves	19,2	61,3	54,0	0,0468	0,3051
<i>Griffonia simplicifolia</i>	seed	140	143	86	0,2404	0,2007
<i>Harpagophytum procumbens</i>	root	n.a.	n.a.	n.a.	0,6055	0,4155
<i>Harungana madagascariensis</i>	leaves	191	99	86	0,748	0,053
<i>Hibiscus sabdariffa</i>	calix epicalix	274,5	81,2	35,8	0,0205	>1
<i>Hoodia gordonii</i>	roots	687	351	137	n.a.	0,3830
<i>Hypoxis hemerocallidea</i>	tuber (corm)	61	45	18	>2	>1
<i>Ipomoea pes-caprae</i> ssp. <i>brasiliensis</i>	leaves	45,8	45,0	29,5	0,0087	0,0394
<i>Kigelia africana</i>	fruit	45	14	14	>2	0,149
<i>Mondia whitei</i>	roots	35,7	25,3	11,7	0,0142	0,0089
<i>Moringa oleifera</i>	leaves	181	115	48	>2	0,152
<i>Nauclea latifolia</i>	bark	n.a.	n.a.	n.a.	n.a.	>1
<i>Pelargonium sidoides</i>	leaves	86	72	40	0,4466	0,3954
<i>Prunus africana</i>	bark	92	93	25	>2	>1
<i>Ravenala madagascariensis</i>	leaves	17,4	20,0	8,2	0,1484	>1
<i>Rauvolfia vomitoria</i>	bark	284	152	101	n.a.	0,0084
<i>Sceletium tortuosum</i>	whole plant	110	32	78	>2	0,4092
<i>Siphonochilus aethiopicus</i>	root	68	26	17	1,77	0,0137
<i>Strophanthus gratus</i>	seeds	137,4	60,6	216,9	0,6601	>1

<i>Sutherlandia frutescens</i>	leaves and twigs	66	35	14	0,3439	0,1815
<i>Terminalia sericea</i>	leaves	62,7	24,2	17,3	0,2032	0,1550
<i>Toddalia asiatica</i>	leaves	34,5	29,5	19,5	0,1989	0,0864
<i>Trichilia emetica</i>	leaves	82,0	55,9	23,4	0,1389	0,0933
<i>Vernonia colorata</i>	leaves	36,6	34,1	12,8	0,0748	0,0359
<i>Vernonia amygdalina</i>	leaves	247	196	109	n.a.	>1
<i>Voacanga africana</i>	leaves	18,8	35,1	24,1	0,0848	0,0801
<i>Warburgia salutaris</i>	leaves	107	24	20	0,304	0,0754
<i>Xylopia aethiopica</i>	roots	n.a.	n.a.	n.a.	n.a.	0,0108
<i>Xysmalobium undulatum</i>	roots	90,7	85,2	24,4	0,0836	0,0261

Note: missing data will be available in the online version of the monographs, see www.aamps.org for more information

APPENDIX 2 - Standard specifications*

Microbiology:

Salmonella spp. – negative

Escherichia coli – negative

Aerobic bacteria – not more than 10⁵/g or ml

Fungi – not more than 10⁴/g or ml

Enterobacteria and Gram-negative bacteria – not more than 10³/g or ml

Total ash: Not more than 5%

Acid-insoluble ash: Not more than 1%

Water-soluble extractive: Not less than 20%

Foreign matter: Not more than 2%

Pesticide residues:

In accordance with national requirements.

Aldrin and dieldrin – not more than 0.05 mg/kg.

Aflatoxins (B2, G1, G2, and B1): 4 ppb

Heavy metals:

Lead in final dosage form – not more than 5 ppm,

Cadmium in final dosage form – not more than 0.3 mg/kg.

* these values correspond with guidelines as published by WHO (1998) and are for general guidance only. National or other pharmacopoeial standards may need to be adhered to and may impose different standards.

APPENDIX 3 - Full monograph template

Name	Carcinogenicity Sensitizing Potential Clinical Safety Data
GENERAL DESCRIPTION	KEY (PROPOSED) USAGE
Scientific Name with Author	Therapeutic Indications
Synonyms	Dosage
Family	Method and Duration
Vernacular Names	of Administration
Botanical Description	Contraindications
Origin and Distribution	Special Warnings and
Plant Part Used	Precautions for Use
Possible Alternative Source Species	Effects on Ability to Drive and Use
ETHNOBOTANICAL INFORMATION	Machines
Major Ethnopharmacological Uses	Interactions
Other Relevant Uses	Pregnancy and Lactation
CHEMICAL CONSTITUENTS	Adverse Effects
Compounds	Overdose
QUALITY CONTROL	Evaluation of Efficacy
Identification	TRADE INFORMATION
Organoleptic Properties	Volume of production in the country
Macroscopic Characteristics	Volume of domestic consumption
Microscopic Characteristics	Volume of export
Solubility	Average price
Moisture Content	Nature of plant material
TLC / HPLC / GC	Nature of plant products
Markers and Quantitative Methods	Processing and storage
NIR Spectroscopy	REGULATORY INFORMATION
DNA finger printing	Pharmacopoeias / Monographs
Purity Tests / Requirements	Regulatory / Registration Status
Adulterants and Adulterations	Patents
Standard Preparations	Traditional Information
Standard Specifications	POSSIBLE DEVELOPMENTS
PHARMACOLOGICAL PROPERTIES	Outlook on Further Uses
Pharmacodynamic Properties	and Research
In Vitro Experiments	REFERENCES
In Vivo Experiments	
Clinical Studies	
Pharmacokinetic Properties	
SAFETY DATA	
Ethnic Use Safety Data	
Preclinical Safety Data	
Single Dose Toxicity	
Repeated Dose Toxicity	
Mutagenic Potential	

The *African Herbal Pharmacopoeia* is the result of five years of intensive research by more than thirty experts in African medicinal plants and herbal medicines.

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