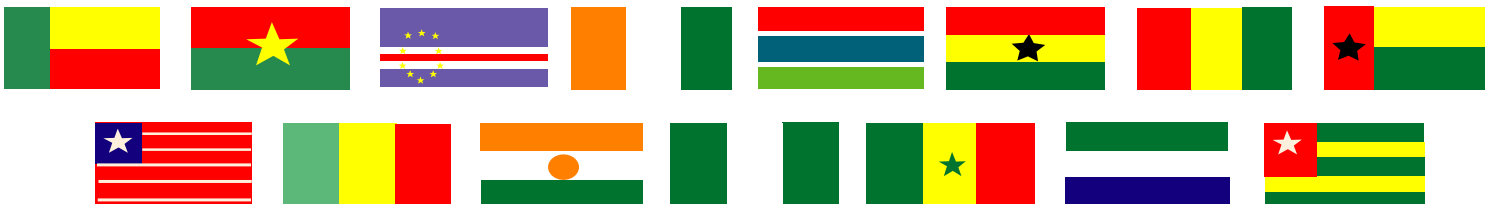




**WEST AFRICAN HEALTH ORGANISATION  
(WAHO)**

# **WEST AFRICAN HERBAL PHARMACOPOEIA**





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**@2020 WAHO**

WEST AFRICAN HEALTH ORGANISATION (WAHO)

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## FOREWORD

Globally, the use of traditional medicine (TM), particularly herbal medicines, has surged over the past two decades, with many people now resorting to it for treatment of various health conditions. For example, in Europe, the use of TM ranges from 42% in Belgium to 90% in the United Kingdom; and from 42% in the USA in adults and 70% in Canada. In Africa, TM usage ranges from 60% in Uganda and the United Republic of Tanzania; 70% in Ghana and Rwanda; to 80% in Benin and 90% in Burundi and Ethiopia.

The fast-growing demand for TM worldwide therefore calls for harmonized monographic standards to safeguard the safety and quality of the products, to foster consumer confidence and to improve access to essential medicines.

In 1978, the World Health Assembly adopted resolution WHA31.33 on Medicinal Plants, which called upon WHO to coordinate the efforts of Member States to develop and apply scientific criteria and methods for proof of safety and efficacy of medicinal plant products, and develop appropriate international standards and specifications, especially for galenicals and manufacturing practices. In pursuance of this policy directive, WHO published guidelines for registration of TM products and monographs on selected medicinal plants. WHO monographs, however, are not pharmacopoeia monographs; rather they are comprehensive scientific references, which aim to: (a) provide scientific information on the safety, efficacy, and quality control of widely used medicinal plants; (b) provide models to assist Member States in developing their own monographs or formularies for these and other herbal medicines; and (c) facilitate information exchange among countries.

In 2013, the West African Health Organisation (WAHO) published the First Volume of the West African Herbal Pharmacopoeia (WAHP), a legally-binding collection of standards and quality specifications for medicines used in the Economic Community of West African States (ECOWAS). The First Volume of the WAHP contains 55 monographs of medicinal plants commonly used in the region for treating the six WHO designated priority diseases (HIV/AIDS, tuberculosis, hypertension, diabetes, malaria and sickle cell anaemia).

Interestingly, unlike the First Volume, the Second Volume contains 30 monographs, primarily focused on medicinal plants with antiviral potential against some of the most deadly viral infections of the modern era. For example, the medicinal plants: *Euphorbia poissonii*, *Fluggea virosa* and *Piliostigma thonningii* have been shown to have the ability to treat opportunistic infections related to HIV/AIDS.

Nevertheless, there appears to be no research studies on the therapeutic potential of any of the plants featured in the Second Volume of the WAHP for treating emerging or re-emerging diseases. But, since a number of these plants have chemical constituents that are antiviral, it is likely that a well formulated composition containing some of them may prove useful in the treatment of emerging and re-emerging diseases.

As part of WHO's response to the novel coronavirus outbreak, the WHO Blueprint for R&D has been activated to accelerate the development of diagnostics, vaccines and therapeutics. The Blueprint also recognizes behavioural change interventions, and initiatives to fill critical gaps in scientific knowledge, to allow the design of better disease control measures.

This therefore provides an opportunity for West African TM research scientists to pursue rigorous R&D activities aimed at contributing to the search for effective remedies for treating emerging diseases, such as Nipah virus, Crimean-Congo haemorrhagic fever, avian influenza A (H5N1), Ebola virus disease, Marburg, Lassa fever, Middle East Respiratory Syndrome (MERS), Severe Acute Respiratory Syndrome (SARS), and Rift Valley fever.

The first and Second Volumes of the WAHP are useful additions to the Herbal Pharmacopoeias available in West Africa, developed by national governments of Ghana (1992, 2007 and 2015), Nigeria (2008) and Cote d'Ivoire (2019), as well as a Multilingual Dictionary and Monographs of African medicinal plants for eight West African Member States (2012).

Preparing the Second Volume of the WAHP involved a fourteen-member Pharmacopoeia Development Committee selected from the ECOWAS Member States. The members of this Committee comprised of experts in Anthropology/Sociology; Botany/Ethnobotany; Communication Experts; Information Technology Specialists; Pharmacognosy; Pharmacology; Public Health; Research; Toxicology; as well as Traditional Medicine Practitioners and Medical Herbalists. They were assisted by eight ex-officio members including staff from WHO/AFRO.

It is my hope that other RECs will follow the footsteps of ECOWAS to develop herbal pharmacopoeias for ensuring the identity, purity, and quality of medicinal plants in their sub-regions. I recommend that the ECOWAS Member States use the monographs of the Second Volume of the WAHP for quality control, training and local manufacturing of TM products. This will contribute to improving access to essential medicines in accordance with one of the objectives of UHC which is now more urgent than before, given the frequent pandemic of emerging infectious diseases.

**Dr Matshidiso Rebecca Moeti**  
**WHO Regional Director for Africa**

## PREFACE

In its 2014-2023 Traditional Medicine Strategic Document, the World Health Organization (WHO) sets out two major objectives - to support countries seeking to harness the contribution of traditional medicine to health and wellbeing, and to promote the safe and effective use of traditional medicine through regulation.

Since 2010 however, the West African Health Organisation (WAHO) has championed the recognition and promotion of rational traditional medicine practices by Member States of the Economic Community of West African States (ECOWAS). Member States are encouraged and supported to document, validate and regulate the use of plant and herbal medicines in the region. Traditional medicine is now included as a module in the training curriculum of many medical schools in the region, and a dedicated traditional medicine day is celebrated annually in each country.

This deliberate policy is a reflection of the political determination of ECOWAS Heads of State and Government to improve access in the region to quality medicinal plants with established therapeutic benefits. As part of the policy, WAHO published in 2013 the first West Africa Pharmacopoeia of Herbal Medicines which was adopted in April 2014 by the 15th Ordinary Session of the Assembly of ECOWAS Health Ministers in Monrovia, Liberia. It identifies several traditional herbs and plants of medicinal efficacy in the region and specifies the criteria and methods of analysis to be used to guarantee the quality, safety and potency of the medicinal plants.

The second volume of the West African Pharmacopoeia of Herbal Medicines is now available. And continuing the practice with the first, this second volume was again prepared by a multidisciplinary team of professionals that included traditional medicine practitioners, herbalists, experts in pharmacognosy, pharmacologists, toxicologists, botanists, ethno-botanists, anthropologists, sociologists and public health physicians. The team was supported by IT and communication specialists. Specific objectives were set for the multidisciplinary team of experts, in line with the instruction of ECOWAS Ministers of Health that WAHO should regularly produce monographs of plants for treating common and newly-emerging diseases in the region. Preparation of the Pharmacopoeia therefore involved documentation of emerging and re-emerging public health diseases in West Africa, a review of the socio-anthropological studies on the uses of medicinal plants for treating such diseases, and a literature survey of all available data on the selected medicinal herbs and plants. A detailed description of the macroscopic and microscopic characteristics, phytochemical analysis, thin layer chromatography fingerprinting, and high performance liquid chromatography fingerprinting is included in the monograph for each herb, as well as the safety profiles.

The successful development of this second volume of the West African Pharmacopoeia of Herbal Medicines clearly demonstrates the wealth of expertise in traditional medicine and plant medicine research in the ECOWAS region. As the second decade of "African Traditional Medicine 2011-2020" comes to a close, I am very proud to be associated with this landmark document, and on behalf of WAHO, I would like to express special gratitude to the members of the Pharmacopoeia Development Expert Committee and the Federations/Associations of Traditional Medicine Practitioners that contributed to its production. Special thanks also go to all those who contributed in diverse ways to this project, for their dedication, devotion and spirit of public service.

I am convinced that this Pharmacopoeia will contribute immensely to the promotion of rational traditional medicine practices in the ECOWAS region, and the search for local solutions to some of the health problems in the region. I am therefore pleased to recommend it to you as a most useful reference in this regard.

**Prof Stanley Okolo**  
**Director General**



## INTRODUCTION

In many countries around the world, traditional medical knowledge is only passed on by oral tradition, with very little or no documentation. However, since the latter part of the 20<sup>th</sup> Century, due to the realisation of the immense potential of traditional medicine, and the growing demand of indigenous knowledge holders for fair and equitable share of benefits derived from the commercialization of their products, there have been calls for documentation and protection of traditional medical knowledge.

In the light of this, as far back as 1986, the fourth International Conference of Drug Regulatory Authorities held in Tokyo, called on WHO to compile a list of medicinal plants and to establish international specifications for the most widely used ones. Subsequently, several WHO Member States made efforts to provide safe and efficacious herbal medicines for their populations.

An herbal pharmacopoeia is a document that provides information, which enables the proper identification of a medicinal plant. It contains the basic description of the plant including nomenclature, parts used, chemical constituents, therapeutic actions and indications, contraindications and side effects, as well as dosage and dosage forms. Essentially, an herbal pharmacopoeia aims to promote the rational use of herbal medicines of proven efficacy and safety by providing information on standards of identity, quality and safety, ethnomedical uses and scientific studies.

The development of an herbal pharmacopoeia is both capital and labour-intensive. It requires careful planning and mobilisation of the requisite resources, such as technical expertise, finance, access to credible scientific data, as well as sensitisation of the Member States. This explains why to date in West Africa, only Ghana (1992 and 2007) and Nigeria (2008) and very recently Cote d'Ivoire (2018) have been able to develop national herbal pharmacopoeias.

It is against this background that the West African Health Organisation (WAHO) sought to publish Herbal Pharmacopoeias (2013 and 2020) to serve as reference documents on the safety and efficacy of some of the region's medicinal plants.

By the publication of the first volume of this important document, ECOWAS became the first Regional Economic block to take one of the most pragmatic steps towards the attainment of the Alma-Ata Declaration of 1978. Volume 1 of the West African Herbal Pharmacopoeia, which was published in 2013, was widely acknowledged by the Member States and other relevant stakeholders as a landmark document, culminating in the adoption of a resolution for its use, at the Fifteenth Ordinary Session of the Assembly of ECOWAS Health Ministers, held in Monrovia-Liberia in 2014.

Mindful of the rampant outbreaks of disease epidemics in the ECOWAS region, and the fact that traditional medicine remains the first line of treatment for the region's populations, the Honourable Ministers of Health in adopting this resolution, recommended that WAHO should take immediate steps to develop Herbal Pharmacopoeias targeted specifically at newly-emerging diseases. This recommendation was made against the backdrop of the outbreak of the Ebola virus epidemic (2013–2016), which caused major loss of life and socioeconomic disruption in the region, mainly in Guinea, Liberia and Sierra Leone.

Indeed, despite the huge advances in medical science with the development of powerful diagnostic and therapeutic tools and vaccines, it is still extremely difficult to contain infectious diseases that affect the health and economic stability of societies, due to the ease of world travel and increased globalisation.

Besides the Ebola virus disease, other emerging infectious diseases that have become public health concerns include HIV infections, severe acute respiratory syndrome (SARS), Lyme disease, pandemic H1N1 influenza, Lassa fever, dengue fever, West Nile virus, Zika virus and very recently the coronavirus (COVID-19) pandemic.

Traditionally, many infectious diseases are treated with antibiotics and antiviral agents. However, due to the emergence of antimicrobial-resistant strains and mutant microorganisms, antimicrobial resistance (AMR)-the resistance of bacteria, parasites, viruses and fungi to antimicrobial drugs previously effective for treatment of infections they caused – is now a serious worldwide threat to public health. Today, nearly all pathogenic microorganisms, including bacteria, fungi and viruses, have developed sophisticated resistance to multiple antimicrobial agents, with a resultant increase in morbidity and mortality from otherwise treatable infections.

Consequently, in response to the call of the Honourable Ministers of Health of ECOWAS to compile monographs of medicinal plants for treating newly-emerging diseases, in December 2017, WAHO constituted a group of experts drawn from the Member States, to develop herbal monographs, primarily focused on plants for treating infectious diseases of viral origin, for the second volume of the West African Herbal Pharmacopoeia.

Guided by previous experience, it was decided from the outset that membership of this group would be restricted to dedicated experts with the requisite pedigree in traditional medicine promotion, practice and research. A consultative meeting was therefore held in Lome-Togo in June 2016, to launch the project, during which was established, a fourteen-member Pharmacopoeial Development Committee, comprising of plant medicine researchers, pharmacognocists, pharmacologists, toxicologists; botanists/ethnobotanists; anthropologists/ sociologists; traditional medicine practitioners/medical herbalists; public health physicians; Information Technology specialists and communication experts, to be assisted by eight ex-officio members (See Annex 1).

During the meeting, the Terms of Reference of the Committee as well as a template for preparing the monographs, and the list of plants to feature in this volume, were proposed and adopted. Two working groups (one Anglophone and one Francophone), were then constituted, with clear Terms of Reference to make proposals for preparing the different monographs. After nearly two days of intensive work, the outcome of the meeting were:

- Validation of a list of 30 selected medicinal plants that will feature in the Pharmacopoeia;
- Sharing of the selected medicinal plants among the Experts;
- Identification of experts to undertake the toxicological, phytochemical, microscopic and macroscopic studies;
- Selection of experts to review the ethnobotanical use and socio-anthropological aspects of medicinal plants; and
- Identification of possible sources for the collection of information and scientific data required for the development of the Pharmacopoeia.

Subsequently, after nearly two years of work, the Expert Committee completed its work, which was then finalised to include a preface, a foreword, an introduction, and monographs of 30 medicinal plants.

Each of the monographs has microscopic and macroscopic characterisations, phytochemical analysis, one or two photos, Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC) fingerprinting, as well as safety profiles.

Each monograph is structured as follows:

*Names:* botanical name with author; family; synonyms; common names; vernacular names (not more than 3 per country). For all the monographs, the botanical name is chosen for the title.

*General information (summary):* plant description; ethnomedical uses; scientific, clinical and safety data

*Description of the plant:* whole and plant parts, especially parts with medicinal properties; fresh and dried parts if dried parts are used; pictures (good quality, high resolution); herbarium specimen number; habitat and geographical distribution; definition of the plant medicine ( plant material of interest).

*Chemical constituents:* active and non-active constituent, but chemical structures are for only those compounds which are known to contribute the plant's activity.

*Biological and pharmacological activities:* experimental data; clinical data (where available).

*Safety data:* acute toxicity; sub chronic and chronic toxicity (where necessary); contraindications; precautions; adverse effects.

*Therapeutic indications:* authenticated claims.

*Therapeutic actions:* based on biological and pharmacological data.

*Tests for identity and purity:* moisture content; ash values; extractive values; chromatographic fingerprints; macroscopy and microscopy (qualitative and quantitative)-whole and powdered samples.

*Dosages:* obtained from such reputable texts as the United States Pharmacopoeia, which expresses the dose of infusions and decoctions as a weight to volume ratio of 1:20 (i.e. 1 part dried herb to 20 parts water). Thus the traditional therapeutic dose for infusions/decoctions is taken as 30 g dried herb in 600 ml of water, 60-200 ml three times a day, while the concentrations of tinctures are expressed as a weight to volume ratio (w:v.). In general, many herbal medicine practitioners prefer to prescribe drop doses of 1:5 (i.e. 1 kg of herb in 5 litres of solvent) or even more dilute tinctures with formulations usually prescribed as 2.5-5 ml three times daily). Thus, except in a few unique cases, 1:5 tinctures are recommended throughout the text.

*Storage conditions:* based on information obtained from other texts.

*References:* scientific journals; books; technical reports; institutional publications; theses; information from Traditional Medicine best practices.

The West African Herbal Pharmacopoeia will serve traditional medicine practitioners, socio- anthropologists, consumers, research scientists, programme managers, physicians, pharmacists, research students, academic institutions, health policy makers, development partners and non-governmental Organizations involved in the development of traditional medicine

# MONOGRAPHS

**Botanical name**

*Abrus precatorius* Linn. Subsp. *africanus* VERDC.

**Family**

Fabaceae - Faboideae

**Synonyms**

Abruzzo (L.) Wright, *Abrus cyaneus* R. Vig., *Abruzzo maculatus* Noronha, *Abrus minor* Desv., *Abrus pauciflorus* Desv., *Abrus squamulosus* E. Mey., *Abruzzo tunguensis* Lima, *Glycine abrus* L.

**Common Names**

Indian liquor, Jequirity, Prayer beads (English), Cascavelle, Liane réglisse, Jéquirity (French)

**Common Local Names**

**Benin:** Fon-Viviman; Adja-Assiunkouvi; Bariba-Babanyerou

**Burkina Faso:** Moore -Lim-tiiga

**Côte d'Ivoire:** Agni-Tamaboa ; Ashanti-Tamaboa ; Baoulé- Alobogna

**Gambia:** Mandinka-Fanto

**Ghana:** Akan- Obreku-ani; Ewe- Adenkiodzi, Dedekuada; Ga- Hinmetsofa, Nmeibia

**Guinea Bissau:** Créole-N'fentu

**Mali:** Bambara -N'tè bilèni; Peulh- gitégélodé

**Niger:** Djerma/Zarma – Gurmundi; Haoussa - Idon zakana

**Nigeria:** Hausa- Idon zakara; Igbo- Nkpulu-anya-nnunu; Yoruba-Oju Ologbo

**Senegal:** Serer- Ngid fangool; Socé/Madingue – Dolimo, Ndolinu, dolign'hou

**Togo:** Ewé- Dzedzekudze ; Ouatchi- Dzedzekudze ; Mina- Djedjinkudjin

**Description of the plant**

Creeping, woody plant. The alternate leaves are compound paripinnate up to 1-32 foliate with a rachis 7 to 8 cm long. The leaflets, small and numerous, are opposite, oblong to obovate, short-stalked, rounded at the base with rounded to emarginate apex. Leaf is slightly pubescent on the lower surface. The blade is glabrous. White to light pink flowers occur in cymose inflorescences. Pods are up to 3 cm long, 1 to 1.2 cm, wide tomentose and papillose with 5 to 6 round seeds sometimes hard and shiny. Pods are dehiscent, oblong, slightly swollen and sparsely pubescent. The seed colour can be white, but most often they are bright red with a black spot near their umbilicus (Kueté, 2014).



A- *Abrus precatorius* L plant, B – flower, C – immature fruit, D and E - fruits showing seeds

### Herbarium specimen number

Benin: 2339 (AP)

Burkina Faso: 3784 (OAU)

Ghana: GH 024/KNUST

Côte d'Ivoire: 12527 (CNF)

Mali: 0865 / DMT

Nigeria: FHI111921

OOAS : 0865/DMT

Senegal: IFAN 20

Togo 05586 – Faculty of Sciences, University of Lome

### Habitat and geographical distribution

*Abrus precatorius* grows wild in small woods, forests, shrub savannas, coastal sand, fields, clearings and sometimes in hedgerows. It is a widespread plant in the tropical and subtropical regions of Africa.

### Plant material of interest

Leaf

### Other parts used

Leafy stem and roots

### Definition of plant material of interest

*Abrus precatorius* consists of the fresh or dried leaves of *Abrus precatorius* Linn. (Fabaceae -Faboideae).

### Ethnomedical uses

The hot-water extract of fresh roots is administered orally in West Africa as antispasmodic and anticonvulsant (Adesina, 1982). The aqueous extract of the leaf and stem is also taken orally as an aphrodisiac, and to facilitate delivery in women (Kerharo and Bouquet, 1950). Also in West Africa the dried roots are used to prepare a decoction administered orally to treat schistosomiasis, gonorrhoea, chest pains and as an antiemetic and antiparasitic against tapeworm. Several African tribes use the seed powder as an oral contraceptive (Garaniya and Bapodra, 2014). The aqueous seed extract is used in the treatment of cancers of the epithelial tissue. The entire plant is used in the treatment of venereal diseases, headaches and snake bites (Iwu, 2014).

### Biological and pharmacological activities

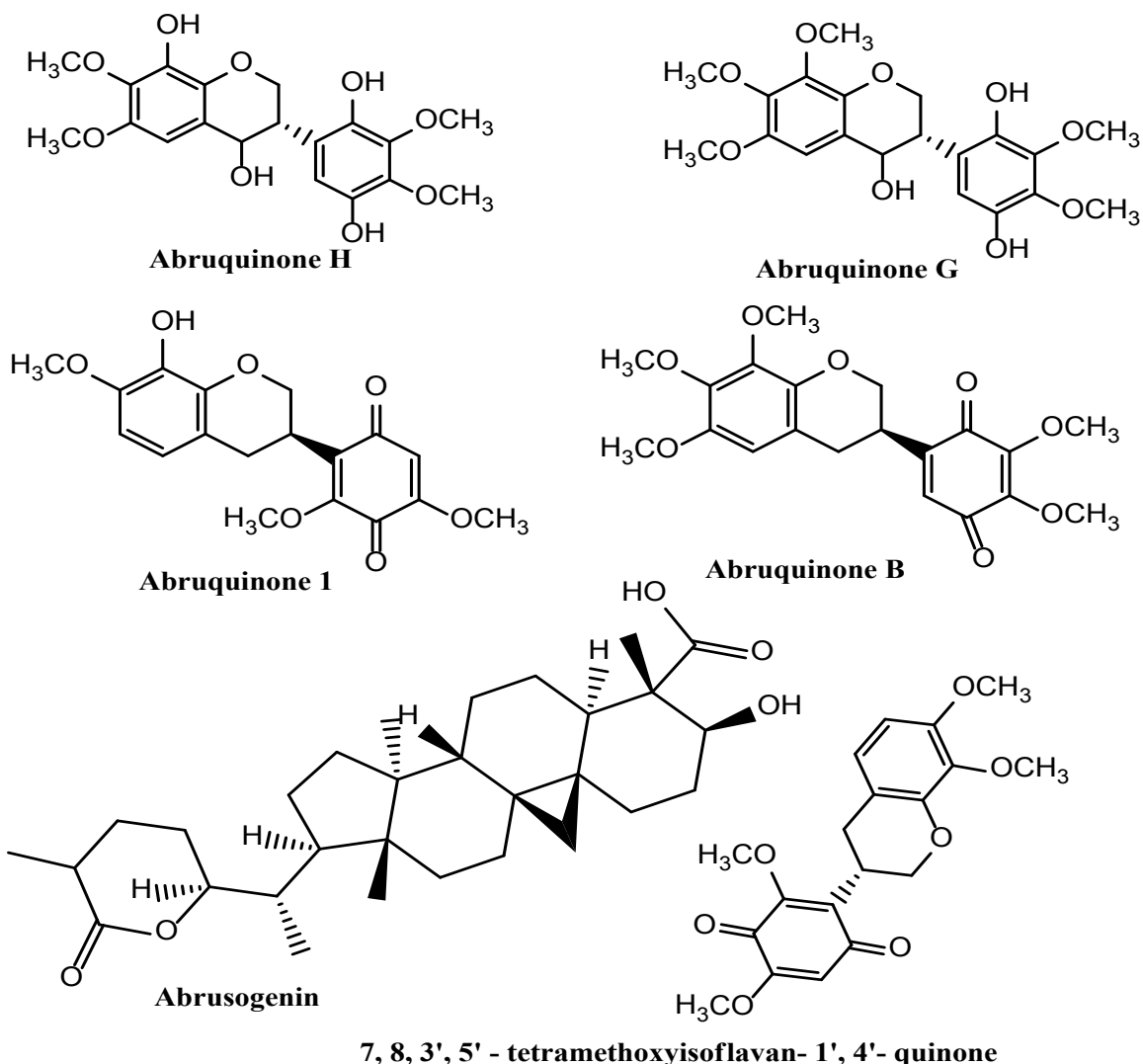
Methanolic extracts of the leaves of the plant showed hypoglycaemic and insulin secreting activity in streptozotocin-induced diabetic rats (Umamahesh and Veeresham, 2016). Similarly, leaf extracts have shown *in vitro* anti- $\alpha$ -amylase activity suggesting that the plant has anti-hyperglycaemic properties (Yonemoto *et al.*, 2014). Palvai *et al.* (2014) established that the plant has antioxidant potential. Alcoholic leaf extracts have been shown to be bronchodilators both *in vitro* and *in vivo* (Mensah *et al.*, 2011). The plant (seeds and aerial parts) has a protective effect against alcohol- and paracetamol-induced renal damage (Ligha *et al.*, 2009, Sohn *et al.*, 2009). Extracts from seeds and aerial parts have been shown to be active on several bacterial species (Adelowotan *et al.*, 2008, Ouattara *et al.*, 2012). Studies have shown that the plant exerts antidepressant and antiepileptic effects (Adesina, 1982), and as a neuromuscular inhibitor (Wambebe and Amosun, 1984). Anbu *et al.* (2011) have shown that extracts from *A. precatorius* increase survival time in mice with cancerous tumours. The work of Sandhya *et al.* (2012) showed that aqueous leaf extracts (300 mg / kg) exert a remarkable effect on hair growth in the Wistar rat.

### Clinical data

Not available

### Chemical constituents

Alkaloids: abrasine, hyalophorin, N, N-dimethyltryptophan-methocation-methyl ester, picatorine, prescicine, trigonellin (William and Li, 1970) abrin (very toxic and found in seeds), urease (Ibrahim, 1980, Kinjo *et al.*, 1991); terpenoids: oleanolic acid, abruslactone A,  $\beta$ -amyrin, cycloartenol, hederagenin (Choi *et al.*, 1989), abrusoside, abrusoside A, B, C, D, abrusgenic acid, abruslactone (Choi *et al.*, 1989, Chang *et al.*, 1982, Chang *et al.*, 1983); saponins: abras-saponin I and II, methyl abrusgenate, glycyrrhizin (Akinloye and Adalumo, 1981). Others include isoflavoquinones: abruquinones A, B, C, D, E, F, O, G (Kuo *et al.*, 1995



### Test for identity and purity

#### Aerial part or leafy stem

**Moisture content:** air dried coarse powder does not lose more than 4.8% at 105°C.

**Total ash:** not more than 6.5%

**Acid insoluble ash:** not more than 1.0%

**Water soluble extractive:** not less than 14.0%

**Ethanol soluble extractive (70%):** not less than 4.0%

### Chromatographic fingerprinting

#### Thin layer chromatography

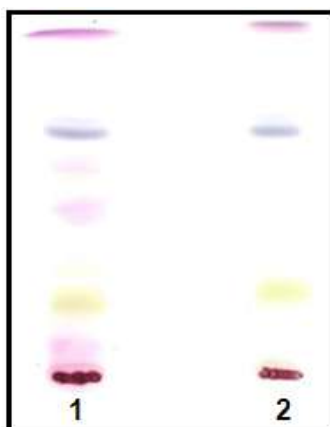
**Preparation:** About 5 g of the powdered leafy stem were extracted with ethyl acetate by cold maceration, filtered and the filtrate concentrated to a small volume. A small spot was then applied to the TLC plate for analysis.



**Chromatographic conditions:** Analytical TLC on silica gel G60 F254, 0.25 mm layer in hexane/ethyl acetate (7:3) as the mobile phase.

**Detection:** Visualized in daylight after spraying with anisaldehyde-sulphuric acid (Lane 1) and vanillin-sulphuric acid reagents (Lane 2) (Stahl, 1969) and heating to 110°C for 10 mins.

The TLC chromatogram showed three prominent spots with R<sub>f</sub>s of 0.97 (purple), 0.70 (ash) and 0.23 (yellow) when sprayed with both anisaldehyde and vanillin reagents. Two additional spots appeared in the chromatogram sprayed with anisaldehyde at R<sub>f</sub>s of 0.61 (pink) and 0.49 (pink)



### High Performance Liquid Chromatography

**Sample preparation:** About 10 mg of the hydroethanolic extract of *A. precatorius* L. (aerial part) were reconstituted in 3 mL acetonitrile in a 10 mL volumetric flask with sonication for 17 minutes. The resulting solution was then diluted to volume with the mobile phase under chromatographic conditions. It was centrifuged to obtain a clear test solution which was subsequently filtered through a 0.45 µm filter into an HPLC vial and analyzed.

### Chromatographic system

#### Optimized chromatographic conditions

**Mode:** LC

**Column:** YMC ODS, 4.6 x 150 mm, 5 µm

**Column temperature:** Ambient – 30°C

**Mobile phase:** Acetonitrile: water (60:40 v/v)

**Elution mode:** Isocratic

**Injection volume:** 20 µL

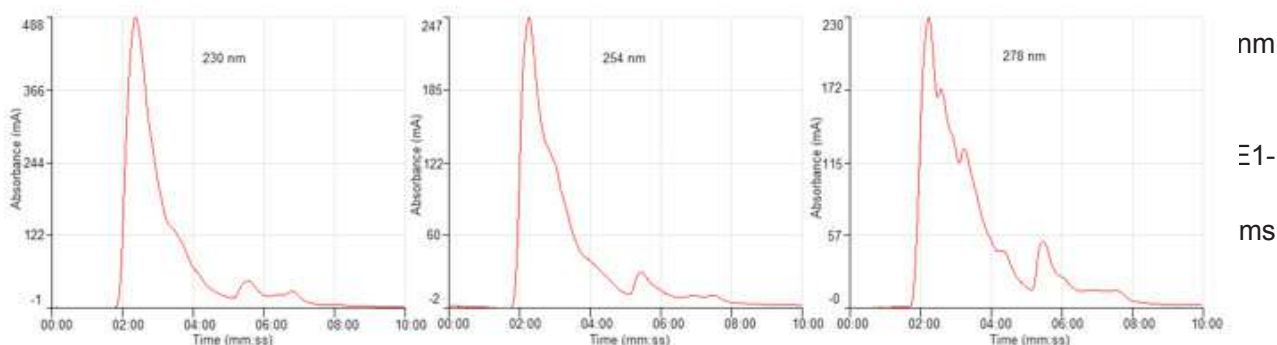
**Flow rate:** 0.5 ml/minute

**Detection wavelengths:** 230 nm, 254 nm and 278 nm.

### System Suitability parameters

**Number of peaks:** 230 nm (3), 254 nm (2), 278 nm (3)

**Retention time (s):** 230 nm (rt1-2.22 min, rt2- 5.33 min, rt3-6.48 min), 254 nm (rt1- 2.16 min, 5.27 min), 278 nm (rt1-2.13 min, rt2-2.34 min, rt3 – 2.46 min, rt4-3.13 min, rt5- 4.17 min, rt6- 5.27 min)



## HPLC chromatogram

## FT-IR

A small amount of the dried hydroethanolic extract (70%) was placed on the sample area of the Perkin Elmer UATR Fourier transform infrared (FT-IR) spectrometer and scanned between  $4000-400\text{ cm}^{-1}$  with a resolving power of  $4\text{ cm}^{-1}$  and a cumulative scanning limitation of 24 times. Principal peaks appeared at wavenumbers  $3316.72$  (broad),  $2923.22$ ,  $2853.07$  and  $1601.82\text{ cm}^{-1}$ .

## Macroscopy

The leaf is a compound, lacks a terminal leaflet, alternately arranged, green in colour and having up to 5 -15 pairs of oblong leaflets. The leaf bears prostrate hairs. The leaflet stalk is about 1 mm long. Each leaf blade is about  $16-25 \times 6-8\text{ mm}$ . The petiole is deeply grooved on the upper surface. The lateral and reticulate veins are well defined. Leaflets are rounded at the base and rounded to emarginate at the apex.

## Microscopy

Upper surface has wavy epidermal cells and numerous appressed unicellular trichomes  $125-185-292\text{ }\mu\text{m}$  with acute apex and thick walls. The lower surface has numerous unicellular trichomes  $119-168-250\text{ }\mu\text{m}$  similar to those on the upper surface. Epidermal cells are wavy, and there are anomocytic stomata with up to five subsidiary cells. The veins of both surfaces are lined with calcium oxalate sheaths.

## Transverse section

## Powdered plant material

Powdered leaf is dark green in colour; consists of fragments of trichomes and unicellular appressed trichomes, numerous starch granules of irregular shape, fragments of upper and lower surface of the leaf show wavy cell walls and anomocytic stomata, fragments of veins with elongated rectangular epidermal cells. Bundles of fibres with rows of polygonal calcium oxalate prisms, which are four- to six-sided. Portions of the transverse section of the leaf through the lamina showing palisade and spongy mesophyll cells.

## Therapeutic actions

Antimicrobial, analgesic, antitussive, anticonvulsant, anthelmintic, antiviral, antifungal, antiinflammatory, antispasmodic, contraceptive, hypoglycaemic, hepatoprotective.

### Therapeutic indications

Microbial infections, pain, cough, parasitosis, inflammatory diseases, contraception, diabetes, memory disorders, liver and kidney diseases.

### Safety data

LD<sub>50</sub> of the aqueous leaf extract by oral route was estimated to be beyond 3000 mg/kg in rats. Treatment did not affect the central or autonomic nervous system, and target organs such as the liver, kidney, heart, lungs. WBC and RBC were not affected. There were mild elevations in Mean Corpuscular Volume (MCV), haemoglobin, haematocrit at high doses of treatment (300-1000 mg/kg). It may, however, induce mild leucopenia as a result of lowered granulocytes (neutrophils and MID cells) with a corresponding increase in granulocytes (lymphocytes). Treatment decreased thrombocyte count. Liver enzymes decreased slightly, which may contribute to the reported hepatoprotective effects of the plant, but serum proteins were not generally affected. Bilirubin levels did not change. Abrus extract (>300 mg/kg) increases clotting time in rabbits. There was no significant change in sleeping time up to 1000 mg/kg. Hepatocytes, Kupffers cells, central and hepatic vein appeared normal. There were no signs of tubular or glomerular necrosis in kidneys. Extract did not affect urea or creatinine or the urea creatinine ratio. Seeds of Abrus may possess more toxic compounds than the leaves used in this study. Other authors have noted changes in haematological, liver enzymes and total proteins with the use of the seed extract. Leaf extract causing mild neutropenia supports an early report on the extracts' ability to inhibit milk-induced leukocytosis in mice. The decrease in thrombocytes in rats and the subsequent increase in clotting time in rabbits suggest that it may have potential in the management of thrombotic episodes. It also suggests that caution must be taken in patients with coagulative and haemorrhagic disorders.

### Precautions for use

Do not administer seeds untreated by heat.

### Adverse effects

Hypoglycaemia, contraception.

### Contraindications

Should not be used by pregnant or lactating women, and in haemorrhagic disorders

### Dosage form

Infusion, decoction, tincture

### Dosage

**Infusion:** 20-30 g of dry leaves per litre of water; take 2-3 teacups day

**Decoction:** 30 g of dried plant material in 900 mL water; simmer until reduced to 600 mL; 1-3 tablespoonfuls daily

**Tincture:** 1:5 30% fresh alcohol, take 2-5 ml twice daily

## Storage

Store in a cool, dry place away from light.

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**Botanical name**

*Acanthospermum hispidum* (DC)

**Family**

Asteraceae

**Synonyms**

*Acanthospermum humile* var. *hispidum* (DC.) Kuntze

**Common Names**

Star bur, goat's head, bristly starbur, hispid starbur, horn spine (English), herbe-savane (French), carrapicho de carneiro (Portugese).

**Common Local names**

**Benin:** Fon-kponomi; Yoruba-Tchakatou; Batonu-Saroutange

**Burkina Faso:** Moré/Mossi - Kurkur gôse; Bambara - Suraka voni

**Côte d'Ivoire:** Bété – Kokodoegbagla; Gouro – Bohuederi; Malinké - Lukoubassa moni

**Ghana:** Lobi- Bongore Tatulatugin

**Guinea:** Malinké/Dioula - Soulaka wani,

**Mali:** Bambara- Suraka wôni; Dogon- degiri ku; Peulh-dagasalum

**Nigeria:** Yoruba - Dagunro, Urhobo- gorogoro, Hausa-Kaashinyaawo

**Senegal:** Socé-Suraka wôni; Wolof-Dagiganar; Peuhl- Dagasalum; Serer- Sakarkasâg

**Sierra Leone:** Sahiligbin

**Togo:** Adja-Gongadé; Ewé-Afegba; Mina-Ahonglon

**Description of the plant**

The plant is a grass and grows to 1 m high; stems and branches densely pubescent, cylindrical, green when young and brown in the adult plant. Tap root up to 20 cm deep, with a slightly sweet aroma (Asase et al., 2005). Leaves are sessile, entire, simple, opposite and pubescent without appendages; oval, oblong elliptical in shape, symmetrical, acute attenuated at the base, acute acuminate at the apex, entire margin, slightly sinuous, the main vein protrudes on the lower surface and has 7 to 8 pairs of lateral veins. The petiole is marginal, glabrous, 4-7 mm long and 1-2 mm wide. The small bunch of Inflorescence is axillary, with small, yellow, unisexual flowers. Achenes fruit, triangular, elongated and covered by long shredded hairs (Araújo et al., 2013).



A – *Acanthospermum hispidum* DC leaves and fruits: B – flowers, C – whole plant

#### Herbarium specimen number

Benin: 2340(AP)

Burkina Faso: 3400 (OAU); MSLS 1338 (CNSF)

Côte d'Ivoire: 16762 / CNF / RCI

Ghana: GH 101/KNUST

Mali: 2533 / DMT

Nigeria: FHI111917

Senegal : UCAD 1088, IFAN ETH 1

Togo: TG 15178

#### Habitat and geographical distribution

*Acanthospermum hispidum* is a pantropical species, native to tropical America (Holm *et al.*, 1997, Akoègniniu *et al.*, 2006). It is a ruderal plant found in sandy soils; it invades inhabited and cultivated places, roadsides and tracks (Diarra, 2006).

#### Plant material of interest

Roots

#### Other parts used

Leafy stem, and whole plant

## Definition of plant material of interest

*Acanthospermum hispidum* is the fresh or dried root of *Acanthospermum hispidum* DC. (Asteraceae)

## Ethnomedical uses

*Acanthospermum hispidum* is used in traditional medicine for the treatment of jaundice, malaria, vomiting, headache, abdominal pain, seizures, stomach upset, constipation eruptive fever, snake bite, epilepsy, blennorrhoea, hepatobiliary disorders, malaria, microbial and viral infections. It is also used for the treatment of skin disorders, coughs and bronchitis (Adjanohoun *et al.*, 1989). In Benin, a decoction and maceration of the leaves are used orally against abdominal pain. The macerate is used orally to treat female infertility (Adjanohoun *et al.*, 1989). In Togo, leaf sap is used as eye drops in ocular conditions and in local applications against migraines and headaches. Masticated leaves are used to treat animal bites and stings. A decoction of the leafy stem is used orally in the treatment of liver disease (Adjanohoun *et al.*, 1986), urinary tract infections and typhoid fever. In Togo, a decoction of the plant is used to treat urinary tract infections and typhoid fever. In Nigeria, leaf decoction is used orally in the treatment of malaria (Lyamah and Idu, 2015). Whole plant maceration taken orally and in a steam bath is used for the treatment of malaria and liver disease. Macerate is used against haemorrhoids (Adjanohoun *et al.*, 1981). The entire plant is used in the treatment of liver diseases in Benin (Adjanohoun *et al.*, 1989) and malaria in Ghana (Asase *et al.*, 2005). *A. hispidum* appears to contain phytoconstituents that may be useful as adjuvants for antibiotic formulations (Adu *et al.*, 2011). The plant also has immunomodulatory, antibacterial and antifungal properties (Chakraborty *et al.*, 2012).

The juice of the fresh plant is indicated orally and externally in the treatment of headache, vomiting, severe gastralgia, convulsions, syncope, tonsillitis, snake bites, jaundice, genitourinary infections, dysentery, viral hepatitis, malaria, rheumatism, sinusitis, conjunctivitis, epilepsy, migraine, wounds and stomach disorders (Kpodar *et al.*, 2016). Decoction of the entire plant is used orally and externally in the treatment of urethritis, cough, epilepsy, constipation, eruptive fevers, and all conditions treated by the juice (Gbekley *et al.*, 2015; Agbodeka *et al.*, 2017). In Cote d'Ivoire, it is used as an antimalarial, antihypertensive, antispasmodic, vermifuge and as an abortifacient. The Peuhls of Boundou in Senegal use it in combination with *Combretum glutinosum* and *Gardenia triacantha* in a medico-magic preparation for the treatment of hepatobiliary diseases (Kheraro and Adam, 1974; Adjanohoun and Aké-Assi, 1970).

## Biological and pharmacological activities

The ethanolic extract of the leaves and flowering tops exhibited potent antimicrobial activity against a number of pathogenic microorganisms including *Streptococcus pyogenes*, *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Clostridium histolyticum*, *Neisseria gonorrhoea*, *Microsporium gypseum*, *Cladosporides cladosporioides*, Pseudorabies virus and *Trichophyton mentagrophytes* (Fleischer *et al.*, 2003; Araújo *et al.*, 2008; Adu *et al.*, 2011). The root also showed promising activity against *Staphylococcus aureus*, *Salmonella species* and *E. coli* (Araújo *et al.*, 2008). The leaves have antiparasitic and molluscidal (*Biomphalaria glabrata*) activities. Acanthospermolide extracted from the plant exhibited both *in vitro* and *in vivo* anti-cancer activity as well as consistent antiplasmodial activity (Araújo *et al.*, 2008). Two sesquiterpene lactones isolated from the plant have been shown to exhibit *in vitro* antiplasmodial activity on chloroquine-sensitive *Plasmodium falciparum* strain 3D7 (IC<sub>50</sub> between 2.9 ± 0.5 and 2.23 ± 0.09 μM). These two sesquiterpene lactones also exhibited *in vitro* anti-trypanosomiasis activity on *Trypanosoma brucei brucei* and *in vitro* antileishmaniasis activity against *Leishmania mexicana mexicana* (Ganfou *et al.*, 2012). The crude hydro-acidified extract also showed some *in vivo* antimalarial activity on *Plasmodium berghei berghei*. Deepa *et al.* (2007) reported antitumour activity of the 50% aqueous ethanolic extract of the plant on Dalton ascites lymphoma in mice.



Thus, it was suggested that *A. hispidum* has potential for the development of herbal medicines for the treatment of cancer. Sawadogo *et al* (2012) also reported that the methanolic extract of the flowering stems showed significant cytotoxic activity against some cell lines.

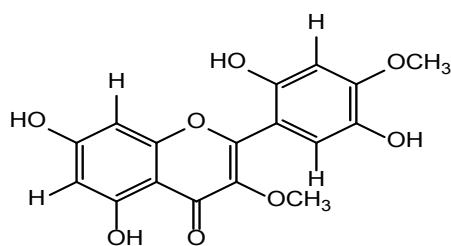
Agunu *et al.* (2005) showed that the hydromethanolic extracts of *A. hispidum* leaves and stem had pharmacological action against diarrhoea. Their study showed that the extract at low doses induced a smooth muscle relaxant effect on the rabbit jejunum. The leaf extract of the plant showed antiviral activity on alpha herpes viruses, pseudo rabies virus (PRV) and bovine herpes virus 1 (Artur *et al.*, 1997). Acanthospermal B, a major sesquiterpene lactone of *A. hispidum*, is a selective antibacterial agent against *Enterococcus faecalis* and *Staphylococcus aureus*, but inactive on Gram-negative bacteria and *Lactobacillus*. This compound was found to be active on Methicillin-resistant *Staphylococcus aureus*, which is one of the main microorganisms involved in chronic infections in humans (Mario *et al.*, 2011). Studies have shown that 230 mg/kg of a decoction of the aerial parts of *A. hispidum*, displayed significantly higher peripheral analgesic activity than paracetamol at a dose of 100 mg/kg in mice. The decoction of the aerial parts of *A. hispidum* at doses of 115 mg/kg and 230 mg/kg also showed antiinflammatory activity in the mouse carrageenan oedema test (Diarra, 2006).

### Clinical data

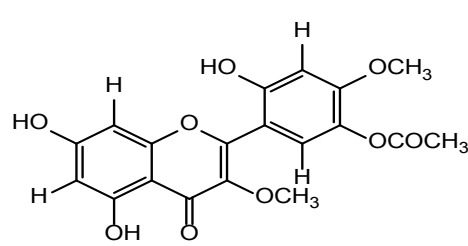
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### Chemical constituents

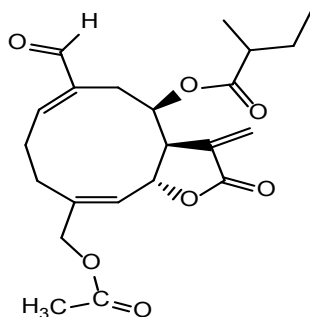
Sesquiterpene lactones such as guaianolides, germacranolides, melampolides, heliangolides, Pseudoguaianolides, hypocretenolides, eudesmanolides, 15-acetoxy-8 $\beta$ -[(2-methylbutyryloxy)]-14-oxo-4,5-cis-acanthospermolide, 9 $\alpha$ -acetoxy-15-hydroxy-8 $\beta$ -(2-methylbutyryloxy)-14-oxo-4,5-trans-acanthospermolide (Jakupovic *et al.* 1986; Cartagena *et al.*, 2000; Ganfon *et al.*, 2012); flavones (eg. 5,7,2',5'-tetrahydroxy-3,4'-dimethoxyflavone and 5'-acetoxy-5,7,2'-trihydroxy-3, 4'-dimethoxyflavone) (Edewor and Olajire, 2011), N-butyl eicosante, N-heptacosanol (Mathur *et al.*, 1976), acanthospermal- $\beta$ -galactosidopyranoside (Ramachandrana *et al.*, 1976; Geran *et al.*, 1972; Jakupov *et al.*, 1986; Herz & Kalyanarama, 1975)



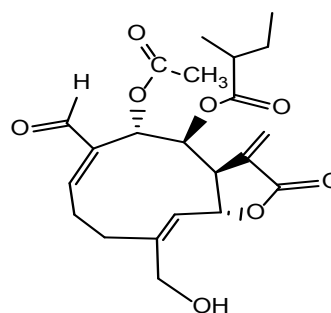
5,7,2',5'-tetrahydroxy-3,4'-dimethoxyflavone



5'-acetoxy-5,7,2'-trihydroxy-3, 4' dimethoxyflavone



15-acetoxy-8 $\beta$ -[(2-methylbutyryloxy)]-14-oxo-4,5-cis-acanthospermolide



9 $\alpha$ -acetoxy-15-hydroxy-8 $\beta$ -(2-methylbutyryloxy)-14-oxo-4,5-trans-acanthospermolide

### Test for identity and purity

Moisture content: air dried coarse powder does not lose more than 6.2% at 105°C.

Total ash: not more than 15.9%

Acid insoluble ash: not more than 6.7%

Water Soluble extractive: not less than 7.0%

Ethanol soluble extractive (70%): not less than 6.0%

### Chromatographic fingerprint

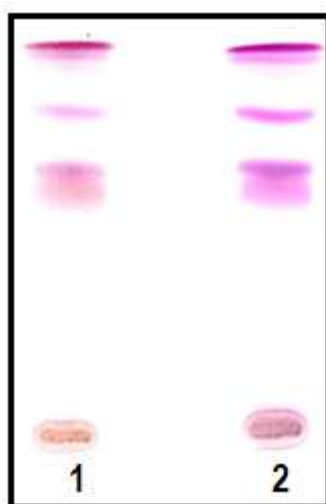
#### *Thin Layer Chromatography (TLC)*

**Preparation:** About 5 g of the powdered root were extracted with ethyl acetate by cold maceration, filtered and the filtrate concentrated to a small volume. A small spot was then applied to the TLC plate for analysis.

**Chromatographic conditions:** Analytical TLC on silica gel G60 F254, 0.25mm layer in hexane/ethyl acetate (7:3) as the mobile phase.

**Detection:** Visualized in daylight after spraying with anisaldehyde-sulphuric acid (**Lane 1**) and vanillin-sulphuric acid reagents (**Lane 2**) (Stahl, 1969) and heating to 110°C for 10 mins

The TLC chromatogram showed three prominent pink spots with R<sub>f</sub>s of 0.82 , 0.67 and 0.62 when sprayed with both anisaldehyde and vanillin



#### *High Performance Liquid Chromatography*

**Sample preparation:** About 10 mg of the hydro-ethanolic extract of *A. hispidum* root were reconstituted in 3 mL acetonitrile in a 10 mL volumetric flask with sonication for 17 minutes. The resulting solution was then diluted to volume with the mobile phase under chromatographic conditions. It was centrifuged to obtain a clear test solution which was subsequently filtered through a 0.45µm filter into an HPLC vial and analyzed.

#### Chromatographic system

##### Optimized chromatographic conditions

**Mode:** LC

**Column:** YMC ODS, 4.6 x 150 mm, 5 µm

**Column temperature:** Ambient – 30°C

**Mobile phase:** Acetonitrile: Methanol: Water (60:20:20 v/v/v)

**Elution mode:** Isocratic

**Injection volume:** 20 µL

**Flow rate:** 0.5 mL/minute

**Detection wavelengths:** 230 nm, 254 nm and 278 nm.

### System Suitability parameters

**Number of peaks:** 230 nm (2), 254 nm (2), 278 nm (1)

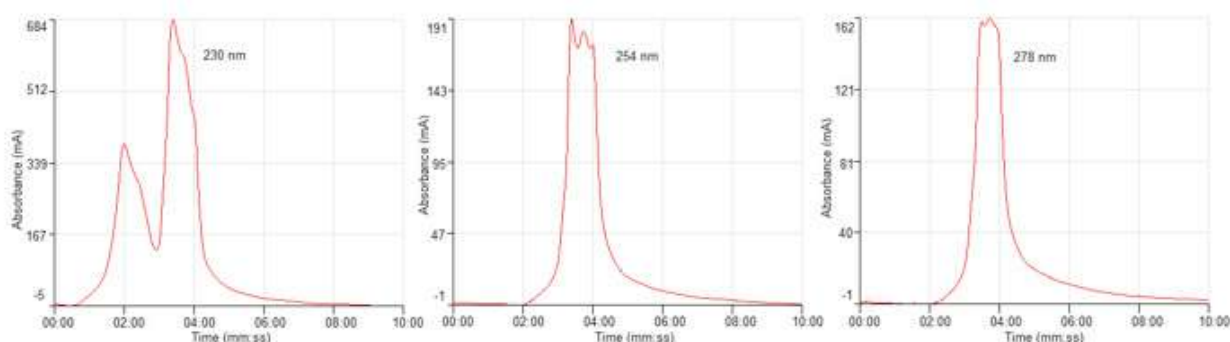
**Retention time (s):** 230 nm (rt1-2.00 min, rt2-3.24 min), 254 nm (rt1-3.23 min, rt2-3.44 min), 278 nm (1)

**Asymmetric factor(s):** 230 nm (af1-1.100, af2-1.733), 254 nm (af1-1.287, af2-1.407), 278 nm (1.153)

**Tailing factor:** NMT 2.0

**Efficiency:** 230 nm (E1-40.05, E2-83.90), 254 nm (E1-2614.88, E2-2351.53), 278 nm (121.76)

**Acceptance criteria:** Sample solution of hydroethanolic crude extract of *Acanthospermum hispidum* DC. (root) conforms to the system suitability parameters.



HPLC chromatogram

### FT-IR

A small amount of the dried hydroethanolic extract (70%) was placed on the sample area of the Perkin Elmer UATR Fourier transform infrared (FT-IR) spectrometer and scanned between 4000-400  $\text{cm}^{-1}$  with a resolving power of 4  $\text{cm}^{-1}$  and a cumulative scanning limitation of 24 times. Principal peaks appeared at wavenumbers 3279.97 (broad), 2923.94, 2853.31 and 1568.38  $\text{cm}^{-1}$

### Macroscopy

Light brown coloured roots tortuous in shape. The fracture is brittle and fibrous; has slightly sweet aroma.

### Microscopy

#### *Transverse section of root*

Consists of a layer of rows of rectangular shaped cork cells, followed by irregular shaped parenchyma. Interspersing the next layer of parenchyma are large schizogenous glands. This is followed by rows of large parenchyma with polygonal shape up to the xylem area. The xylem area is made up of large xylem vessels with xylem parenchyma traversed by medullary rays up to ten cells wide which taper off at the pith.

### Powdered plant material

Powder is light brown in colour. Powder is characterised by bundles of annular and spiral xylem vessels and fibres; fragments of cork and parenchyma cells with calcium oxalate crystals occurring.

### Therapeutic actions

Antipyretic; hypoallergenic; sudorific, depurative; astringent, anthelmintic, antalgic, abortive, antihypertensive, antitussive, diuretic, haemostatic, anti-dysenteric, expectorant.

### Therapeutic indications

Fever, allergic bronchitis, cough, urinary diseases, gonorrhoea, dysmenorrhoea, dysentery.

### Safety data

LD<sub>50</sub> of the aqueous dried root extract by oral route was estimated to be beyond 3000 mg/kg in rats. There were no signs of CNS depression/stimulation or autonomic effects. No significant changes were noted with highly perfused organs such as the liver, kidney, heart, lungs. The relative organ to body ratios of spleen, thymus, and adrenals did not change. Aqueous root extract of *A. hispidum* did not affect RBC indices such as HCT, MCV, MCH, MCHC. It neither stimulated erythropoiesis nor induced anaemia. It however increased white cell count at all tested doses, suggesting that it has immunostimulating activity. Agranulocyte (lymphocytes) proportion increased significantly whereas granulocyte (neutrophils, MID cells) decreased. Thrombocyte counts were relatively higher compared to control. *Acanthospermum* root extract had minimal effect on the enzymes of naïve rats. Doses below 300 mg/kg appeared to suppress AST and ALT, suggesting a possible hepatoprotective activity. However, this effect was not apparent at the high dose of 1000 mg/kg. Slight elevations were also noted with ALP and GGT.

Total protein levels did not change, neither urea nor creatinine, but mild renal necrosis were noticed around the glomerulus. Pentobarbitone induced sleeping time was prolonged at doses above 1000 mg/kg. Mild fibrosis in the liver with sparse inflammation and renal necrosis were noticed.

### Precautions for use

Use samples without seeds. Do not exceed the recommended dose. Driving is not recommended for patients during treatment.

### Adverse effects

Possible depression of the central nervous system.

### Contraindications

Pregnant women at any stage of pregnancy, children under two years of age, people with diabetes, liver dysfunction (cirrhosis, hepatitis, alcoholism) or kidney problems (Araújo, *et al.*, 2008).

### Dosage form

Decoction, infusion, tincture

## Dosage

Decoction: 30 g of dried plant material in 900 mL water; simmer until reduced to 600 mL; 1-3 tablespoonfuls daily

Infusion: 30 g of dried aerial part in 600 mL of water; 3-4 teacups daily

Tincture: 1:5 30% fresh alcohol, take 2-5 ml twice daily

## Storage

The product should be stored in a cool place, protected from light, in its original packaging. Keep out of reach of children.

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**Botanical name***Anacardium occidentale* Linn**Family**

Anacardiaceae

**Synonyms***Anacardium curatellifolium* A. St.Hil.**Common names**

Cashew (English), Anacardier, acajou, pommier cajou, pommed'cajou, noix d'cajou, anacardes pomme acajou (French), Caju, Cajueiro (Portugese)

**Common Local names****Benin:** Dendi-Yuburu Somba; Yiko; Fon- Akaju, Lakaju, Gbosama; Yoruba - Ekaju Kanju**Burkina Faso:** Malinké/Dioula –Balambala; Dioula – Acajou; Fulfuldé –Acajou**Côte d'Ivoire:** Bété - Baisou**Gambia:** Madinka-Kasuwo; Wolof- Darkassau; Fulah- Ndarkassu**Ghana:** Twi- Atea; Ga- Atea; Ewe-Yevutsia, Atsia**Guinea :** Pular -yalagè porto; Maninka –sömö, Sossoe – koussou;yagalé**Guinea Bissau:** Créole-caju, Pulaar- lalaguei; Mandiak-Caju**Mali:** Bambara- Somon Jibarani; Malinké/Dioula- Jibarani; Senoufo- Komigason**Niger :** Djerma-Sayintourizé**Nigeria:** Hausa- Kaju, Kanju; Igbo- Okpokpo; Yoruba - Kaju, Kasu**Senegal:** Diola– Bukaaju; Socé – Finzâ ; Wolof-Darkassou**Sierra Leone:** Krio – kushu; Mende – kundi ; Temne - an lil a potho**Togo:** Ewé– Kadzu; Mina -Yovotchan**Description of the plant**

*Anacardium occidentale* Linn is a perennial, well branched tree or shorted tortuous trunk, with height varying from 8 to 15 m, a large hemispherical regular crown span that can reach 20 m, sometimes reaching down to the ground. Short and tortuous trunk; bark, rough, gray, with a pinkish slice; branch more or less pubescent, grey to brown. The mature leaves are evergreen, mainly thick and oval or elongated, pointed or round tipped, cunate at the base, compound or simple, alternate or rarely oppositely arranged; some have terminal leaflets. The leaf has pinnate venation with prominent secondary veins. Leaf has the smell of turpentine when crumpled. The red-greenish flowers have bracts, occur at the end of a branch or stem or at the angle from where the leaves join the stem. They often have bisexual and male flowers on same plant and bisexual and female flowers on others or flowers having both stamens perfect pistils. The calyx has 3 to 7 sepals and same number of petals and occasionally no petals. Mature fruits are mainly oval, yellowish, reddish or dark-red when ripe, fleshy, pear shaped, mostly drupe and rarely opening. The seed is kidney shaped and located on the edge of the fruit with hard or leathery covering (Marlos *et al.*, 2007; Wagner, 2007; Mshana *et al.*, 2000).





A – *Anacardium occidentale* L, B D - unripe fruit, C – flowers, E – ripe fruit

**Herbarium specimen number:**

Bénin : 2461(AP)  
 Burkina Faso : 3249 (OAU)  
 Côte d'Ivoire : 8548 (CNF)  
 Ghana : GH051/KNUST  
 Mali : 1341/DMT  
 Nigeria: UPFH 110  
 Senegal : IFAN 464  
 Togo : TG 15178

**Habitat and geographical distribution**

Native to Brazil, Mexico and United States of America. Exotic to Cambodia, Gambia, India, Indonesia, Kenya, Malaysia, Mozambique, Myanmar, Phillipines, Sri Lanka, Sudan, Tanzania, Thailand, Uganda and Vietnam. Major production is from Vietnam, Nigeria, India and Cote d'Ivoire (Orwa *et al.*, 2009). The tree is now cultivated in all tropical countries where it has been dispersed and also used in reforestation (Mshana *et al.*, 2000).

## Plant material of interest

Leaf and Stem bark

## Other parts used

Seeds, fruit

## Definition of plant material of interest

*Anacardium occidentale* Linn is the dried or fresh leaves or stem bark of *Anacardium occidentale* Linn (Anacardiaceae).

## Ethnomedical uses

The stem bark is used to stop tooth bleeding (Arokoyo *et al.*, 2015), and as antidiabetic, antiinflammatory (Tedong *et al.*, 2007) and antihypertensive (Tchikaya *et al.*, 2011). A decoction of the cashew nut shell is used to treat leprosy, elephantiasis, psoriasis, ringworm, diabetes, warts and corns (Mbatchou and Kosoono, 2012). Leaves and bark are used as a decoction for diarrhoea, swelling and mouth ulcers. Roots are used to treat yaws (Mshana *et al.*, 2000). Infusion of the leaves and the bark relieves toothache, sore gums and dysentery (Godghate *et al.*, 2013).

## Biological and pharmacological activities

In an *in vitro* antihypertensive test, ethanolic extracts of *A. occidentale* leaves inhibited the contraction (induced by cumulative addition of phenylephrine) of isolated rat aorta (Agung *et al.*, 2013). The ethanolic extract of *A. occidentale* exhibited antimicrobial activity, which was attributed to the 2-hydroxy-6-pentadecylbenzoic acid constituent of the plant (Agedah *et al.*, 2010). Anacardic acid and cardanol tested on HeLa cancer cell lines using (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), showed some anticancer activity (Ola *et al.*, 2008). *A. occidentale* aqueous leaf extract was found to be nontoxic at concentrations up to 20 mg/mL in hepatoma cells (Hep G2). The leaf extract was tested for its ability to reduce cholesterol in cell culture at 2.5, 10.5 and 20mg/mL. The extract did not increase the concentration of low density lipoprotein receptor, but rather significantly increased the concentration of farnesyl-diphosphate, farnesyltransferase, apolipoprotein A1, lecithin – cholesterol acyltransferase, scavenger receptor B1, ATP binding cassette transporter and hepatic lipase; showing that the aqueous extract may be involved in reversing cholesterol transport processes to reduce metabolism of cholesterol in HepG2 cells (Hasan *et al.*, 2015). Anti-obesity effect of cashew nuts was evaluated in male swiss albino rats using Ayurslim (a herbal formulation comprising of *Garcinia cambogia*, *Commiphora wightii*, *Gymnema sylvestre*, *Terminalis chebula* and *Trigonella foenum-graecum* manufactured by Himmalaya Drug company, Bangalore) at 3mg/kg p.o. twice daily, as standard drug. Rats treated at 100,150 and 200 mg/kg twice daily for 40 days showed significant reduction in body weight, locomotor activities, fat pad weights (kidney fat, mesenteric fat and uterine fat), cholesterol, triglycerides, LDL, VLDL level and increased HDL level. The results showed that cashew nut had potent anti-obesity activity (Asdaq and Malsawmtluangi, 2015). The methanolic leaf extract of *A. occidentalis* was investigated for its effects on activities of glucose-6-phosphate dehydrogenase (G-6-PDH), thiobarbituric acid reactive substances (TBARS) and anti-oxidant enzymes (glutathione peroxidase, GPx and superoxide dismutase (SOD) in the testicular homogenate of streptozocin induced diabetic rats. Wistar rats were treated with 300 mg/kg of body weight of extract. The study showed that the extract improved the level of TBARS and activities of G-6-PDH, SOD and GPx in the testis of treated rats (Ukwenya *et al.*, 2012). Methanolic extract of the stem bark of *A. occidentalis* was investigated for its homeostatic properties. Albino rats were given 100, 200, and 400 mg/kg of the methanolic

extract of the stem bark intraperitoneally once a day for two weeks. Blood samples were examined and showed the extract significantly decreased ( $p > 0.05$ ) the bleeding time, prothrombin time, clotting time and activated partial thromboplastin time respectively in a dose dependent manner. Platelet count significantly increased ( $p > 0.05$ ) (Arokoyo *et al.*, 2015). Aqueous extract of the defatted stem bark of *A. occidentale* was investigated for its hypotensive activity. The extract applied intravenously in doses of 12, 40, 90, and 167 mg/kg body weight to rabbits, produced a significant dose-dependent decrease in blood pressure of previously normotensive rabbits up to 89% vs control. Atropine (1 mg/ml) pretreatment did not reverse the hypotensive effects elicited by the extract. The extract applied to isolated rat heart preparations in concentrations of 0.01, 0.1, 1.0 and 10  $\mu\text{g/ml}$  caused negative inotropic and chronotropic effects. Atropine pretreatment of heart preparations at (0.1  $\mu\text{g/ml}$ ) did not reverse the negative effects. The extract induced strong hypotensive and cardio-inhibitory effects in animal models (Tchkaya *et al.*, 2011). Ethanol extracts of the leaves, stem bark and flowers were evaluated for antimicrobial activity against *Streptococcus mutans*, *Lactobacillus acidophilus*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Helicobacter pylori*, *Salmonella choleraesuis* *Candida albicans* and *Candida tropicalis*. The flower extract was the most effective against all the fourteen organisms (Amaral da Silva, *et al.*, 2016). In a test for anticonvulsant activity, the methanolic and aqueous leaf extracts of *A. occidentale* at 250 and 500mg/kg body weight (p.o), protected the animals from pentylenetetrazole –induced tonic seizures and significantly delayed onset of tonic seizures (Ghori *et al.*, 2011).

Several studies have confirmed the antidiabetic activity of the leaves (Sokeng *et al.*, 2001). Decoction of fresh leaves given to rats at 4.5 ml/kg body weight lowered blood glucose level and showed a significant decrease in triglyceride levels (Brijesh and Kamath, 2016). Aqueous and methanolic extracts reduced blood glucose, total cholesterol, triglycerides, total protein, activity of alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase in alloxan – diabetic rats, increasing activity of Superoxide dismutase catalase and malonyldialdehyde level (Elekofehinti *et al.*, 2016). Ethanolic extract of the leaves administered at 100 mg/kg to rats showed 8.01% and 19.25% decrease in fasting blood glucose levels on day 15 and 30, respectively. The effect of the treatments was comparable to pioglitazone (Jaiswal *et al.*, 2016). The active antidiabetic principle in cashew seeds, leaf and stem bark, anacardic acid was administered to rodents at 50  $\mu\text{g/ml}$ , incubated for 18 hours and glucose uptake was measured. Seed extracts had significant effect only at 100  $\mu\text{g/ml}$ . Glucose uptake was significantly elevated in cells incubated with high doses of the extracts and insulin, compared to either the insulin or the extracts alone (Godstime *et al.*, 2014). In a study of the effects of three plant extracts against the anthracnose disease of cowpeas, plots sprayed with leaf extract of *A. occidentale* gave the lowest yield loss (31.83%), indicating that it can be used to spray cowpea farms to reduce losses (Eno *et al.*, 2016). Methanolic leaf extracts showed utmost DPPH radical scavenging activity (83.36%) inhibition at 1000  $\mu\text{g/mL}$ . It also exhibited, inhibition of lipid peroxidation induced by  $\text{FeSO}_4$  in sheep liver homogenate in a concentration dependent manner, and prohibited the free radical mediated DNA damage in various concentrations, with the highest being 1000  $\mu\text{g/mL}$  with 58.26% inhibition (Udand Rao *et al.*, 2016). Ethanolic and aqueous extracts of the leaves and bark were examined for antimicrobial and anti-inflammatory activity. The results showed the ethanolic extracts were more efficacious than the aqueous extracts in inhibiting the carrageenan induced paw oedema in rats in a non-dose dependent manner. No significant difference was found between the ethanolic extract of the leaves and bark. Antibacterial activity was higher in the ethanolic extract than in the aqueous extract for both leaves and bark, with the bark extract displaying a significantly ( $P < 0.05$ ) higher activity compared to the leaves. The cytotoxic effects of leaf extract were determined by microculture tetrazolium assay on human gingival fibroblast and Chinese hamster lung fibroblast (V79) cell lines. Cashew leaf extract significantly produced a larger zone of inhibition against test pathogens when compared to povidone-iodine–based mouth rinses. Although the minimum inhibitory concentration and minimum bacterial/fungicidal concentration values of mouth rinses were effective in lower concentrations, plant extracts significantly suppressed the biofilms of oral pathogens. The leaf extract was less cytotoxic compared to the mouth rinses (Anand *et al.*, 2015). Methanolic leaf and bark

extracts were tested against drug resistant clinical isolates of urinary tract infection, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Staphylococcus aureus* and *Enterococcus faecalis*. The leaf extract showed high inhibitory activity. Oil from the seeds of *A. occidentale* was tested on male albino rats for sexual behaviour at 0.5, 1.0 and 1.5 mls. Sildenafil citrate, 0.5 mg/ml, was the positive control. The results showed significant increase in mount (21.1, 22.0 and 24.28%) and intromission frequencies (18.79, 22.23 and 26.49%) and decrease in mount latency (36.38, 29.56 and 22.75%) in a dose-dependent manner (Mbatchou and Kosoono, 2012). Evaluation of the chloroform-ethanol extracts of cashew kernel at a dose of 21 mg/kg and 84 mg/kg on electrolyte imbalance in castor oil-induced diarrhoeal rats showed the extracts significantly reduced the concentration of sodium and potassium ions in the intestinal solution compared to control animals induced with castor oil only (Omoboyowa *et al.*, 2015). Vanderlinde *et al.*, in 2009, confirmed the antiinflammatory and antinociceptive activity of the acetone extract. Ethyl acetate fraction of water/acetone stem bark extract assessed for antiinflammatory activity in mice at 12.5; 25; 50 and 100 mg/kg /weight p.o.), showed a reduction in oedema, and at 50 and 100 mg/kg, antiinflammatory response was observed (Vanderlinde *et al.*, 2009; Araujo Vilar *et al.*, 2016). Effect of aqueous extract of stem bark at 20,40, 80 mg/kg administered intraperitoneally to rabbit colon, cannulated and perfused *in situ* with iso-osmotic medium at 0.3 ml/min, showed a dose-dependent stimulation of sodium absorption and no effect on chloride transport. This confirmed the extract's activity in stimulating sodium and water absorption, and its effective use in the treatment of diarrhoea. Stem bark methanolic extract at a dose of 200.0 mg/kg body weight was administered continuously to fructose-induced diabetic rats. Results showed that the extract may be a safe, alternative antihyperglycaemic agent that has beneficial effect by improving plasma glucose and lipids (Olatunji *et al.*, 2005). *In vivo* assessment of the antigenotoxicity and anticlastogenicity of cashew apple juice against cyclophosphamide-induced genotoxicity and mutagenicity in mice showed that both the cashew juice and the processed juice decreased the average number of cells with chromosome aberrations in bone marrow by 53 and 65% respectively (De Carvalho *et al.*, 2011). Gum from the trunk was found to protect against gastrointestinal damage via mechanisms that involve the inhibition of inflammation and increase in the amount of adherent mucus in the mucosa (Carvalho *et al.*, 2016). Cashew gum (30, 60, 90 mg/kg, p.o.) showed a significant antidiarrhoeal effect in rats with castor oil-induced diarrhoea. A dose of 60 mg/kg of cashew gum exhibited significant antidiarrhoeal activity in rats. Also treatment with the gum, at a dose similar to loperamide (5 mg/kg, p.o.), reduced the distance travelled by a charcoal meal in the 30 min gastrointestinal transit model by interacting with opioid receptors, in cholera toxin-induced secretory diarrhoea. It significantly inhibited the intestinal fluid secretion and decreased Cl<sup>-</sup> ion loss in the cholera toxin treated isolated loops model of live mice by competitively binding to cholera –GM1 receptors. These results indicate that the gum has antidiarrhoeal activity in acute inflammatory, and secretory diarrhoea models (Araujo, *et al.*, 2015). Hydroalcoholic extract of the fruit was studied for its neuroprotective properties against behavioural and biochemical parameters induced by subcutaneous injection of rotenone in rats. Doses of 150 mg and 600 mg/kg p.o., improved rotenone-induced dysfunctional behaviour (locomotor, musculature coordination and memory retention). The extracts also attenuated the increase in lipid peroxidation by the systemic administration of rotenone (Linard-Medeiros, *et al.*, 2015). A functional beverage prepared by mixing 50% cashew apple pulp, 50% yacon extract together with 0.06% of stevioside, administered to alloxan-induced diabetic male wistar rats, showed a decrease in glucose levels, promoted growth of lactobacilli in faecal material and increased catalase activity in the liver, indicating that yacon and cashew apple have important hypoglycaemic activity. Juice from ripe cashew apple concentrated to 1/10<sup>th</sup> of original volume was tested against five urinary tract pathogens. Activity was highest against *Pseudomonas aeruginosa* and *Enterococcus faecalis* and lowest against *Escherichia coli* (Vivek *et al.*, 2013).

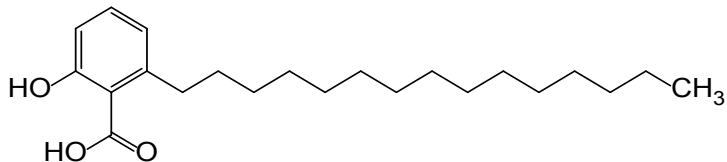
### Clinical data

Effect of consumption of cashew drink on postprandial glucose-insulin response in type 2 diabetics was investigated. A drink was developed with cashew pseudo fruit 60% (v/v), sucralose, annatto, citric acid and sodium benzoate. Consumption of cashew drink improved glucose-insulin response of patients in the study

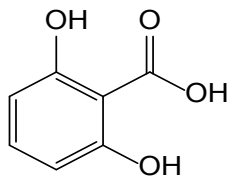
(Dominguez *et al.*, 2012).

### Chemical constituents

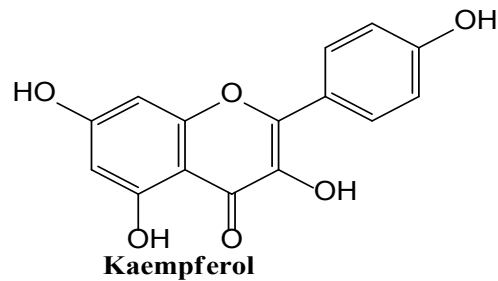
Phenolic compounds (myricetin, quercetin, kaempferol, rhamnetin, cyanidin and delphinidin) (Paramashivappa *et al.*, 2001; Assuncao *et al.*, 2003); 2-hydroxy-6-pentadecylbenzoic acid, cardanol, salicylic acid (Agedah *et al.* 2010; Terdong *et al.*, 2010); ethyl gallate and hyperoside (Subramanian *et al.*, 1969).



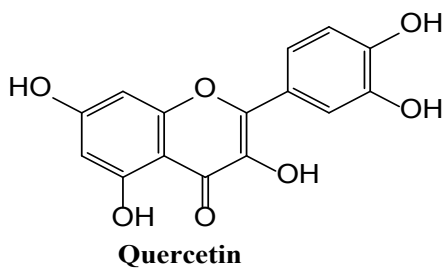
**2-hydroxy-6-pentadecylbenzoic acid**



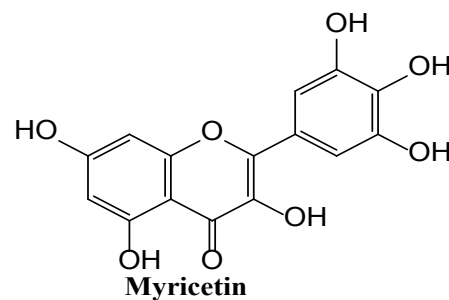
**2,6-dihydroxybenzoic acid**



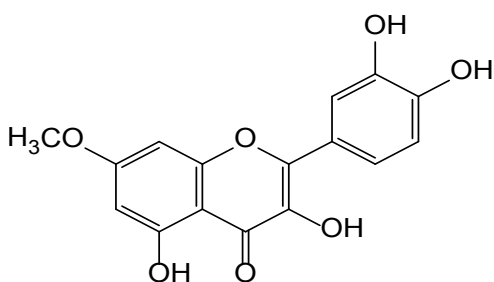
**Kaempferol**



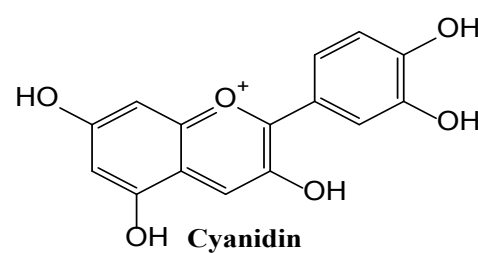
**Quercetin**



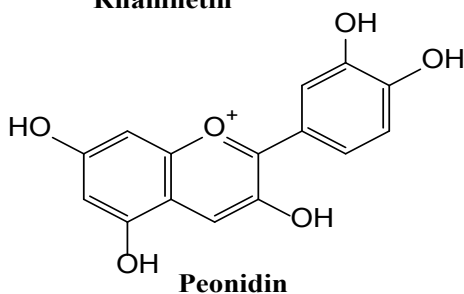
**Myricetin**



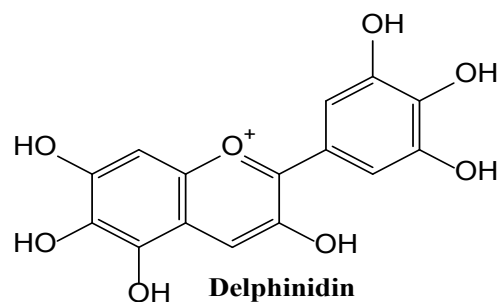
**Rhamnetin**



**Cyanidin**



**Peonidin**



**Delphinidin**

### Test for identity and purity

Moisture content: Air dried coarse powder does not lose more than 5.3% (leaves), 6.08 % (stem bark) at 105°C.

Total ash: not more than 10.3% (leaves), 2.6%

Ash insoluble in acid: not more than 0.5% (leaves) 0.70% (stem bark)

Water soluble extractive: not less than 7.0% (leaves), 11.34% (stem bark)

Ethanol soluble extractive (70%): not less than 01.0% (leaves), 14.75% (stem bark)

### Chromatographic fingerprint

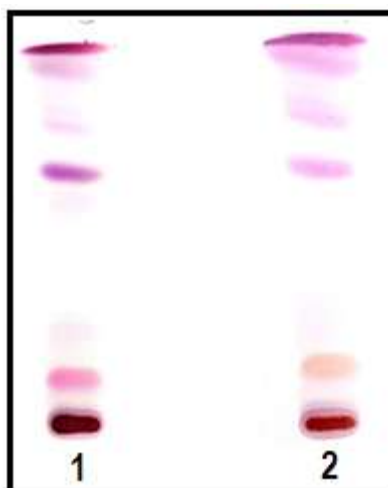
#### Thin Layer Chromatography

**Preparation:** About 5 g of the powdered stem bark were extracted with ethyl acetate by cold maceration, filtered and the filtrate concentrated to a small volume. A small spot was then applied to the TLC plate for analysis.

**Chromatographic conditions:** Analytical TLC on silica gel G60 F254, 0.25 mm layer in hexane/ethyl acetate (7:3) as the mobile phase.

**Detection:** Visualized in daylight after spraying with anisaldehyde-sulphuric acid (Lane 1) and vanillin-sulphuric acid reagents (Lane 2) (Stahl, 1969), heating to 110°C for 10 mins.

The TLC chromatogram showed a prominent spot with R<sub>f</sub> of 0.68 (purple) when sprayed with both anisaldehyde and vanillin reagents. An additional spot each, appeared with R<sub>f</sub> of 0.12 with colours pink and peach when sprayed with anisaldehyde and vanillin respectively.



#### High Performance Liquid Chromatography

**Sample preparation:** About 10 mg of the hydro-ethanolic extract of *Anacardium occidentale* stem bark were reconstituted in 3 mL acetonitrile in a 10 mL volumetric flask with sonication for 17 minutes. The solution was then diluted to volume with the mobile phase under chromatographic conditions. It was centrifuged to obtain a clear test solution which was subsequently filtered through a 0.45 µm filter into an HPLC vial and analyzed.

## Chromatographic system

### Optimized chromatographic conditions

**Mode:** LC

**Column:** YMC ODS, 4.6 x 150mm, 5 $\mu$ m

**Column temperature:** Ambient – 30oC

**Mobile phase:** Acetonitrile: water (60:40 v/v)

**Elution mode :** Isocratic

**Injection volume :** 20  $\mu$ L

**Flow rate:** 0.5mL/minute

**Detection wavelengths:** 230 nm, 254 nm and 278 nm.

### System Suitability parameters

**Number of peaks:** 230 nm (1), 254 nm (1), 278 nm (1)

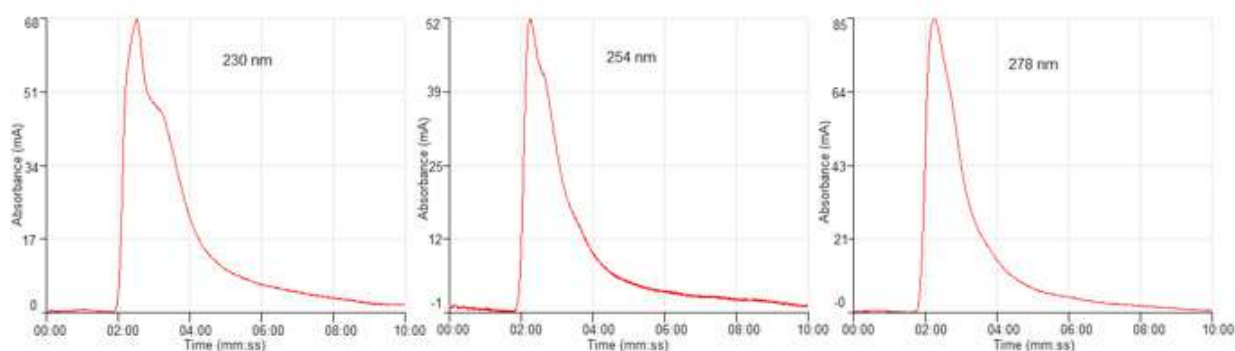
**Retention time (s):** 230 nm (2.31 min), 254 nm (2.14 min), 278 nm (2.14 min)

**Asymmetric factor(s):** 230 nm (1.011), 254 nm (0.665), 278 nm (0.629)

**Tailing factor:** NMT 2.0

**Efficiency:** 230 nm (18.11), 254 nm (31.69), 278 nm (28.93)

**Acceptance criteria:** Sample solution of hydroethanolic crude extract of *Anacardium occidentale* L. (stem bark) conforms to the system suitability parameters



HPLC chromatogram of *Anacardium occidentale*

## FT-IR

A small amount of the dried hydro-ethanolic extract (70%) was placed on the sample area of the Perkin Elmer UATR Fourier transform infrared (FT-IR) spectrometer and scanned between 4000-400  $\text{cm}^{-1}$  with a resolving power of 4  $\text{cm}^{-1}$  and a cumulative scanning limitation of 24 times. Principal peaks appeared at wavenumbers 3252.05 (broad), 1687.87 and 1606.93  $\text{cm}^{-1}$

## Macroscopy

The mature leaves are evergreen, mainly thick and oval or elongated in shape, pointed or round tipped and cuneate at the base. The margin is smooth and entire and venation is pinnate.

Stem bark: the outer surface of the bark is dark brown and darker than the inner surface. There are striations in the inner surface and the fracture is short in the outer part and fibrous in the inner part.

## Microscopy

### *Leaf*

Lower surface of the leaf has numerous paracytic stomata elevated above the surrounding epidermal cells. The surrounding subsidiary cells are wing-shaped and have striations which are perpendicular to the axis of the stomata. The normal epidermal cells are extremely wavy without out any definite form to the cells. Epidermal walls are bead-like in formation. Occasional glandular four celled, trichomes occur. Numerous calcium oxalate cluster crystals and prism are visible beneath the epidermal cells. Upper surface has thick walled polygonal cells, paracytic stomata and trichomes are absent.

Upper epidermis of the transverse section through the midrib is followed by a layer of collenchyma cells with calcium oxalate cluster crystals. Next is a layer of collenchyma of several rows of cells followed by a row of parenchyma with calcium oxalate crystals scattered all along the row, which occurs only at the upper section of the transverse section. This is followed by the vascular system which consists of a layer of phloem fibres and phloem surrounding the xylem and shaped like a womb. The xylem vessels are large and form a very thick layer at the centre of which are large parenchyma cells with thick walls. The phloem layer is interspersed with oval shaped vacuoles. The circle of phloem is bound by collenchyma all round. The laminar shows one layer of palisade with rectangular cells and spongy tightly packed mesophyll interspersed with vascular bundles.

### *Stem bark*

Cork layer with cork cells of several layers followed by layer of parenchyma with groups of sclereids. This is followed by an almost continuous layer of sclereids with yellowish colour. There are large schizolysegenous glands in an almost continuous row; others are scattered in the ground tissue. The medullary rays start from after the layer with sclereids to the cambium. Medullary ray consists of rows of two to several cells. The phelloderm consists of alternate bands of fibres and sieve elements. There are calcium oxalate crystals enclosed in parenchyma cells.

### *Powdered plant material*

Leaf powder is a dark green colour; fragments of leaf showing paracytic stomata and cork cells, unicellular fibres with acute apex, upper surface with striated polygonal cells, fragments of spongy mesophyll and palisade cells.

Stem bark powder is dark brown in colour with characteristic odour. There are fragments and bundles of fibre; fragments of parenchyma cells; numerous groups of sclereids and calcium oxalate cluster crystals.

## Therapeutic actions

Antihyperglycaemic, antibacterial, antidiarrhoea

## Therapeutic indications

Diabetes, infections, diarrhoea

## Safety data

LD<sub>50</sub> by oral route was estimated to be beyond 3000 mg/kg in rats. Treatment in rats 0-1000 mg/kg, did not



affect the central nervous system (CNS) and the autonomic nervous system (ANS). No significant changes were noted with liver, kidneys and the spleen. Anacardium did not significantly affect RBC and WBC indices but marginally decreased platelet count in treated animals particularly at high doses. Clotting time increased significantly following treatment beyond 300 mg/kg for 10 days. *A. occidentale* extract did not affect liver enzymes, total serum proteins or bilirubin. Conjugated bilirubin decreased leading to a decline in total bilirubin. There was no significant effect on pentobarbitone sleeping time in rabbits. The reduced platelet count and increased bleeding time seen in this study is consistent with an earlier report by Olajide *et al.* (2013), which showed that Anacardium stem bark inhibits many mediators of inflammation in the arachidonic acid metabolic pathway such as NF- $\kappa$ B, COX-2, iNOS. Because of its significant antiinflammatory effect through COX-2 and significant antiplatelet activity, Anacardium may exhibit ulcerogenic properties if overused. Other members of the same family such as *Semecarpus anacardium*, have also been shown to be ulcerogenic.

### Precautions for use

Use with caution in patients with coagulation and haemorrhagic disorders. Avoid use in ulcer patients.

### Adverse effects

May cause decrease in platelet count and increase in bleeding time. It may also be ulcerogenic.

### Contraindications

Pregnant women, lactation and children under six years of age. Ulcer patients

### Dosage forms

Powder and decoction, infusion, tincture

### Dosage

Decoction: 30 g of dried plant material in 900 mL water; simmer until reduced to 600 mL; 1-3 tablespoonfuls daily

Infusion: 30 g of dried aerial part in 600 mL of water; 3-4 teacups daily

Tincture: 1:5, 45% ethanol; 5 ml three times daily

### Storage

Store in cool, dry place away from light.

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**Botanical name***Annona senegalensis* Pers.**Family**

Annonaceae

**Synonyms***Annona senegalensis* Pers. var. *senegalensis***Common Names**

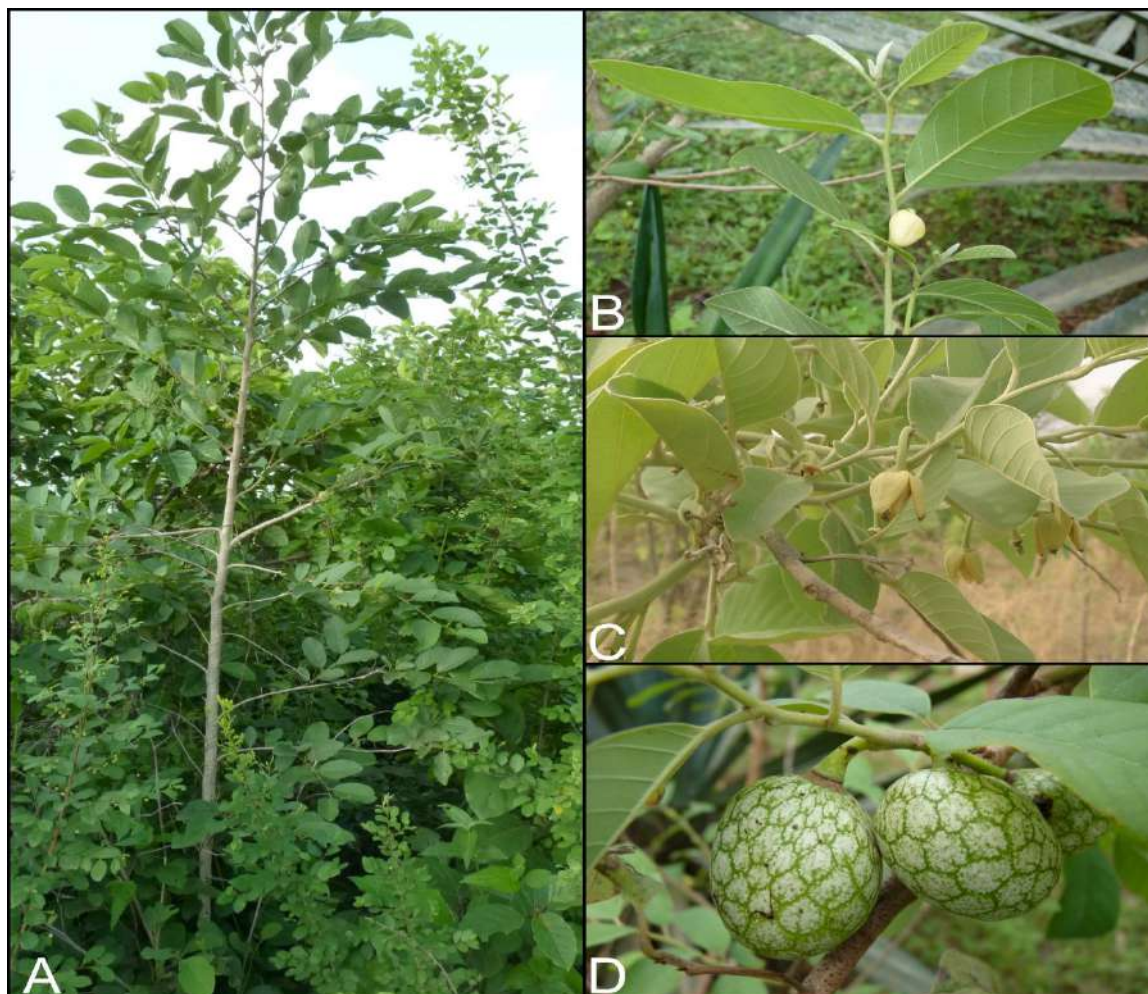
Wild custard apple, African custard apple (English), wild corossolier, pomme cannelle du Sénégal (French).

**Common Local names**

**Benin:** Fon – Wenglema; Yoruba- Arere; Dendi- Batako  
**Burkina Faso:** Dioula-Manden sunsun; Haoussa – Gwadda; Moré-Baataama  
**Côte d'Ivoire:** Baoulé – Amlon; Dioula – Sunkungo ; Sénoufo- Damourana  
**Gambia:** Madinka – Sinkungo; Wolof – digirt, dugor; Fulah – Dokumi, Dukmi  
**Ghana:** Dagaare – Batanga; Ewe – Anyikle; Twi– Abodoma  
**Guinea:** Malinké- Sunsuningbé ; Soussou – Sundyi ;Peuhl-Dukummé.  
**Guinea Bissau:** Balanta- Bore ; Pulaar- Ducume ; Mandinka – Sucum  
**Mali:** Bambara- Maden sunsun; Peuhl – Dakumi; Senoufo- Namurungo, Namklgho.  
**Niger:** Djerma – Mufa; Gwandara –Gwandardaji; Haoussa - Gwanda.  
**Nigeria:** Hausa - Gwandar daajii; Igbo - Uburu ocha; Yoruba – Abo, ibobo  
**Senegal:** Diola-Bore; Mandingue - Sunk, Wolof-Dugar  
**Sierra Leone:** Mandinka – walisa; Temne – Amomina; Susu- Dalonke Korɛɛ-na  
**Togo:** Ewe-Zogbenyiglin; Akasselem-Dipussa; Moba-Baglanwoalouk

**Description of the plant**

It is a bushy shrub of variable size depending on the type of biotope where it is located, ranging from 1 to 2 m high or more. Its summit is irregular; the bark is grey in colour and smooth with pink slice, more or less pubescent. The leaves are whole and arranged alternately, coriaceous, pubescent to glabrescent; the blade has an oval shape, 7-12 cm long and 6-8 cm wide. The base is rounded or slightly re-entrant with an obtuse corner. The leaves have 6 to 8 regular lateral veins parallel and protruding below. The petiole of each leaf is 10 to 12 mm long. The flowers are solitary or arranged in groups of two or three in the axil of a leaf, suspended under the branches by a pedicel about 2 cm long, greenish to yellowish, waxy, bell-shaped and up to 2 cm long. The fruit is a globose or ovoid and fleshy berry; dark orange at maturity, with many smooth protuberances and pineapple odour: sweet, edible, in which the seeds are drowned. *A. senegalensis* flowers from February to May, while the fruit matures in late May to June.



A – *Annona senegalensis* Pers, B –leaves, C- flowering stage and D – matured fruits

#### Herbarium specimen number:

Benin: 2338 (AP)

Burkina Faso: BUR-326 (CNSF), 5099 (OAU)

Côte d'Ivoire : CNF14105

Ghana: GH 077/KNUST

Mali : 0012/DMT

Nigeria: FHI111916

Senegal : UCAD 64, IFAN 122

Togo : TG 01885

#### Habitat and geographical distribution

Shrub of Sudanian savannahs, on stony soils, on banks of gravel on the banks and on fallow and fallow land (Arbonnier, 2002). The species is found throughout West Africa from Senegal to East Africa and extending to Madagascar. *A. senegalensis* is cultivated in northern Nigeria (Alqasim, 2013). *A. senegalensis* is spread by seeds. Seed scarification improves germination rates of nursery plants. Natural regeneration by seed is usually good, especially on recently cultivated or burned areas. There is also a vegetative natural regeneration in the species through suckering which is a stimulation of the root following an injury. The seeds of *A. senegalensis* seem orthodox. However, they are susceptible to insect attack and lose viability within 6 months after storage.

### Plant material of interest

Leaves and fruit

### Other part used

Roots, stem and leafy stems

### Definition of plant material of interest

*A. senegalensis* consists of the fresh and dried leaves or fruits of *Annona senegalensis* Pers (Annonaceae)

### Ethnomedical uses

*A. senegalensis* is a multipurpose plant with a strong traditional use for the maintenance of health of African populations. Traditionally, the plant is used as a stimulant, analgesic, and for the treatment of dysentery. The plant also has antioxidant, antimicrobial, antidiarrhoeal, antiinflammatory, antiparasitic, anticonvulsant, antimalarial, antitripsmatic, antisnake venom and antinociceptive effects (Alqasim, 2013). In several African countries, the bark of this plant is used to treat worm infestation, diarrhoea, dysentery, gastroenteritis, snake bite, toothache and respiratory infections. The bark of the roots mixed with garlic and placed in the house, is an effective snake repellent. The gum of the bark is used to seal cuts and wounds. The leaves are used to treat pneumonia and as a tonic to promote general wellbeing. The roots are used for upset stomachs, venereal diseases, colds and dizziness (Orwa *et al.*, 2009).

The fruit obtained from the plant is widely used locally in the treatment of two common energy deficiency syndromes, kwashiorkor and marasmus. Dalziel (1937) reported the plant to be of great medicinal value and used in indigenous medicine for headaches and body aches (Arnold and Gulumian, 1984; Chhabra 1987), and eyelid swelling (Klaus and Adala, 1994).

### Biological and pharmacological activities

The methanolic stem bark extract of *A. senegalensis* was studied for its effect on intestinal transit time, using *in vivo* and *in vitro* models. Oral administration of 5000 mg/kg of the extract to mice fed with char meal, decreased intestinal transit time by reducing spontaneous contractions of the intestine. The results provide a scientific basis for the use of *A. senegalensis* stem bark extract in the treatment of diarrhoea (Suleiman *et al.*, 2008). *A. senegalensis* showed antimicrobial activity against the pathogenic bacterial strains *Staphylococcus aureus*, *Shigella flexneri*, *Salmonella paratyphi* and *Pseudomonas aeruginosa*. The result showed that the ethanolic and aqueous leaf extracts had significant zone inhibition (Johnson and Olatoye 2002), while the methanolic extract showed significant antimicrobial activity against clinical isolates of *S. enteritidis*, *S. dysenteriae* and *E. coli* (Awa, *et al.*, 2012). Using the Agar-well diffusion method, the various solvent extracts of *A. senegalensis* leaf showed antimicrobial activity against pathogenic microorganisms such as *Staphylococcus aureus*, *Shigella flexneri*, *Salmonella paratyphi* and *Pseudomonas aeruginosa* (Johnson and Olatoye, 2002). *A. senegalensis* aqueous leaf extract showed significant antioxidant and drug detoxification activity, when evaluated by carbon tetrachloride-induced hepatocellular lesions in rats using DPPH, superoxide, hydrogen peroxide, 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonate), (ABTS) and ferric ion models (Ajboye *et al.*, 2010). Sahpaz *et al.*, (1994) reported the cytotoxic activity of *A. senegalensis* against normal cell lines (VERO) and human epidermoid carcinoma (KB). It also showed antiparasitic activities against *Trypanosoma brucei brucei*, *Leishmania donovani* and *Leishmania major*. The antiinflammatory activities of the leaf extract was also evaluated in rodent models of inflammation. The extract induced a significant decrease in the number of inflammatory cells. This effect was attributed to higher concentrations of tannins and phenolic compounds in the plant extract (Yeo, *et al.*, 2011). Yeo *et al.* (2011)

also reported the anti-convulsive activities of the root bark extract on pilocarpine-induced convulsions in animals. The results gave credence to the use of *A. senegalensis* in the treatment of epilepsy and seizures.

In other studies, the methanol extract of *A. senegalensis* was shown to have a higher antimalarial activity against *Plasmodium berghei*, than the standard reference drug chloroquine disphosphate (Ajaiyeoba, *et al.*, 2006). Aqueous extract of *A. senegalensis* demonstrated trypanocidal activity against *Trypanosoma brucei* in infected mice (Ogbadoyi, *et al.*, 2007). Adzu *et al.*, (2005) tested the potency of the methanol extract of the root bark of *A. senegalensis* on brine shrimp (*Artemia saline* Leach) and against cobra venom (*Naja nigricottlis nigricottlis* Wetch) using rodents. Reduction in induced hyperthermia, directly detoxifying the snake venom from 16 to 33% demonstrated the plant's potential in the management of snakebites. However, it did not restore the biochemical functions of the liver. The methanolic extract of *A. senegalensis* showed antinociceptive activity using the hotplate test, the acetic acid twist test, and the late phase of formalin-induced nociception. The analgesic effect of the methanolic extract justified its folkloric use in the treatment of rheumatic pain (Adzu, *et al.*, 2003

### Clinical data

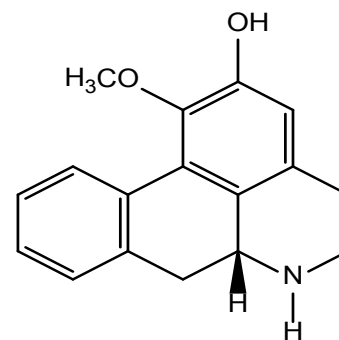
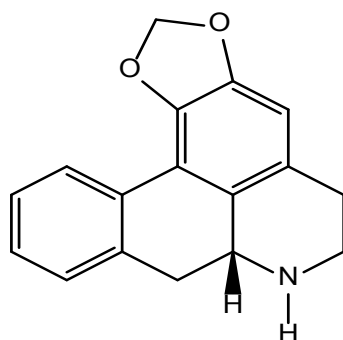
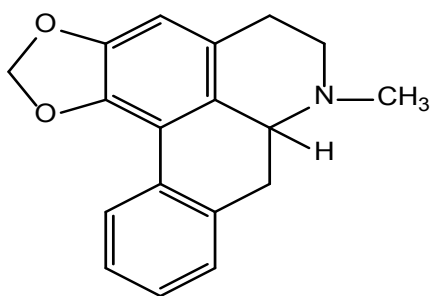
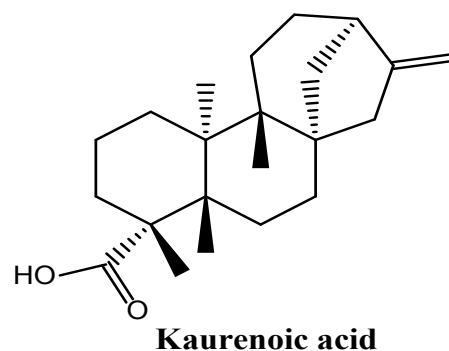
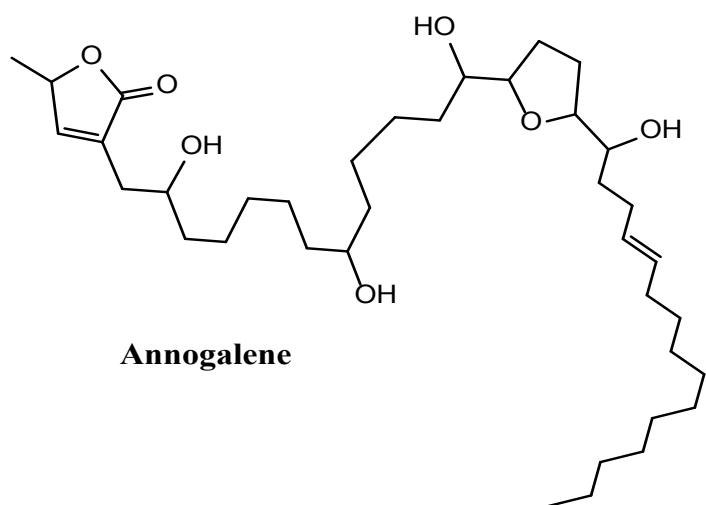
*A. senegalensis* had an effect on digestive strongles (*Haemonchus. contortus*, *trychostrongylus*, *strongylus*) in sheep (Nguessan *et al.*, 2017). Administration of the leaves resulted in a reduction in osteoprotegerin (OPG) from the fifth day, roots from the tenth day and stems from the twentieth day. In addition, the leaves generally exhibited higher reduction rates than the stems and roots. This greater leaf activity could probably be explained by the fact that the leaves are the main seat of biosynthesis and storage of the active ingredients responsible for the biological properties of plants (Bitsindou, 1996). These results confirm those already found *in vitro* from alcoholic extracts of the leaves, roots and whole plant during the hatching of eggs and the larval stages of *H. contortus* (Alawa 2003; Fall *et al.*, 2016). The use of *A. senegalensis* in traditional therapy as anthelmintic in small ruminants in veterinary medicine to treat intestinal worms and gastrointestinal disorders (Koné *et al.*, 2006) is therefore justified. However, under the conditions of use of the breeders (aqueous extracts), the leaves seem to be more effective than the roots whereas in the north of Côte d'Ivoire, it is root decoctions which are used to treat worms, intestinal and gastrointestinal disorders (Koné *et al.*, 2006). Anthelmintic activity of *A. senegalensis* against gastrointestinal nematodes is thought to be due to the presence of an acetogenic squamocin in this plant, which is more potent than levamisole (Fall *et al.*, 2008; Okhale *et al.*, 2016 ). In addition, the reduction rates observed in organs treated with organ extracts from *A. senegalensis* during the first days were lower than those of albendazole. These results confirm the assertion of Githiori *et al.* (2006) that herbal remedies have, in most cases, lower levels of parasitism than those of synthetic anthelmintics in *in vivo* control tests. It must be recognised, however, that in this study the doses applied were low (22 mg/kg body weight) compared to those used in some trials conducted on other plants. For example, in a study by Kaboré (2009) to evaluate the anthelmintic activity of *Anogeissus leiocarpus* and *Daniellia oliveri* in mall ruminants, the oral doses administered were 160 and 242.5 mg/kg of live weight respectively. Sacramento *et al.* (2010) obtained a significant reduction of more than 75% in OPG in brushcutters using papaya seeds at a dose of 100 mg/kg of PV. In addition, after the 20th day, the reduction rates were statistically the same for albendazole and the extracts from the various organs. This is due to the fact that from the 20th day, the NGI eggs returned to the faeces of batch AI. These eggs appear approximately 20 days after the treatment, which corresponds roughly to the pre-patent period of the eggs (Menzies, 2010). This means that the eggs found around the 20th day of batch AI come from infective L3 larvae ingested by sheep after treatment with albendazole. With regard to cestodes (*Taenia* sp), the sporadic appearance of the eggs a few days after the test seems to indicate the ineffectiveness of *A. senegalensis* on these parasites.

### Chemical constituents

Alkaloids (roemerine, anonaine, norantenine and isocorydine); annonaceous acetogenins (molvizarin,



asimicin, rolliniastatin, squamocin, annogalene and annosenegalin)(Sahpaz *et al.*, 1996; Zeng *et al.*, 1996); essential oils (citronellal, citronellol, geranial, thymol,  $\beta$ -caryophyllene, carvacrol, p-cymene,  $\alpha$ -phellandrene,  $\alpha$ -pinene, Z-sabinol, limonene (Nkounkou-Loumpangou *et al.*, 2010); diterpenoid kaurenoic acid (Okoye *et al.*, 2012).



### Test for identity and purity

#### Leaves

Moisture content: air dried coarse powder does not lose more than 5.4% at 105°C.

Total ash: not more than 6.3%

Ash insoluble in acid: not more than 0.8%

Water soluble extractives: not less than 8.0%

Ethanol soluble extractive (70% Ethanol): not less than 11.0%

### Chromatographic fingerprint

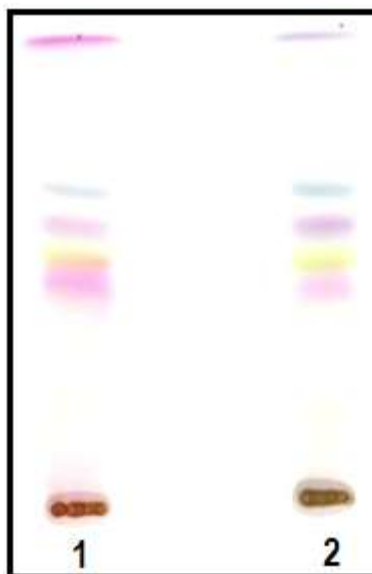
#### Thin Layer Chromatography

**Preparation:** About 5 g of the powdered leaves were extracted with ethyl acetate by cold maceration, filtered and the filtrate concentrated to a small volume. A small spot was then applied to the TLC plate for analysis.

**Chromatographic conditions:** Analytical TLC on silica gel G60 F254, 0.25mm layer in hexane/ethyl acetate (7:3) as the mobile phase.

**Detection:** Visualized in daylight after spraying with anisaldehyde-sulphuric acid (Lane 1) and vanillin-sulphuric acid reagents (Lane 2) (Stahl, 1969), heating to 110°C for 10 mins.

The TLC chromatogram showed two prominent spots with R<sub>f</sub>s of 0.68 (light blue) and 0.47 (pink) when sprayed with both anisaldehyde and vanillin. Two additional spots appeared in the chromatogram sprayed with anisaldehyde at R<sub>f</sub>s of 0.60 (pink) and 0.52 (yellowish brown). Similar spots appeared in the chromatogram sprayed with vanillin at R<sub>f</sub>s of 0.60 (purple) and 0.52 (yellow)



### High Performance Liquid Chromatography

**Sample preparation:** About 10 mg of the hydro-ethanolic extract of *A. senegalensis* leaves were reconstituted in 3 mL acetonitrile in a 10 mL volumetric flask with sonication for 17 minutes. The resulting solution was then diluted to volume with the mobile phase under chromatographic conditions. It was centrifuged to obtain a clear test solution which was subsequently filtered through a 0.45µm filter into an HPLC vial and analyzed.

### Chromatographic system

#### Optimized chromatographic conditions

**Mode:** LC

**Column:** YMC ODS, 4.6 x 150 mm, 5 µm

**Column temperature:** Ambient – 30°C

**Mobile phase:** Acetonitrile: water (60:40 v/v)

**Elution mode:** Isocratic

**Injection volume:** 20 µL

**Flow rate:** 0.5mL/minute

**Detection wavelengths:** 230 nm, 254 nm and 278 nm.

#### System Suitability parameters

**Number of peaks:** 230 nm (1), 254 nm (1), 278 nm (2)

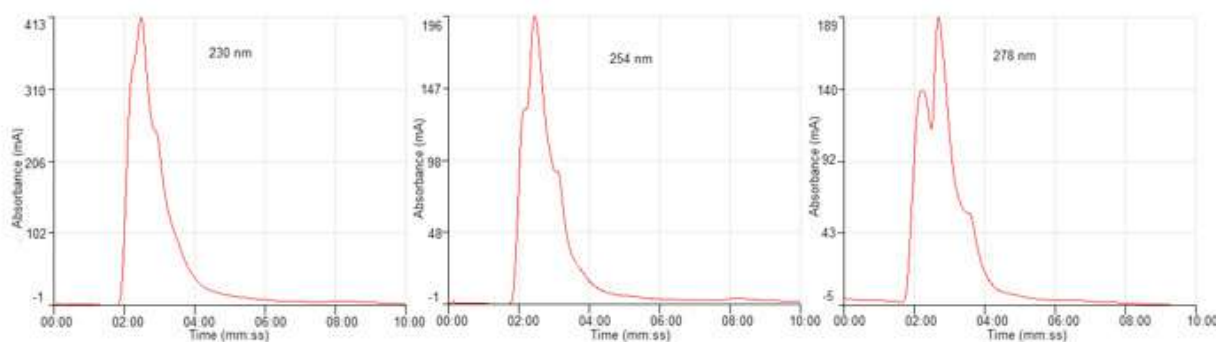
**Retention time (s):** 230 nm (2.28 min), 254 nm (2.27 min), 278 nm (rt1-2.13 min, rt2-2.41 min)

**Asymmetric factor(s):** 230 nm (1.208), 254 nm (1.108), 278 nm (af1-1.688, af2-1.261)

**Tailing factor:** NMT 2.0

**Efficiency:** 230 nm (44.90), 254 nm (296.59), 278 nm (E1-138.04)

**Acceptance criteria:** Sample solution of hydro-ethanolic crude extract of *A. senegalensis* Pers. (Leaves) conforms to the system suitability parameters.



## FT-IR

A small amount of the dried hydroethanolic extract (70%) was placed on the sample area of the Perkin Elmer UATR Fourier transform infrared (FT-IR) spectrometer and scanned between 4000-400  $\text{cm}^{-1}$  with a resolving power of 4  $\text{cm}^{-1}$  and a cumulative scanning limitation of 24 times. Principal peaks appeared at wavenumbers 3292.31 (broad), 2923.66, 1603.61 and 1035.46  $\text{cm}^{-1}$

## Macroscopy

The leaf is pubescent to glabrescent; blade oval in shape, 7-12 cm long and 6-8 cm wide; rounded at the base or slightly re-entrant with an obtuse corner, 6 to 8 regular lateral veins parallel and protruding below. Leaf petiole measures 10 to 12 mm long; flowers solitary or arranged in groups of two or three in the axil of the leaf, suspended under the branches by a pedicel about 2 cm long, greenish to yellowish, waxy, bell-shaped and up to 2 cm long. The fruit is a berry, globose or ovoid in shape and fleshy; dark-orange when mature, with many smooth protuberances and pineapple odor, sweet, edible, in which the seeds are drowned

## Microscopy

Lower epidermal cells of the greenish leaf are polygonal, stomata anomocytic with 4 subsidiary cells; upper epidermis also has polygonal cells with appressed hairs, uniseriate 3-4 celled each, long unicellular trichomes with acute apex found along the veins; no stomata; twisted fibres of 1-celled, vascular bundles with annular xylem vessels, one palisade cell layer and spongy mesophyll.

Seed testa consists of polygonal cells, cell contents include aleurone grains in parenchymatous tissue, tested positive for proteins with picric acid and iodine solutions; inner tissues show groups of sclereids, thick-walled, irregular shape with yellow lumen content; annular or spiral xylem vessels carry single or groups of xylem fibres.

## Powdered drug

Fragments of leaf epidermis showing anomocytic stomata with four subsidiary cells and polygonal epidermal cells. There are annular xylem vessels, fragments of upper epidermal cells with polygonal cells and no

stomata, with uniseriate three to four celled, curved trichomes. Spongy mesophyll and fragments through the transverse section showing a layer of palisade cells. Long unicellular trichomes with acute apex are scattered along the veins which have rectangular cells. There are long unicellular trichomes and long unicellular fibres.

Numerous groups of stone cells, which are thick walled, irregular in shape and have yellowish coloured content, characterize the powdered fruit; large oval shaped parenchyma cells; parenchyma cells with numerous circular aleurone grains; fibres and groups of fibres attached to annular and spiral xylem vessels. Bundles of veins, which consist of xylem vessels, occur. Fragments of the testa of the seeds with polygonal cells are evident

### Therapeutic actions

Antiplasmodic, trypanocidal, antibacterial

### Therapeutic indications

Bacterial and worm infestations (Johnson and Olatoye 2002).

### Safety data

LD<sub>50</sub> by oral route was estimated to be beyond 3000 mg/kg in rats. There were no signs of CNS depression/stimulation or autonomic effects following treatment with aqueous extract 0-1000 mg/kg). Target organs including the liver were not affected by the treatment. The extract had no effect on haematological indices. It caused an increase in AST but not ALT, ALP and GGT. It did not increase serum bilirubin, but it appears to rather decrease conjugated bilirubin leading to an insignificant decrease in total bilirubin. Renal function was not affected. It increased pentobarbitone sleeping time very slightly at doses of up to 1000 mg/kg. No histopathological changes were observed in the liver and kidney. Similar findings had been reported on the stem bark. The authors however, noticed an increase in total WBC, but a decrease in neutrophils (Okoye *et al.*, 2012). In a study by Yeo *et al.* (2011), it was observed that there were minute decreases in WBC across all doses tested without a change in the proportions of granulocytes and agranulocyte. It is not clear if the decrease in WBC seen in this study is related to its pharmacological actions, but care must be taken during concomitant administration of other CNS drugs.

### Precautions for use

Care should be taken with concurrent administration of other CNS drugs.

### Adverse effects

Could increase effects of sedatives

### Contraindications

Contraindicated in pregnant women

### Dosage forms

Decoction, powder, infusion, tincture

## Dosage

Decoction: 30 g of dried plant material in 900 mL water; simmer until reduced to 600 mL; 1-3 tablespoonfuls daily

Infusion: 30 g of dried aerial part in 600 mL of water; 3-4 teacups daily

Tincture: 1:5, 45% ethanol; 5 ml three times daily

## Storage

Store in a cool, dry place away from light.

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**Botanical name***Calotropis procera* (Aiton) W.T. Aiton**Family**

Asclepiadiaceae

**Synonyms***Asclepia procera* Aiton**Common names**

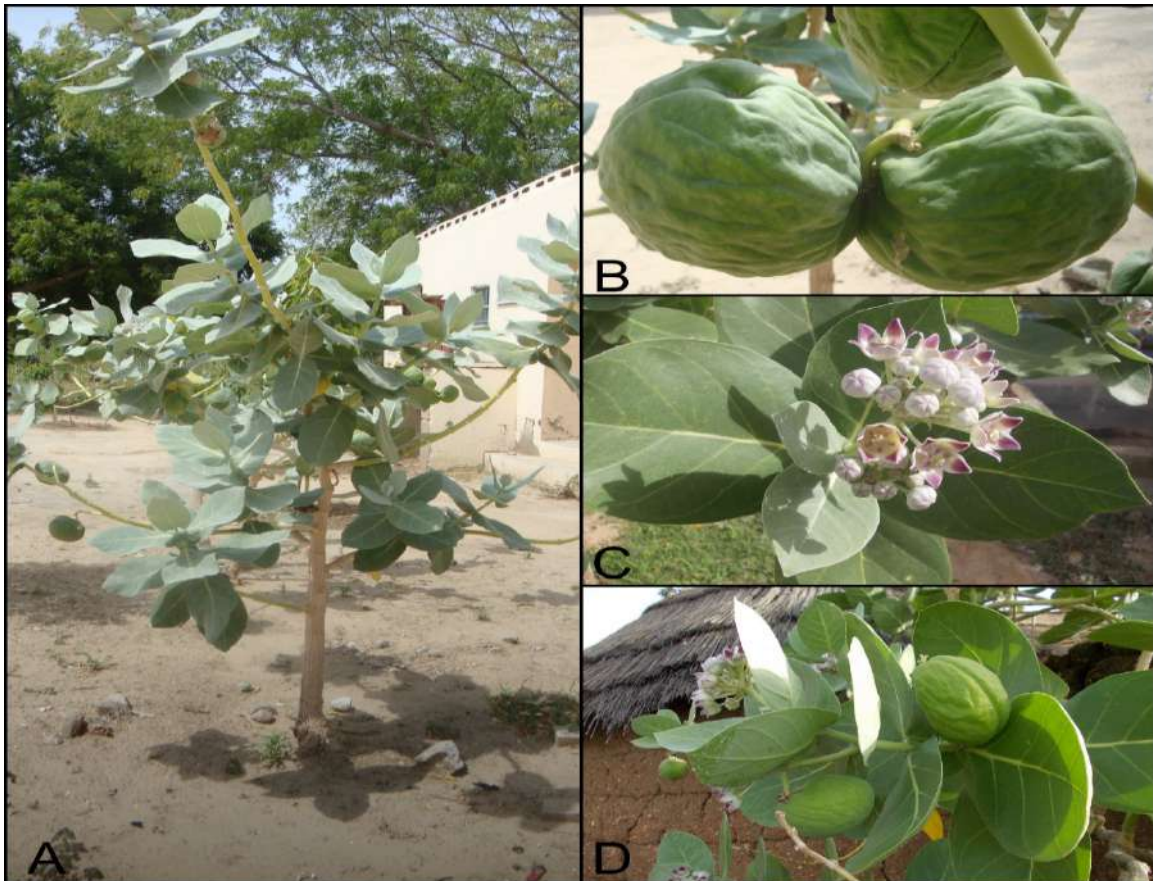
Auricula tree, dead sea apple, Sodom apple, swallow wort, calotrope cabbage tree, rubber tree, small crown flower, rooster tree, French cotton (English), Arbre a soie; arbre a soie du Senegal; pomme de Sodome (French) (Burkill, 1985; Mshana *et al.*, 2000), Algodão-de-seda, bombardeira (Portugese)

**Common Local names****Benin:** Fon – Kpɔntwe; Yoruba- bom bomu; Dendi- Saagei**Burkina Faso:** Dioula-Fogofogo; Fulfulé-Bamambi; Moré-Putrupuugu**Côte d'Ivoire:** Dioula-Toumo tigi ; Malinke-Togo logo; Fulfulde-Ganganpi**Gambia:** Pulaar-Bawane; Manding- Kupamango; Wolof - Faftan**Ghana:** Ga-Gbekebii awuo ; Twi-Mpatu ; Ewe-Gboloba.**Guinea:** Malinké – Mpompompogolo; Pular - Pampam**Guinea-Bissau:** *Balanta* – Bagueuóne; *Crioulo* – Bombardeira ; *Diola-Flup* - Belápse**Mali:** Bambara – Popompogolo; Dogon- Pounpoun; Malinke – Tounroumba; Peulh-Sabaïe, Bamambé**Niger:** Haoussa-Tounfafia ; Djerma-Sageye ; Tamacheck- Toerza**Nigeria:** Hausa – tumfafiyya; Yoruba – bomubomu; Igbo - otosi**Senegal:** Wolof-Poftan; Peulh-Kupapa; Diola-Bupumba pumb**Sierra Leone:** Krio English – Kōtin; Mende – Puu vande**Togo:** Ewe-Wagachibgé; Akposso-Wuagansiti; Mina-Wangashigbé**Description of the plant**

*Calotropis procera* is a xerophytic perennial shrub or small tree. It is single, or many stemmed soft-wooded shrub that grows up to 6 m high, with a crown diameter of up to 7 m, occurring in arid conditions. Young stems are greyish green in colour, smooth in texture, with a covering of whitish coloured hairs. Mature stems are deeply fissured, with a cork-like bark that is light brown in colour. Though the plant does not grow very tall, the stems may reach nearly 1 m in girth and are often thick enough to serve as joists support for mud houses where other timber may be lacking. The leaves are oblong to broadly obovate, cordate to clasping heart shaped at the base, abruptly and shortly acuminate to blunt at the apex, up to 30 cm long and 15 cm broad, tough, glaucous and with no leaf stalk. The leaf blades are light to dark green with nearly white veins, slightly leathery with a fine coat of soft hairs that rub off. Leaves are pubescent when young and glabrous on both sides on maturity. They have a waxy appearance and contain a milky white sap. The inflorescence is umbelliform, pedunculate, extra-axillary and flowers are numerous. Each cluster contains 3-15 flowers, which are surrounded by involucre of several small oblong, pointed scaly caduceus bracts. The main stalk, the peduncle is 20-55 mm long, and each flower corolla and calyx are five lobed; sepals 7-8 mm long are



ovate acute, hairy outside. The petals of the flowers are 2-3 cm wide, white with purple tips internally; the flowers have a crown like centre. Androecium has five stamens, gynandrous, anther dithecous, coherent. Gynoecium is bicarpillary, apocarpus and styles are united at their apex. Stigmas are peltate with five lateral stigmatic surfaces. Anthers are adnate to the stigma forming a gynosteggium. The fruits are greenish with blue or purple colour and sub-globose, to obliquely ovoid in shape, inflated and up to 10 cm long and 8 cm in diameter. Its apex is rounded, green, spongy and smooth. Fruiting takes place throughout the year. Seeds are numerous (350-500) per fruit, they are flat, obovate, 6 x 5 mm or more, with silky white pappus of 3 cm or longer (Burkill, 1985; Mshana *et al.*, 2002; Murti *et al.*, 2010; Sharma *et al.*, 2011; Hassan *et al.*, 2015).



A: plant, B: matured fruit, C: fleurs, D: unripe fruit

#### Herbarium specimen number

Benin: 2336 (AP)  
 Burkina Faso: 3423 (OUA) CNSF-450  
 Côte d'Ivoire: CNF18128  
 Ghana: GH 118/KNUST  
 Mali: 2901 / DMT  
 Nigeria: UPFH 111  
 Senegal: IFAN 96  
 Togo: TG 02210

#### Habitat and geographical distribution

Paleotropical plant, widespread through mostly dry regions of inter-tropical Africa (Mshana *et al.*, 2002; Hassan *et al.*, 2015; Parihar and Balekar, 2016). Native to West, North, and East Africa and Madagascar,

as well as Asia (Southern Asia, Indo China to Malaysia and Macronesia) and common in the Middle East (Arabian peninsular). It has naturalized in Australia, Central, North and South America and the West Indies (Parrota, 2001). It grows on a variety of soils from fine-to-coarse, with varying degrees of salinity, and in dry habitats where rainfall is limited to 150 to 1000 mm. It is also found in areas of excessive drained soil with as much as 2000 mm of annual precipitation, and commonly on roadsides and beachfront dunes. It is also found in elevated areas of up to 1,000 m. It is easy to propagate and manage (Parrota, 2001). The plant is to some extent an anthropogene, occurring commonly around villages, perhaps planted, but not necessarily tended. Its presence in the bush may mark an abandoned village site and exhausted soil, but also said to indicate subsoil water (Burkill, 1985).

### Plant material of interest

Leaves

### Other parts used

Roots, fruits, latex

### Definition of plant material of interest

*Calotropis procera* is the fresh or dried leaves of *Calotropis procera* (Aiton) W.T. Aiton (Asclepiadaceae)

### Ethnomedical uses

Stalk is used to treat boils and parotitis, while the stem is used for conjunctivitis, ringworm, ulcer and dracontiasis. Stem latex is used to treat dystocia, catarrh, ringworm and sinusitis. Roots are used thought to be effective against leprosy, diarrhoea, skin ulcers, abdominal pain, toothache and lactation failure. Powder of the root mixed with goat milk is applied to the nose to treat epilepsy. Secretions from the root bark are used for skin diseases, cough, intestinal worms, ascites and anasarca and enlargement of the abdominal viscera. Leaves are used for dracontiasis, migraine, dystocia, female infertility and catarrh. The leaves are boiled and oily preparations used to treat paralysis. Tender leaves are used for migraine. The bark is used for sickle cell anaemia, cholera, Guinea worm infestation and indigestion. The leaves, roots and flowers are used to treat snake bite, dermatitis and constipation. Stem and roots are used for yaws. The whole plant is used to treat dermatitis and constipation. The milky juice was considered a drastic purgative and caustic applied to toothache. The juice is also given to women to induce abortion. Tanners use the juice to remove hair from hides. Flowers are used to improve digestion, catarrh and to increase appetite, and the flowering tops used to treat asthma. The raw latex has been considered toxic, but reports of its toxicity may be exaggerated, A safe, effective dose could be obtained by scooping out the seeds and pulp from a halved ripe fruit and drinking sheep, goat or camel milk from the remaining green skin "cup". Poultices made from the leaves are used to treat rheumatism (Verma *et al.*, 2010; Mshana *et al.*, 2002).

### Biological and pharmacological activities

In a test for analgesic activity, the dried latex of *C. procera* at a dose of 415 mg/kg against acetic acid-induced writhing was more active compared to an oral dose of aspirin at 100 mg/kg. Dried latex at 830 mg/kg showed marginal analgesia in the tail-flick model that was comparable to aspirin (Quazi *et al.*, 2013). The latex protein fraction tested at doses of 12.5, 25, and 50 mg/kg for its antinociceptive effects in three different models of nociception; acetic acid-, formalin-induced abdominal constrictions and hotplate test in mice showed antinociceptive activity in a dose-dependent manner (Dewan *et al.*, 2000). Antipyretic effect of extracts of *C. procera* has also been documented (Soares *et al.*, 2005; Gupta *et al.*, 2012). Aqueous and

chloroform extracts of the roots of *C. procera* root were evaluated for anticonvulsant activity using the maximal electroshock seizures (MES) and the pentylene tetrazol tests. The extracts inhibited lithium, – pilocarpine and electrical kindling induced convulsions, with the chloroform extract showing significant activity (Quazi *et al.*, 2013). Latex proteins of *C. procera* administered at high doses (50 or 100 mg/kg) and diazepam (2 mg/kg as standard) caused significant increases in latencies to convulsions and death in PTZ-induced seizure model. The latex proteins and diazepam caused a decrease in sleep time compared to control group. Latex proteins have a CNS–depressant activity shown by the potentiation of sleeping time induced by phenobarbital and anticonvulsant action (Lima *et al.*, 2012). Aqueous extract of the leaves of *C. procera* evaluated for anticonvulsant activity using the MES test in rats at 250 mg, 500 mg/kg doses and phenytoin 25 mg/kg as standard, showed significant decrease in the duration of hind limb extension and convulsions compared with the control (Madhyastha *et al.*, 2016). Ethanolic extracts of different parts of *C. procera* showed  $IC_{50}$  values ranging from 0.11 to 0.47 mg/ml against *Plasmodium falciparum* MRC20 chloroquine-sensitive strain and from 0.52 to 1.22 mg/ml against MRC 76 chloroquine-resistant strain. The flower and bud extracts were the most effective, though they were 220 and 440 times less effective than chloroquine (Sharma and Sharma, 2000; Meena, *et al.*, 2010). Evaluation of *C. procera* for larvicidal activity showed various parts were effective in both antifeeding and larvicidal activity. Latex constituents showed toxicity to egg hatching and larvae of *Ae. aegypti*. The whole latex caused 100% mortality of 3rd instars within 5 mins. The latex has larvicidal activity against three important vectors *Ae. aegypti*, *Ae. stephensis* and *Culex quinquefasciatus* of dengue, malaria and lymphatic filariasis respectively (Singhi, *et al.*, 2004; Ramos *et al.*, 2006; Rahuman *et al.*, 2009). Alkaloidal extracts of *C. procera* evaluated against 5<sup>th</sup> instar larvae on ovarian growth of *Schistocerca gregaria* showed a mortality rate of 100% on the 15<sup>th</sup> day after treatment (Abbassi, *et al.*, 2004). Aqueous extracts of the leaves, flowers and roots were found to be effective repellent and antifeedant against *Henosepilachna elaterii*. Extracts of leaves, flowers were found active against two species of termites *Heterotermes indicola* and *Coptotermes heimi*. Extracts were more active against *C. heimi* during feeding stage (Quazi *et al.*, 2013). Larvae of *Culex pipiens* were treated with concentrations of 1, 0.5, 0.25 and 0.125% of latex and leaf extract and monitored at 24, 48 and 72 hours. No larvae survived at 1% latex concentration (Anjum *et al.*, 2016). Leaf extracts at 20, 40, 60, 80 and 100% were tested against *Tribolium castanem* cereals, stored grain pest. After three months storage of the wheat grains, maximum repellency was observed with 80 and 100% concentration. The result showed less spoilage, insignificant reduction in grain weight and a decrease in insect population (Abbasi *et al.*, 2012). Fresh and aqueous extracts of dried latex showed a dose dependent inhibition of spontaneous motility and pin pricks by earthworms. Higher doses of 100 mg/ml of aqueous extract of dry latex and 100% fresh latex showed comparable effects to 3% piperazine. The effects of the latex however were not reversible, while with piperazine the worms recovered completely after six hours (Al-Quarawi *et al.*, 2001). In sheep, infected with single oral dose of 12000 *Haemonchus contortus* the latex was given at 0.01 ml or 0.02 ml/Kg body weight. Egg production was significantly reduced, but not completely suppressed. Fewer adult worms were found in the abomasum and the latex showed a concentration dependent larvicidal activity *in vitro* within 20 mins of application. (AL-Qurawi *et al.*, 2001). Aqueous and methanolic extracts of *C. procera* flowers were investigated *in vivo* and *in vitro* for anthelmintic activity in sheep in comparison to levamisole. Extracts showed activity against live *Haemonchus contortus* as evident by their mortality and temporary paralysis. The crude powder, aqueous and methanolic extracts of the flowers were administered to sheep naturally infected with mixed species of gastrointestinal nematodes. In sheep treated with aqueous extract and crude powder at 3g/kg body weight, egg count reduction of 88.4 and 77.8% was recorded on day 7 and 10 post treatment respectively. The methanol extract was least effective (20.9%). Activities were however lower than levamisole 7.5 mg/kg (98.8-100%) (Iqbal *et al.*, 2005; Quazi *et al.*, 2013; Calvacante *et al.*, 2016). The latex of *C. procera* was evaluated by various methods for its antiinflammatory activities. Aqueous and methanolic extracts of the dried latex had more pronounced activity against carrageenan-induced inflammation than phenyl butazone, and comparable activity to chlorpheniramine and phenylbutazone in inflammation induced by histamine and PGE<sub>2</sub>. The mechanism of action showed that the dried latex exerted its antiinflammatory activity by inhibiting histamine, bradykinin and PGE<sub>2</sub>. (Kumar and Basu, 1994; Arya and

Kumar, 2005). Antiinflammatory activity of petroleum ether, acetone, methanol and aqueous extracts of dry latex were tested in carrageenan-induced rat paw oedema model. Acetone and aqueous extracts showed the greatest activity (Sangraula *et al.*, 2002). Dried latex showed significant activity in inhibiting carrageenan- and Freund's adjuvant-induced oedema formation, cotton pellet- and carrageenan-induced granuloma formation, fluid exudation, delayed onset of UV-induced erythema and intensity. Dried latex has shown activity comparable to standard antiinflammatory drugs. The effect of dried latex was comparable to rofecoxib a selective COX-2 inhibitor and phenyl butazone, a non-selective COX 1 inhibitor. Methanolic *C. procera* latex extract compared favourably, and markedly reduced cell influx, release of mediators, and oxidative stress which are associated with arthritic conditions (Kumar and Roy, 2007). Root extract of *C. procera* was found to be active against COCO320 tumour cells and inhibited the proliferation of Hep2 cancer cells by apoptotic and disruption of cell cycle based mechanism (Mathur *et al.*, 2009). Dried latex was evaluated in several anti-tumour studies. Evaluation of its activity in X15-Myc transgenic mouse model of hepatocellular carcinoma showed significant level of vascular endothelial growth factor in treated mice in comparison to the control. In cell cultures of Huh-7 (Hepatoma cells), dried latex caused extensive cell death in AML12 (non-transformed hepatocytes cells) (Smit *et al.*, 1995; Choedon *et al.*, 2006). The protein fraction of the latex was investigated in increasing concentrations against MCF-7 (Breast cancer cell line) for 24 hr and analysed by MTT assay. The protein fraction caused a decrease of cell growth, with an IC<sub>50</sub> after 24 hr of 88.33 ug/ml (Olievera *et al.*, 2007). Latex protein also showed cytotoxicity to SF295 and MDA-MB-435 cell lines with IC<sub>50</sub> of 0.42 and 1.36 ug/ml respectively. In the *Allium cepa* root meristem model, dried latex inhibited the growth of roots and mitotic activity in a dose-dependent manner (Sehgal *et al.*, 2006). Methanolic extract of *C. procera* was tested against SK-MEL-2 cells. Cell viability in the treated cells decreased at 0, 5, 10, 20 and 40 ug/mL with IC<sub>50</sub> at 20 ug/mL at 24 hr. At 20 ug/mL the extract caused a 50 ± 5% inhibition of cell growth, compared with paclitaxel and ouabain which showed 60.3 ± 5% and 53.3 ± 5% respectively (Joshi *et al.*, 2015). All the parts of *C. procera* have been investigated for antimicrobial activity. Proceragenin a cardenolide of *C. procera* was found active against *Pseudomonas pseudomallei*, which causes melioidosis. Extracts of the stem bark showed significant activity against fungi *Trichophyton rubrum* and *Microsporum gypseum*. The leaf extracts inhibited all the test organisms. The hexane and petroleum ether extracts of the root were significantly active against *M. gypseum* and *Aspergillus niger*. The aqueous extracts of all plant parts were active against all test organisms (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*). Hexane and petroleum ether extracts of the roots showed significant growth inhibition of *M. gypseum* and *A. niger* and the pathogenic organism *S. aureus*. Aqueous stem extracts also showed activity against *Epidermophyton floccosum* and *Trichophyton gypseum* at 4.0 mg/ml and 0.5 and 0.9 mg/ml MIC, and minimum fungal concentration (MFC) of 2.0 and 4.0 mg/ml respectively (Yesmin *et al.*, 2008). Aqueous, hexane and petroleum ether extracts of leaves, stem bark, and roots were screened for antifungal activity. The organic solvent extracts of leaves and stem bark were significantly active against *T. rubrum* and *M. gypseum*, with the root hexane and petroleum ether extracts being active against *M. gypseum* and *A. niger*. Aqueous, ethanol and chloroform extracts of leaf and latex investigated on six bacteria, *Pseudomonas aeruginosa*, *Escherichia coli*, *S. aureus*, *S. albus*, *S. pyogenes*, *S. pneumonia*, fungi *A. niger*, *A. flavus* and *M. boudardii* and the yeast *C. albicans* by the agar well diffusion and paper disc methods, showed that the ethanol extract was most active followed by the chloroform and the aqueous extracts. The latex was most active against *E. coli*. All bacteria were inhibited by the extracts except for *P. aeruginosa* and *S. pyogenes*. The ethanol extract of the latex showed the most pronounced activity against *C. albicans* with MIC 5.0 to 20 mg/ml for fungi (Yesmin *et al.*, 2008). *C. procera* leaves were investigated for activity against *E. coli*, *S. aureus*, *Streptococcus pyogenes*, *A. niger*, *Penicillium fellutanum* and *Candida* sp. isolated from commercial motor cycle helmets in Lagos, Nigeria. Aqueous and ethanol extracts were active against all test organisms. Chloroform extracts had lower activity and did not inhibit *S. aureus* and *E. coli*. Ethanol extract was most active (Adama *et al.*, 2013). Endophytic fungi of *C. procera* were studied and evaluated for their antibacterial activity. The dominant endophyte found were *Phaeoramularia calotropidis* (63.5%), *Guignardia bidwellii* (21.1%) The fungi were active against only gram positive bacteria, and none was active against the pathogens tested (Nascimento *et al.*, 2015). In another study ethanolic leaf extracts of *C. procera* showed significant

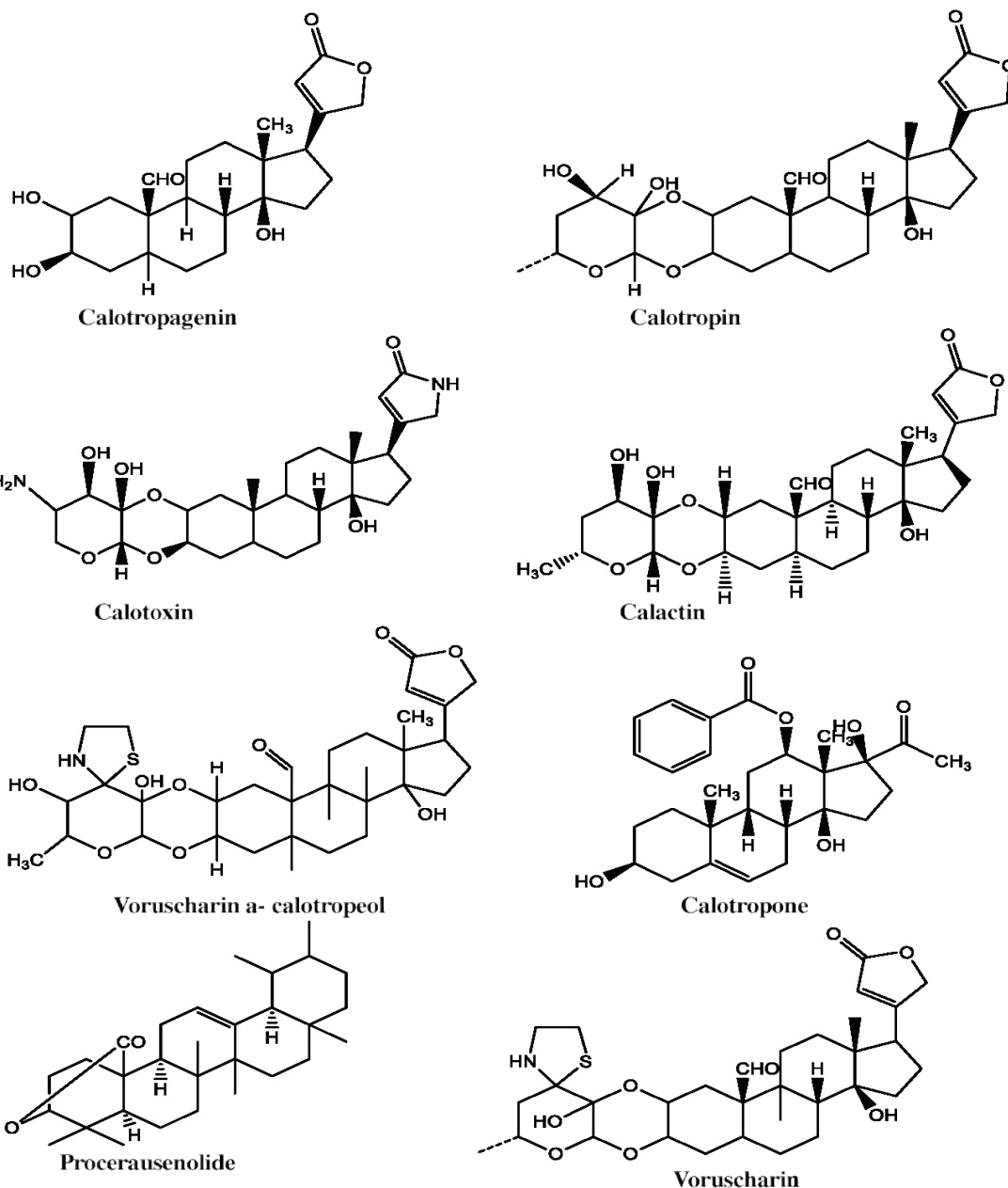
activity against *E.coli* and *A. niger* (Pattnaik *et al.*, 2016). Root bark extract of *C. procera* was investigated at doses of 50, 100 and 200 mg/kg for immunomodulatory activity using immunological tests in mice, delayed type hypersensitivity, humoral mediated antibody titer, vascular permeability, haematological profile and cyclophosphamide-induced myelosuppression. The extract was active and stimulated the defence system by modulating several immunological parameters. The latex protein extract was found to protect against *Listeria monocytogenes* in experimental infections (Ramos *et al.*, 2007; Nascimento *et al.*, 2016). Kumar *et al.*, in 2001, assessed the dried latex for anti-diarrhoeal activity. Dried latex caused a significant decrease in frequency of defaecation and severity of diarrhoea at a single dose of 500mg/kg. In rats treated with castor oil, 80% were protected from diarrhoea. It also produced a decrease in intestinal transit by 27-37% in comparison to normal rats treated with castor oil. Dried latex inhibited castor oil-induced enteropooling, but did not alter electrolyte concentration in the intestinal fluid as compared to rats treated with castor oil. Aqueous extract of *C. procera* investigated for muscle relaxant activity *in vitro* using trachea smooth muscle chain of the guinea pig at 50, 100 and 200 µg/ml, showed dose dependent relaxant activity (Iwalewa *et al.*, 2005). Antioxidant potential of the field grown and tissue-cultured *C. procera* were analysed for free scavenging activity by DPPH. Roots and leaf extracts and latex were investigated. Extracts of lyophilized latex showed the highest activity (IC<sub>50</sub> 0.06 mg/ml), with the root extracts showing the lowest activity (IC<sub>50</sub> 0.27 mg/ml) (Roy *et al.*, 2005). About 20 µl of 1.0% sterile solution of the latex of *C. procera* was evaluated on guinea pigs by topical application for wound healing activity. The solution was applied twice daily for seven days to full thickness excision wounds of 8.0 mm diameter on the back of guinea pigs. The latex helped the process of healing by significantly increasing collagen, DNA and protein synthesis and epithelization (Rasit *et al.*, 1999). Tsala *et al* (2015) in another study, investigated the healing action of the bark extract on surgical wounds. Dried latex showed activity comparable to glibenclamide when assessed for ability to stimulate increase in hepatic levels of the endogenous antioxidants, superoxide dismutase, catalase and glutathione. Levels of thiobarbituric acid reactive substances were reduced in alloxan-induced diabetic rats (Roy *et al.*, 2005). Using *in vivo* ulcer models, *C. procera* inhibited aspirin, reserpine, alcohol and serotonin-induced gastric ulcerations in rats, and also protected aspirin-induced ulceration in the gastric mucosa of pyloric-ligated rats. In guinea pigs, extracts also protected histamine-induced duodenal ulcers (Basu *et al.*, 1996). Ethanolic extract of roots of *C. procera* showed strong anti-implantation activity (100%) in albino rats, and uterotropic activity at a dose of 250 mg/kg (25% of the LD<sub>50</sub>). Kamath and Rana, 2002). Seventy percent (70%) hydroethanolic extract of flowers was investigated for hepatoprotective effect in paracetamol-induced hepatitis in rats. The hydroethanolic extract at 200 mg and 400 mg/kg restored the altered levels of biochemical markers (SGPT, SGOT, ALP, bilirubin, cholesterol, AHL, levels and tissue GSH) to near normal in a dose-dependent manner (Setty *et al.*, 2007). The extract was also evaluated for protection against isoproterenol- (at 20 mg/100 mg body weight, sc.) induced myocardial infarction in albino rats (Mueen *et al.*, 2004). Pre-treatment with the extract at 300 mg/kg body weight, given thrice a day for 30 days, reduced the elevated marker enzyme levels in serum and heart homogenates and showed marked myocardial necrotic damage. The dried latex and its methanolic extract protected rats and significantly reduced joint inflammation (50-80%) and associated hyperalgesia. The activity of the methanolic extract was comparable to rofecoxib. Both significantly improved motility and stair climbing ability of the rats (Kumar and Roy, 2007).

**Clinical data:** Not available

### Chemical constituents

Cardenolides (calotropin, calotropagenin, uscharin, uscharidin, proceroside, calactin, calotoxin, coroglaucigenin, uzarigenin, coroglaucigenin or frugoside) (Parihar and Balekar 2016); rutin (Tiwari *et al.*, 1978),  $\alpha$ - and  $\beta$ -amyrin, cyaindin-3-rhamnoglucoside, cycloart-23-en-3 $\beta$ ,25-diol, cyclosadol, multiflorenol, procestrol, quercetin-3-rutinoside, Beta-sitosterol, beta-sitost-4en-3one, and stigmasterol, cyanidin-3-rhamnoglucose, calotropenyl acetate, ergost-5—en-3-ol (Dwivedi *et al.*, 2014),  $\alpha$ -calotropeol,

3-epimoretenol, gigantol, giganteol, isogiganteol,  $\alpha$ -lactucerylacetate,  $\alpha$ -lactuceryl isovalerate, lupeol, syriogenin, taraxast-20- $\alpha$ -(30)-en-(4-methyl-3-pentenoate), voruscharin (Mohamed *et al.*, 2015; Sweidan and Abu, 2015), labdane -type diterpenic galactosides, labdan-18ol-B- D-galactofuranoside and labdan-3-B-ol-11,15-olide-18,20-dioic acid-3-B-D-galactofuranoside, bezolisoleneolone, benzolleneolone; fatty acids and amino acid s (Pattnaik, 2016).



## Test for identity and purity

### Leaves

Moisture content: Air dried coarse powder does not lose more than 04.2% at 105°C.

Total ash: not more than 18.5%

Acid insoluble ash: not more than 0.9%

Water soluble extractive: not less than 24.0%

Ethanol soluble extractives (70%): not less than 16.0%

## Chromatographic fingerprint

### Thin Layer Chromatography

**Preparation:** About 5 g of the powdered leaves were extracted with ethyl acetate by cold maceration, filtered and the filtrate concentrated to a small volume. A small spot was then applied to the TLC plate for analysis.

**Chromatographic conditions:** Analytical TLC on silica gel G60 F254, 0.25mm layer in hexane/ethyl acetate (7:3) as the mobile phase. A small spot was then applied to the TLC plate for analysis.

**Detection:** Visualized in daylight after spraying with anisaldehyde-sulphuric acid (Lane 1) and vanillin-sulphuric acid reagents (Lane 2) (Stahl, 1969), heating to 110°C for 10 mins.

The TLC chromatogram showed six prominent spots with R<sub>f</sub>s of 0.76 (pink), 0.68 (light blue), 0.61 (purple), 0.54 (yellow), 0.39 (pink) and 0.21 (yellow) when sprayed with both anisaldehyde and vanillin.



### High Performance Liquid Chromatography

**Sample preparation:** About 10 mg of the hydroethanolic extract of *C. procera* leaves were reconstituted in 3 mL acetonitrile in a 10 mL volumetric flask with sonication for 17 minutes. The solution was then diluted to volume with the mobile phase under chromatographic conditions. The resulting solution was then centrifuged to obtain a clear test solution, which was subsequently filtered through a 0.45 µm filter into an HPLC vial and analyzed.

## Chromatographic system

### Optimized chromatographic conditions

**Mode:** LC

**Column:** YMC ODS, 4.6 x 150 mm, 5  $\mu$ m

**Column temperature:** Ambient – 30°C

**Mobile phase:** Acetonitrile: water (60:40 v/v)

**Elution mode :** Isocratic

**Injection volume :** 20  $\mu$ L

**Flow rate:** 0.5 mL/minute

**Detection wavelengths:** 230 nm, 254 nm and 278 nm.

### System Suitability parameters

**Number of peaks:** 230 nm (1), 254 nm (1), 278 nm (2)

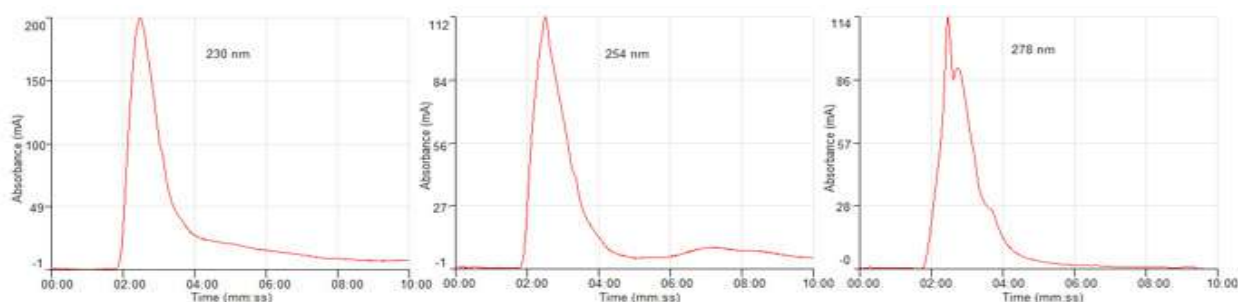
**Retention time (s):** 230 nm (2.28 min), 254 nm (2.30 min), 278 nm (rt1-2.28 min, rt2-2.45 min)

**Asymmetric factor(s):** 230 nm (0.892), 254 nm (0.990), 278 nm (af1-0.562, af2-1.647)

**Tailing factor:** NMT 2.0

**Efficiency:** 230 nm (48.40), 254 nm (36.63), 278 nm (E1- 953.37, E2-216.65)

**Acceptance criteria:** Sample solution of hydro-ethanolic crude extract of *C. procera* (Aiton) W.T.Aiton (leaves) conforms to the system suitability parameters.



## FT-IR

A small amount of the dried hydro-ethanolic extract (70%) was placed on the sample area of the Perkin Elmer UATR Fourier transform infrared (FT-IR) spectrometer and scanned between 4000-400  $\text{cm}^{-1}$  with a resolving power of 4  $\text{cm}^{-1}$  and a cumulative scanning limitation of 24 times. Principal peaks appeared at wavenumbers 3261.09, 2925.37 and 1587.24  $\text{cm}^{-1}$ .

## Macroscopy

The leaves are oblong to broadly obovate, cordate to clasping heart shape at the base, abruptly and shortly acuminate to blunt at the apex, up to 30 cm long and 15 cm broad, tough, glaucous and with no leaf stalk. The leaf blades are light to dark green with nearly white veins, slightly leathery and have a fine coat of soft hairs that rub off. Leaves are pubescent when young and glabrous on both sides on maturity. They have a waxy appearance.



## Microscopy

### Leaf

Consists of polygonal epidermal cells and anomocytic stomata with four to six subsidiary cells. Occasional cyclocytic stomata and trichome stumps are seen. Many of the trichomes are very long and twisted and form the white fluff seen on the surfaces of the leaves. They are easily broken off and cannot be seen whole. Upper surface consists of polygonal cells and anomocytic stomata. Cyclocytic stomata are absent. Transverse section shows three large sections of collateral vascular bundles which form an arc in the midrib. The leaf is dorsiventral; collenchyma within which secretory glands can be seen are both above and below the arc of vascular bundles.

### Powdered plant material

The powdered leaf is bright green in colour with a characteristic odour. Powder consists of fragments of upper and lower surface of leaf showing stomata and epidermal cells; unicellular fibres; annular xylem vessels; fragments of spongy mesophyll and palisade; tracheids and long twisted trichomes in contortous which are characteristic.

### Therapeutic actions

Antimicrobial, antioxidant, antidiarrhoeal, antiinflammatory antidiabetic, anthelmintic, antitumour, lactagogue.

### Therapeutic indications

Diarrhoea, inflammation, skin infections, worm infestations.

### Safety data

LD<sub>50</sub> of the aqueous leaf extract by oral route was estimated to be beyond 3000 mg/kg in rats. No signs of CNS depression/stimulation or autonomic effects were observed at doses of 0-1000 mg/kg. The extract did not cause target organ damage including the liver, kidney and the spleen. Calotropis did not stimulate RBC proliferation, nor the haemoglobin content. There was an increase in MCV and HCT, raising concern of possible induction of macrocytic anaemia. However, this observation did not reflect as a decrease in mean cell hemoglobin content or the RDW-CV. Calotropis extract did not affect WBC count, but significantly increased the proportion of MID cells in WBC cell counts. It caused a decrease in platelet count at all doses (0-1000 mg/kg). Liver transaminase enzymes were not affected by treatment with the aqueous extract. Total serum protein was reduced. Bilirubin levels did not change except at 1000 mg/kg, which showed an elevation. Calotropis caused a dose-dependent decrease in urea and a slight decrease in creatinine. Clotting time in rabbits increased marginally (> 300 mg/kg for 10 days). No histopathological changes were seen in the liver and kidney of treated animals. The increase in HCT (Packed Cell volume) and elevations in WBC corroborates well with earlier findings by Ajagbonna *et al.*, (1999). Some components of calotropis extract have been shown to be pro-inflammatory and immunomodulatory because they activate murine monocytes and macrophage to protect against microbial invasion. The increase in MID cells in the haematological studies may be related to this specific property of Calotropis. There were decreases in platelet counts in both studies, but further studies showed that it may prolong bleeding time marginally only with prolonged usage. Calotropis also affects serum proteins, and this may be linked to the decrease in serum urea and mild elevation in AST. Ajagbonna *et al.*, (1999) reports that the decrease in serum proteins may be due to an effect on albumin. However, the present findings suggests that the decrease was largely due to decreases in globulins.

### Precautions for use

Caution should be exercised during long-term use in patients prone to coagulation disorders and bleeding episodes. *C. procera* can increase the effects of digoxin if taken with it, and therefore should not be taken together. *C. procera* may have diuretic activity, which could affect lithium levels in the body by decreasing the excretion rate. When potassium is low in the body, the side effects from use of *C. procera* on the heart can increase. *C. procera* should be used with caution when stimulant laxatives such as bisacodyl, cascara, castor oil and senna and diuretics are being used.

### Adverse effects

*Calotropis* is not safe in doses beyond the recommended dose. At high doses it can cause diarrhoea, convulsions vomiting, slow heart beat and death (Mossa *et al.*, 1991).

### Contraindications

It is unsafe to use *C. procera* during pregnancy and breast-feeding. Do not use for children below six years of age.

### Dosage forms

Decoction, infusion, tincture, powders.

### Dosage

Decoction: 30 g of dried plant material in 900 mL water; simmer until reduced to 600 mL; 1-3 tablespoonfuls daily

Infusion: 30 g of dried aerial part in 600 mL of water; 3-4 teacups daily

Tincture: 1:5, 45% ethanol; 5 ml three times daily

### Storage

Store in a cool dry place away from light.

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**Botanical name***Cassia sieberiana***Family**

Fabaceae - Ceasalpinoideae

**Synonyms***Cassia kotshcyana* Oliv.**Common Names**

African laburnum, West African laburnum; drumstick tree (English) Casse du Sénégal; Casse de Siéber; casse flûte (French).

**Common Local names****Benin:** Fon-Alladamanondo, Yoruba-Efo, Dendi-Tina**Burkina Faso:** Dioula-Sinjan; Fufuldé-Gama; Moré-Balepsado**Côte d'Ivoire:** Baoulé - Diongobaka; Dioula-gbé, Malinké - Sissenouvo.**Gambia:** Madinka - Sinjango**Ghana:** Akan – Osanya; Mole – Aonga; Dagbani – Kul phariyo**Guinea:** Guéré - Zone vagma, Peuhl – Sindjagor; Soussou – Bamba**Guinea Bissau:** Creole-Babosa**Mali:** Bambara- Sinjan; Dogon- Irborolo; Peulh- Gana-fadahi; Malinké– Sinzan**Niger:** Djerma- Sinsan ; Haoussa – Thidiaye ; Peulh - Sinsangohi**Nigeria:** Hausa-Margaa ; Yoruba-Aridan ; Igbo-Ugba**Senegal:** Mandeng-Sindian; Diola-Busayet; Sérére-Selo selum**Sierra Leone:** Creole – Canafistra**Togo:** Akasselem – Mikeli; Ewé – Gatigati; Moba - Pangpapumu**Description of the plant**

*C. sieberiana* is a shrub or small tree growing up to 15-20 m tall. It has a fissured grey to brown bark with blackish stripes. The young branches bear dense short hairs. The leaves are paripinnate compound with 5-14 pairs of leaflets arranged spirally (Burkill, 1985). The leaflets are elliptical to ovate (3.5-10 cm x 2-5 cm) with a rounded to acute apex. The flower is an inflorescence (axillary pendulous raceme) up to 35 - 45) cm long, bisexual and slightly zygomorphic; bears 5-8 mm long slightly hairy elliptic sepals. The petals are oblong to almost circular, bright yellow about 2-3.5 cm long. There are 10 free stamens with 3 hooked at the base. The plant has a cylindrical black fruit with a pod 40-60 (-90) cm x 1.5 cm, transversely partitioned, dehiscent by two valves. It is many seeded embedded in a yellow pulp. The seeds are rusty to dark brown ellipsoid, 8-9 mm long (Schmelzer *et al.*, 2008; Burkill, 1985).



A and B – *Cassia sieberiana* DC, C – flowers, D- fruit, E – leaves

### Herbarium specimen number

Benin: 2347 (AP)  
 Burkina Faso: MSAD 669 (CNSF), 459 (AUO);  
 Côte d'ivoire: 16811 (CNF)  
 Ghana: GH 122/KNUST  
 Mali: 971DMT  
 Nigeria: UPFH 112  
 Senegal: IFAN 75  
 Togo: TG 12522

### Habitat and geographical distribution

It is widespread in Western tropical Africa extending from Senegal and Gambia in the West to DR Congo and Uganda in the East. Commonly found in wooded grassland and moist savannah areas, it thrives in lateritic soils, roadsides and gallery forest (Von Maydell, 1990).

### Plant material of interest

Root



### Other part used

Leaves, pods and stem bark

### Definition of plant material of interest

*Cassia sieberiana* root consists of the fresh or dried root bark and rootlets of *Cassia sieberiana* DC. (Fabaceae – Ceasalpinoideae)

### Ethnomedical uses

The entire plant is used as a purgative and diuretic. An infusion of the plant is given as a remedy to treat a number of childhood diseases in Senegal. Powder from different parts of the plant is applied to the teeth to treat toothache (Burkill, 1985). A mixture of the powder with butter is used for skin diseases. The powdered leaves are taken with food to manage gonorrhoea (Sam *et al.*, 2011), while an infusion of the leaves sweetened with honey is taken for stomachache, ulcers and diarrhoea. A steam bath of leafy twigs boiled in water is prescribed for malaria and fever. Boiled and squeezed fresh leaves are applied as poultice in burns. The twigs are used to treat sleeping sickness (Schmelzer *et al.*, 2008), while a decoction of the roots is used for haemorrhoids, schistosomiasis, leprosy, dropsy and bloody dysentery. In Cote d'Ivoire, the root decoction is used to treat intestinal worms including tapeworms. A small amount of dried root decoction is taken at the end of each meal for malaria prophylaxis. The root decoction is also used for body massage. Crushed roots are rubbed on the temples to treat headache (Mshana *et al.*, 2000), an an infusion of the root bark is used for venereal diseases, sterility and dysmenorrhoea. Capsules made from the root bark are prescribed for AIDS in Burkina Faso. The yellow pulp around the seeds and an infusion of the pods is taken as a laxative (Schmelzer *et al.*, 2008).

### Biological and pharmacological activities

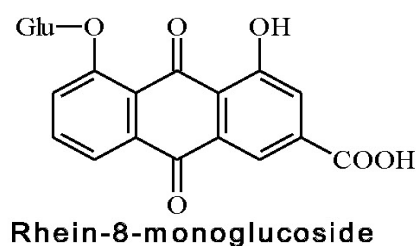
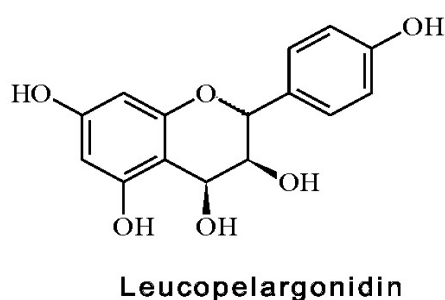
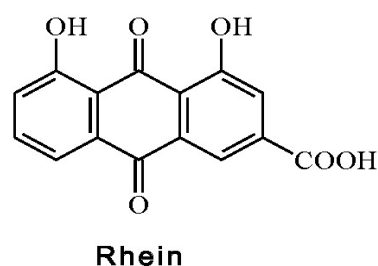
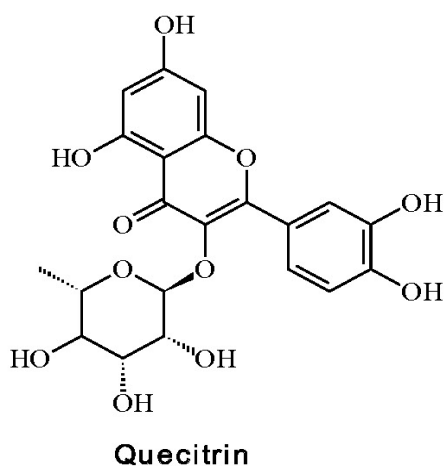
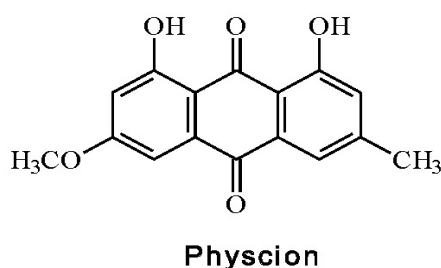
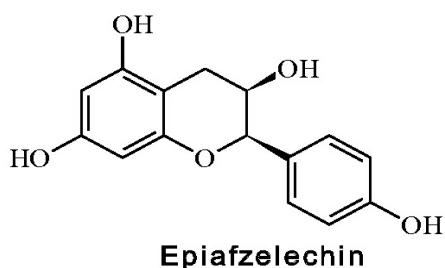
The plant has been explored for a wide range of pharmacological activities. Roots, stem and leaf extracts have been found to be active against *Staphylococcus lutea*, *Mycobacterium phlei*, *Bacillus subtilis* and *Proteus sp.*, but not *Staphylococcus albus*, *Pseudomonas aeruginosa* or *Escherichia coli*. They also showed antiviral activity against *Herpes simplex* virus type 1 (Schmelzer *et al.*, 2008). The laxative activities of the roots and stem bark of *C. sieberiana* was demonstrated in male albino rats using *Senna alexandrina* (the official senna leaf) as the reference standard. *C. sieberiana* roots exhibited 80% of the potency of *S. alexandrina* at 700 mg/kg body weight (Ajayi *et al.*, 2014). The purgative action of the plant was ascribed to its anthraquinones (Schmelzer *et al.*, 2008). Nartey *et al.* (2012) demonstrated the antioxidant and gastric cytoprotective properties of the root bark extract. The extract was found to possess significant ferric reducing antioxidant effect as well as hydroxyl radical scavenging activity. It also showed DPPH scavenging activity and dose-dependently prevented lipid peroxidation and free radical generation. The root bark extract dose-dependently increased gastric mucosal PGE<sub>2</sub> and PGI<sub>2</sub> levels, while aqueous root and leaf extracts demonstrated antinociceptive and antiinflammatory activities *in vivo*. *C. sieberiana* extract (10 - 40 mg/kg, *p.o.*) caused dose-dependent antinociceptive effects in rats on the hotplate. However, the analgesic action of *C. sieberiana* (40 mg/kg, *p.o.*) was less than that of morphine (Duwiejua *et al.*, 2007). In another study, the ethanolic root and stem bark extract showed antiplasmodial activity against chloroquine-sensitive strain of *Plasmodium berghei* NK65 in mice (Abdulrazak *et al.*, 2015).

### Clinical data

Not available

## Chemical constituents

Anthraquinones: physcion, rhein, chrysophanol (Sam *et al.*, 2011); flavones (quercitrin and isoquercitrin); phenolics: (-)-epiafzelechin (Waterman and Faulkner, 1979), epicatechol and leucopelargonidin (Paris and Etchepare, 1967).



## Test for identity and purity

**Moisture content:** air dried coarse powder does not lose more than 4.9% (root), 5.3% (leaves) and 4.8% (stem bark) at 105°C.

**Total ash:** not more than 7.9% w/w (root), 7.6% (leaves) and 4.0% (stem bark)

**Ash insoluble in acid:** not more than 5.15% (root) 1.8% (leaves) and 0.5% (Stem bark)

**Water soluble extractive:** not less than 20.5% (root), 11.0% (leaves) and 9.0% (stem bark)

**Ethanol soluble extractive (70%):** not less than 14.38% (root) 4.0% (leaves) and 8.0% (stem bark)

## Chromatographic fingerprint

### *Thin Layer Chromatography*

**Preparation:** About, 5 g of the powdered roots were extracted with ethyl acetate by cold maceration, filtered and the filtrate concentrated to a small volume. A small spot was then applied to the TLC plate for analysis.

**Chromatographic conditions:** Analytical TLC on silica gel G60 F254, 0.25 mm layer in hexane/ethyl acetate (7:3) as the mobile phase.

**Detection:** Visualized in daylight after spraying with anisaldehyde-sulphuric acid (Lane 1) and vanillin-sulphuric acid reagents (Lane 2) (Stahl, 1969), heating to 110°C for 10 mins.

The TLC chromatogram showed three prominent spots with R<sub>f</sub>s of 0.96 (pink), 0.87 (pink) and 0.59 (pink) when sprayed with both anisaldehyde and vanillin reagents.



### *High Performance Liquid Chromatography*

**Sample preparation:** About 10 mg of the hydroethanolic extract of *Cassia sieberiana* roots were reconstituted in 3 mL acetonitrile in a 10 mL volumetric flask with sonication for 17 minutes. The solution was then diluted to volume with the mobile phase under chromatographic conditions. It was then centrifuged to obtain a clear test solution which was subsequently filtered through a 0.45 µm filter into an HPLC vial and analyzed.

### Chromatographic system

#### Optimized chromatographic conditions

**Mode:** LC

**Column:** YMC ODS, 4.6 x 150 mm, 5 µm

**Column temperature:** Ambient – 30°C

**Mobile phase:** Acetonitrile: Methanol: Water (60:20:20 v/v/v)

**Elution mode :** Isocratic

**Injection volume :** 20 µL

**Flow rate:** 0.5 mL/minute

**Detection wavelengths:** 230 nm, 254 nm and 278 nm.

## System Suitability parameters

**Number of peaks:** 230 nm (1), 254 nm (1), 278 nm (2)

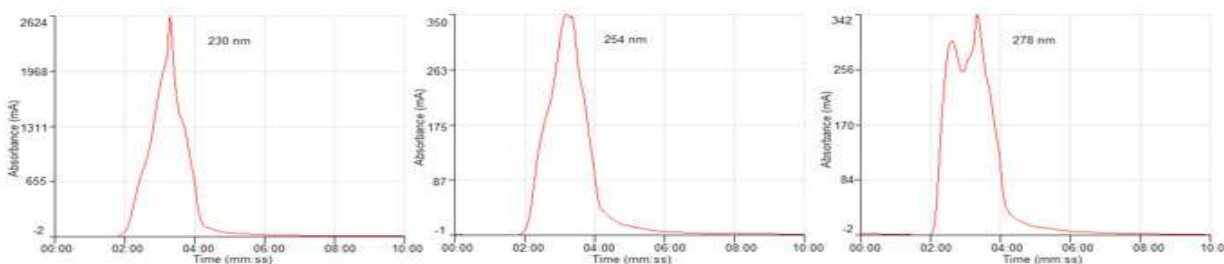
**Retention time (s):** 230 nm (3.17 min), 254 nm (3.12 min), 278 nm (rt1-2.36 min, rt2- 3.20 min)

**Asymmetric factor(s):** 230 nm (0.606), 254 nm (1.341), 278 nm (af1- 1.726, af2- 1.185)

**Tailing factor:** NMT 2.0

**Efficiency:** 230 nm (95.08), 254 nm (40.42), 278 nm (E1- 164.92, E2-197.37)

**Acceptance criteria:** Sample solution of hydroethanolic crude extract of *C. sieberiana* DC. (Root) conforms to the system suitability parameters.



## HPLC chromatogram of *Cassia sieberiana* hydroethanolic root extract

### FT-IR

A small amount of the dried hydroethanolic extract (70%) was placed on the sample area of the Perkin Elmer UATR Fourier transform infrared (FT-IR) spectrometer and scanned between 4000-400  $\text{cm}^{-1}$  with a resolving power of 4  $\text{cm}^{-1}$  and a cumulative scanning limitation of 24 times. Principal peaks appeared at wavenumbers 3240.41 and 1606.064  $\text{cm}^{-1}$ .

### Macroscopy

#### Root

The roots are cylindrical and branched, sometimes tortuous, with rootlets. The roots are dark brown in colour, with a fibrous fracture, and a bitter taste.

#### Leaf

The leaves are paripinnate compound with 5-14 pairs of leaflets arranged spirally. The leaflets are elliptical to ovate in shape with a rounded to acute apex. The leaflets have a short petiole, an entire margin and pinnate venation. Leaflets are a dark green colour on the upper surface, with a lighter shade on the undersurface. Leaves are glabrous and papery to touch.

### Microscopy

#### Leaf

Lower surface is covered with numerous straight unicellular trichomes, and anomocytic stomata. Upper surface has polygonal thick walled epidermal cells interspaced with cells in rows of two to four each containing a prism of calcium oxalate. There are curved unicellular, straight unicellular, and uniseriate trichomes. The midrib section of the transverse section of the leaf shows a vascular system which is almost circular, forming a deep arc. The xylem is surrounded by the phloem making the vascular system amphicribal. The midrib

starts with the epidermal cells followed by rows of polygonal collenchyma cells. The vascular system is completely lined by a row of calcium oxalate prisms. At the upper surface of the midrib, beneath the row of calcium oxalate prisms are phloem cells not bound by phloem fibres. At the centre of the midrib section, are parenchyma cells containing calcium oxalate, surrounded by xylem. Towards the lower surface are large collenchyma cells before the lower epidermal cells. The lamina has up to two rows of palisade cells, which are not uniform throughout the leaf lamina. The vascular bundle is surrounded by calcium oxalate prisms. Spongy mesophyll cells fill the lamina with little intercellular spaces.

### **Root**

### **Powdered plant material**

The powdered leaf is dark green in colour with a characteristic odour. It consists of bundles of phloem fibres with sheaths of prism crystals. The upper epidermis has fragments of polygonal cells. There are many single unicellular fibres with tapering ends; unicellular curved and numerous unicellular straight and many appressed trichomes; annular xylem vessels and fragments of spongy mesophyll occur.

### **Therapeutic actions**

Laxative, antimicrobial, antinociceptive, antimalarial.

### **Therapeutic indications**

Constipation, infections, malaria.

### **Safety data**

### **Animal studies**

LD<sub>50</sub> by oral route was estimated to be beyond 3000 mg/kg in rats. There were no signs of CNS depression/stimulation or autonomic effects in all the doses tested. No significant changes were noted with highly perfused organs such as the liver, kidney, heart and lungs. The relative organ to body ratios of spleen, thymus, and adrenals were not significantly affected by treatment with the aqueous extract. There were no significant changes in haematological parameters. Treatment with the aqueous extract did not affect liver enzymes, serum proteins or bilirubin concentration. Renal function remained normal. Pentobarbitone sleeping time did not change in treated animals compared to control groups. No histopathological changes were observed in major organs including the liver and the kidney. Other reports indicate that chronic administration (greater than 6 weeks) is associated with elevations in liver enzymes, urea and creatinine and significant weight losses in rats. Its threshold for hepatotoxicity appears to be lower than that of nephrotoxicity. Furthermore, due to its habitual use as a purgative and diuretic, there is a risk of dehydration especially in infants and geriatric patients. The presence of cardiac glycosides suggests that it may cause serum potassium alteration, which can be detrimental in some patients.

### **Precautions for use**

If drowsiness, dizziness, hypotension or a head ache is experienced when using *C. sieberiana*, driving or operating heavy machinery, may be ill-advised. If used for more than 6 weeks, there is a possibility of elevated liver enzymes, urea and creatinine and weight loss.

**Adverse effects**

May cause drowsiness, dehydration

**Contraindications**

Contraindicated in pregnant women and children under six years.

**Dosage forms**

Decoction, infusion, tincture, powders.

**Dosage**

Decoction: 30 g of dried plant material in 900 mL water; simmer until reduced to 600 mL; 1-3 tablespoonfuls daily

Infusion: 30 g of dried aerial part in 600 mL of water; 3-4 teacups daily

Tincture: 1:5, 45% ethanol; 5 ml three times daily

**Storage**

Store in a cool dry place away from direct heat and light

**References**

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**Botanical name**

*Chromolaena odorata* (L.) R.M. King & H. Robinson

**Family**

Asteraceae

**Synonyms**

*Eupatorium conyzoides* Vahl, *Eupatorium odoratum* L., *Osmia odorata* (L.) Schultz-Bip.

**Common Names**

Chromolaena, Armstrong's weed, bitter bush, Christmas bush, Jack in the bush, kingweed, paraffin bush, Siam weed (English); fleurit-Noël, herbe du Laos (French); chimuyo, crucito, hierba de chiva (Spanish) (PIER, 2003).

**Common Local names**

**Benin:** Yoruba – agatou ; Fon – Ketla

**Côte d'Ivoire:** Bété - Koussou; Malinké - Flota

**Ghana:** Akan-Acheampong

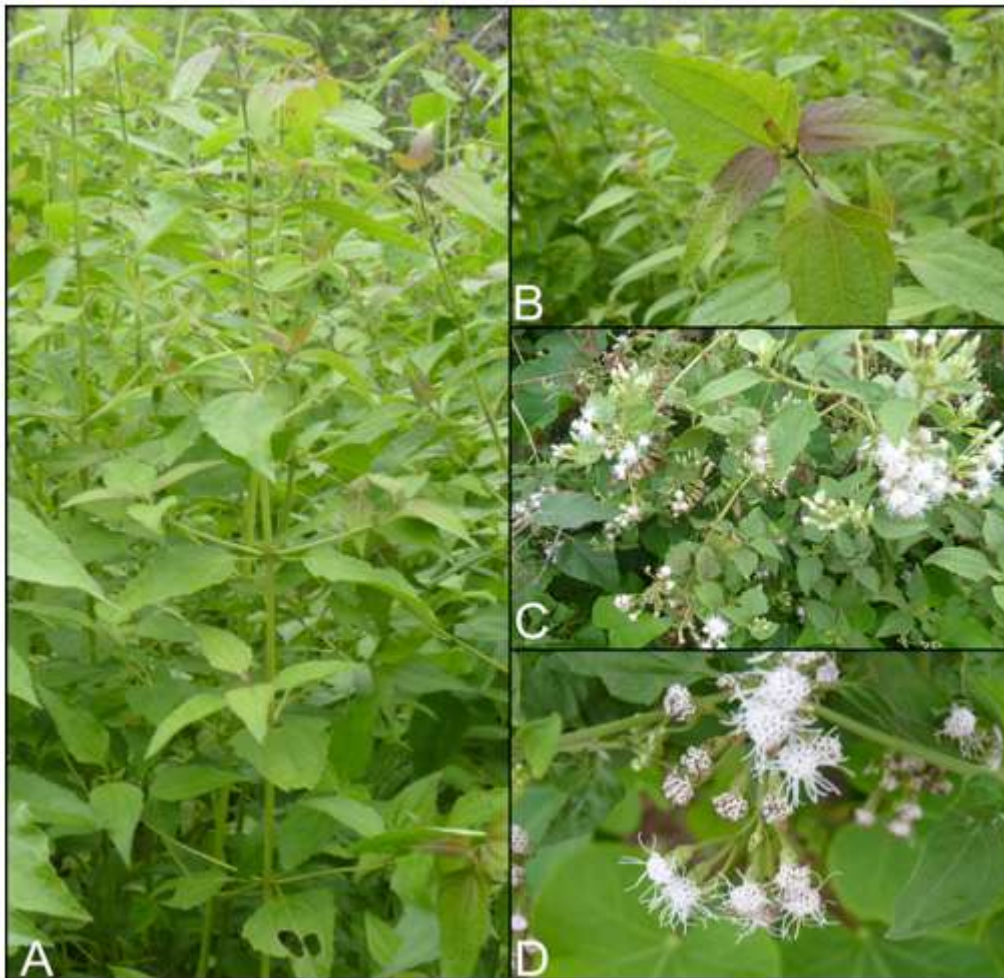
**Nigeria:** Igbo - Obu inenawa; Yoruba - Ewe Awolowo; Hausa - Obiarakara

**Togo:** Ewé - Lologu

**Description of the plant**

*Chromolaena odorata* is an erect or sprawling shrub forming thickets and usually growing 1.5 to 3 m tall in the open. However, it may grow higher (6-20 m) when climbing over trees and other taller vegetation (Cruttwell-McFadyen, 1989). The slender stems are generally yellowish-green and somewhat hairy (pubescent). These stems grow up to 7 m or more in length, and several are usually produced from the rootstock (crown). The stems branch freely, with lateral branches developing in pairs from the axillary buds. The older stems are brown and woody near the base; tips and young shoots are green and succulent (Cruttwell-McFadyen, 2004; Vanderwoude *et al.*, 2005). The root system is fibrous and does not penetrate beyond 20-30 cm in most soils (GISD, 2006). The oppositely arranged leaves (5-12 cm long and 3-7 cm wide) are triangular or egg-shaped in outline with an ovate base and acute apex. They are hairy (pubescent) on both surfaces and have serrated margins. These leaves are borne on stalks (petioles) up to 6 cm long (usually 10-15 mm), and give off a strong odour when crushed. The flower heads (capitula) do not have any petals and are borne in terminal panicles. These flower heads (about 10 mm long and 3 mm wide) are pale pink or pale mauve in colour (sometimes appearing whitish when older) and consist of numerous (15-30) tiny flowers. Several layers of overlapping slender bracts (an involucre) 8-9 mm long, surrounds the flowers (10-12 mm long). Each flower is borne on a stalk 10-30 mm long. The black or dark brown 'seeds' (achenes) are 4-5 mm long and topped with a ring (pappus) of white to brownish coloured hairs (5-6 mm long) (Navie and Adkins, 2008).





A – *Chromolaena odorata* (L.) R.M. King & H. Rob, B – leaves, C and D – flower

#### Herbarium specimen number

Benin : 2334 (AP)

Burkina Faso : CNSF-555

Cote d'Ivoire: 17825 (CNF)

Ghana: GH 134/KNUST

Nigeria: UPFH 113

Senegal : IFAN 3812

#### Habitat and geographical distribution

An opportunistic weed that invades tropical and subtropical regions. Commonly found on banks of watercourses, bushland, forest margins, roadsides, disturbed sites, waste areas, neglected pastures, crops and plantations (Gautier, 1992). It can grow on most soils, but prefers well-drained sites and will not grow in water-logged or saline soils (PIER, 2003). *C. odorata* is naturally distributed in tropical and southern Africa, tropical Asia and some oceanic islands with warm climates. It has recently been introduced to East Africa with the species been noted in parts of western Kenya, western Uganda and North-western Tanzania (GISD, 2006).

#### Plant material of interest

Leaves

## Other part used

Flowers

## Definition of plant material of interest

*Chromolaena odorata* consists of the fresh or dried leaves of *Chromolaena odorata* (L.) R.M. King & H. Robinson. (Asteraceae)

## Ethnomedical uses

The leaves are used as antibiotic, antimalarial and febrifuge in traditional medicine. An infusion of the leaves is taken to cleanse the blood (DeFilipps *et al.*, 2004). The young leaves are crushed, and the resulting liquid used to treat skin wounds. The leaves are used to treat eye disorders (Dansie *et al.*, 2009). *C. odorata* has been extensively studied for its profound wound healing activity (Ayyanar and Ignacimuthu, 2009). The stems and branches are crushed and combined with the wood-pulp of *Cecropia obtusa* and a seed of *Theobroma cacao*, kneaded in carapa oil, and locally applied in a plaster to treat wounds (DeFilipps *et al.*, 2004). It is mainly used by for the treatment of cuts and wounds. In Ghana, a leaf decoction is taken orally for the management of diarrhoea, while the leaf poultice is used for skin ulcers (Sam *et al.*, 2013).

## Biological and pharmacological activities

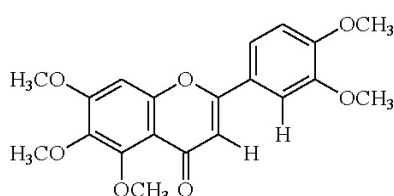
The methanolic leaf extract of *C. odorata* exhibited hypoglycaemic, hypolipidaemic, antianaemic and possibly immune-stimulating properties in alloxan-induced diabetic rats (Adedapo *et al.*, 2016). *In vitro* studies of leaf extracts demonstrated enhanced proliferation of fibroblasts, endothelial cells and keratinocytes, stimulation of keratinocyte migration, up-regulation of production by keratinocytes of extracellular matrix proteins and basement membrane components, and inhibition of collagen lattice contraction by fibroblasts (Afolabi *et al.*, 2007). Other studies demonstrated increased expression of several components of the adhesion complex and fibronectin by human keratinocytes (Phan *et al.*, 2000; Panda *et al.*, 2010). It was also found to accelerate haemostatic and wound healing activities by altering the expression of genes, including heme oxygenase-1 (HO-1), thromboxane synthase (TXS), and matrix metalloproteinase-9 (MMP-9) (Pandith *et al.*, 2013). The antioxidant property of the leaf extract, which helps in conserving the fibroblast and keratinocyte proliferation on the site, was found to contribute to its wound healing activity (Phan *et al.*, 2001; Mahmood *et al.*, 2005). This was demonstrated using lactate dehydrogenase release colorimetric assay involving cultured fibroblasts and keratinocytes. The antiinflammatory, analgesic and antipyretic activities of *Chromolaena* was demonstrated using the hot plate and formalin paw licking tests for analgesic activities; carrageenan paw oedema and cotton pellet granuloma for antiinflammatory activities; and Brewer's yeast induced pyrexia for antipyretic tests. The extract produced consistent analgesic, antiinflammatory and antipyretic activities (Ogunbiyi *et al.*, 2008). *C. odorata* was also found to exhibit antimicrobial activity against a number of gram positive and negative organisms (Taleb-Contini *et al.*, 2003). It was found to be particularly active against staphylococci with appreciable anti-fungal activity (Taleb-Contini *et al.*, 2003; Vital and Windell, 2009). It also showed moderate activity against *Mycobacterium tuberculosis* (Suksamrarn *et al.*, 2004). The dichloromethane/water extract of the plant showed anti-HSV-1 and antimalarial activity (Pisutthanan *et al.*, 2005). Oral intake of *Chromolaena* extract in combination with honey, was also found to be cytoprotective when used in stomach ulcer lesions in rats (Nur Jannah *et al.*, 2006).

## Clinical data

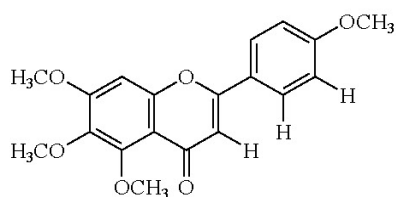
Not available

## Chemical constituents

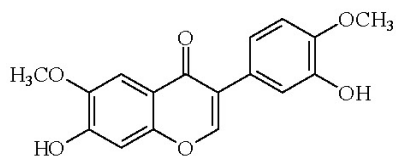
Sinensetin, scutellarein tetramethyl ether (Suksamrarn *et al.*, 2004; Atindehou *et al.*, 2013), 5-hydroxy-7,4'-dimethoxyflavanone, 2'-hydroxy-4,4',5',6'-tetramethoxychalcone, cadalene (Kouame *et al.*, 2013), odoratin, 3 $\beta$ -acetyloleanolic acid, ursolic acid, ombuin, 4,2'-dihydroxy-4',5',6'-trimethoxychalcone, (-)-pinoresinol, austrocortinin, tianshic acid, cleomiscosin D, (-)-medioresinol, (-)-syringaresinol and cleomiscosin A (Zhang *et al.*, 2012); flavonoids: akuranetin, persicogenin, 5,6,7,4'-tetramethoxyflavanone, 4'-hydroxy-5,6,7-trimethoxyflavanone, acacetin, luteolin, tamarixetin, eupatillin, kaempferide, protocatechuic acid; p-coumaric acid, p-hydroxybenzoic acid, ferulic acid, vanillic acid, sinensetin and rhamsetin (Phan *et al.*, 2001b; Suksamrarn *et al.*, 2004); essential oils:  $\alpha$ -pinene,  $\beta$ -pinene, germacrene D,  $\beta$ -copaen-4 $\alpha$ -ol, (E)-caryophyllene, and geijerene/ pregeijerene (Owolabi *et al.*, 2010).



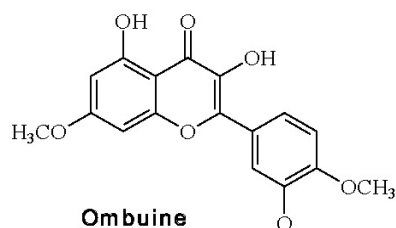
**Sinensetin**



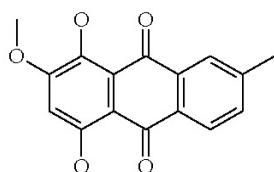
**Scutellarein tetramethyl ether**



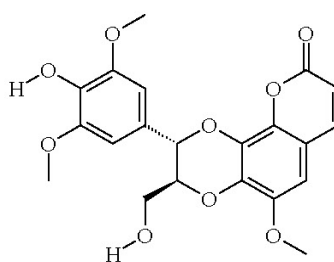
**Odoratin**



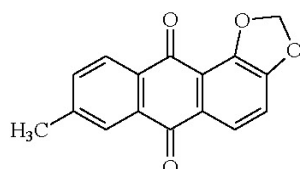
**Ombuine**



**Austrocortinin**



**Cleomiscosin D**



**1,2-methylenedioxy-6-methylanthraquinone**

## Test for identity and purity

**Moisture content:** air dried coarse powder does not lose more than 6.2% at 105°C.

**Total ash:** not more than 10.7%

**Acid insoluble ash:** not more than 2.2%

**Water soluble extractives:** not less than 9.0%

**Ethanol soluble extractives (70%):** not less than 3.0%.

### Chromatographic fingerprint

#### *Thin Layer Chromatography*

**Preparation:** About 5 g of the powdered leaves were extracted with ethyl acetate by cold maceration, filtered and the filtrate concentrated to a small volume. A small spot was then applied to the TLC plate for analysis.

**Chromatographic conditions:** Analytical TLC on silica gel G60 F254, 0.25 mm layer in hexane/ethyl acetate (7:3) as the mobile phase.

**Detection:** Visualized in daylight after spraying with anisaldehyde-sulphuric acid (Lane 1) and vanillin-sulphuric acid reagents (Lane 2) (Stahl, 1969), heating to 110°C for 10 mins.

The TLC chromatogram showed a prominent spot with Rf of 0.78 (pink) when sprayed with both anisaldehyde and vanillin. Three additional spots with Rfs of 0.59 (purple), 0.31 (yellow) and 0.12 (yellow) appeared in the chromatogram sprayed with anisaldehyde. Similarly, three additional spots with Rfs of 0.59 (ash), 0.31 (pink) and 0.22 (yellow) appeared in the chromatogram sprayed with vanillin.



#### *High Performance Liquid Chromatography*

**Sample preparation:** About 10 mg of the hydroethanolic extract of *C. odorata* leaves were reconstituted in 3 mL acetonitrile in a 10 mL volumetric flask with sonication for 17 minutes. The solution was then diluted to volume with the mobile phase under chromatographic conditions. It was centrifuged to obtain a clear test solution which was subsequently filtered through a 0.45 µm filter into an HPLC vial and analyzed.

## Chromatographic system

### Optimized chromatographic conditions

**Mode:** LC

**Column:** YMC ODS, 4.6 x 150 mm, 5  $\mu$ m

**Column temperature:** Ambient – 30°C

**Mobile phase:** Acetonitrile: water (60:40 v/v)

**Elution mode:** Isocratic

**Injection volume:** 20  $\mu$ L

**Flow rate:** 0.5 mL/min

**Detection wavelengths:** 230 nm, 254 nm and 278 nm.

### System Suitability parameters

**Number of peaks:** 230 nm (5), 254 nm (6), 278 nm (5)

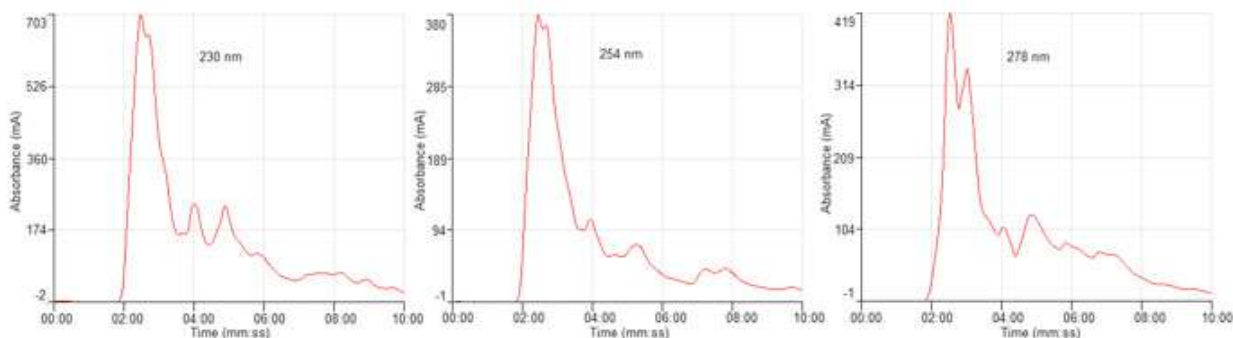
**Retention time (s):** 230 nm (rt1-2.28 min, rt2-2.42 min, rt3-4.00 min, rt4-4.53 min, rt5-5.46 min), 254 nm (rt1-2.27 min, rt2-2.40 min, rt3-3.56 min, rt4-5.16 min, rt5-7.15 min, rt6- 7.47 min), 278nm (rt1-2.31 min, rt2-3.01 min, rt3-4.03 min, rt4-4.51 min, rt5-5.51 min)

**Asymmetric factor(s):** 230 nm (af1-1.666, af2-1.963, af3-1.335, af4-0.892, af5-1.374), 254 nm (af1-1.712, af2-1.659, af3-0.979, af4-1.480, af5-1.579, af6-1.231), 278 nm (af1-1.243, af2-1.264, af3-1.600, af4-1.140, af5-1.189)

**Tailing factor:** NMT 2.0

**Efficiency:** 230 nm (E1-236.82, E2-876.32, E3-989.90, E4-1531.26, E5-1121.33), 254 nm (E1- 219.72, E2-1002.33, E3-1049.28, E4-724.08, E5-2207.64, E6-2184.28), 278 nm (E1-475.20, E2-496.18, E3-1059.30, E4-409.21, E5-4666.48)

**Acceptance criteria:** Sample solution of hydroethanolic crude extract of *C. odorata* (L.) R.M.King & H.Rob. (Leaves) conforms to the system suitability parameters.



### HPLC chromatogram of *Chromolaena odorata* hydroethanolic leaf extract

### FT-IR

A small amount of the dried hydroethanolic extract (70%) was placed on the sample area of the Perkin Elmer UATR Fourier transform infrared (FT-IR) spectrometer and scanned between 4000-400  $\text{cm}^{-1}$  with a resolving power of 4  $\text{cm}^{-1}$  and a cumulative scanning limitation of 24 times. Principal peaks appeared at wavenumbers 3252.01, 2927.86 and 1604.50  $\text{cm}^{-1}$

## Macroscopy

Leaves are 3-nerved from base and arrangement is opposite –decussate. The leaves are simple, with an obovate to deltoid-ovate shape. Leaf base is acute to truncate and the leaf margin is coarsely serrated. They are hairy (pubescent) on both surfaces and have serrated margins. The leaves are borne on stalks (petioles) up to 6 cm long (usually 10-15 mm), and give off a strong odour when crushed.

## Microscopy

Lower surface is made up of numerous anomocytic stomata with wavy walled subsidiary cells. The subsidiary cells are connected to more than one stomata. There are three to five subsidiary cells, with four celled glandular trichomes, unicellular, uniseriate trichomes (2-3), spread over the surface. The apex is elongated, pointed, short curled uniseriate, up to 4-celled trichomes. The upper surface is made up of less wavy, more polygonal angled epidermal cells, fewer glandular trichomes, longer uniseriate trichomes than on the lower surface with up to seven cells and curled uniseriate trichomes with up to five cells. The transverse section is kidney shaped, and the upper midrib section is concave. The ground tissue of the upper and lower portion of the midrib is collenchymatous. There are three groups of vascular bundles each consisting of a large, collateral (main) median vascular bundle, with a slightly lateral vascular bundle on either side. On the adaxial section are two small vascular strands, each with a few xylem and phloem elements. These also occur in the abaxial or lower section of the midrib. The mesophyll consists of short palisade layer of cells and spongy mesophyll tissue. The parenchyma cells surround the vascular bundle and are thin-walled. There are many trichomes arising from the epidermis; they are uniseriate, long, straight, some curved, and some having curled tips. They occur on both surfaces.

## Powdered plant material

Powdered leaf is dark green in colour with aromatic odour. Powder consists of many types of trichomes, uniseriate, warty, up to ten cells with acute apex, unicellular curved trichomes, four celled, unicellular and glandular trichomes. There are unicellular fibres with twisted ends, fragments of vessels, and wavy epidermal cells, anomocytic stomata with three to five subsidiary cells.

## Therapeutic actions

Haemostatic, wound healing, antimicrobial.

## Therapeutic indications

Wounds, ulcers, infections

## Safety data

LD<sub>50</sub> by oral route was estimated to be beyond 3000 mg/kg. There were no signs of CNS depression/stimulation or autonomic effects at doses of 0-1000 mg/kg. No significant changes were noted with highly perfused organs such as the liver, kidney, heart and lungs. The relative organ to body ratios of spleen, thymus, and adrenals were not significantly affected by treatment. Extract of *Chromolaena* did not significantly affect RBC count or indices. It did not induce leucocytosis, but affected the proportion of granulocytes and agranulocytes in WBC. Significant increases in MID cells and a decrease in lymphocytes were noted, but neutrophils were not affected with treatment. *Chromolena* appears to affect liver enzymes of treated rats. It significantly caused a decrease in ALP with mild elevations in AST. At the highest dose of 1000 mg/kg, it caused a decrease in GGT levels and serum proteins. Its effect on bilirubin may be more beneficial than

detrimental. In naïve rats, *Chromolaena* appears to cause a dose dependent decrease in total as well as conjugated and unconjugated bilirubin. Renal function was unaffected. Pentobarbitone sleeping times were slightly prolonged up to doses of 1000mg/kg. It did not affect urea, creatinine and no histopathological changes were observed in major organs including the liver and kidney. Chromolena appears to affect liver enzymes of treated rats even in naïve treated animals. This effect has been reported by other authors and could be partly due to its high phenolic content. ALP was particularly affected significantly in the study. There are concerns about the presence of pyrrolizidine alkaloids in inducing liver and kidney carcinomas. However, this effect was not seen in our study. Nuclear factor erythroid 2-related factor 2 (Nrf2) alters hepatic drug metabolizing enzymes and xenobiotic transporters. The decrease in liver enzymes caused by Chromolena extracts, and the ability of the compound chromomoric acid, to activate nuclear factor erythroid 2-related factor 2 (Nrf2), suggests that concomitant administration may cause some degree of interaction.

### Precautions for use

Oral intake of Chromolena should not be encouraged. Caution should be taken especially in combination with orthodox or other herbal remedies

### Adverse effects

Not known

### Contraindications

Should not be taken by people with liver problems even at safe doses.

### Dosage forms

Decoction, powder, juice from leaves, tincture

### Dosage

Decoction: 30 g of dried plant material in 900 mL water; simmer until reduced to 600 mL; 1-3 tablespoonfuls daily

Tincture: 1:5 in 45% alcohol; 5mL three times:

### Storage

Plant material should be stored in a cool and dry place in an amber bottle away from light.

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**Botanical name***Chrysanthellum indicum***Family**

Asteraceae

**Synonyms**

*Chrysanthellum americanum* (L.) Vatke; *Chrysanthellum americanum* auct., *Hinterhubera kotschyi* Sch. Bip. ex Hochst.

**Common Names**

Chrysanthellum, Grass with golden flowers, golden chamomile (English)

**Common Local names**

**Benin:** Fon - guv□ desa; Yoruba – Oyigi; Dendi - Kparoko natara

**Burkina Faso:** Bambara – Furakuna; Moré - Kaan-nao; Dioula - Timitimi

**Ghana:** Moore - Niba

**Mali:** Bambara-Tôri tèkè

**Niger:** Haoussa - Goshin ba'ana; Yorouba - Abilere

**Nigeria:** Yoruba – Abilere; Igbo - Agadi-isi-awo; Hausa - Zazargiwa mai yad'o

**Senegal:** Manding-fura kuna

**Description of the plant**

It is an annual plant, glabrous, with branching erect stems, slightly aromatic and about 30 cm high. Leaves are alternate, bi- or tripennatized, 3 to 5 cm long and 2.5 to 4 cm wide. The general outline of the limb is triangular, 3 cm to 5 cm long and of equivalent width. The lobes are mucronate at the top and each has a midrib. The petiole varies from 2 cm to more than 4 cm. Inflorescences are bright yellow, axillary and terminal, capitula borne by peduncles 0.5 to 6 cm long; involucre bracts in 1 or 2 series; 8 to 12 yellow or orange-yellow peripheral florets, with bidentate ligule; internal yellow flowers. Winged achenes, 2 to 5 mm long. This annual herb has a thin stem about 15 cm and leaves in small numbers, cut and alternated (Chapano and Mugarisanwa, 2003).



A, B,C: Aerial part of *Chrysanthellum indicum*

#### Herbarium specimen number

Benin: 2349 (AP)

Burkina Faso: BUR-285 (OAU); 785 (OAU), CNSF-645

Côte d'Ivoire: CNF 19483

Mali: 806/DMT

Nigeria: KASU/PCG.091

Senegal: IFAN 187

#### Habitat and geographical distribution

Native to tropical America, *Chrysanthellum* grows wild in Africa and South America. Pantropical species, represented in tropical and southern Africa, in Madagascar, especially in the Sudano-Guinean region. It is found in the lowlands in the Sudanian zone. The species is indicative of well-structured and fertile soils; generally prefers soils with sandy upper horizons (Le Bourgeois and Merlier, 1995). *C. indicum* is easily propagated by seeds, which are usually harvested between September and October. They are kept in jars and sown on the fly at the beginning of the winter season. The species prefers mostly sandy soils.

#### Plant material of interest

Whole plant

## Other part used

Flowers

## Definition of plant material of interest

*Chrysanthellum indicum* consists of the whole plant fresh or dried of *Chrysanthemum indicum* DC (Asteraceae) collected after flowering.

## Ethnomedical uses

*Chrysanthellum* is a very good hepatoprotector and is useful in cases of food or alcohol poisoning. It is also used to treat cirrhosis and viral hepatitis. Being a choreletic, this plant promotes the secretion of bile, stimulates the digestive system and plays a detoxifying role. It is antilithiasis and is particularly effective against kidney and gallstones, reducing its size and facilitating evacuation. It is also effective against vascular diseases such as heavy legs, rosacea, arteritis, varicose veins and haemorrhoids. It is a potent hyperlipidaemic, which in combination with a proper diet, lowers cholesterol levels.

## Biological and pharmacological activities

Virtually unknown some decades ago, *C. indicum* could be a major hepatovascular remedy, endowed with significant antilithiasis, hypoglycaemic and anti-inflammatory properties. *C. indicum* showed hypolipidaemic activity compared to clofibrate used as a reference drug, with more dramatic results on triglycerides than cholesterol (Lievre *et al.*, 1984). According to Nacoulma-Ouédraogo, (1996), the decoction of leafy stems is used to treat gonorrhoea, yellow fever, haematuric jaundice, dystonia, alcoholism, anuria, malaria, gallstones, renal colic, urolithiasis, dyspepsia and intestinal fermentations. *Chrysanthellum* acts on the microcirculation of capillaries and thus treats heavy legs, rosacea and retinal disorders. A study of the vasculotropic properties of the plant showed it to be only slightly active, per os, in the rat, although superior to rutin and its derivatives; the results in humans were significantly better (Glawe, *et al.*, 1979; Lievre and Guillot, 1983). *C. indicum* had significant effects on microcirculation similar to vitamin P. It also had a vasodilating activity, acting directly on the vascular wall (Ghédira and Goetz, 2017). The plant extract showed weak anti-oedematous activity in rat when administered orally, but proved to be superior to that of phenylbutazone and aspirin when administered peritoneally (Glawe *et al.*, 1979). Similarly, oral administration of the extract showed weak antiemetic activity (Lievre and Guillot 1983; Glawe *et al.*, 1979; Combiér *et al.*, 1977). Intravenous administration of *C. americanum* produced hypotensive and bradycardic effects (Lievre and Guillot, 1983). A concentration dependent contraction of the rabbit jejunum, guinea pig ileum, and rat duodenum was produced by aqueous extract of *C. indicum* DC. (subsp. *Afroamericanum* B.L. Turner). The contraction evoked by the extract was abolished by atropine but was not attenuated by mepyramine and pirenzepine. These actions suggest the presence of constituents in the extract whose action are mediated through muscarinic receptors. Intraperitoneal LD<sub>50</sub> of the extract in mice was 282.2 ± 5.2 mg/kg (Amos *et al.*, 2000). Aqueous extract of *C. indicum* (CI) was studied on calcium activation and mobilization using the rat portal vein. The extract showed a concentration-dependent contraction of the portal vein. Potassium chloride (KCl), norepinephrine and chloride (Cl) evoked sustained contraction of the portal vein. The contractions evoked by these agents were reduced significantly in calcium-free medium. The times-to-peak of KCl, NA and Cl were similar in normal physiological salt solution (PSS), but in Ca-free medium, the times-to-peak for KCl and Cl were greatly increased. The contractions induced by Cl were blocked by verapamil, but not inhibited by chlorpropamide and prazosin. It was suggested that the aqueous extract utilizes extracellular calcium pools to bring about contractile response which might be mediated through the activation of potassium -sensitive channels (Amos *et al.*, 2003). The methanolic extract of *C. indicum*

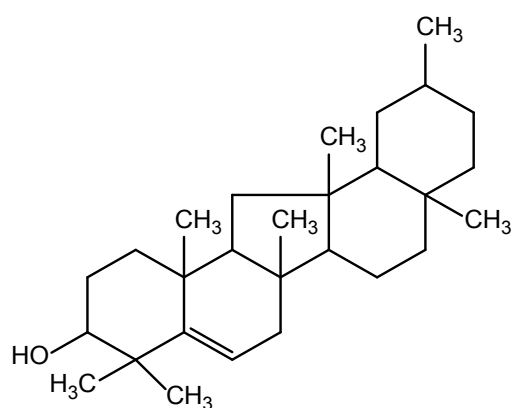
Linn. was evaluated for its behavioural effects on spontaneous motor activity (SMA), amphetamine and apomorphine-induced stereotype behaviour, pentobarbitone-induced hypnosis, exploratory activity and haloperidol-induced catalepsy in mice and rats. The extract significantly decreased spontaneous motor activity (SMA), antagonized apomorphine, and amphetamine-induced stereotyped behaviour in a dose dependent manner. There was no effect on the onset of pentobarbitone-induced sleep, but it significantly prolonged its duration and enhanced haloperidol-induced catalepsy. Exploratory activity was decreased in mice and had no effect on motor coordination. Results showed that the methanol extract of *C. indicum* contains psychoactive substance(s) with potential antipsychotic properties. The LD<sub>50</sub> in mice, intraperitoneal and oral acute toxicity values were found to be 288.5 and 2154 mg/kg body weight respectively (Yaro *et al.*, 2007). Intraperitoneal administration of 100, 200 and 400 mg/Kg of the methanolic extract in alloxan-induced diabetic Wistar rats showed no significant difference in blood glucose levels at day 1 when compared to negative control. However, after 3, 5, 7 and 9 days of extract administration, there was a significant ( $p < 0.05$ ) decrease in glucose levels for all three doses when compared to negative control (normal saline). Three doses of extracts administered showed both hypoglycaemic and antihyperglycaemic ( $P < 0.05$ ) effects in Wistar rats (Tanko *et al.*, 2011).

### Clinical data

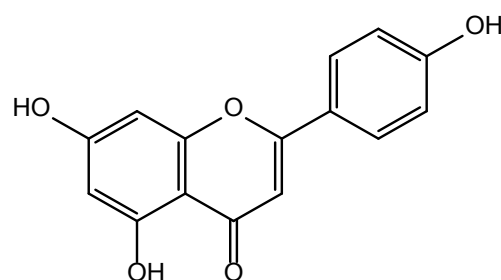
A study in humans to investigate the hepatoprotective effect of *C. indicum* showed significant improvement in liver function (Lievre and Guillot, 1983). When several patients with lithiasis (cystine lithiasis) were treated with *Chrysanthellum* extracts, chronic patients had no relapse after eight (8) months of treatment (Becchi *et al.*, 1979). *C. indicum* has a well-documented effect on vascular wall permeability, with the ability to increase the mechanical resistance of capillaries. The efficacy and safety of a cream containing 1% *C. indicum* extract with vitamin P properties were investigated in the treatment of rosacea. The study included 246 patients having clinically diagnosed moderate rosacea. Patients were randomly allocated to *C. indicum* extract-based cream ( $n = 125$ ) and placebo ( $n = 121$ ) groups. Patients applied the cream to their face for 12 weeks twice daily. The patients were examined at the end of each 4-week period. On days 0, 28, 56 and 84, the severity of erythema, surface of erythema, and overall severity of rosacea were recorded. Treatment with the *C. indicum* extract-based cream resulted in significant improvement ( $P < 0.05$ ) in severity of erythema, and overall rosacea severity compared with baseline and placebo. The investigator and patient overall efficacy assessment scores were significant ( $P = 0.046$  and  $P = 0.001$ , respectively) compared with placebo scores. Adverse reactions were not different from those observed with the placebo group. It was concluded that *C. indicum* extract-based cream is an effective and well-tolerated topical agent for the treatment of moderate rosacea. The mode of action of the extract suggested that additional efficacy might be expected from combination with other topical treatments (Rigopoulos *et al.*, 2005).

### Chemical constituents

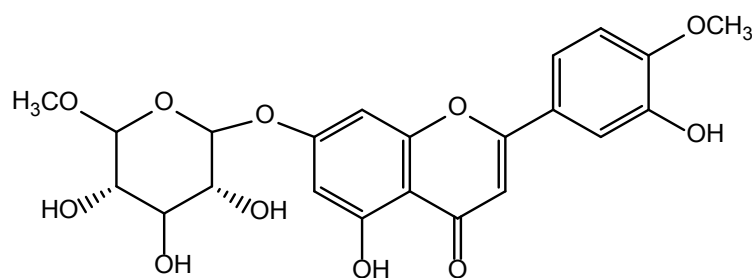
Flavonoids: apigenin, acacetin-7-O-beta-D-glucopyranoside and apigenein-7-O-beta-D-glucopyranoside (Lu *et al.*, 2009), chrysanthellins A and B, maritimetine, marin, chrysenol (Momoh and Idris, 2014), eriodictyol and flavonomarine; saponosides; caffeic acid derivatives chlorogenic acid, caffeic acid and quinic acid depside; essential oil (chrysanthenone); alkaloids steroids (Van Der Ploeg and Heuvelink 2006).



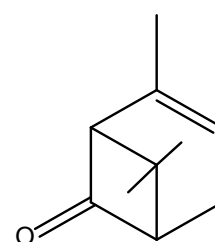
Chrysenol



Apigenin



Acacetin-7-O-beta- D-glucopyranoside



Chrysanthenone

### Test for identity and purity

**Moisture content:** air dried coarse powder does not lose more than 7.3% (leaves), 6.0% (stem bark), 5.0% (roots) at 105°C.

**Total ash:** not more than 5.4% (leaves), 6.8% (stem bark) and 4.4% (roots)

**Acid insoluble ash:** not more than 0.5% (leaves), 0.2% (stem bark), 0.5% (roots)

**Water soluble extractive:** not less than 8.0% (leaves), 7.0% (stem bark), 11% (roots)

**Ethanol soluble extractive (70%):** not less than 8.0% (leaves), 7.0% (stem bark), 18.0% (roots) at 105°C.

### Chromatographic fingerprint

#### Thin Liquid Chromatography

**Preparation:** About 5 g of the powdered aerial part were extracted with ethyl acetate by cold maceration, filtered and the filtrate concentrated to a small volume. A small spot was then applied to the TLC plate for analysis.

**Chromatographic conditions:** Analytical TLC on silica gel G60 F254, 0.25 mm layer in hexane/ethyl acetate (7:3) as the mobile phase.

**Detection:** Visualized in daylight after spraying with anisaldehyde-sulphuric acid (Lane 1) and vanillin-sulphuric acid reagents (Lane 2) (Stahl, 1969), heating to 110°C for 10 mins.

The TLC chromatogram showed five prominent spots with R<sub>f</sub>s of 0.83 (pink), 0.80 (purple), 0.70 (purple), 0.65 (yellow) and 0.59 (pink) when sprayed with both anisaldehyde and vanillin.



### High Performance Liquid Chromatography

#### Sample preparation

About 10 mg of the hydroethanolic extract of *C. indicum* aerial part were reconstituted in 3 mL acetonitrile in a 10 mL volumetric flask with sonication for 17 minutes. The solution was then diluted to volume with the mobile phase under chromatographic conditions. It was centrifuged to obtain a clear test solution, which was subsequently filtered through a 0.45  $\mu$ m filter into an HPLC vial and analyzed.

#### Chromatographic system

##### Optimized chromatographic conditions

**Mode:** LC

**Column:** YMC ODS, 4.6 x 150 mm, 5  $\mu$ m

**Column temperature:** Ambient – 30°C

**Mobile phase:** Acetonitrile: water (60:40 v/v)

**Elution mode :** Isocratic

**Injection volume :** 20  $\mu$ L

**Flow rate:** 0.5 mL/minute

**Detection wavelengths:** 230 nm, 254 nm and 278 nm.

#### System Suitability parameters

**Number of peaks:** 230 nm (1), 254 nm (2), 278 nm (2)

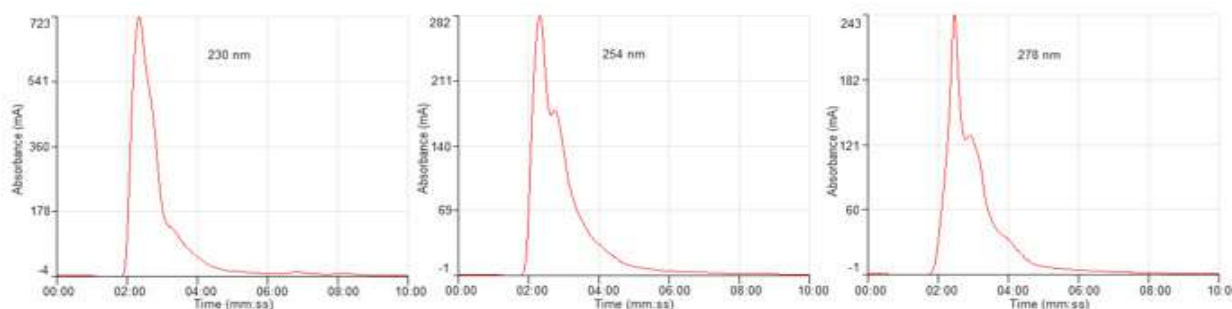
**Retention time (s):** 230 nm (rt1-2.20 min), 254 nm (rt1-2.18 min, rt2-2.44 min), 278 nm (rt1-2.27 min, rt2-2.54 min)

**Asymmetric factor(s):** 230 nm (af1-1.372), 254 nm (af1-1.426, af2-1.919), 278 nm (af1-0.616, af2-1.440)

**Tailing factor:** NMT 2.0

**Efficiency:** 230 nm (E1-67.16), 254 nm (E1-182.54, E2-366.21), 278 nm (E1-420.54, E2-1234.30)

**Acceptance criteria:** Sample solution of hydro-ethanolic crude extract of *C. indicum* DC. Aerial part conforms to the system suitability parameters.



## FT-IR

A small amount of the dried hydroethanolic extract (70%) was placed on the sample area of the Perkin Elmer UATR Fourier transform infrared (FT-IR) spectrometer and scanned between 4000-400  $\text{cm}^{-1}$ , with a resolving power of 4  $\text{cm}^{-1}$  and a cumulative scanning limitation of 24 times. Principal peaks appeared at wavenumbers 3279.29, 2927.86, 1567.13 and 1370.80  $\text{cm}^{-1}$ .

## Microscopy

### *Powdered plant material*

Powdered material is brown in colour, with characteristic odour of the flowers. The powder is characterised by numerous fragments of petals of the flower showing hexagonal epidermal cells with numerous calcium oxalate prisms with irregular shape, other fragments showing rows of canal like tubes filled with brownish content interspersed with rows of clear cells; fragments of filament of the stamens with thick rectangular cells with dark walls. Numerous fragments of petiole of leaf and flowers showing groups of fibres and annular and spiral vessels; fragments of trichomes showing uniseriate appressed trichomes with acute apex; fibres occur singly and in groups, they are unicellular and have acute apex; fragments of the herbaceous stem showing a central vascular bundle in longitudinal section with rectangular parenchymatous cells.

### Therapeutic actions

Antipsychotic, anitlithic, hypolipidaemic, hepatoprotective.

### Therapeutic indications

Insufficiency of biliary secretion; hepatic poisoning of various origins; digestive disorders due to excess food and alcoholism; pre-cirrhosis; compensated cirrhosis; lithiases; retinal or choroidal diseases of vascular origin; vascular fragility and permeability; enterocolitis; rosacea; circulatory problems; neuropsychiatric diseases.

### Safety data

The  $\text{LD}_{50}$ , by oral route, was estimated to be beyond 3000 mg/kg in rats. Central and autonomic nervous systems were not affected by treatment with the aqueous extract. There was no evidence of damage to the liver, kidney, heart, lungs, spleen, thymus, and adrenals following treatment at doses of 0-1000 mg/kg). Extract of *C. indicum* did not significantly affect RBC count or indices. It did not affect total WBC but the proportion of MID cells within WBC appeared to have been elevated. There was no change in platelet count in treated rats, orenzyme markers of liver damage, serum proteins and bilirubin levels. Pentobarbitone sleeping time also did not change in animals treated at doses of up to 1000 mg/kg. This plant appears to be



relatively safe in normal doses. It is unlikely to affect organ systems in the body within recommended doses.

### Precautions for use

Because of its sesquiterpene lactone content, *Chrysanthellum* is not recommended for persons allergic to plants of the Asteraceae family (Honore-Thorez, 1985; Jung, 2005).

### Adverse effects

Nausea, vomiting, headache or stomach pain, and benign biliary colitis.

### Contraindications

*Chrystanthellum* is contraindicated in children under 6, pregnant women and people with biliary dyspepsia.

### Dosage form

Powder, decoction, infusions, tincture, creams

### Dosage

Decoction: 30 g of dried plant material in 900 mL water; simmer until reduced to 600 mL; 1-3 tablespoonfuls daily

Infusion: 30 g of dried aerial part in 600 mL of water; 3-4 teacups daily

Tincture: 1:5, 45% ethanol; 5 ml three times daily

### Storage

Store in cool, dry place away from light.

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**Botanical name**

*Citrus paradisi* Macfadyen

**Family**

Rutaceae

**Synonyms**

None

**Common names**

Grapefruit, Melogold grapefruit (English); Pamplemousse (French); Toranja (Portugese).

**Common Local names:**

Benin: Fon/Goun-Gbo

Burkina Faso: Dioula - Lèmùrùkuna

Ghana: Fante - Ankama, Twi – Ankaadwea; Ewe - Mumoe

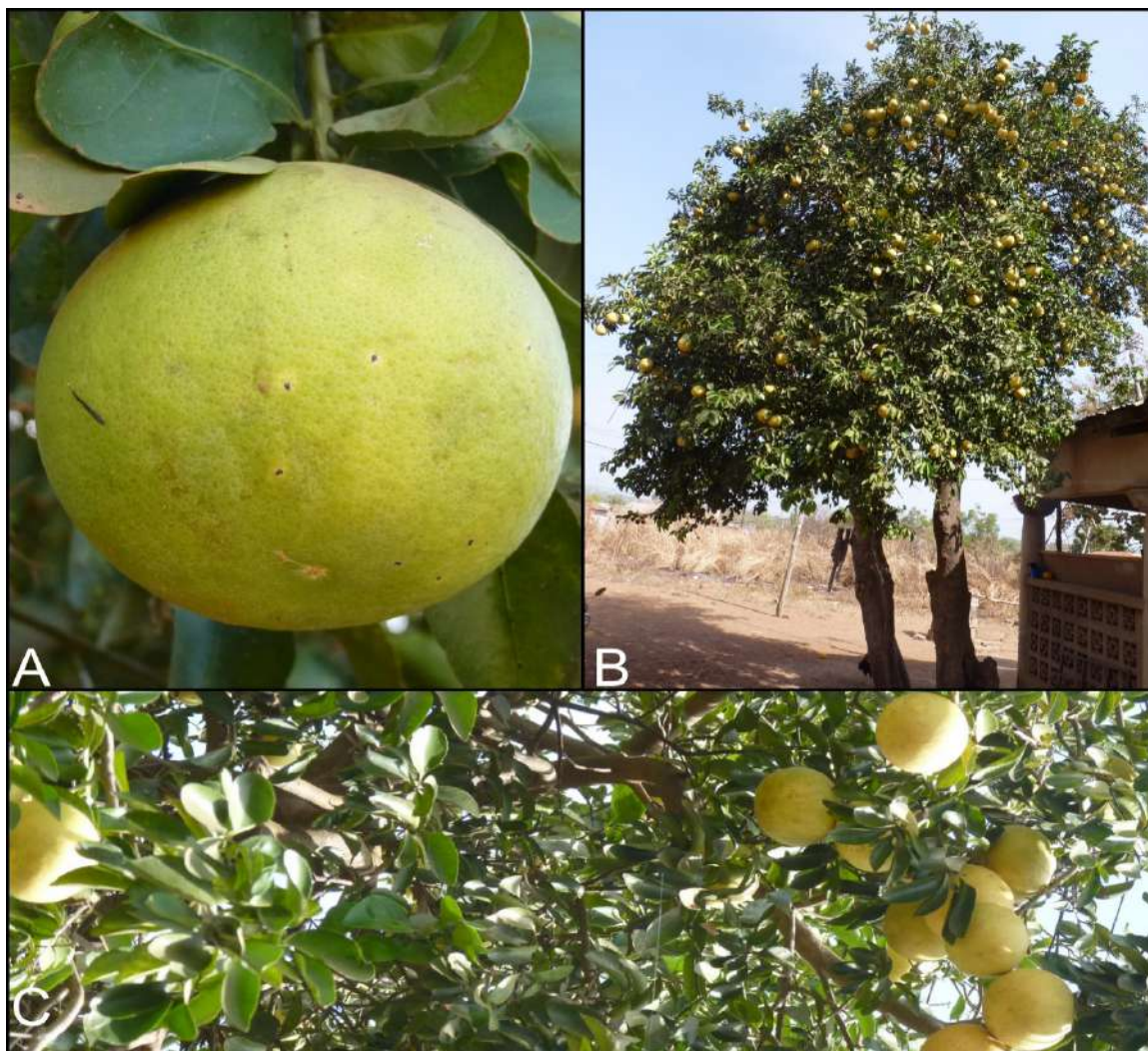
Niger : Hausa-Lémou ; Djerma-Lémou kaina

Nigeria: Hausa – Garehul; Yoruba - eso girepufurutu; Igbo - mkpuru osisi grepu

Togo: Ewé – Agbaklonti

**Description of the plant**

*Citrus paradisi* tree reaches up to 4.5-6 m and even 13.7 m with age. It has a rounded top of spreading branches. The evergreen leaves are ovate, 7.5-15 cm long and 4.5-7.5 cm wide, dark green above, lighter beneath, with minute, rounded teeth on the margins. Leaves are dotted with tiny oil glands and the petiole has broad, oblanceolate wings (Duke *et al.*, 2012). The white, 4-petalled flowers are 4.5-5.0 cm across and borne singly or in clusters in the leaf axis. Fruits are nearly round or oblate to slightly pear shaped, 10-15 cm wide with smooth finely dotted peel, 1.0 cm thick, pale-lemon, sometimes blushed with pink, and outwardly aromatic. The flowers are white spongy and bitter inside, the centre may be solid or semi-hollow, divided into about 18 boxes or more that can be detached separately. Some fruits are seedless. Some may have up to 90 white, elliptical, pointed seeds about 1.25 cm in length (Gupta *et al.*, 2010a).



A – *Citrus paradise* fruit, B- *Citrus paradise* tree, C- fruits and leaves

#### Herbarium specimen number

Benin: 2352 (AP)

Côte d'ivoire 1765B (CNF)

Ghana: GH 194/KNUST

Mali 2250 DMT

Nigeria: UPFH 114

#### Habitat and geographical distribution

Grape fruit is native to the island of Barbados. Other varieties of grapefruit were developed mainly in Florida and Texas USA. Grapefruits are commercially grown in Spain, Morocco, Israel, Jordan, South Africa, Brazil, Mexico, Jamaica and Asia (Gupta *et al.*, 2010a).

#### Plant material of interest

Leaves

#### Other parts used

Fruit, seed, peel

## Definition of plant material of interest

*Citrus paradisi* Macfad is the fresh or dried leaves of *Citrus paradisi* Macf. (Rutaceae)

## Ethnomedical uses

The plant has been used in ethnomedicine as antibacterial, antifungal, antiinflammatory, antioxidant, antiviral, astringent, preservative, anti-cancer, and for cellular regeneration, lowering of cholesterol, cleansing, detoxification, rheumatoid arthritis and weight loss (Gupta *et al.*, 2010a). *C. paradisi* has also been used traditionally to reduce stress and anxiety (Gupta *et al.*, 2010b). The fruit peels have been used in traditional medicine in Sudan to treat catarrh and malaria. Grapefruit seed is used in the treatment of urinary tract infections (Osungunna and Onawunmi, 2016).

## Biological and pharmacological activities

The essential oil of the fruit peel of *C. paradisi* has been shown to possess insecticidal effects against *Aedes aegypti* and *Aedes albopictus*, with LC<sub>50</sub> of 47.3 ppm and 85.1 ppm respectively (Ivoke *et al.*, 2016). Antimicrobial assays of the oil against *Paenibacillus larvae* gave Minimum inhibitory concentration (MIC) and Minimal bactericide concentration (MBC) of 385.0 mg/l and 770.0 mg/L respectively (Fuselli *et al.*, 2008). When the aqueous and methanolic extracts of the unripe fruits were tested against *Salmonella typhi* with MICs of 0.1 mg/ml and 0.01 mg/ml respectively, the methanolic extract was found to be more active than the aqueous extract. Mixtures of the extract with extracts of unripe *Carica papaya* fruit, *Citrus aurantifolia*, *Gossypium* species leaves, *Cocus nucifera* chaff, *Ananas sativus*, *Euphorbia heterophylla*, *Carica papaya* brown leaves and *Cymbopogon citratus*, decreased the MIC to 0.01 and 0.0001 mg/ml in the aqueous and methanolic extracts respectively (Oluduro and Omoboye, 2010). Essential oil tested against *Propioni bacterium acne* gave MBC of 0.25% v/v (Zu *et al.*, 2010). Peel oil also showed effectiveness against *Escherichia cloacae*, *Streptococcus* sp. and *P. fluorescence* (Javed *et al.*, 2011). When the essential oil was tested against five strains of *Staphylococcus*, the oil showed bactericidal activity at 2-4% v/v, with limited or no activity against biofilm formation (Adukwu *et al.*, 2012). Ethanol extracted seed oil tested against *Aedes albopictus* larvicidal activity was found to have LD<sub>50</sub> of 1,322.23 ppm at 24 hours, 998.03 ppm at 48 hours and 645.25 ppm at 72 hours (Hafeez *et al.*, 2011). Oil from the peel of *C. paradisi* variety *shamber* showed activity against five pathogenic bacterial strains viz *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Proteus vulgaris*, *Staphylococcus epidermidis* and two fungi *Aspergillus flavus* and *Trichophyton alba* (Khan *et al.*, 2012). Oil extracted by soxhlet extraction was tested against *Escherichia coli* on chicken nuggets. It was found that with increasing concentration of the oil, the MIC decreased (Imran *et al.*, 2013). Hydrodistilled oil from the peel of *C. paradisi* was tested against clinical bacterial isolates *Bacillus cereus*, *Enterococcus faecalis*, *Escherichia coli*, *E.coli* ATCC 25292, *Klebsiella pneumonia*, *Pseudococcus* sp, *Salmonella typhimurium*, *Shigella flexneri*, *Staphylococcus aureus*, *Staphylococcus aureus* ATCC 29213 and fungal isolates *Aspergillus niger*, *Candida albicans* and *Penicillium chrysogenum*. Inhibition of the test isolates depended on the solvent used to dissolve the oil. Methanolic oil mixture inhibited all the isolates, while ethanolic mixture inhibited the bacteria and *C. albicans* (Okunnowo *et al.*, 2013).

Steam distilled oil of grapefruit peel was evaluated against *Aedes aegypti* for ovicidal and larvicidal potency. Egg hatching was completely inhibited at 400 ppm and development of 1st to 2<sup>nd</sup> larval stage was inhibited at 100 ppm. The LC<sub>50</sub> and LC<sub>90</sub> values obtained for 2<sup>nd</sup> instar larval stage were 180.460, 334.629 ppm, respectively and for 4<sup>th</sup> instar 210.937 and 349.489 ppm respectively after a 24-hour exposure (Ivoke *et al.*, 2013). Cold pressed oil and hydrodistilled oil were not active against *Pseudomonas aeruginosa* at 20 mg/ml, but were found very active against *S. enterica* subsp even when compared with streptomycin (Ou *et al.*, 2015). Oil tested against *Anopheles stephensi* larvae, for its larvicidal activity, gave LC<sub>50</sub> and LC<sub>90</sub> against four instar larvae at 35.71 ppm and 70.23 ppm respectively. At 80 ppm, more than 90% of the larvae

were killed (Sanel-Dehkordi *et al.*, 2016). In another study, oil extracted by cold pressing was tested against *E. coli*, *Staphylococcus aureus*, *Lactococcus lactis* subsp. *Lactis*, *L. lactis* subsp. *Diacetylactis*, *Leuconostoc mesenteroides* subsp. *dextrannicum* and *Lactobacillus plantarum*. The lowest concentration of oil required to inhibit *L. lactis* subsp. *lactis* was 4.29 ppm (Vasek *et al.*, 2015). Peel oil was tested against 3<sup>rd</sup> instar of *Anopheles gambiae* larvae at concentrations ranging from 40–400 ppm. LC<sub>50</sub> of the oil was 76 ppm in methanol and 82 ppm in ethanol (Okunowo *et al.*, 2016). Methanolic extracts of white and pink peel of *C. paradisii* were examined for the effect of processing on their antioxidant activities and total polyphenol index. Freeze drying enhanced antioxidant activity determined by three methods: 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, 2,2'-azino-bis(3-ethylbenz thiazoline-6-sulphonic acid (ABTS) assay, and Ferric ion Reducing Antioxidant Power (FRAP) assay. Fresh and oven dried grapefruit peel extracts exerted a strong cytoprotective effect on SH-SY5Y neuroblastoma cell lines at concentrations within 0.1–0.25 mg/ml (Castro-Vaquez *et al.*, 2016). Antioxidant properties were further confirmed by investigation of *C. paradisii* cocktail cultivar (Canan *et al.*, 2016). Two phenolic extracts of the grapefruit peel, viz free phenolics extracted with 80% acetone, and bound phenolics extracted from the alkaline and acid-hydrolysed residue with ethyl acetate, were evaluated for their interaction with enzymes. The phenolic extracts inhibited  $\alpha$ -amylase,  $\alpha$ -glucosidase and ACE enzyme activities. The free phenolics had significantly ( $P < 0.05$ ) higher activity against  $\alpha$ -amylase and  $\alpha$ -glucosidase. The extracts were also more active against  $\alpha$ -glucosidase than  $\alpha$ -amylase. Both extracts inhibited nitroprusside-induced peroxidation in the pancreas in a dose-dependent manner (Obob and Ademosun, 2010). Investigation of the effect of the essential oil on sexual competitiveness of the Mexican fruit fly *Anastrepha ludens* (Diptera: Tephritidae) males showed that exposure of wild male to the smell of grapefruit oil, significantly increased their mating success, but had no effect on copulation duration (Morato *et al.*, 2015). Grapefruit juice antioxidant activity was estimated for four cultivars and found to range from 34.51 to 128.37  $\times 10^{-3}$  using the DPPH method (Kelebek, 2010). Grapefruit furanocoumarins have been shown to exhibit several biological activities including antioxidative, antiinflammatory and anticancer effects, as well as promoting bone health *in vitro* and *in vivo*. *In vitro* inhibitory properties of six cultivars on proliferation and growth of cancer cells were tested against K562 (human chronic myelogenous leukemia), NCI-H460 (Human lung cancer) and MCF-7 (Human breast adenocarcinoma) cell lines. All tested grapefruit juices showed evidence of antiproliferative activity against the cancer cell lines. Ruby red and Foster grape juices showed the highest inhibitory activity against the growth of NCI-H460 (100% and 80.87% respectively) and MCF-7 (98.36% and 66.2% respectively) cell lines at 5%v/v fresh juice diluted in cell culture medium (Hung *et al.*, 2016). The curative effect of aqueous extract of the zest of *C. paradisii* on cisplatin-induced testicular degeneration was studied for 8 weeks. Cisplatin only treated mice showed significant decrease in testis weight, testis volume, sperm count, sperm motility and normal sperm morphology. Also there was marked degeneration and atrophied tubules with absence of late stage germ cells evidenced by significant reduction in tubular diameter, perimeter, length and width in germinal epithelia height, cross-sectional area, number of profiles per unit area, and numerical density and PAS-positive materials and Ki67 cells of seminiferous tubules. All these parameters were attenuated in the groups that were post-treated with the aqueous extract of *C. paradisii*, suggesting that it has the potential of abating cisplatin-induced testicular toxicity in Wistar rats (Akunna *et al.*, 2016). Infusion of the fruit of *C. paradisii* was evaluated for its modulatory activity in lipid metabolism and insulin resistance, blood pressure regulation and renal alterations in obese male Sprague-Dawley rats. The infusion in diet-induced obese rats, suppressed hepatic tissue fat accumulation and significantly down-regulated mRNA levels of two hepatic lipogenesis genes, sterol regulatory element binding protein 1c (SREBP1c), and fatty acid synthase compared to obese controls. The infusion improved insulin resistance, and reduced blood pressure (Gamboa-Gomez *et al.*, 2014; 2015). *C. sinensis* and *C. paradisii* juice at three doses and in combination were evaluated for their effect on plasma insulin and blood glucose levels in normal and alloxan-induced diabetic rats. *C. paradisii* caused a highly significant decrease in blood glucose and a highly significant increase in plasma insulin levels at 0.05 ml/kg. The combination dose of both juices also showed the highly significant reduction in blood glucose and increase in plasma insulin, compared to the control. The juices may therefore be used in combination to effect decrease in blood glucose and elevate plasma insulin levels (Malick and Khan, 2015). Rats fed with a diet rich in

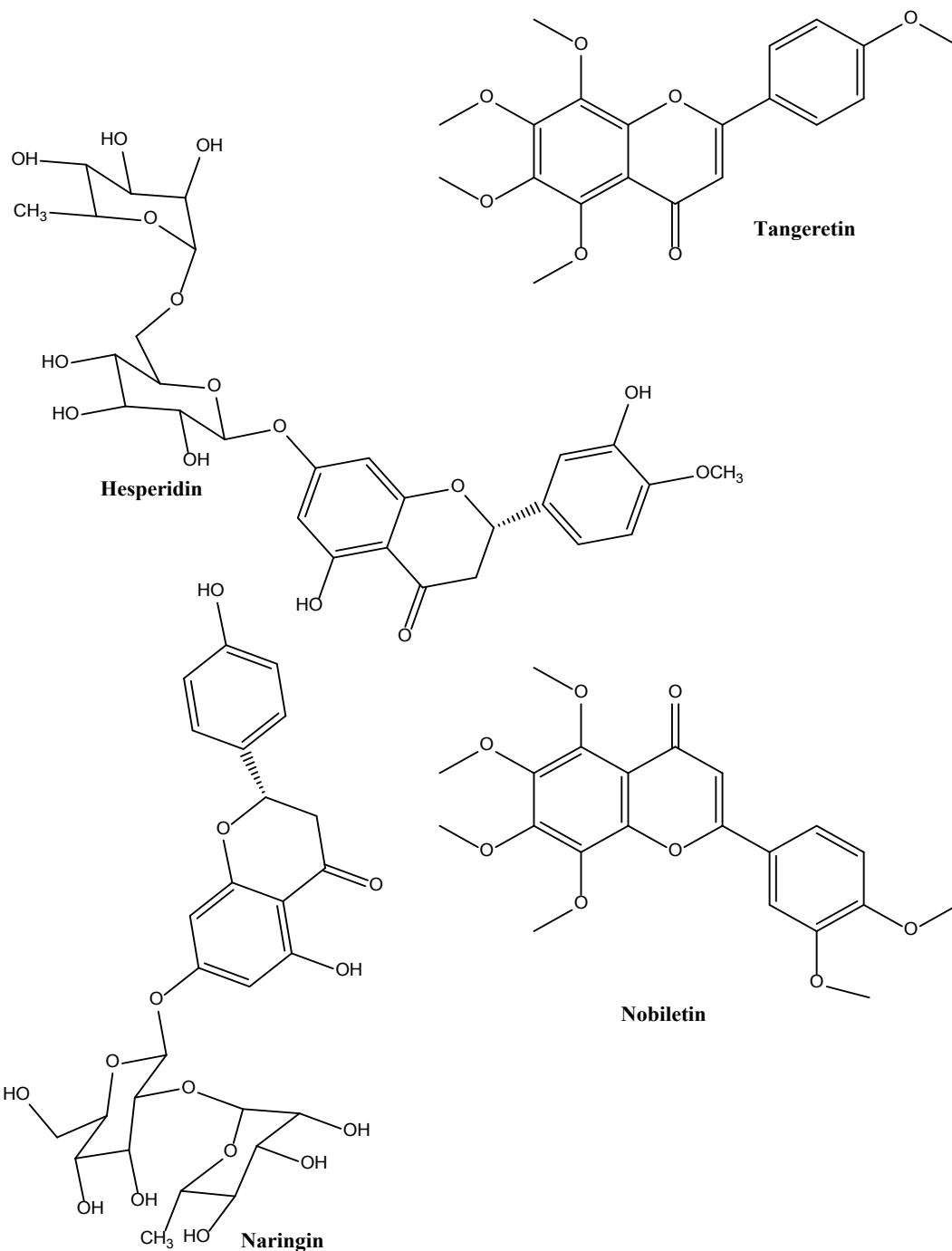
cholesterol were used in evaluating the juice of *C. paradisi* and *C. sinensis* for hypolipidaemic effects, with atorvastatin as the standard. Levels of cholesterol, triglycerides (TGs) and low density lipoprotein (LDL), were decreased at all three doses of *C. sinensis* and *C. paradisi*, but an increase in high-density lipoprotein (HDL) was only significant at 0.8 ml/kg and 0.3 ml/kg respectively. A combination of the two also showed a highly significant decrease in cholesterol, LDL, TGs and significant elevation in HDL. The two plants showed antihyperlipidaemic activity (Mallick and Khan, 2016). Anti-anxiety activity of the petroleum ether, chloroform, methanol and aqueous leaf extracts of *C. paradisi* varieties Duncan and Star Ruby were evaluated using elevated plus maze (EPM) model in Swiss albino mice. Albino mice were treated orally with extracts of doses 100, 200 and 400 mg/kg. Diazepam (2 mg/kg P.O) was used as positive control and behaviour observed on the EPM. Methanol extracts of the leaves at 100 mg/kg markedly increased the average time spent in the arms of the EPM. This effect was comparable to that produced by diazepam (Gupta *et al.*, 2010a; Gupta *et al.*, 2010b). The petroleum ether, chloroform, methanol and water extracts of the leaves of *C. paradisi* were evaluated using light dark model and hole board methods in Swiss albino mice. The methanol extract at 100 and 200 mg/kg p.o. had marked activity, comparable to that produced by standard drug diazepam at 2 mg/kg p.o. (Gupta *et al.*, 2015).

### Clinical data

Diets supplemented with red grapefruits significantly decreased plasma lipid levels, especially triglycerides in patients suffering from coronary atherosclerosis and related hyperlipidaemia. A study population of patients who had undergone coronary bypass surgery due to coronary artery disease were recruited for the study. Fifty-seven male patients between the ages of 47 and 68 years were examined. No lipid lowering medicine was used during the 30 days of the investigation. The fifty-seven patients were randomly divided into three groups; two experimental EG1 and EG2 and one control. All patients were given the usual Israeli diet recommended for patients. The diets of EG1 and EG2 were supplemented once a day by one equal in weight with fresh red or blond grapefruits respectively. Before and after the completion of the diet, the patients were examined. Systolic and diastolic blood pressure, heart rate and weight were recorded. Blood samples were collected a day before and a day after the investigation after an over night fast. Results obtained showed decrease by 15.5% and 7.6% total cholesterol; 20.3 and 10.7% low density lipid cholesterol; 27.2% and 5.6% total triglycerides for the experimental group of red grapefruits and blond grapefruits respectively. Only the patients who took blond grapefruits registered a significant decrease in hypertriglyceridemia. It was therefore recommended that red grapefruit should be included in atherosclerosis preventive diet (Park *et al.*, 2009). A study was carried out over two months to investigate the efficacy of a combination of rosemary (*R. officinale*) and grapefruit (*C. paradisi*) in decreasing susceptibility to UV exposure (redness and lipoperoxides) and in improving skin wrinkling and elasticity. The test product was a commercially available mixture of dried leaves of rosemary and grapefruit extract (Nutroxsun™). A randomised parallel group study was carried out on 9 subjects. A pilot, randomised crossover study with five subjects was also carried out. Female subjects having skin phototype I to III showing mild to moderate chronic or photo ageing clinical signs were enrolled in both studies. Both the long term and short term tests subjects were assigned to randomly receive 100 mg or 250 mg Nutroxsun or placebo (100% Maltodextrin). In the short term study, subjects received the first dose (100 or 250 mg) of the test product or placebo 15-30 mins before UVB exposure to 1 MED. Two supplementary doses were given 24 and 48 hr after UV exposure. In the long term study subjects received 100 mg Nutroxsun, 250 mg Nutroxsun or the placebo once a day at breakfast. Skin redness after UVB exposure to 1 minimal erythemal dose (MED) was assessed in the pilot study. MED, lipoprotein (malondialdehyde) skin content, wrinkle depth (image analysis) and skin elasticity (suction and elongation method) were also measured in the main study. Treated subjects showed a decrease of the UVB and UVA induced skin alterations (decreased skin redness and lipoperoxides) and an improvement of skin wrinkling and elasticity. No differences were found between the 100 mg and 250 mg extract, indicating a plateau effect from 100 mg dose of extract. Some positive effects were noted in as short as 2 weeks of product consumption (Nobile *et al.*, 2016).

## Chemical constituents

Flavonoids and limonoids including hesperetin, naringenin, narirutin, naringin, hesperidin, neoheperidin, didymin, poncirin (Kelebek , 2010; Goulas and Manganaris, 2012), nonanal, nootkatone,  $\beta$ -pinene,  $\alpha$ -phellandrene, 3-carene, ocimene, octanol, trans-linalool oxide, cis-pmentha-2-8-dien-1-ol,  $\alpha$ -pinene, limonene, linalool, geraniol citronellal, alpha-terpineol, nerol, dodecanal,  $\alpha$ -humulene (Bennett and Hasagava, 1989) and mercaptan (Buettner and Schieberie, 1999); phenolics: hydroxybenzoic acids, gallic, protocatechuic, p-hydroxybenzoic and vanillic acids; hydroxycinnamic acids, caffeic, chlorogenic acid, p-coumaric, ferulic and sinapic acids; furocoumarins (bergamottin, expoxybergamottin and 6', 7'-dihydroxybergamottin); (Fuselli *et al.*, 2008), limonin, nomilin (Hafeez *et al.*, 2011).





### Test for identity and purity

About 50 g fresh peel of *C. paradisii* were hydrodistilled using a Clavenger-like apparatus for three hours at 70°C, yielded 0.85± 0.14% (w/w) based on the fresh weight.

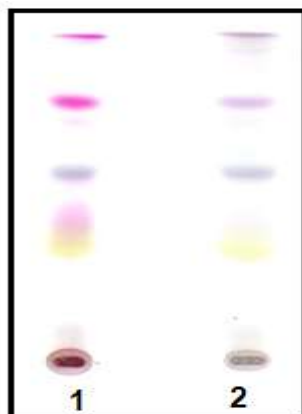
### Chromatographic fingerprint

#### *Thin Layer Chromatography*

**Preparation:** About 5 g of the powdered leaves were extracted with ethyl acetate by cold maceration, filtered and the filtrate concentrated to a small volume. A small spot was then applied to the TLC plate for analysis.

**Chromatographic conditions:** Analytical TLC on silica gel G60 F254, 0.25 mm layer in hexane/ethyl acetate (7:3) as the mobile phase.

**Detection:** Visualized in daylight after spraying with anisaldehyde-sulphuric acid (Lane 1) and vanillin-sulphuric acid reagents (Lane 2) (Stahl, 1969), heating to 110°C for 10 mins. The TLC chromatogram showed three prominent spots with R<sub>f</sub>s of 0.58 (mauve), 0.45 (pink) and 0.35 (yellow) when sprayed with both anisaldehyde and vanillin. An additional spot with R<sub>f</sub> 0.78 with colours of pink and purple appeared in the chromatogram sprayed with anisaldehyde and vanillin respectively.



#### *High Performance Liquid Chromatography*

**Sample preparation:** A sample of about 10 mg of the hydro-ethanolic extract of *C. paradisii* leaves was reconstituted in 3 mL acetonitrile in a 10 mL volumetric flask with sonication for 17 minutes. It was then diluted to volume with the mobile phase under chromatographic conditions. It was centrifuged to obtain a clear test solution which was subsequently filtered through a 0.45 µm filter into an HPLC vial and analyzed.

### Chromatographic system

#### Optimized chromatographic conditions

**Mode:** LC

**Column:** YMC ODS, 4.6 x 150 mm, 5 µm

**Column temperature:** Ambient – 30°C

**Mobile phase:** Acetonitrile: Methanol: Water (60:20:20 v/v/v)

**Elution mode :** Isocratic

**Injection volume :** 20  $\mu$ L

**Flow rate:** 0.5 mL/minute

**Detection wavelengths:** 230 nm, 254 nm and 278 nm.

### System Suitability parameters

**Number of peaks:** 230 nm (1), 254 nm (1), 278 nm (1)

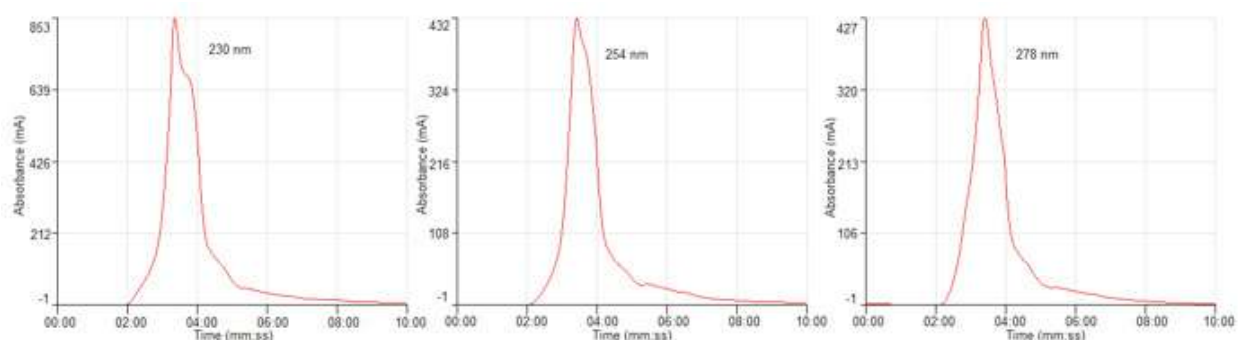
**Retention time (s):** 230 nm (3.21 min), 254 nm (3.25 min), 278 nm (3.24 min)

**Asymmetric factor(s):** 230 nm (0.832), 254 nm (0.723), 278 nm (0.653)

**Tailing factor:** NMT 2.0

**Efficiency:** 230 nm (76.74), 254 nm (92.47), 278 nm (82.53)

**Acceptance criteria:** Sample solution of hydroethanolic crude extract of *C. paradisii* Macfad. (Leaves) conforms to the system suitability parameters.



### HPLC chromatogram of hydroethanolic leaf extract of *Citrus paradisii*

#### FT-IR

A small amount of the dried hydroethanolic extract (70%) was placed on the sample area of the Perkin Elmer UATR Fourier transform infrared (FT-IR) spectrometer and scanned between 4000-400  $\text{cm}^{-1}$  with a resolving power of 4  $\text{cm}^{-1}$  and a cumulative scanning limitation of 24 times. Principal peaks appeared at wavenumbers 3300.73, 2923.80, 2853.46 and 1599.00  $\text{cm}^{-1}$

### Macroscopy

The evergreen leaves are ovate, 7.5-15 cm long and 4.5-7.5 cm wide, dark green above, lighter beneath, with minute, rounded teeth on the margins. Leaves are dotted with tiny oil glands and the petiole has broad, oblanceolate wings.

### Microscopy

Upper epidermis made up of polygonal cells. Numerous circular cells each filled with a monoclinic prism of calcium oxalate are beneath the epidermal layer. Scattered all over are circular, multicellular, shizogenous oil glands. There are no stomata. Lower surface epidermis is made up of polygonal cells and actinocytic stomata are numerous. Numerous circular cells each filled with a monoclinic prism of calcium oxalate. Multicellular circular shizogenous oil glands and smaller unicellular circular oil gland with yellowish content are present. The transverse section shows concentric – amphicribal vascular bundle xylem in a circular form, surrounded by phloem and phloem fibres, with calcium oxalate crystals forming a ring around the phloem. Below the upper and lower epidermis are collenchyma and parenchyma with calcium oxalate prisms; large schizogenous oil glands between the vascular bundles and epidermis of both surfaces. Along the lamina below both epidermis are a row of parenchyma cells which, are spherical and contain calcium oxalate prisms; there is a row of palisade following the upper epidermal cells; interrupting the epidermis of both surfaces are large multicellular schizogenous oil glands.

### Powdered plant material

The leaf powdered plant material is dark green and has a very strong lemon odour. Consists of fragments of lower surface epidermis showing actinocytic stomata; numerous monoclinic calcium oxalate prisms; bundles of fibers with rows prisms; fragments of spongy mesophyll and columnar palisade cells and fragments of upper surface showing polygonal cells.

### Therapeutic actions

Antioxidant, antianxiety, antibacterial, antifungal, antiviral, antiinflammatory, astringent and preservative, cholesterol lowering.

### Therapeutic indications

Inflammation, stress, malaria, high cholesterol

### Safety data

LD<sub>50</sub> by oral route was estimated to be beyond 3000 mg/kg in rats. There were no signs of CNS depression/ stimulation or autonomic effects at doses of 0-1000 mg/kg. No significant changes were noted with highly perfused organs such as the liver, kidney, heart and lungs. The relative organ to body ratios of spleen, thymus, and adrenals were not significantly affected by treatment. The effect of *C. paradisii* was very minimal and statistically insignificant. It induced very mild leucopaenia and also decreased platelet counts at all doses tested. Differentially, leucopaenia was due to decreased agranulocytes. Treatment with Citrus resulted in increased ALT but not AST, ALP and GGT. Citrus caused mild elevation in total protein. Increases were dose dependent on serum albumin. Citrus did not affect bilirubin, urea and creatinine significantly. Pentobarbitone sleeping time was significantly prolonged by Citrus treatment (1000 mg/kg *i.p*). No histopathological changes were seen in livers and kidney. The extract is relatively safe on the haematological system.

**Precautions for use**

Oils from *C. paradisii* leaf should be used with caution. Care should be taken during concurrent administration of the leaf extract with CNS drugs.

**Adverse effects**

Not Known

**Contraindications**

Not known

**Dosage forms**

Decoction, infusion, tincture

**Doasage**

Decoction: 30 g of dried plant material in 900 mL water; simmer until reduced to 600 mL; 1-3 tablespoons daily

Infusion: 30 g of dried aerial part in 600 mL of water; 3-4 teacups daily

Tincture: 1:5, 45% ethanol; 5 ml three times daily

**Storage**

Store in a cool dry place away from light

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**Botanical name***Cochlospermum tinctorium* Perr. ex A. Rich.**Family**

Cochlospermaceae; Bixaceae

**Synonyms***Cochlospermum niloticum* Oliv.**Common Names**

Cotton plant (English), Plante de coton (French).

**Common Local names****Benin:** Dendi – Kpararu; Fon - Atinyi v□kanfun; Yoruba - Aw□ owu.**Burkina Faso:** Dioula-N'dribala ; Moré-Sôasga ; Fulfuldé-Njadere**Côte d'Ivoire:** Baoulé-Kadiendi diéssé; Dioula-Bédiara korandi; Senoufo-Tikwélégué.**Gambia:** Pulaar - *Dafe*, Manding - *Foosea***Ghana:** Dagbani - Biberetugu, Twi – Kokrosabia; Ewe - Kakalito**Guinea:** Pular-Rèmè; Maninka-Truban; Soussou-Filira gèsè**Guinea-Bissau:** Pulaar – Jándèrè**Mali:** Bambara-Ndribala; Dioula-Tiriba; Peuhl-Njadere ndilibara; dâduré**Niger:** Djerma – Bagarbey; Haoussa – Lawaga**Nigeria:** Yoruba – Sewetu; Hausa – Balagande; Igbo – Nkalike**Senegal:** Bambara – Ndilibara; Mandingo – Turubga; Diola - Bu lulumay.**Togo:** Ewé– Dzogbedhèti; Kotokoli- Kulobonku; Moba – Nyongmonsavi**Description of the plant**

A perennial savanna plant, 50 to 100 cm high, with a very fibrous thick strain, bringing out each year unbranched cylindrical aerial stems. Finely pubescent or glabrescent. Bark of fibrous aerial stem is detachable in fibrous bands, with characteristic odour. Leaves are alternate, petiolate, palmate, with 5 closely lanceolate and finely denticulate lobes, 5-10 cm long, 5-10 mm wide, glabrous. Flowers are large, pointed at the apex, rounded at the base, up to 10 to 12 cm wide, actinomorphic, appearing at ground level after the passage of bush fires; petals 5, yellow; stamens numerous, with linear threads 10 mm long; anthers yellow, about 5 mm long, basifixed; ovary globose, greenish-white, glabrous, about 4 mm long. Fruits are dehiscent, capsular, ovoid, up to 6 cm long, opening with 4 valves. Seeds densely covered with long hairs (Kerharo and Adam, 1974).



A – *Cochlospermum tinctorium* Perr, B – leaves, C – flowers, D – C fruit

### Herbarium specimen number

Benin: 2358 (AP)

Burkina Faso: BUR – 537

Côte d'Ivoire: 9704 (CNF)

Ghana: GH 802

Guinea: 37HK442 (CRVPM - Dubréka)

Mali: 0375 (DMT)

Nigeria: UPFH 115

Senegal: IFAN HG 763

### Habitat and geographical distribution

The species is a plant of the open savannah zones, preferring mainly gravel soils with little or no wood, growing in groups more or less extensive, often scattered when the tree vegetation becomes dominant. *C. tinctorium* grows in dry savannas, with a preference for devastated, rocky and burned areas at altitudes between 300 and 1500 m (Burkill, 1985; 2000).

### Plant material of interest

Rhizome



**Other part used**

Leaf

**Definition of plant material of interest**

*Cochlospermum tinctorium* consists of the fresh or dry rhizome of *Cochlospermum tinctorium* Perr. ex A. Rich. (Cochlospermaceae)

**Ethnomedical uses**

Apart from its very popular use in West Africa for the treatment of malaria, jaundice and bilious fevers mainly haematuria, the yellow rhizome is also used to treat oedema, urinary incontinence, dysmenorrhoea, epilepsy, schistosomiasis, pneumonia, bronchial affections, conjunctivitis, diarrhoea, indigestion, stomach upset, haemorrhoids and skin conditions. In Nigeria, it is drunk with fruits and tamarind to treat snake bites. In Côte d'Ivoire and Burkina Faso, rhizome powder is used topically for wound healing (Jansen, 2005). Also in Côte d'Ivoire, leaf pulp is used as a wet dressing to ripen abscesses and boils whereas a decoction of twigs or rhizomes is consumed, or poured into bath water to treat genital disorders, urinary, renal or intercostal pain. The body is washed with an aqueous extract of the rhizome not only to treat skin diseases, but also for prophylaxis. In Nigeria, the rhizome is chewed as a tonic, and also widely used in veterinary medicine. Seed oil is used to treat leprosy. In Mali, the infused root is used in the treatment of gastric ulcer. In addition, its decorative flowers make it a potential ornamental plant (Kerharo and Adam, 1974; Inngjerdigen *et al.*, 2014; Diarra *et al.*, 2015; Lamien-Meda *et al.*, 2015).

**Biological and pharmacological activities**

The methanol and aqueous extracts of *C. tinctorium* showed analgesic, antiinflammatory and antibacterial properties. At high concentrations, cochloxanthin and dihydrocochloxanthin showed moderate antimicrobial activity against *Candida albicans*, *Aspergillus fumigatus*, *Staphylococcus aureus* and *Escherichia coli* (Ahmed *et al.*, 2011; Baillin *et al.*, 2002). Choloretic and hepatoprotective properties of the plant have been observed *in vivo* (Sere *et al.*, 1983). Tests on mice have shown that arjunolic acid and its methoxylated or acetylated derivatives have more pronounced inhibitory effects on skin tumour promoters than natural products previously described as such (Diallo *et al.*, 1987; Diallo *et al.*, 1989; Diallo *et al.*, 1992). The hepatoprotective effect of the rhizome seems to be related to the presence of phenolic and polyphenolic compounds (gallic and ellagic acids, ellagitannins, flavonoids) in the active fraction. The aqueous extract of the rhizome showed an IC<sub>50</sub> of 1 - 2 µg/mL against *Plasmodium falciparum* (Benoit *et al.*, 1995). The extract of the root has antiinflammatory, vasoconstrictor, and decongestant properties (Nacoulma-Ouedraogo, 1996). The aqueous crude extracts and the essential oil of the leaves are cytotoxic to human K562 cells, with an IC<sub>50</sub> between 33 and 2000 µg/mL (Benoit-Vical, 1999). Polymers contained in the aqueous extract have been described as possessing antiulcer, immunomodulatory and antioxidant activities (Nergard *et al.*, 2005). The roots were reported to treat jaundice, gastrointestinal diseases, malaria, schistosomiasis and dysurea. Aqueous extract (25, 50 and 100 mg/kg, body weight) significantly inhibited HCl/ethanol-induced gastric lesions in mice. The extract showed DPPH-radical scavenging and immunomodulating activities *in vitro*. Ahmed *et al.*, (2011) reported the analgesic and antiinflammatory activities of the aqueous methanol leaf (20–80 mg/kg), root (7.5–30 mg/kg), and root bark (20–80 mg/kg) extracts of *C. tinctorium*, using acetic acid-induced writhing and hot plate tests in mice ; the antiinflammatory activity was also investigated using carrageenan-induced paw oedema in rats. The extracts significantly and dose-dependently inhibited the acetic acid-induced writhing in mice with the highest protection produced by the leaf extract at the dose of 80 mg/kg (96.65%), which was greater than that of the standard agent, ketoprofen (82.30%) at 10 mg/kg. The extracts did not significantly increase mean latency of response in the hot plate test, but the aqueous methanolic root bark extract at the dose of 20 mg/kg significantly ( $P < 0.05$ ) increased the mean latency

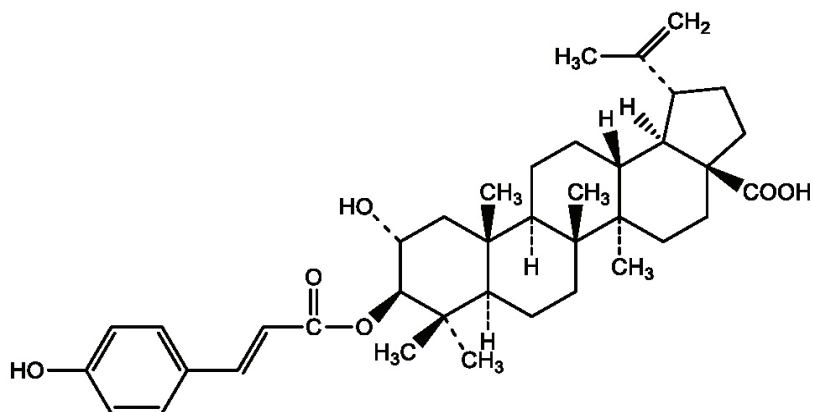
of pain response. Extracts of the root and root bark of the plant showed a non dose-dependent protection against carrageenan-induced oedema, and the leaf extract significantly and dose-dependently inhibited carrageenan-induced hind paw oedema at the end of the third hour. An ethanolic extract of the rhizome showed pronounced antiplasmodial activity (1-2 µg/ml), with 3-OEp-coumaroylalphitolic acid as the most active compound (IC<sub>50</sub>: 2.3 µM) (Baillin *et al.*, 2002). An alcoholic extract of the rhizome yielded an IC<sub>50</sub> of 17 µg/mL. The compounds, cochloxanthin and dihydrocochloxanthin significantly reduced the haemolytic potency of saponins at 33 and 11 µg/mL, respectively. The antiplasmodial activity of leaf extracts has been described as moderate (Lamien-Meda *et al.*, 2015). The aqueous root extract showed antiulcer activity by 30% inhibition of *H. pylori* adhesion (Inngjerdingen *et al.*, 2014). Tannin content of the plant have shown remarkable antihepatotoxic activity and gallic acid in particular inhibits the production of oxygen-free radicals in leucocytes (Baillin *et al.*, 2015). Hepatoprotective effects of aqueous root extracts of *C. tinctorium* against carbon tetrachloride- (CCl<sub>4</sub>) induced acute hepatic injury were investigated in rats. In carbon tetrachloride intoxicated rats, significantly (P < 0.05) higher levels of total, direct and indirect transaminases, alkaline phosphatase, albumin and bilirubin were observed. These were reduced to almost normal levels in rats treated with the extract. The hepatoprotective activity of *C. tinctorium* was confirmed when compared with untreated groups by histopathological examination. The lethal dose (LD<sub>50</sub>) was greater than 5000 mg/kg body weight for both leaf and root extracts of *C. tinctorium* (Adam *et al.*, 2017).

### Clinical data

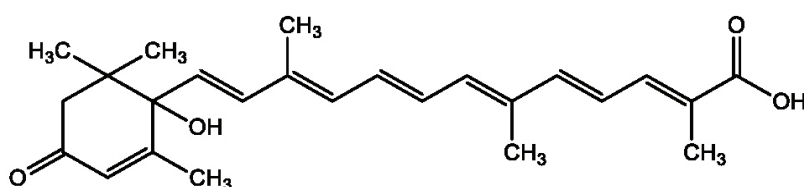
The treatment of 24 patients with HBsAg (Hepatitis B) by a combination of *Combretum micranthum* G. Don (Combretaceae) leaves and *C. tinctorium* roots resulted in clinical and biochemical improvement in the patient's condition from the first month of treatment. The disappearance of HBsAg was noted in 4.17% of patients after three months of follow-up (Mouzouvi *et al.*, 2014).

### Chemical constituents

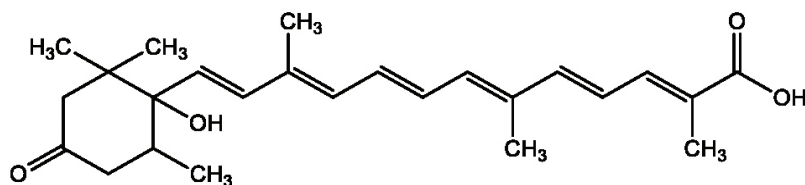
Carotenoids; mucilages, sugars, acetogenins, tannins (gallic acid, ellagic acid and ellagitannin), essential oils (3-hexadecanone), arjunolic acid, apocarotenoids (cochloxanthin and hydrocochloxanthin) (Diallo and Vanhaelen, 1987); triacylbenzenes (cochlospermins A, B, C, D and 1,3-Bis (tetradecanoyl) -5-hexadecanoylbenzene), 2-tridecanone, 1-dodecanol, 1-tetradecanol, nonadecanol, 2-pentadecanone, 3-octadecanone, 1-hydroxy-3-hexadecanone, 3-hexadecanone, 1-O-acetyl-3-hexadecanone, 1-hydroxy-3-octadecanone, alphitolic acid, 1-hydroxytetradecan-3-one, β-bisabolene (Achenbach, 1989 ; Diallo *et al.*, 1991; Diallo *et al.*, 1989; Ballin *et al.*, 2002).



3-O-p-coumaroyl alphitolic acid



Cochloxanthin



dihydrocochloxanthin

### Test for identity and purity

#### *Rhizomes*

**Moisture content:** Air dried coarse powder does not lose more than 7.7% at 105°C.

**Total ash:** not more than 11.3%

**Acid insoluble ash:** not more than 1.0%

**Water soluble extractive:** not less than 20.0%

**Ethanol soluble extractable (70%):** not less than 20.0%

#### Chromatographic fingerprint

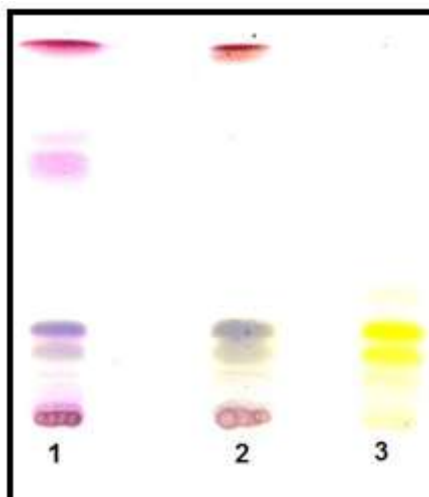
##### *Thin Layer Chromatography*

**Preparation:** A sample of 5 g of the powdered rhizomes was extracted with ethyl acetate by cold maceration, filtered and the filtrate concentrated to a small volume. A small spot was then applied to the TLC plate for analysis.

**Chromatographic conditions:** Analytical TLC on silica gel G60 F254, 0.25mm layer in hexane/ethyl acetate (7:3) as the mobile phase.

**Detection:** Visualized in daylight after spraying with anisaldehyde-sulphuric acid (Lane 1) and vanillin-sulphuric acid reagents (Lane 2) (Stahl, 1969), heating to 110°C for 10 mins.

The TLC chromatogram showed two characteristic spots at R<sub>f</sub>s of 0.24 and 0.18 with colours mauve and blue-black when sprayed with anisaldehyde and vanillin respectively. The spots appeared yellow in visible light without spraying (Lane 3). Two additional spots at R<sub>f</sub>s of 0.79 (pink), 0.76 (pink) appeared in the chromatogram sprayed with anisaldehyde.



### High Performance Liquid Chromatography

**Sample preparation:** About 10 mg of the hydro-ethanolic extract of *C. tinctorium* rhizome were reconstituted in 3 mL acetonitrile in a 10 mL volumetric flask with sonication for 17 minutes. The solution was then diluted to volume with the mobile phase under chromatographic conditions. It was centrifuged to obtain a clear test solution which was subsequently filtered through a 0.45 µm filter into an HPLC vial and analyzed.

### Chromatographic system

#### Optimized chromatographic conditions

**Mode:** LC

**Column:** YMC ODS, 4.6 x 150 mm, 5 µm

**Column temperature:** Ambient -30°C

**Mobile phase:** Acetonitrile: Methanol: Water (60:20:20 v/v/v)

**Elution mode:** Isocratic

**Injection volume:** 20 µL

**Flow rate:** 0.5mL/minute

**Detection wavelengths:** 230 nm, 254 nm and 278 nm.

### System Suitability parameters

**Number of peaks:** 230 nm (1), 254 nm (1), 278 nm (1)

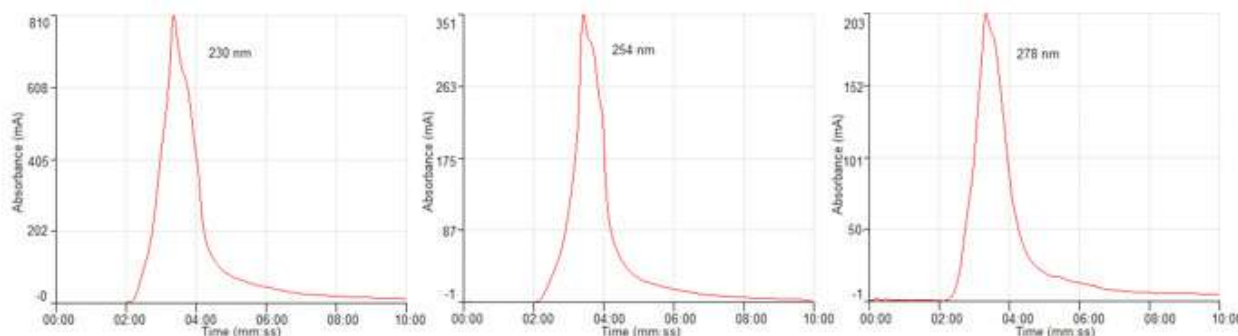
**Retention time (s):** 230 nm (3.21 min), 254 nm (3.25 min), 278 nm (3.19 min)

**Asymmetric factor(s):** 230 nm (0.843), 254 nm (0.638), 278 nm (0.761)

**Tailing factor:** NMT 2.0

**Efficiency:** 230 nm (63.501), 254 nm (97.36), 278 nm (73.82)

**Acceptance criteria:** Sample solution of hydro-ethanolic crude extract of *Cochlospermum tinctorium* Perr. (Rhizome) conforms to the system suitability parameters.



## FT-IR

A small amount of the dried hydroethanolic extract (70%) was placed on the sample area of the Perkin Elmer UATR Fourier transform infrared (FT-IR) spectrometer and scanned between 4000-400  $\text{cm}^{-1}$  with a resolving power of 4  $\text{cm}^{-1}$  and a cumulative scanning limitation of 24 times. Principal peaks appeared at wavenumbers 3281.81, 2923.08 (strong), 2853.18 and 1708.64  $\text{cm}^{-1}$ .

## Macroscopy

The root has a grey to black outer bark, the wood of the root is non-malleable (brittle). A cut of the fresh root shows a whitish and floury surface which is quickly stained by an orange exudate; it turns yellow with time. The powder of the root is yellow, with a strong odour, weakly astringent and slightly bitter taste. The fracture is very brittle and short. The bark is covered with hairs. Stem is brown in colour and very hairy. It has a hollow centre and is cylindrical in shape.

## Microscopy

The transverse section of the stem shows a layer of rows of cork cells with squashed rectangular cells. Depending on the age of the stem, there could be more than ten rows of cells. Arising from the cork layer are long, curved, unicellular trichomes. A layer of thin walled parenchyma cells follows the cork layer. Next, is a layer with schizogenous glands and followed by vascular bundles interspersed with large parenchyma. The last layer consists of several rows of irregularly shaped cells which form the outline of the hollow in the stem.

## Powdered plant material

Presence of tissue fragment with cells, groups of fibres, starch grains, sclereids, isolated fibres, wood fragment, fragment of the epidermis, crystals of calcium oxalate, fragments of large vessels (Sangaré, 2005). The stem powder is brown in colour characterized by many fragments of appressed unicellular trichomes, which are curved; large annular xylem vessels and numerous bundles of fibres occur; fragments of cork cells showing the surface and transverse view; sclerenchymatous cells with thin walls and rectangular shape.

## Therapeutic actions

Wound healing, antimalarial, antiviral

**Therapeutic indications**

Malaria, liver diseases (hepatitis), wound dressing

**Safety data**

Administration of 100 mg to 2 g/kg of powder dissolved in 2 mL of water for ten days in rats (average weight), showed no signs of intoxication. No adverse effects on the liver were also found. However, a slight decrease in bilirubin from the sixth to the tenth day of treatment and mild weight gain were observed. LD<sub>50</sub> by oral route was estimated to be beyond 3000 mg/kg in rats. There were no signs of CNS depression/stimulation or autonomic effects at doses of 0-1000 mg/kg. No significant changes were noted in the morphology of the liver, kidney, heart and the lungs. The relative organ to body ratios of spleen, thymus, and adrenals remained unchanged after treatment. *C. tinctorium* did not affect RBCs, HB. It significantly elevated WBC. Differential cell counts showed that the elevation occurred in lymphocytes with corresponding decreases in neutrophils. It caused a significant decrease in AST, but did not affect ALT, ALP, GGT. It appears to elevate serum albumin and globulins and hence total protein, but the effect was not statistically significant. Renal function was normal and bilirubin levels were unaffected. Histopathology examination did not indicate possible damage to target organs. This plant extract did not appear to be potentially toxic. There is a possible link between the decreased AST and increased serum proteins. It affects pentobarbitone sleeping time indicating possible inhibition of drug metabolizing enzymes.

**Precautions for use**

Care should be taken when used concomitantly with sedatives.

**Adverse effects**

Not known

**Contraindications**

Not known

**Dosage forms**

Decoction, infusion, powder, tincture

**Dosage**

Decoction: 30 g of dried plant material in 900 mL water; simmer until reduced to 600 mL; 1-3 tablespoonfuls daily

Infusion: 30 g of dried aerial part in 600 mL of water; 3-4 teacups daily

Tincture: 1:5, 45% ethanol; 5 ml three times daily

**Storage**

To be stored in a cool, dry place away from light.

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**Botanical name***Combretum glutinosum* Perr. ex DC.**Family**

Combretaceae

**Synonyms***Combretum leonense* Engl. & Diels, *Combretum passargei* Engl. & Diels, *Combretum relictum* (Aubrév.)**Common Names**

Elephant wood (English), Bois d'éléphant, chigommier (Français).

**Common Local names**

**Benin:** Fon – Doso; Yoruba- Bodomi; Dendi– Bwagosa  
**Burkina Faso:** Dioula – Katakidum ; Fulfuldé - Dooki; Morée - koèguenga  
**Côte d'Ivoire:** Malinké – Naniaragbwé; Manding- Naniaragbwé  
**Gambia:** Pulaar – Buki; Manding – jambakatar; Wolof – rat  
**Ghana:** Grusi-Kasena - Vakogu, vokoŋ; Nankanni - urinperiga  
**Guinea:** Malinké– Tiangara; Peuhl – Doki ; Manding– demba  
**Mali:** Bambara – Tiangara, Cangwèrèbilen; Dogon- Andanga; Peulh- Dooki  
**Niger:** Beriberi – Kedagav; Hausa – dalo; Songhai - dèeli-ñá'  
**Nigeria:** Fula-Fulfulde – Boodi; Hausa - dageera; Yoruba - daguro  
**Senegal:** Wolof - rat; Diola – kalâkudun; Manding – dâbakatâ  
**Togo:** Ewé – Atisèsê; Yanga – Makpiob; Yoruba - bodomi

**Description of the plant**

Small bushy shrub, more or less evergreen, usually 8-10 (-12) m high, rounded and open crown. Bark, rough, fissured on the surface, with red to orange slice. Velvety twig with tomentose, greyish appearance. Leaves opposite, verticillate by three or sometimes subopposite, of variable shape and size, leathery, glaucous to greyish and more or less densely pubescent below (pubescence always visible under a magnifying glass) (Arbonnier, 2004). Elliptical, oval or obovate blade with sometimes wavy margins, apex more or less pointed or apiculated, sometimes notched or mucronate, rounded corner or sometimes indented, 9-18 x 4-8 cm. Petiole tomentose, 5-10 (-15) long. Nerves pinnate, projecting on both sides, at (7- 8-12 (-15) pairs of pubescent secondary veins connecting to the apex. Reticulated nerve and tomentose. Leaves appear from November to February; flowers from December to April and fruits from late December (Malgras, 1992). Inflorescence, raceme spiciform, axillary, usually more or less tomentose, about 4-5 cm long. Flower greenish yellow to pale yellow, 2.5-3 mm in diameter, 4 petals. Fruit, elliptic samara with 4 wings, indented at the base and at the top, 2.5 - 4.0 x 1.5 – 3.0 cm, glabrous or shortly pubescent, more or less sticky (mostly in the centre), reddish becoming beige or yellowish. The trunk is often twisted and covered with a rough bark. The leaves are very polymorphic on the same tree. They are sticky and very deeply reticulated on the underside with a whitish or sometimes almost glabrous pubescence. The tomentose coating of twigs, always visible with a magnifying glass, is a characteristic of the species (Burkill, 1985, 2000).



A - *Combretum glutinosum* Guill. & Perr. Whole plant, B – leaves, C – leaves and immature fruit, D- fruits and flowers, E – fruits

#### Herbarium specimen number

Côte d'Ivoire: CNF 6127  
 Mali: 760/DMT (Mali)  
 Nigeria: KASU/PCG/096  
 Senegal: IFAN 83  
 Togo: TG 00461

#### Habitat and geographical distribution

The plant is native to tropical Africa in the Sudano-Guinean regions, from Senegal to Sudan; found in wooded savannahs. It grows from Mauritania to Uganda; widespread, often abundant and gregarious (Marquet and Jansen, 2005).

#### Plant material of interest

Fresh or dried leaves

#### Other part used

Stems and roots

#### Definition of plant material of interest

*Combretum glutinosum* consists of the fresh or dried leaves of *Combretum glutinosum* Perr. ex DC.

## Ethnomedical uses

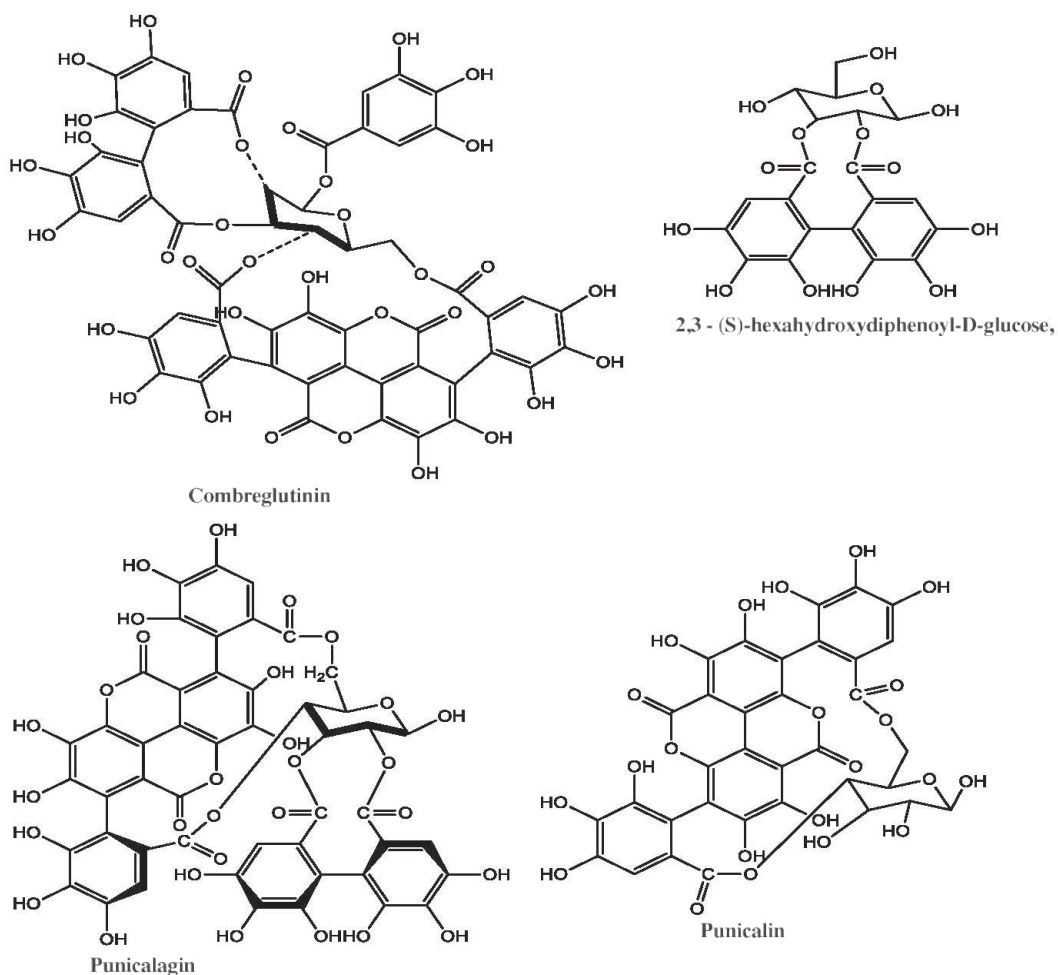
The plant is used for the treatment of common ailments (Kerharo and Adam, 1974) including hepatobiliary diseases, urinary disorders, oedema, arterial hypertension, cough, malaria, infantile gastritis and proteinuria (Fortin *et al.*, 1990). The leaf decoction or infusion is used as a diuretic, cholagogue, depurative and febrifuge at a dose of 5 leaves per litre of water. The leaf buds are crushed and mixed with cold red millet porridge for the treatment of dysentery (Fortin *et al.*, 1990). The crushed green leaves are applied to wounds. An infusion of the leaves may also be used for wound cleansing. They are also given for bronchitis, malaria, anaemia, migraine, blood effusions, and colds (Maydell, 1980). The decoction of the tender leaves are used to treat cough and fever in children, and in baths and lotions for the treatment of wounds. The decoction is also used in bathing and fumigation for fatigue and chest pains. Decocted leafy twigs are used in the treatment of jaundice, malaria, infantile gastritis and conjunctivitis (Malgras, 1992). Oral intake of a 24-hour macerated pulverized leaf with rock salt, treats bilious haemoglobinuric fever. Tender leaves are chewed and the juice swallowed, to treat dysenteric amoebiasis. Macerated crushed leaves are administered on an empty stomach to treat constipation. Regularly drinking and bathing with the infused leaves during pregnancy and some time before it, has been found to be effective in women prone to repeated abortions. In case of snake bite, the tender leaves are chewed and the juice swallowed, then the residue is applied to the wound (Traoré, 1999). In Senegal, leaves have a high reputation for the treatment of chest and stomach disorders (Marquet and Jansen, 2005). In The Gambia and Nigeria, leaf maceration is taken as a purgative (Marquet and Jansen, 2005). In Cote d'Ivoire, the Maninka take the leaf decoction in baths to treat general fatigue. The dried and crushed leaves are used in post-circumcision haemorrhages (Burkill, 1985). The leaf decoction is also used as a diuretic and hypotensive at the dosage of 30 g of leaves in a litre of water (Pousset, 2004). Trunk, stalk and root bark are used as anthelmintic and aphrodisiac. Bark infusion is used in Senegal to stop vomiting and as an aphrodisiac (Fortin *et al.*, 1990). Crushed bark is used to treat wounds. Peuls in Nigeria use bark-infused bath for influenza and rheumatism (Fortin *et al.*, 1990). Root extracts are used against stomach diseases and coughs (Fortin *et al.*, 1990). Decoction of the roots is used against kidney pain of various origins, as well as gonorrhoea (Maydell, 1980). Dried and crushed immature fruits are active on syphilitic cankers (Fortin *et al.*, 1990). Crushed green seeds are used for the treatment of wounds, syphilis and veterinary medicine (Maydell, 1980). The gum is used as a laxative and antidiarrhoeal. The plant is also often used in combination with other plants in the treatment of bilharziasis, leprosy, sexual impotence, and mental illness (Maydell, 1980).

## Biological and pharmacological activities

The plant extract has shown considerable ameliorative effect in renal lithiasis and hepatitis (Kerharo and Adam, 1974). Chloroform and methanolic extracts of the leaves and stems inhibited the growth of *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, but the aqueous extract was inactive (Yahaya *et al.*, 2012). The extracts demonstrated antitussive and antispasmodial activity (Pousset, 2004). The methanolic and hydromethanolic extracts of the leaves demonstrated an antiplasmodial activity *in vitro* on the chloroquine-resistant *Plasmodium falciparum* strain W2, with  $IC_{50}$  of  $53 \pm 0.01$  and  $IC_{50}$  of  $43.6 \pm 3.33$   $\mu\text{g/ml}$  (Ouattara *et al.*, 2006). The extracts exhibited antibacterial activity against *Staphylococcus aureus* TCC 6538, with minimal inhibitory concentration of 1.41 mg/ml (Sore *et al.*, 2012). The ethanolic extracts of the leafy stems and roots exhibited cercaricidal activity against *Shistosoma mansoni*, with a lethal concentration of 90% of 3.71 ppm after 3 hours of exposure for the stem extract and respectively of 5 and 23.64 ppm after 6 hours hours of exposure for leaves and roots (Albagouri *et al.* 2014). Polar extracts of the plant showed antitrypanosomal activity on *Trypanosoma brucei brucei* (Traore *et al.*, 2014). Aqueous extracts of the leaves exhibited dose-dependent antidiarrhoeal activity in albino rats, with 80.4% inhibition at the 600mg/kg dose (Garba and Mota'a 2015).

## Chemical constituents

Tannins: combreglutinin, 2, 3-hexahydroxydiphenoyl-D-glucose, hydrolyzable tannins (punicalin and punicalgin), gallic acid, ellagic acid, ferulic acid, leucocyanidins and leucodelphinidols (Traoré 1999; Hilou *et al.*, 2014).



## Test for identity and purity

**Moisture content:** Air dried coarse leaf powder does not lose more than 06.5% at 105°C.

**Total ash:** not more than 08.8%

**Acid insoluble ash:** not more than 0.3%

**Water soluble extractives:** not less than 14.0%

**Ethanol soluble extractive (70% ethanol):** not less than 04.0%

## Chromatographic fingerprint

### Thin Layer Chromatography

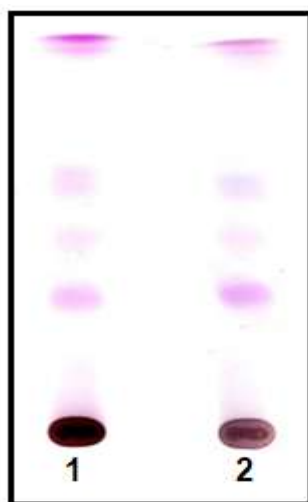
**Preparation:** About 5 g of the powdered leaves were extracted with ethyl acetate by cold maceration,

filtered and the filtrate concentrated to a small volume. A small spot was then applied to the TLC plate for analysis.

**Chromatographic conditions:** Analytical TLC on silica gel G60 F254, 0.25 mm layer in hexane/ethyl acetate (7:3) as the mobile phase.

**Detection:** Visualized in daylight after spraying with anisaldehyde-sulphuric acid (Lane 1) and vanillin-sulphuric acid reagents (Lane 2) (Stahl, 1969), heating to 110°C for 10 mins.

The TLC chromatogram showed three prominent spots with R<sub>f</sub>s of 0.49 (pink), 0.65 (purple) and 0.35 (purple) appeared in the chromatogram sprayed with both anisaldehyde and vanillin.



### High Performance Liquid Chromatography

**Sample preparation:** About 10 mg of the hydroethanolic extract of *C. glutinosum* leaves were reconstituted in 3 mL acetonitrile in a 10 mL volumetric flask with sonication for 17 minutes. The solution was then diluted to volume with the mobile phase under chromatographic conditions. It was centrifuged to obtain a clear test solution which was subsequently filtered through a 0.45 µm filter into an HPLC vial and analyzed.

### Chromatographic system

#### Optimized chromatographic conditions

**Mode:** LC

**Column:** YMC ODS, 4.6 x 150 mm, 5 µm

**Column temperature:** Ambient – 30°C

**Mobile phase:** Acetonitrile: water (60:40 v/v)

**Elution mode:** Isocratic

**Injection volume:** 20 µL

**Flow rate:** 0.5 mL/minute

**Detection wavelengths:** 230 nm, 254 nm and 278 nm.

### System Suitability parameters

**Number of peaks:** 230 nm (2), 254 nm (2), 278 nm (2)

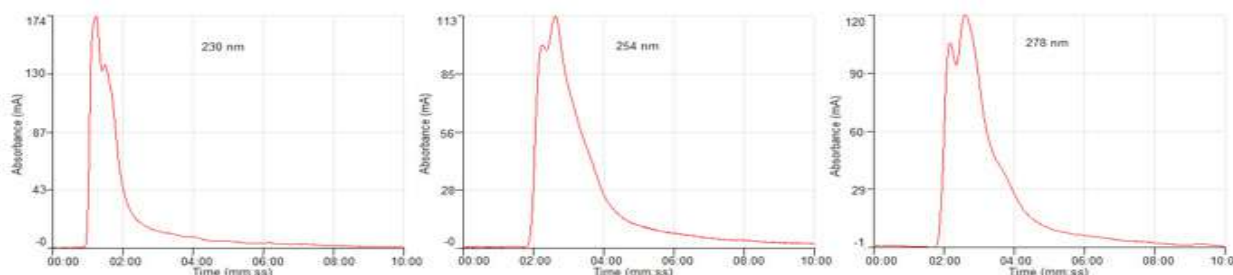
**Retention time (s):** 230 nm (rt1-2.29 min, rt2-3.00 min), 254 nm (rt1-2.15 min, rt2-2.38 min), 278 nm (rt1-2.10 min, rt2-2.35 min)

**Asymmetric factor(s):** 230 nm (af1-1.818, rt2-1.274), 254 nm (af1-1.830, af2-0.587), 278 nm (af1-1.495, af2-1.513)

**Tailing factor:** NMT 2.0

**Efficiency:** 230nm (E1-106.35, E2-573.66), 254 nm (E1-320.61, E2-304.74), 278 nm (E1-369.80, E2-123.75)

**Acceptance criteria:** Sample solution of hydroethanolic crude extract of *C. glutinosum* Guill. & Perr. (Leaves) conforms to the system suitability parameters.



### FT-IR

A small amount of the dried hydroethanolic extract (70%) was placed on the sample area of the Perkin Elmer UATR Fourier transform infrared (FT-IR) spectrometer and scanned between 4000-400  $\text{cm}^{-1}$  with a resolving power of 4  $\text{cm}^{-1}$ , and a cumulative scanning limitation of 24 times. Principal peaks appeared at wavenumbers 3350.89 (very broad) and 1614.41  $\text{cm}^{-1}$ .

### Macroscopy

Leaves are opposite, verticillate by three or sometimes subopposite and of variable shape and size. They are leathery, glaucous to greyish, more or less densely pubescent, elliptical, oval or obovate in shape with sometimes wavy margins. Apex more or less pointed or apiculated, sometimes notched, mucronate or rounded.

### Microscopy

The transverse sections of the median vein have numerous curved, unicellular and sometimes intertwined trichomes on the upper part and around the vein. Below the upper epidermis is a clear section of collenchyma cells. The vascular system is cup-shaped. The phloem fibres form an outer layer that surrounds the phloem and the xylem. There is another part of the phloem in the xylem divided in the middle by parenchyma cells. The vascular system is surrounded by collenchyma cells that are larger in the near section of the vascular system and whose size decreases towards the epidermis. The laminar portion shows only the upper palisade and consists of well-fitting columnar cells. Spongy parenchymal cells have an oval to irregular shape and are very tight. There are trichomes from the epidermis of both surfaces.

### Powdered plant material

The leaf powder has many fragments of the lower surface of the epidermis, with long, distinct unicellular trichomes; broken trichomes; broken fibres; upper epidermal surface fragments with thick-walled polygonal cells; fragments of vascular bundles showing spiral and annular xylem vessels.

**Therapeutic actions**

Antitussive, diuretic, hypotensive, hepatoprotective, antihelminthic, antibleorrhagic, antiemetic, cholagogue, expectorant, laxative, depurative and febrifuge.

**Therapeutic indications**

Hepatobiliary disorders, urinary disorders, oedema, arterial hypertension, cough and spasm, malaria, infantile gastritis, diarrhoea and dysentery, constipation, fever.

**Safety data**

LD<sub>50</sub> of the leaf extract by oral route was estimated to be beyond 3000 mg/kg in rats. CNS and ANS were not affected at doses of 0-1000 mg/kg. No significant changes were noted with the liver, kidney, heart and lungs. The relative organ to body ratios of spleen and thymus did not change. Extract of *C. glutinosum* did not significantly affect RBC count or indices and WBC and platelet count in treated rats. Combretum extract had no effect on enzyme markers of liver damage, serum proteins and bilirubin. It had marginal effect on pentobarbitone-induced sleeping time. There was no evidence of damage to target organs in the body including the liver and kidney. Combretum is a purported haematinic, although this study did not show significant changes in RBC counts and indices. The lack of toxicity seen in this study corroborates well with others reported by Alowanou *et al.*, (2015).

**Precautions for use**

Care must be taken in using it with extracts that can cause hypoglycaemia and hypotension.

**Adverse effects**

Constipation, hypotension and hypoglycemia

**Contraindication**

Not known

**Dosage form**

Decoction, infusion, tincture

**Dosage**

Decoction: 30 g of dried plant material in 900 mL water; simmer until reduced to 600 mL; 1-3 tablespoonfuls daily

Infusion: 30 g of dried aerial part in 600 mL of water; 3-4 teacups daily

Tincture: 1:5, 45% ethanol; 5 ml three times daily

**Storage**

Store in a cool, dry place away from light.

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**Botanical name***Daniellia oliveri* (Rolfe) Hutch. & Dalz.**Family**

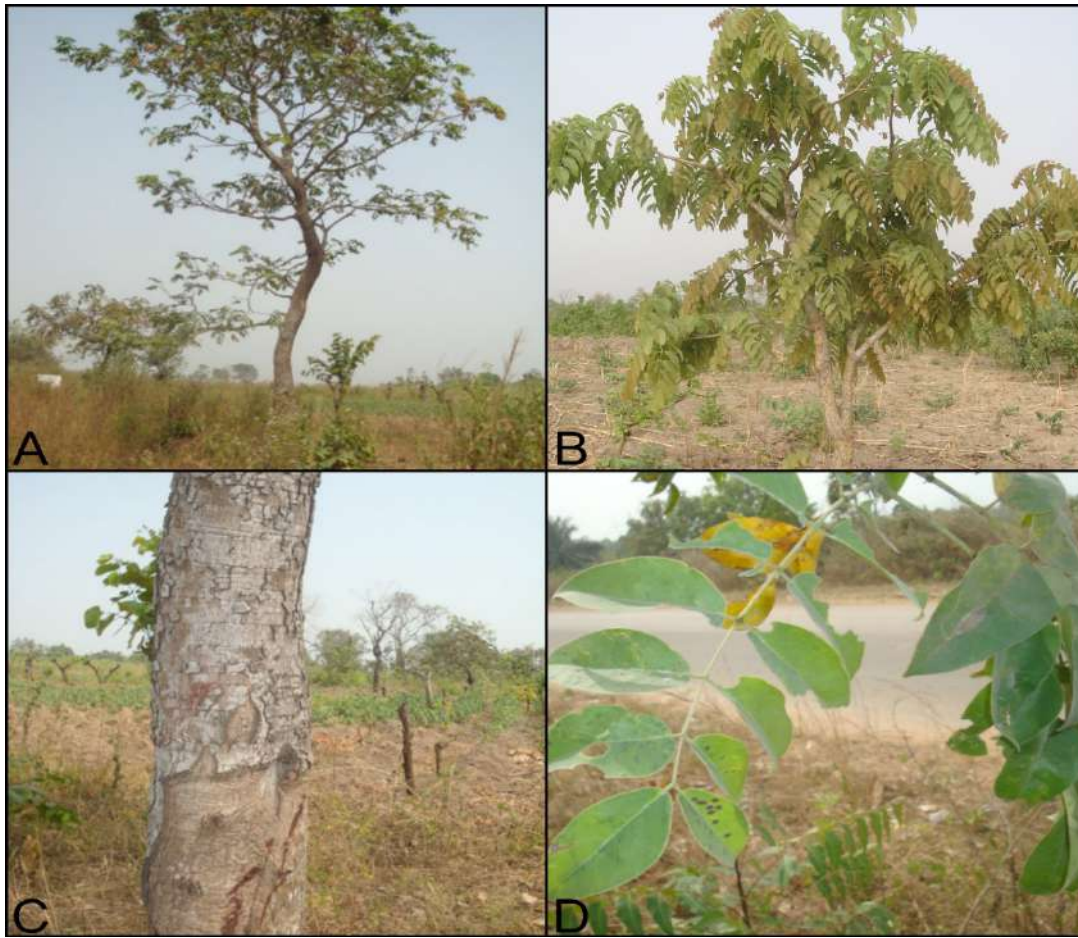
Fabaceae-Caesalpinioideae

**Synonyms***Daniellia thurifera* var. *chevalieri* J.Léonard - quoad fol., *Paradaniellia oliveri* Rolfe**Common Names**

African copaiba balsani tree, African copaiba Balsam, West African copal, Ilorin balsam (English), Copalier africain de balsam, Santan, Téré benthe (French).

**Common Local names****Benin:** Dendi – Falmey; Fon – Za; Yoruba - Iya**Burkina Faso:** Bambara - Sana; Dioula - Sana yiri; Moré- Aonga.**Côte d'Ivoire:** Dioula- Sanam brou; Haoussa – Mudié; Senoufo - Sourotchiqué**Gambia:** Mandinka – Tallo; Wolof - dettah**Ghana:** Mole - Aonga; Twi – Osanya; Konkomba - nialé**Guinea:** Malinké– Sandan ; Peuhl – Tièwi; Soussou - Ouloungui.**Guinea-Bissau:** Mandeng-Santan**Mali:** Bambara – Sanan; Dogon- Kédjè; Peulh- Kaha, Kalahi**Niger:** Djerma– Farmè; Haoussa - Mage.**Nigeria:** Hausa – Maje; Igbo – Agba; Yoruba - Iya**Senegal:** Mandeng-Santan; Sérère-Samban; Pular-Tewi**Togo:** Akasselem – Dényen ; Ewé – Lifiti; Gourmantché - Onyabugu**Description of the plant**

Large tree with spreading and quite dense crown, 15-25 m high. It is often low-branched with straight cylindrical bole up to 200 cm in diameter, and lacking buttresses. The bark has a smooth surface with a greyish white colour, which becomes scaly when matured. The foliage is pink to red at the time of leafing. The scaly bark desquamates in more or less circular plates. The branches are glabrous. Leaves are alternate, paripinnately compound with (3–) 6–11 pairs of leaflets. Leaflets are opposite, oblong-ovate to lanceolate, up to 15(–21) cm × 7(–10) cm, basal and apical leaflets smaller than middle ones. The base is cuneate, asymmetrical and the apex acuminate. The texture is papery to leathery, margins slightly wavy, sparsely to densely short-hairy to nearly glabrous, with few translucent glandular dots, pinnately veined with 9–17 pairs of lateral veins. The petiole has a small gland, which is more or less black. The inflorescence is a short axillary panicle. Flowers are white, greenish-white, or creamy. Fruits are obovate flat pod with two rigid papery valves. Seeds are brown, obovate and more or less flat, hanging and retained at one of the valves by a funiculus of 12-15 mm long (Arbonnier, 2002).



A and B – *Daniellia oliveri* (Rolfe) Hutch & Daiziel, C – Trunk showing stem bark, D – leaf

#### Herbarium specimen number

Benin: 2353 (AP)  
 Burkina Faso: CNSF-347 (OUA), Guinko 115 (OUA)  
 Côte d'Ivoire: 14221  
 Ghana: GH 244/KNUST  
 Mali: 190/DMT  
 Nigeria: UPFH 116  
 Senegal: IFAN AM 659  
 Togo: TG 00145

#### Habitat and geographical distribution

It is a large savanna and Sudano-Guinean woodland tree. Species grow on all types of soil spreading from Senegal to Cameroon, the Central African Republic, Zaire, Sudan and Angola (Arbonnier, 2002).

#### Plant material of interest

Stem bark and gum

#### Other parts used

Roots, leaves

## Definition of plant material of interest

Daniellia consists of the gum and fresh or dried stem bark of *Daniellia oliveri* (Rolfe) Hutch & Daiziel

## Ethnomedical uses

According to Arbonnier (2000), all parts of the plant are used in traditional medicine. The gum and stem bark of *D. oliveri*, and to a lesser extent the roots and leaves, are widely used in traditional medicine in almost all African countries. The gum, bark and leaves are burned and the smoke inhaled to treat headache and migraine. The smoke is also used as mosquito repellent. The gum, chewed and swallowed is used to treat diarrhoea (Arbonnier, 2002). In Cote d'Ivoire, the gum is considered an aphrodisiac and diuretic, and is chewed for cough, headache, tachycardia and menstrual pains. It is also used externally for itching and other skin conditions. The gum and bark are used in the manufacture of several internal and external preparations, sometimes with other parts of the plant to treat venereal diseases, ulcers and lesions, circumcision injuries, leprosy, dysentery, colic, menstrual disorders, cough, colds, angina, bronchitis, tuberculosis, kidney disease, appendicitis, headache, sore throat, rheumatism, pain due to fever, hernia, dental pain and snake bites. The root is considered a diuretic and the decoction is taken for venereal diseases, amenorrhoea, anxiety and dementia. The leafy twigs are also added to bath water and a steam bath is taken in case of fever and jaundice, and as a tonic. Decoction of leafy twigs with salt is prescribed as a purgative against constipation and stomach pains. The infusion of leaf buds is taken for migraine and feverishness in children during dental flares. Young crushed leaves are also applied to wounds, burns and abscesses to mature, as well as to relieve body pains. A gargle with a decoction of leaves is used to treat dental pain and the diction is drunk against colic. The large stipules are used to cover wounds, ulcers and as tampons for women during their menstrual period. In Burkina Faso, a stem bark decoction is administered to sheep and goats to treat worm infestation (Adjanohoon and Aké, 1971). In Nigeria, available literature suggests that the plant is used in the treatment of tumours, vaginal fistulas, abscesses and diabetes. Also in Nigeria, the gum is applied to the skin of horses to treat scabies (Jegede *et al.*, 2006). In Burkina Faso, the leaves of *Daniellia oliveri* are used in the management of syncope, malaria, convulsions, jaundice, conjunctivitis, ulcers and mycoses. The bark is effective in severe enteralgia, colic, intestinal obstruction, anorexia, anaemia, bronchitis, pneumonia, haemorrhoids, haemorrhages, metrorrhagia, kidney pain, dysentery, gonorrhoea and dysmenorrhoea. The roots are used to treat dysmenorrhoea, stomach ache, metrorrhagia, syphilis, obstinate constipation, dystocia and helminthiasis. The fruit, especially the pulp, treats avitaminosis C and B, vertigo and infectious diseases. The roots serve as diuretics and is also effective against gonorrhoea, female sterility, dysmenorrhoea, anxiety and madness. The bark is useful in the treatment of migraines, headaches, body aches, wounds, ulcers, skin diseases and dental caries, leprosy, snake bites, menstrual disorders, hydrocele and tuberculosis. The twigs are used to treat fever, jaundice, and cough, while the stipules treat wounds and ulcers. The leaves are used in cases of burns, constipation, jaundice, infertility, difficult deliveries, worms and glaucoma. The resin treats scabies, bronchitis, lumbago, body aches and hernia (Arbonnier, 2000).

## Biological and pharmacological activities

The methanolic extract of the stem bark has a relaxing effect on smooth muscle (Onwukaema and Udoh, 1999). Stem bark maceration showed anti-inflammatory properties in rats (Jegede *et al.*, 2006). The ethanolic leaf extract exhibited antidiarrhoeal activity in rats (Ahmadu *et al.*, 2007). The antimicrobial effect of the leaves of *D. oliveri* was more pronounced on the bacteria with an MIC of 1.875 mg/ml compared to the yeast. Trichloromethane extracts of stem and root barks and hydroethanolic stem bark extract proved to be active against bacteria. On yeast, only the hydroethanolic extracts of the trunk and aqueous extract of the root bark showed good activity with a MIC of 1.875 mg/ml. The ethyl acetate extract of the root had an MIC of 30 mg/ml on yeast and 7.5 mg/ml on bacteria, which were lower than those obtained by El-Mahmood *et al.*, (2008). The latter, showed an MIC equal to 50 mg/ml on *S. aureus* for the aqueous extracts, and

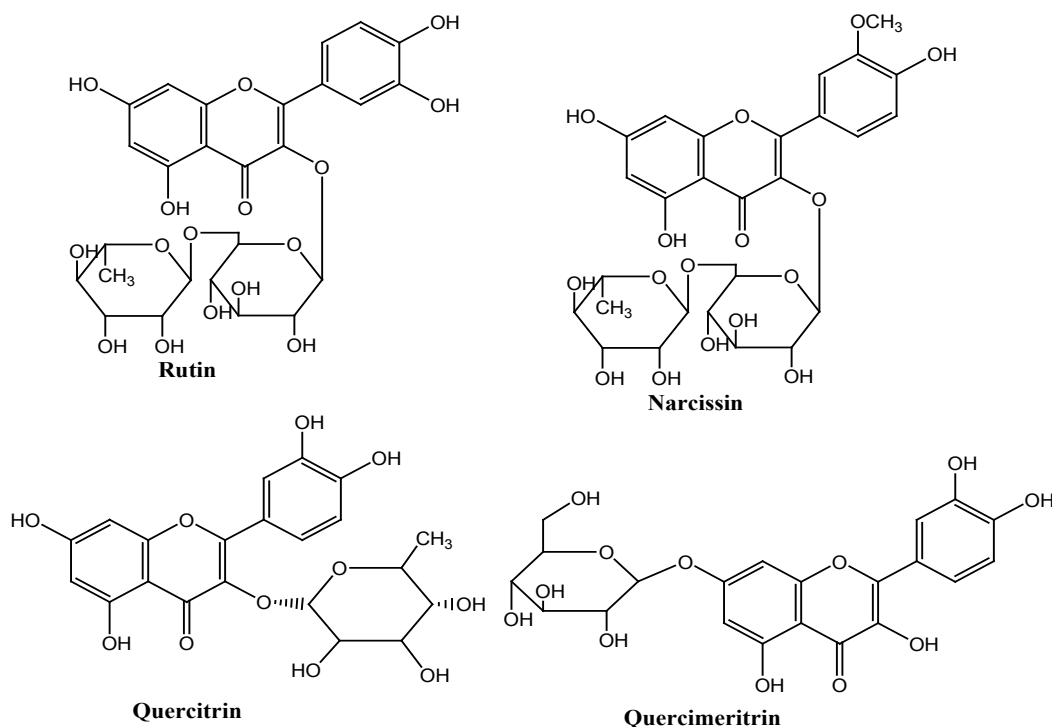
25 mg/ml for the ethanolic leaf extracts and trunk bark. For the roots, the MIC was 12.5 mg/ml for the aqueous extract and 6.25 mg/ml for the ethanolic extract. These differences in results may be due to the methods used to perform the antimicrobial tests not being the same. The cardiac glycosides present in the methanolic extract of the bark have been shown to be non-competitive muscarinic receptor antagonists. Methanolic extracts of leaves and bark have been shown to have neuromuscular blocking properties in rats (Ahmadu *et al.*, 2003; Kaita *et al.*, 2003; Balogun *et al.*, 2007; Osakwe *et al.*, 2004). Different water and ethanol extracts from leaves, bark and roots revealed moderate to significant *in vitro* antibacterial action against a range of pathogenic bacteria. The leaf ethanol extract also showed clear antifungal activity against *Tricophyton rubrum*. A crude bark extract was found to have significant dose-dependent anthelmintic activity on the gastrointestinal parasite *Haemonchus contortus in vitro*. A pot experiment showed that a leaf mulch of *D. oliveri* had an inhibitory effect on the germination of soybean, cowpea, maize, sorghum and millet (Ahmadu *et al.*, 2003; Kaita *et al.*, 2003; Balogun and Adebayo, 2007). The aqueous extracts of the bark of *D. oliveri* have dose-dependent antinociceptive activities after intraperitoneal injection in rats and mice. The extract revealed a non-dose-dependent antiinflammatory activity. The effect was significant at doses of 100 and 200 mg/kg in the rat (Ahmadu *et al.*, 2003; Kaita *et al.*, 2003; Balogun *et al.*, 2007; El Mahmood *et al.*, 2008; Osakwe *et al.*, 2004). The ethanol extract reduced feed consumption and the production of urine and faeces in the rat. The extract also revealed competitive antagonism on histamine-induced contraction of the guinea pig ileum and non-competitive inhibition of acetylcholine-induced contraction of the right muscle of the frog's abdomen (Onwukaeme *et al.*, 1999).

### Clinical data

Not available

### Chemical constituents

Sesquiterpenoids:  $\delta$ -cadinene,  $\alpha$ -copaene and germacrene D; flavonoid glycosides: rutin, quercitrin, narcissin and quercimeritrin (Ahmadu *et al.*, (2004); daniellic acid (illurinic acid), a diterpene oleoresin, ozic acid and ozol (Schwob *et al.* 2008; Adubiaro *et al.*, 2011; Ahmadu *et al.*, 2004; Onwukaem, 1995; Onwukaeme *et al.*, 1999).



### Test for identity and purity

**Moisture content:** Air dried coarse powder; does not lose more than 6.2% (leaves) and 6.5% (stem bark) at 105°C.

**Total ash:** not more than 8.9% (leaves) and 4.6% (stem bark)

**Acid insoluble ash:** not more than 0.5% (leaves) and 0.2% (stem bark)

**Water soluble extractive:** not less than 6.0% (leaves) and 9.0% (stem bark)

**Ethanol soluble extractive (70%):** not less than 8% (leaves) and 12.0% (stem bark)

### Chromatographic fingerprint

#### *Thin Layer Chromatography*

**Preparation:** About 5 g of the powdered stem bark were extracted with ethyl acetate by cold maceration, filtered and the filtrate concentrated to a small volume. A small spot was then applied to the TLC plate for analysis.

**Chromatographic conditions:** Analytical TLC on silica gel G60 F254, 0.25 mm layer in hexane/ethyl acetate (7:3) as the mobile phase. A small spot was then applied to the TLC plate for analysis.

**Detection:** Visualized in daylight after spraying with anisaldehyde-sulphuric acid (Lane 1) and vanillin-sulphuric acid reagents (Lane 2) (Stahl, 1969), heating to 110°C for 10 mins.

The TLC chromatogram showed five prominent spots with R<sub>f</sub>s of 0.91 (pink), 0.81 (light blue), 0.74 (pink), 0.44 (pink) and 0.24 (pink) when sprayed with both anisaldehyde and vanillin



#### *High Performance Liquid Chromatography*

**Sample preparation:** About 10 mg of the hydroethanolic extract of *D. oliveri* stem bark were reconstituted in 3 mL acetonitrile in a 10 mL volumetric flask with sonication for 17 minutes. The solution was then diluted to volume with the mobile phase under chromatographic conditions. It was centrifuged to obtain a clear test solution, which was subsequently filtered through a 0.45 µm filter into an HPLC vial and analyzed.

## Chromatographic system

### Optimized chromatographic conditions

**Mode:** LC

**Column:** YMC ODS, 4.6 x 150mm, 5 $\mu$ m

**Column temperature:** Ambient – 30°C

**Mobile phase:** Acetonitrile: Methanol: Water (60:20:20 v/v/v)

**Elution mode :** Isocratic

**Injection volume :** 20  $\mu$ L

**Flow rate:** 0.5mL/minute

**Detection wavelengths:** 230 nm, 254 nm and 278 nm.

### System Suitability parameters

**Number of peaks:** 230 nm (1), 254 nm (2), 278 nm (1)

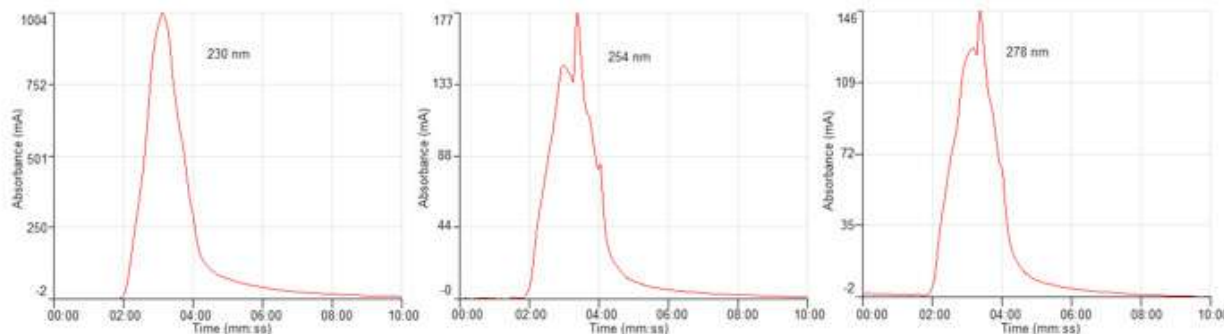
**Retention time (s):** 230 nm (3.07 min), 254 nm (rt1-2.59 min, rt2-3.22 min), 278 nm (3.22 min)

**Asymmetric factor(s):** 230 nm (0.991), 254 nm (af1-1.727, af2-1.176), 278 nm (1.225)

**Tailing factor:** NMT 2.0

**Efficiency:** 230 nm (44.18), 254 nm (E1-76.11, E2-2205.13), 278 nm (41.39)

**Acceptance criteria:** Sample solution of hydroethanolic crude extract of *D. oliveri* (Rolfe) Hutch. & Dalziel (Stem Bark) conforms to the system suitability parameters.



## FT-IR

A small amount of the dried hydroethanolic extract (70%) was placed on the sample area of the Perkin Elmer UATR Fourier transform infrared (FT-IR) spectrometer and scanned between 4000-400  $\text{cm}^{-1}$  with a resolving power of 4  $\text{cm}^{-1}$  and a cumulative scanning limitation of 24 times. Principal peaks appeared at wavenumbers 3225.75, 1603.08 and 1517.57  $\text{cm}^{-1}$

## Macroscopy

The bark of *D. oliveri* is scaly, peeling in more or less circular patches. The outer surface has shades of milky-brown to greyish brown colour. The inner surface has a brown colour with no furrows. It breaks with short granular fracture. The gum that is secreted by the heartwood is yellow to dark brown, oily and sticky.

## Microscopy

### *Transverse section*

Consists of rows of cork cells with brownish content followed by section of layers of groups of stone cells scattered within the bed of parenchyma cells. This is followed by a section of rows of sclereids, fibres traversed by medullary rays of large parenchyma cells with slightly polygonal cells up to seven cells or more wide tapering to the end of the inner layer of the transverse section. The groups of sclereids are surrounded by sheaths of calcium oxalate prisms.

### Powdered plant material

Powder consists of numerous stone cells with thick walls occurring singly and in groups, some with yellowish content. There are fragments of thick walled mainly hexagonal shaped cork cells in surface view and fragments in transverse section. Groups of fibres attached to stone cells, with calcium oxalate prisms lining the fibres. Fibres are long and twisted at the ends with acute apex. Cells, each with a prism of calcium oxalate occur in groups. Numerous calcium oxalate prisms are scattered in the powder.

## Therapeutic actions

Antibacterial; antifungal; antimicrobial; antiviral, amenorrhoea; dysmenorrhoea; analgesic; antipyretic; antiinflammatory.

## Therapeutic indications

Tuberculosis, fever, hernia, toothache, snake bites, fever, jaundice, intestinal worms, tumours, diabetes, malaria, syphilis, absent menstruation, inflammation.

## Safety data

LD<sub>50</sub> of the aqueous stem bark extract by oral route was estimated to be beyond 3000 mg/kg. The LD<sub>50</sub> of *D. oliveri* extract was estimated to be above 3000 mg/kg, consistent with the report of Iwueke and Nwodo (2008). There were no signs of CNS depression/stimulation or autonomic effects at doses of 0-1000 mg/kg. No significant changes were noted with organs such as the liver, kidney, heart and lungs. The relative organ to body ratios of spleen, thymus, and adrenals were not affected by treatment. Extract of *D. oliveri* did not affect RBC count. There was an apparent, but statistically significant reduction in haemoglobin and haematocrit, but not in mean cell haemoglobin content. It reduced WBC count mainly due to a decrease in granulocyte (neutrophils and MID) cells with a corresponding increase in lymphocytes. The extract also caused elevations in ALP and GGT, but did not affect total proteins and bilirubin. It decreased urea dose-dependently, but did not affect creatinine. It also altered the blood urea nitrogen to creatinine ratio. There was only a marginal increase in pentobarbitone-induced sleeping time. There were histological evidence of tubular necrosis in the kidney. Other experimental results show that intake of *D. oliveri* extract may predispose the individual to cardiovascular diseases because of the increased serum cholesterol and atherogenic index (Balogun and Adebayo 2008). Decrease in serum urea without a change in creatinine



may indicate a possible inhibition in urea reabsorption at the nephrons due to renal damage. Histopathology of the kidney supports this conclusion.

### Precautions for use

Do not use in patients with kidney or cardiovascular disease.

### Adverse effects

Possible inhibition of renal or cardiac function.

### Contraindication

Patients with cardiac or renal disease.

### Dosage form

Powder, decoction, infusion, tincture

### Dosage

Decoction: 30 g of dried plant material in 900 mL water; simmer until reduced to 600 mL; 1-3 tablespoonfuls daily

Infusion: 30 g of dried aerial part in 600 mL of water; 3-4 teacups daily

Tincture: 1:5, 45% ethanol; 5 ml three times daily

### Storage

Store in a cool, dry place away from light.

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**Botanical name***Euphorbia poissonii* Pax**Family**

Euphorbiaceae

**Synonyms***Synadenium pereskiifolium* (Houllet ex Baill.)**Common Names**

Candle plant (English), Solo (Français).

**Common Local names**

**Benin:** Fon - So Jekpé; Yoruba - Oro adètè; Dendi - Gorigbo  
**Ghana:** Mole – Aoga, Aonga; Twi – Osanya; Adangme – atroku  
**Mali:** Pulaar- Pendiré; Barth - abári e sébuwa; Songhai - taboru  
**Niger:** Dendi – lokoto; Fulfulde – pendire; Gurma - péni  
**Nigeria :** Hausa-Kandari ; Yoruba-Orin idi ; Kanuri-Garuru  
**Togo:** Ewé – Zontchi, Tredzo; Mina – Adikpoé; Tem – Férémon

**Description of the plant**

The plant is an erect, monoecious shrub with several branches (candelabrum) growing up to 2 m tall. The branches are thick, succulent, with leaves persisting at the top and occasionally sub-spiny. The branches are cylindrical, silvery-grey twigs, covered with prominent rounded tubercles and horny shields of thorns up to 8 mm in diameter, grey, and thorny in young plants or with rudimentary spines containing a white latex in all plant parts. The leaves are spirally arranged at the apex of the stem in 8 to 10 rows, single and whole, falling rapidly; the stipules, if present, are modified into small spines, falling rapidly. The petiole is short and thick and the blade is obovate 5 to 14 cm × 3 to 7 cm. It has a long cuneiform base, apex deeply emarginate, fleshy, glabrous, and pinnately veined. The inflorescence is in axillary cymes, numerous at the end of the branches, made up of groups of flowers called “cyathes”; peduncle and short branches; bracts 2, ovate, about 2 mm long, membranous; the cyathium has a diameter of about 8 mm, involucre in the form of a short funnel, green in colour, presence of 5-lobed with widely oval and fringed lobes, 5 glands, elliptic, touching, green, each involucre containing 1 flower female surrounded by many male flowers; the flowers are unisexual. The male flowers are sessile with absent perianth, the stamen is short and red in colour. Female flowers have a curved pedicel 5 to 12 mm long in the fruit. The flowers are heavily pollinated by bees and other insects. The fruit is a deeply lobed capsule, the 3 lobes present are almost globose, glabrous, in which there are 3 seeds. The seeds are ovoid about 2 mm long, smooth, pale grey with some dark markings (Brown *et al.*, 1909-1913; Dalziel, 1937; Burkill, 1994; Arbonnier, 2002).



A – *Euphorbia poissonii* leaves, B – with no leaves, C – *Euphorbia poissonii* Pax

#### Herbarium specimen number

Benin: 2341 (AP)  
 Burkina Faso: CNSF-524  
 Ghana: GH 263/KNUST  
 Nigeria: UPFH 117  
 Togo: TG 03243

#### Habitat and geographical distribution

*E. poissonii* occurs in savannahs, rocky areas, fields, near villages (Tchinda, 2008). It is also found on dry, stony soils, usually in open grassy woodland at 400-700 m altitude (Tchinda, 2008). *E. poissonii* occurs from southern Burkina Faso and Ghana east to Cameroon. It is probably also present in Guinea, Côte d'Ivoire and Mali (Aubréville, 1950).

#### Plant material of interest

Leaves

#### Other part used

Stems, twigs, roots

#### Definition of plant material of interest

The plant consists essentially of the dried leaves of *Euphorbia poissonii* Pax

## Ethnomedical uses

Despite its toxicity, the latex of *E. poissonii* is used in traditional medicine. The latex from all parts of the plant is to treat trypanosomiasis, incurable wounds and guinea worm infestation (Arbonnier, 2002). Latex and roots are used as fish poisons and for hunting (Arbonnier, 2002). In Nigeria, a few drops are applied to wounds of guinea worm and cutaneous papilloma (a benign tumour of the skin). Few drops of latex with sugar cane, palm wine or soup is taken as a purgative (Burkill, 1994). In Cameroon, lumbago (painful lumbar spine) is treated with latex applications extracted from the leaves of *E. poissonii*. The latex is applied to decayed tooth to sooth the pain or to help loosen it to make extraction easier (Adjanohoun *et al*, 1996). A piece of stalk is mixed with the seeds of *Strophantus hispidus* to make arrow poison. In Nigeria, latex would sometimes be added to snuff to make it more pungent. The Hausas pour latex on cereals to catch wild guinea fowl. In West Africa, *E. poissonii* is sometimes planted in gardens as an ornamental or hedge around fields and cemeteries. In Europe and the United States, it is found in pots in the collections of succulents (Adjanohoun *et al*, 1996; Keay, 1958).

## Biological and pharmacological activities

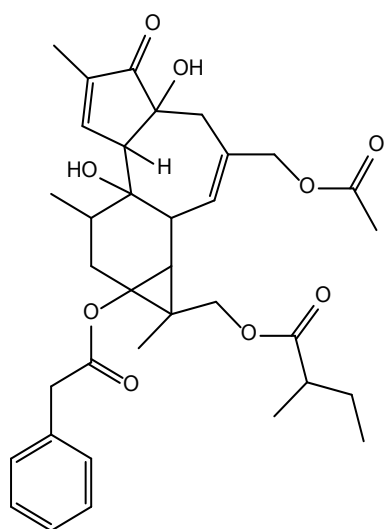
The latex and isolated esters have potent irritating properties and are toxic and carcinogenic. Some of the tiglane compounds, particularly 12-deoxyphorbol derivatives, exhibited anticancer activity (Fakunle *et al.*, 1989; Schmidt, & Evans, 1976 and 1978). Isolated aromatic esters of the daphnane type are more potent irritants in mouse ear sensitization tests than tiglane aromatic esters, especially resiniferatoxin ( $LD_{50} = 0.00021 \text{ nMol/5 } \mu\text{g}$ ) and tinyatoxin ( $LD_{50} = 0.0012 \text{ nMol/5 } \mu\text{g}$ ). The compounds resiniferatoxin and tinyatoxin are very irritating. The irritation peaks after 4 hours before becoming dormant again after 24 hours (Schmidt and Evans, 1976; Graham, 2000). Resiniferatoxin and tinyatoxin are extremely toxic because they bind to the pain receptors in the same way as capsaicin, but much more powerfully. They stimulate the neurons to discharge repeatedly until the neuron dies, triggering a shooting pain and plunging the victim into severe anaphylactic shock. Resiniferatoxin has antihyperactivity of the bladder and antiappetizing and analgesic properties. Derivatives of the compound 19-hydroxyngol, present in the latex has been shown to be cytotoxic against six solid tumour cell lines in humans (carcinoma of the lung, breast, kidney and pancreas, adenocarcinoma of the colon and prostate). Esters derived from 12-deoxyphorbol have shown selective cytotoxicity against the renal cell carcinoma cell line in humans, with a single compound having a potency 10,000 times greater than that of the anticancer drug, adriamycin (Fakunle *et al.*, 1989). 12-Deoxyphorbol 13-phenylacetate (DPP) induced the expression of HIV-1 in latently infected T cells and made them susceptible to immunotoxin clearance. DPP is 20- to 40-fold more potent than phorbol ester, prostratin, and the combination of high potency and antipromotor tumour activity, makes it an attractive candidate for treatment of HIV infection (Schmith and Evans, 1980; Graham, 2000). A root extract of Nigerian origin, showed molluscicidal properties on the freshwater snail *Bulinus globulus* (Schmidt and Evans, 1976; Graham, 2000).

## Clinical data

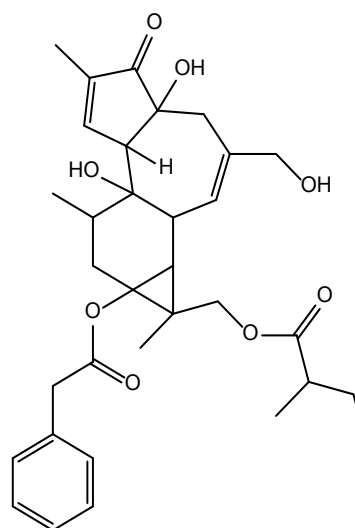
Not available

## Chemical constituents

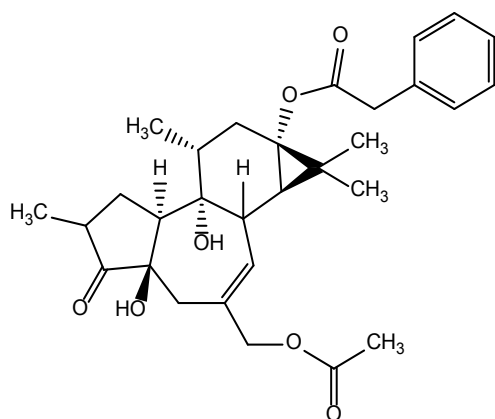
Esters of diterpenic alcohols of the tiglane type (12-deoxyphorbol and 12-deoxy-16-hydroxyphorbol), a diterpene alcohol (resiniferonol), and several esters of a macrocyclic diterpene alcohol, 19-hydroxyngol, euphorianine, monoesters and diesters of 12-deoxyphorbol; daphnane, tiglane esters (Majekodunmi *et al.*, 1996; Fakunle *et al.*, 1989; Schmidt and Evans, 1976 and 1978); 19-hydroxyngol derivatives (Fakunle *et al.*, 1989).



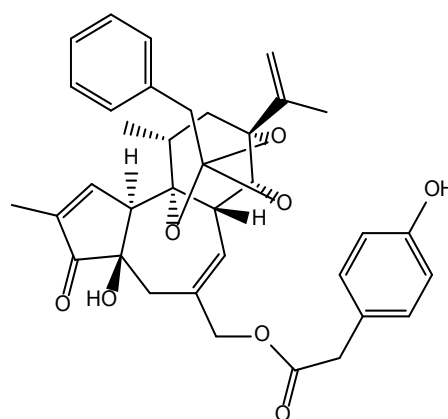
Candletoxin A



Candletoxin B



12-deoxyphorbol-13-O-phenylacetate-20-O-acetate (DPP)



Tinyatoxin

### Test for identity and purity

**Moisture content:** air dried coarse powder does not lose more than 8.5% (leaves) and 11.5% (whole stem) at 105°C.

**Total ash:** not more than 23.1% (leaves) and 17.6% (stem bark)

**Acid insoluble ash:** not more than 5.3% (leaves) and 1.3% (stem)

**Water soluble extractive:** not less than 18.0% (leaves) and 09.0% (stem)

**Ethanol soluble extractive (70%):** not less than 6.0% (leaves) and 5.0% (stem)

### Chromatographic fingerprint

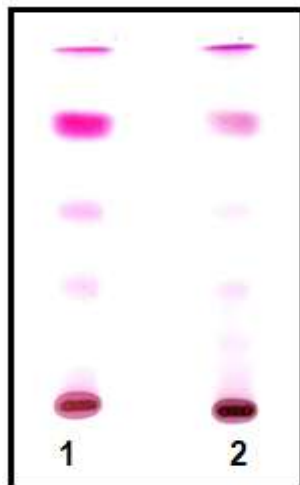
#### Thin Layer Chromatography

**Preparation:** About 5 g of the powdered leaves were extracted with ethyl acetate by cold maceration, filtered and the filtrate concentrated to a small volume. A small spot was then applied to the TLC plate for analysis.

**Chromatographic conditions:** Analytical TLC on silica gel G60 F254, 0.25 mm layer in hexane/ethyl acetate (7:3) as the mobile phase.

**Detection:** Visualized in daylight after spraying with anisaldehyde-sulphuric acid (Lane 1) and vanillin-sulphuric acid reagents (Lane 2) (Stahl, 1969), heating to 110°C for 10 mins.

The TLC chromatogram showed three prominent spots with R<sub>f</sub>s of 0.78 (pink), 0.54 (pink) and 0.4 (pink) when sprayed with both anisaldehyde and vanillin.



### High Performance Liquid Chromatography

**Sample preparation:** A sample of about 10 mg of the hydroethanolic extract of *E. poissoni* leaves was reconstituted in 3 mL acetonitrile in a 10 mL volumetric flask with sonication for 17 minutes. It was then diluted to volume with the mobile phase under chromatographic conditions. It was centrifuged to obtain a clear test solution which was subsequently filtered through a 0.45 µm filter into an HPLC vial and analyzed.

### Chromatographic system

#### Optimized chromatographic conditions

**Mode:** LC

**Column:** YMC ODS, 4.6 x 150 mm, 5 µm

**Column temperature:** Ambient -30°C

**Mobile phase:** Acetonitrile: Methanol: Water (60:20:20 v/v/v)

**Elution mode :** Isocratic

**Injection volume :** 20 µL

**Flow rate:** 0.5 mL/minute

**Detection wavelengths:** 230 nm, 254 nm and 278 nm.

#### System Suitability parameters

**Number of peaks:** 230 nm (1), 254 nm (1), 278 nm (1)

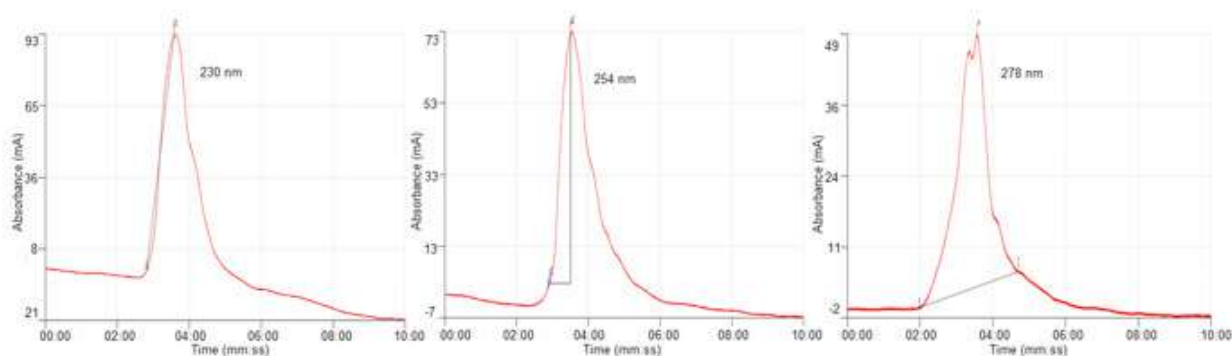
**Retention time (s):** 230 nm (3.36 min), 254 nm (3.33 min), 278 nm (3.34 min)

**Asymmetric factor(s):** 230 nm (0.937), 254 nm (0.756), 278 nm (0.585)

**Tailing factor:** NMT 2.0

**Efficiency:** 230 nm (95.36), 254 nm (102.11), 278 nm (106.87)

**Acceptance criteria:** Sample solution of hydroethanolic crude extract of *E. poissoni* (leaves) conforms to the system suitability parameters.



## FT-IR

A small amount of the dried hydroethanolic extract (70%) was placed on the sample area of the Perkin Elmer UATR Fourier Transform infrared (FT-IR) spectrometer and scanned between  $4000\text{--}400\text{ cm}^{-1}$  with a resolving power of  $4\text{ cm}^{-1}$  and a cumulative scanning limitation of 24 times. Principal peaks appeared at wavenumbers  $3272.20$  (very broad),  $2931.84$  and  $1575.00\text{ cm}^{-1}$  (very strong)

## Macroscopy

Leaf is succulent, thick, glabrous, apex is deeply emarginate and sometimes slightly acute. The leaf is cuneate to spatulate in shape with entire margin. The petiole is short and thick and the blade is obovate  $5$  to  $14\text{ cm} \times 3$  to  $7\text{ cm}$ . It has a long cuneiform base and the venation is pinnate.

## Microscopy

The leaf is amphistomatic with numerous anomocytic stomata on both surfaces. The epidermal cells are non-uniform polygonal cells, mostly five sided. Trichomes are absent from both surfaces.

## Transverse section

Transverse section of the stem shows irregular rows of cork cells with brown contents and thin walls followed by three to four rows of thin walled cork cells with irregular, squashed, rectangular shape. This is followed by one row of cork cells with brown content and two rows of rectangular cells. Cork cell rows are followed by rows up to thirteen or more of six sided polygonal parenchyma cells with thin walls. The leaf transverse section shows a flat lower surface and a slight bulge on the upper surface. Both surfaces are lined with a single row of epidermal cells with very thin cuticle. This is followed by a few rows of rectangular parenchyma cells with thick walled collenchyma cells radiating towards the cortex. The mid-section has a main vascular bundle with xylem completely surrounded by the phloem tissue. Other smaller vascular bundles occur as single row intermittently interspaced by parenchyma cells from the midrib towards the lamina.

## Powder microscopy

The leaf powder shows abundant long twisted, mesh-like fibres with tapering ends. Numerous fragments of leaves bearing anomocytic stomata can also be seen. Reticulate and annular xylem vessels and tracheids occur. The stem powder is light brown coloured and contains mainly cork cells; abundant parenchyma and fibres occur singly and in groups. Fibres are unicellular and slightly twisted.



**Therapeutic actions**

Antiviral against HIV-1 infection, antischistosomiasis, analgesic and anticancer.

**Therapeutic indications**

Pain, HIV-1 infection, trypanosomiasis, prostate disease, urinary incontinence; carcinoma of the lung, breast, kidney and pancreas; adenocarcinoma of the colon and prostate

**Safety data**

The LD<sub>50</sub> by oral route was estimated to be above 3000 mg/kg. CNS depression/stimulation or autonomic effects were not apparent following the treatment. No significant changes were noted with the liver, kidney, heart and lungs. The relative organ to body ratios of spleen, thymus, and adrenals did not change. Haematological indices, serum biochemistry, renal and liver function markers of damage remained normal after treatment. There is no evidence presently to suggest potential toxicity of *Eurphobia* within the recommended doses. However, latex from the fresh leaves is reportedly toxic and a potent irritant to the skin and eye (Basak *et al.*, 2009).

**Precautions for use**

The aqueous extract of the leaves is not toxic at the recommended doses. The latex is caustic and toxic, so its use should be with greater care. The latex is very irritating to the skin and mucous membranes. It can cause blindness on exposure to the eyes. Caution should be taken when administering the aqueous extract to patients with impaired liver function.

**Adverse effects**

Very irritating action of the latex in contact with the skin and the mucous membranes.

**Contraindication**

Pregnancy, breast feeding, active liver diseases

**Dosage form**

Poultice, decoction, infusion, tincture

**Dosage**

Decoction: 30 g of dried plant material in 900 mL water; simmer until reduced to 600 mL; 1-3 tablespoonfuls daily

Infusion: 30 g of dried aerial part in 600 mL of water; 3-4 teacups daily

Tincture: 1:5, 45% ethanol; 5 ml three times daily

**Storage**

Store in a cool, dry place away from light.

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**Botanical name***Flueggea virosa* (Roxb. ex Willd.) Royle**Family**

Phyllanthaceae

**Synonyms***Phyllanthus virosus* Roxb. ex Willd., *Securinega virosa* (Roxb. ex Willd.) Baill., *Flueggea microcarpa* Blume, *Securinega microcarpa* (Blume) Müll.Arg. (Arbonnier, 2009)**Common Names**

White-berry bush, snowberry tree, Chinese waterberry, simple leaf bush weed, common bushweed (English). Balan des savanes (French).

**Common Local Names****Benin:** Bariba – Gaagah; Fon – Adjaya; Gourmantché – Koukrinou**Burkina Faso:** Dioula - Baram-baram; Fulfuldé – Sugurlaagah; Morée – Sugdendaaga**Côte d'Ivoire:** Baoulé-Niassoué; Bété-Génakwo; Dioula-Mokrodama**Gambia:** Manding– Brumbarongo; Wolof - Kell**Ghana:** Akan-Asante – nkanaa; GA - gbekɛbii-able-t[ɔ]; wɔlɔmɔt[ɔ]; Hausa – tsa**Guinea:** Basari - a-nɛembrɛsyɛt; Maninka - barinbarin**Mali:** Bambara- Surukugnègnè Nkoloningé; Dogon- Segele; Minyanka-Jene; Sénoufo-Jeme**Niger:** Arabic - Kartié kartié; Hausa – tsa; *Kanuri* - dagkirto**Nigeria:** Hausa –Gussu ; Igbo-Njisi ntà ; Yoruba-Iranjé ìranjé**Senegal:** Mandeng-Baram baram; Peulh-Sambelgorel; Wolof-Keen.**Sierra Leone:** Mende - Tigwi**Togo:** Akassélem – hesré; Ewé – Kébantchalé; Tem – Kabiyé**Description of the plant**

*Flueggea* is a deciduous, strongly branched, dioecious shrub or small tree growing up to 6 m tall. It has a smooth grey-brown bark occasionally cracked or rough. The branches are erect or arched, the lower ones often having thorny ends. Leaves alternate distichous, simple and entire; stipules lanceolate, 1.5-2 mm long, acute, fringed, deciduous; petiole 3-6 mm long, fluted above, closely winged; almost orbicular to obovate or elliptical, (1-) 2-4 (-6) cm × (0.5-) 1-2 (-3) cm long, wedge-shaped to rounded base, obtuse apex, rounded or emarginate, finely papery, pinnately veined with 5-9 pairs of lateral veins. Inflorescence an axillary fascicle with many flowers in male plants and few in female plants (Berhaut, 1975). Unisexual, regular, 5-mother, fragrant flowers; pedicel up to 9 mm long; sepals slightly unequal, obovate to lanceolate, fringed, pale greenish-yellow; petals absent; male flowers with free stamens, exerted, filaments 2-3 mm long, fleshy disk glands, yellow, rudimentary ovary, 3 styles, up to 2 mm long, fused at base; female flowers with annular disc, weakly 5-lobed, ovary superior, ovoid, 3-celled, styles 3, fused, stigmas 2-fides, spread out horizontally. Fruit a globose capsule, somewhat fleshy and slightly 3-lobed, 3-5 mm in diameter, dehiscent, smooth, glabrous, white, up to 6-seeded. Ovoid seeds, 2-3 mm long, shiny, yellowish brown (Tabuti, 2007).



A – *Flueggea virosa* fruits, B and C- *Flueggea virosa* whole plant

#### Herbarium specimen number

**Benin:** 2342 (AP)

**Burkina Faso:** BUR-129 (CNSF), 1432 (OUA)

**Côte d'Ivoire:** 16025 (CNF)

**Ghana:** GH 313/KNUST

**Mali:** 1356/DMT

**Nigeria:** KASU/PCG/093

**Senegal:** IFAN 35

**Togo:** TG 03763

#### Habitat and geographical distribution

*F. virosa* is common in all kinds of environments: forest edges, shrubby savannas, grassy savannas, wooded savannas and thickets. In dry areas, it is found mainly along watercourses, as well as in marshy environments, sometimes on termite mounds and stony slopes; it is also common in disturbed areas and fallow land, from sea level up to 2300 m altitude (Tabuti, 2007). *F. virosa* is a plant found throughout tropical Africa from Mauritania to Somalia, southern South Africa, and also Madagascar. It is also distributed from Egypt, the Arabian Peninsula and across tropical Asia and Japan, Australia and also Polynesia, via tropical Asia (Ruffo *et al.*, 2002; Arbonnier, 2009).

#### Plant material of interest

Leaves, stems

## Other parts used

Roots

## Definition of plant material of interest

Fresh or dried leaves and stems of *Flueggea virosa* (Roxb, ex Willd.) Royle (Phyllanthaceae)

## Ethnomedical uses

The plant is used for the treatment of fever, malaria and migraines (Malgras, 1992). The leaves are used in the treatment of uro-genital infections (Adjanooun *et al.*, 1986), malaria, hepatic diseases, wounds and also as a laxative or purgative (Adjanooun *et al.*, 1989). It is used together with *Morinda lucida* in the treatment of malaria (Asase *et al.*, 2010). The leaves are used topically for wounds and boils. It is administered orally and as a bath for the treatment of small boils (Inngjerdingen *et al.*, 2004). An infusion of the aerial part is used orally and locally against pruritus and rashes (Adjanooun *et al.*, 1980). The leafy stem is used orally as a laxative and purgative, in the treatment of abdominal pain, and as an ocular instillation in conjunctivitis. (Adjanooun *et al.*, 1986). The root is used for the treatment of malaria, bilious fever, sexual asthenia, renal lithiasis (Kerharo and Adam, 1974), urogenital and hepatic affections (Adjanooun *et al.*, 1981). It is also used to treat snake bites, sexual impotence and haemorrhoids (Malgras, 1992). The powdered root is added to porridge and taken to treat malaria (Sangaré, 2003). The root is also used in the treatment of urinary schistosomiasis.

## Biological and pharmacological activities

Extracts and compounds isolated from the plant showed an antioxidant activity against the DPPH radical with a direct activity on superoxide anion (Sanogo *et al.*, 2009). Acetone extracts of the roots and flavonoids isolated from the leaves also showed strong antioxidant activity (Chauke *et al.*, 2012). Triterpenoids, friedeline, epifriedelanol, stigmasterol, and betulinic acid isolated from *F. virosa* stem extracts demonstrated antiproliferative properties on leukaemic cells (Monkodkaew *et al.*, 2009). Extracts of the leaves and roots have analgesic, antiinflammatory and antipyretic activities. Oral administration of the methanolic extract of the leaves (25 - 100 mg/kg, ip) (Yerima *et al.*, 2009), and aqueous extract of the root (200 - 400 mg/kg), demonstrated antiinflammatory activity in mice, and antipyretic activity in rats (Ezeonwumelu *et al.*, 2012). The methanolic extract of the root bark (6.25 - 25 mg/kg, ip) showed antiinflammatory and analgesic activity (Magaji *et al.*, 2008). The aqueous extracts of the leaves and stems showed analgesic activities, with the latter being more pronounced (Diakit , 2014). The methanolic extract of the leaves demonstrated *in vitro* antiplasmodial activity on *P. falciparum* strain D6 and W2 with an IC<sub>50</sub> of 2.2 µg/mL and 3.6 µg/mL, respectively (Muthaura *et al.*, 2015). The hydromethanol extract (1:1) showed activity on *P. falciparum* W2 strain (IC<sub>50</sub> = 2 µg/mL) (Kaou *et al.*, 2008), and the dichloromethane extract on *P. falciparum* K1 strain (IC<sub>50</sub> = 2.41 µg/mL) (Diallo *et al.*, 2007). The methanolic extract of the leaves administered by gavage at a dose of 100 mg/kg/day for 4 days, demonstrated antiplasmodial activity *in vivo* in mice infected with *Plasmodium berghei* with 70.91 ± 4.53% parasitaemia suppression (Muthaura *et al.*, 2007). The hydromethanol extract (1:1) of the stems had an IC<sub>50</sub> of 5.5 µg/mL on the *P. falciparum* W2 strain (Kaou *et al.*, 2008), while the IC<sub>50</sub> of the aqueous extract of the root was 3 µg/mL on the *P. falciparum* W2 strain (Kaou *et al.*, 2008). The aqueous root extract demonstrated *in vitro* antiplasmodial activity on *P. falciparum* strain K1 with an IC<sub>50</sub> of 8.69 µg/mL (Diallo *et al.*, 2007). The alcoholic extracts of the leaves and the isolated compounds viros curinine and viroallos curinine, tested on leukaemic lymphoid cells *in vitro*, exhibited anti-tumour activity, with significant cytotoxicity *in vitro* with a DE<sub>50</sub> of 2.9 and 0.9 µg/mL, respectively on P.388 cells (Tatematsu *et al.*, 1991). The methanolic extract of the leaves (100 - 300 - 600mg/kg, ip) showed hypoglycaemic activity, by a significant decrease (P < 0.05 - 0.01) in streptozocine-induced hyperglycaemia in rats (Tanko *et al.*,

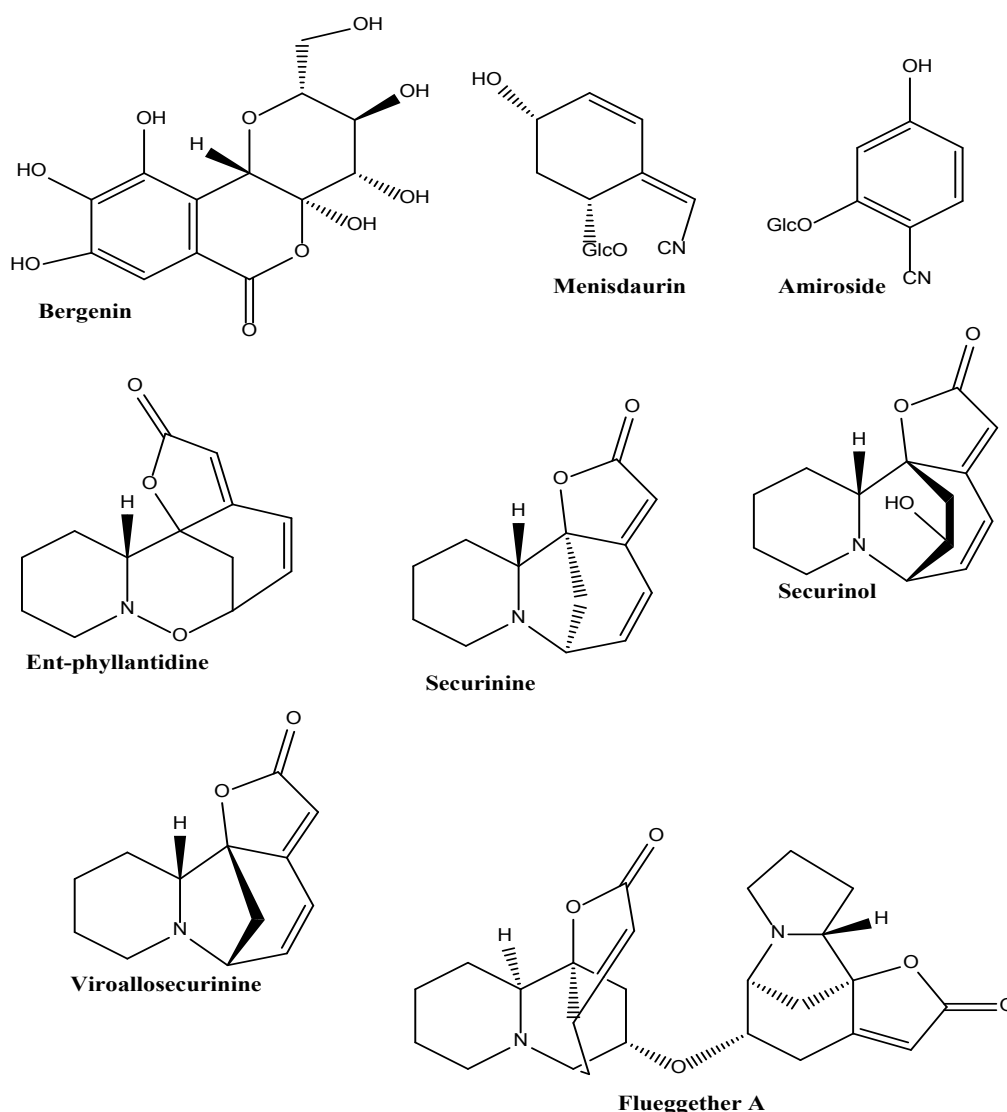
2007). Dinorditerpenes isolated from the roots of *F. virosa* demonstrated *in vitro* antiviral activity by inhibiting hepatitis C virus (Chao *et al.*, 2014). Fluevirosinin isolated from leaves and stems of *F. virosa* inhibited HIV with a  $14.1 \pm 1.2 \mu\text{M}$   $\text{EC}_{50}$  (Zhang *et al.*, 2015), while the ethanolic root extract demonstrated anticonvulsant activity (Sanogo *et al.* 2010, Pedersen *et al.*, 2008)

### Clinical data

Not available

### Chemical constituents

Indolizidine alkaloids: securine-like alkaloids (virosecurinine, viroallosecurinine, norsecurinine, dihydronorsecurinine (virosine); hordenine, N-methyltetrahydro- $\beta$ -carboline; cyanogenetic glycosides menisdaurin, alkaloids ent-phyllanthidine, securinine, securinol, viroallosecurinine; gallicocatechin, epigallocatechin, ethyl glucopyranoside, squalene (Zhang *et al.*, 2015a), flueggether A, virosinine A, virosaines A and B (Zhao *et al.*, 2012), flueggedine (Zhao *et al.*, 2013); 11-O-acetyl bergenin, kaempferol, daucosterol,  $\beta$ -sitosterol (Wang *et al.*, 2008), fluovirosinin (Zhang *et al.*, 2015b), fluevirins (Li *et al.*, 2014).



### Test for identity and purity

**Moisture content:** air dried coarse powder does not lose more than 5.4% at 105°C.

**Total ash:** not more than 8.7%

**Acid insoluble ash:** not more than 0.5%

**Water soluble extractive:** not less than 11.0%

**Ethanol soluble extractive:** not less than 20.0%

### Chromatographic fingerprint

#### *Thin Layer Chromatography*

**Preparation:** About 5 g of the powdered stem bark were extracted with ethyl acetate by cold maceration, filtered and the filtrate concentrated to a small volume. A small spot was then applied to the TLC plate for analysis.

**Chromatographic conditions:** Analytical TLC on silica gel G60 F254, 0.25 mm layer in hexane/ethyl acetate (7:3) as the mobile phase.

**Detection:** Visualized in daylight after spraying with anisaldehyde-sulphuric acid (Lane 1) and vanillin-sulphuric acid reagents (Lane 2) (Stahl, 1969), heating to 110°C for 10 mins.

The TLC chromatogram showed two prominent spots with R<sub>f</sub>s of 0.87 (pink) and 0.62 (pink) when sprayed with anisaldehyde. Only one prominent spot with R<sub>f</sub> of 0.62 (purple) appeared in the chromatogram sprayed with vanillin.



#### *High Performance Liquid Chromatography*

**Sample preparation:** About 10 mg of the hydroethanolic extract of *F. virosa* stem bark were reconstituted in 3 mL acetonitrile in a 10 mL volumetric flask with sonication for 17 mins. The solution was then diluted to volume with the mobile phase under chromatographic conditions. It was centrifuged to obtain a clear test solution which was subsequently filtered through a 0.45 µm filter into an HPLC vial and analyzed.

## Chromatographic system

### Optimized chromatographic conditions

**Mode:** LC

**Column:** YMC ODS, 4.6 x 150 mm, 5 µm

**Column temperature:** Ambient – 30°C

**Mobile phase:** Acetonitrile: Methanol: Water (60:20:20 v/v/v)

**Elution mode :** Isocratic

**Injection volume :** 20 µL

**Flow rate:** 0.5 mL/minute

**Detection wavelengths:** 230 nm, 254 nm and 278 nm.

### System Suitability parameters

**Number of peaks:** 230 nm (1), 254nm (1), 278 nm (1)

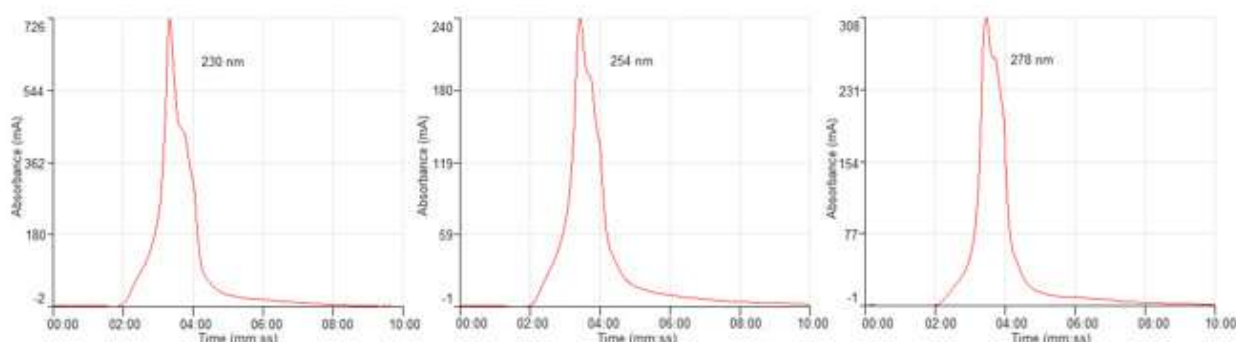
**Retention time (s):** 230 nm (3.19 min), 254 nm (3.26 min), 278 nm (3.28 min)

**Asymmetric factor(s):** 230 nm (0.532), 254 nm (0.716), 278 nm (0.758)

**Tailing factor:** NMT 2.0

**Efficiency:** 230 nm (116.77), 254 nm (114.80), 278 nm (124.46)

**Acceptance criteria:** Sample solution of hydroethanolic crude extract of *Flueggea virosa* (Roxb. Ex Willd.) Royle (stem bark) conforms to the system suitability parameters.



## FT-IR

A small amount of the dried hydroethanolic extract (70%) was placed on the sample area of the Perkin Elmer UATR Fourier Transform infrared (FT-IR) spectrometer and scanned between 4000–400 cm<sup>-1</sup> with a resolving power of 4 cm<sup>-1</sup> and a cumulative scanning limitation of 24 times. Principal peaks appeared at wavenumbers 3287.51, 2923.41, 2852.68, 1708.23 and 1606.23 cm<sup>-1</sup>

## Macroscopy

The stem bark is greyish-brown. Stem is thin with nodes and internodes, smooth and later rough when fully matured. Cross-section shows a brown pith and cork.

## Microscopy

Transverse section shows up to 18 rows of rectangular thin walled cork cells with some portions yellowish brown, followed by phellogen made up of clear rectangular cells. Phelloderm is made up of thin walled parenchyma cells. There are three rows of sclereids and fibres. The first row has small groups of fibres



scattered along the cortex just below the phelloderm. The second row consists of bigger groups of cells occurring more frequently after the first row. The third row of almost continuous groups of sclereids occurs before the cambium. The sclerieds have smooth outer walls and are thick walled. Above and below the third row of stone cells are numerous parenchyma cells containing calcium oxalate prisms and crystals and groups of phloem. The cambium is followed by rows of xylem vessels, fibres and two to three celled rows of rectangular medullary rays up till the pith. Fibres are thick walled and numerous.

### **Powdered plant material**

Light brown in colour; numerous, large, reticulate xylem vessels and tracheids; bundles of fibres with sheaths of calcium oxalate prisms; elongated rectangular thick walled cells containing circular starch granules; fragments of rectangular cork cells with yellowish content; fragments of polygonal parenchyma cells. Round parenchyma cells containing circular starch granules.

### **Therapeutic actions**

Antioxidant, anticancer, antiproliferative, antileukaemic, analgesic, antiinflammatory; antipyretic; antiplasmodial; hypoglycaemic; antiviral (hepatitis C and HIV); anticonvulsant.

### **Therapeutic indications**

Oxidative stress, cancer, leukaemia, pain, fever, malaria, diabetes, hepatitis C, HIV and epilepsy.

### **Safety data**

LD<sub>50</sub> by oral route was estimated to be beyond 3000 mg/kg. CNS and ANS were not affected by the treatment. The hydroalcoholic extract of the stem bark of *F. virosa* did not significantly affect the macro-anatomical structure of the liver, the kidney and the lungs. Organ/body weights of liver, kidney, spleen, thymus adrenals were unaffected by the extract. Flueggea has very minimal effect on the hematopoietic system. It did not affect RBC, HB or the HCT and did not induce anaemia. It induced mild leucopaenia at all doses tested. It appears to marginally affect differential count of WBC, and inhibits agranulocytes (lymphocytes) with a corresponding increase in granulocytes. Increases in MID cells were significant. Flueggea elevates AST marginally and inhibits ALP dose-dependently, but it did not affect GGT and ALT. Flueggea did not increase serum proteins except at the highest dose of 1000 mg/kg. The increase was due to an increase in serum globulin. Flueggea extract did not affect serum bilirubin. It caused marginal decreases in creatinine, but elevated urea creatinine ratio. Flueggea extract (> 300 mg/kg for 10 days) increased clotting time in rabbits. It prolonged the pentobarbitone-induced sleeping time. Hepatocytes, Kupffers cells, central and hepatic vein appeared normal. There were no signs of tubular or glomerular necrosis. Many authors have reported the apparent safety of Flueggea during acute intoxication. However, it must used with care due to its effect on liver enzymes, creatinine and urea creatinine ratio. Adedapo *et al.*, (2007) noted similar changes in liver function enzymes with the use of Flueggea extract. At high doses, Flueggea affects total proteins by increasing serum globulin levels. The present study noticed significant leucopaenia with the use of *F. virosa* whereas, Adedapo *et al.*, (2007) rather noticed leucopaenia amongst other members of the genus, but not this species. It has the propensity to increase bleeding time but this is not likely to be a risk in bleeding disorders in humans. It may also affect renal function. Flueggea may be neuroactive with significant sedative properties.

### **Precautions for use**

Do not combine with hypoglycaemic and hypotensive agents. Driving when on Flueggea medication may be ill advised because of its sedative effect.

**Adverse effects**

Hypoglycaemic, hypotensive, sedative.

**Contraindications**

Pregnancy

**Dosage form**

Decoction, infusion, tincture

**Dosages**

Decoction: 30 g of dried plant material in 900 mL water; simmer until reduced to 600 mL; 1-3 tablespoonfuls daily

Infusion: 30 g of dried aerial part in 600 mL of water; 3-4 teacups daily

Tincture: 1:5, 45% ethanol; 5 ml three times daily

**Storage**

Store in a cool, dry place away from light.

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**Botanical name***Gardenia ternifolia* Schumach. & Thonn**Family**

Rubiaceae

**Synonyms**

*Gardenia medicinalis* Vahl ex Schumach. *Gardenia thunbergia* Hiern (de FTA), *Gardenia jovis-tonantis* Hiern (de FWTA), *Gardenia triacantha* DC

**Common Local names**

**Benin:** Fon – Dakplaasu; Yoruba - Oru wan; Dendi - Babatoru

**Burkina Faso:** Dioula - Goulé kè ; Fulfuldé – Diengali; Moore - Rambrezounga

**Côte d'Ivoire:** Dioula - M'bouré; Malinké - Blé

**Ghana:** Twi – Peteprebi; Ga – Akpetekplebii; Ewe – Flige

**Guinea:** Pular - Bössè ; Maninka – Mburèn

**Mali:** Bambara - M'bouretie, Burèkè; Dogon – Gorogara; Malinké - M'bourékiè

**Niger :** Djerma –N'kondi ; haoussa-gaouden douchi

**Nigeria:** Hausa – Gaude; Yoruba –Gangan; Igbo - Ulimili

**Senegal:** Diola-Kaleg; Peulh-Boséjé; Wolof-Ndibuton

**Togo:** Ewé – Fefe; Ouatchi – Flifè

**Description of the plant**

*Gardenia ternifolia* is an evergreen savannah shrub or small tree that can grow up to 6 m tall. The branches originate from a distinct node at 90° to the main stem and grow in almost 60° to one another. The stem bark is greyish white and smooth. It is thick, brittle, glabrous but externally covered with a greyish dusty coating under which appears the green colour of the bark. The wood is very difficult to cut. The leaves occur in whorls of three, 10-18 cm in length, 7-11 cm in width, margin entire, cuneate at base, rounded at tip, obovate in shape, the midrib is greyish white, protruding on both sides, lateral veins slightly alternate; leaf petiole only 2-3 mm long and 3 mm in diameter. The flowers are solitary, sub-sessile, white or creamy, aging to yellow, about 4 cm in diameter with a tube up to 4-5 cm long and spreading corolla lobes, sweetly scented. It bears a single fruit at the tip of a 4-5 cm long fruit stalk, 3-5 cm. Fruits are subtle, narrowly ellipsoid, up to 8-10 cm long (Baldé and Diallo 1981).



A - *Gardenia ternifolia* whole plant, B – leaves and immature fruits, C – immature fruits, D- mature fruits

#### Herbarium specimen number

Burkina Faso: 3658 (OAU) ; 1062 MSAD (CNSF)

Côte d'Ivoire: 14149 (CNF)

Ghana: GH 404/KNUST

Guinée: 121HK658 (CRVPM – Dubréka)

Mali: 2226/DMT

Nigeria: UPFH 118

Senegal: IFAN 54

Togo: TG07366

#### Habitat and geographical distribution

The plant is found in northern tropical Africa, mostly Sudanian, especially in grassland and forest savannahs, sometimes in thorny scrub and on gravelly soils. It is also found in open forest on wet sites (Adjanohoun and Aké Assi, 1979).

#### Plant material of interest

Leaves and Stem bark

#### Other part used

Root bark

#### Definition of plant material of interest

*Gardenia ternifolia* is composed of the fresh or dry stem and root bark of *Gardenia ternifolia* Schum and Thonn. (Rubiaceae).

## Ethnomedical uses

*G. ternifolia* is used in traditional medicine for the treatment of malaria and jaundice (Baldé and Diallo, 1981; Ahua *et al.*, 2007; Ochieng *et al.*, 2010; Yunana and Dahiru, 2015; Awas *et al.*, 2016, Assase *et al.*, 2005; Nurey, 2017; and Giday *et al.*, 2009). The root powder is rubbed in the small incisions on leprosy spots. It is also used in the treatment of rheumatism and as an antiseptic for ulcers. The leaf infusions are used as baths against syphilis, and as liniment against scapel. Young leaves are palatable to small ruminants (Carrière, 1994). In Guinea, the trunk bark is used in the treatment of asthma and syphilis and for purifying breast milk (Basilevskai, 1969). The decoction of the root bark is used in the treatment of infectious diseases such as sexually transmitted diseases (Magassouba *et al.*, 2007). Maceration of the roots or the paste obtained by mixing the barks of small branches with those of *Detarium microcarpum* is used to treat haemorrhoids in the Democratic Republic of Congo (Makumbelo *et al.*, 2008). In Senegal, root maceration is used to treat ascites, dental caries, wounds (Kerharo and Adams, 1974). In Tanzania, the macerate is used in the treatment of epilepsy and hypertension (Moshi *et al.*, 2003). In Togo, decoction of leaves and stem bark is used orally to treat hypertension (Adjanohoun *et al.*, 1986). Decoction of leaves is used in Benin in the treatment of diabetes (Awede *et al.*, 2015).

## Biological and pharmacological activities

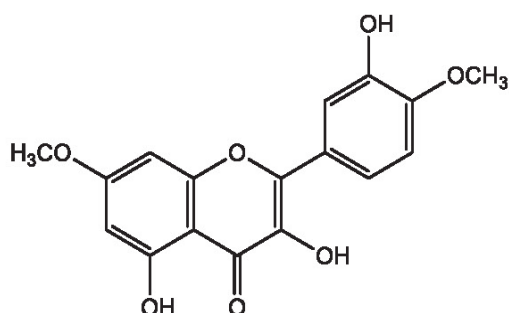
The antiplasmodial activity of *G. ternifolia* is well documented. The dichloromethane leaf extract showed antiplasmodial activity *in vitro* with  $IC_{50} > 12.5 \mu\text{g/mL}$  (Ouattara *et al.*, 2014). The acetone extract of the aerial parts showed antiplasmodial activity against chloroquine-resistant (W2) and chloroquine-sensitive (D6) strains of *Plasmodium falciparum* with respective  $IC_{50}$  values of  $1.06 \mu\text{g/mL}$  and  $0.94 \mu\text{g/mL}$  (Ochieng *et al.*, 2010). The anthocyanin and organic extracts of *G. ternifolia* leaves at a dose of  $6.25 \mu\text{g/mL}$  showed anti-sickling activities with normalization values of 72% and 68% respectively (Ngbolua *et al.*, 2015). The flavonoids (3,5,3'-trihydroxy-7,4'-dimethoxyflavone and 3,5,7-trihydroxy-4'-methoxyflavone) of the aerial parts showed remarkable antioxidant activities with respective  $IC_{50}$  values of  $40.3 \pm 1.55$  and  $75.5 \pm 1.75 \mu\text{M}$ . However these activities were lower than that of quercetin ( $IC_{50} = 20.1 \pm 1.34 \mu\text{M}$ ) used as reference (Awas *et al.*, 2016). The anthocyanin extracts of the leaves of the Congolese species showed considerable antibacterial activity against *Staphylococcus aureus* (MIC =  $62.5 \mu\text{g/mL}$ ) and *Escherichia coli* (MIC =  $125 \mu\text{g/mL}$ ) (Ngbolua *et al.*, 2015). The acetone extract of the aerial parts showed *in vitro* antiplasmodial activity with an  $IC_{50}$  of  $1.06$  and  $0.94 \mu\text{g/ml}^{-1}$  respectively, against a strain of chloroquine-resistant *Plasmodium falciparum* (W2) and a chloroquine-sensitive strain (D6). The same acetone extract showed moderate larvicidal effects against *Aedes aegypti*. Among the active ingredients tested, the most marked effect was noted with naringenin-7-O-methyl-ether (Ochieng *et al.*, 2010). The aqueous extract of the leaves had moderate hepatoprotective effect following carbon tetrachloride- ( $\text{CCl}_4$ ) induced hepatotoxicity in male albino rats (Yunana and Dahiru, 2015). The antidiabetic activity (type 2 diabetes) of the aqueous leaf extract in male rats has been reported (Awede *et al.*, 2015). The chloroform extract of the leaves has been reported as a potential therapeutic agent against prostate cancer (Tshibangu *et al.*, 2016). The isomeric compounds gardenifolin A-H showed cytotoxic effects against human cancer HeLa cell line. Morphological experiments indicate that gardenifolin D (1d) induces apoptosis of HeLa cells at  $25 \mu\text{M}$  (Tshitenge *et al.*, 2017). The aqueous extract of the fruit was highly active against *Theileria lestoquardi*, a protozoan infesting both domestic (goats, sheep) and wild animals (Farah *et al.*, 2012). Larsen *et al.* (2015) showed that *Gardenia* inhibits COX-1 activity.

## Clinical data

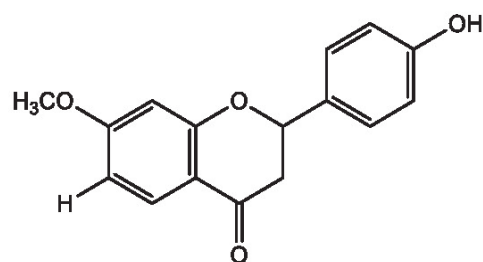
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## Chemical constituents

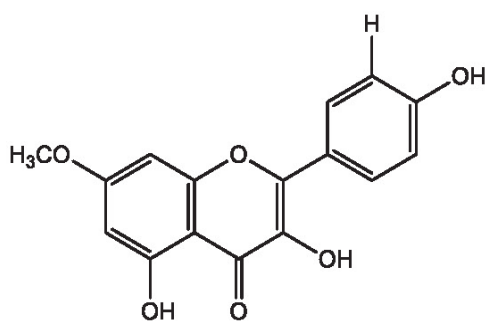
Anthocyanins, leucoanthocyanins, gallic tannins, catechin, coumarins, quinones, saponosides, terpenes, flavonoids (naringenin-7-O-methylether, quercetin-4,7-O-dimethylether, kaempferol 7-O-methylether, 4,5-dihydroxy-6,7-dimethoxyflavanone, naringenin-4,7-O-dimethylether), steroids (stigmasterol,  $\beta$ -sitosterol), mucilage (Ochieng *et al.*, 2010, Ngbolua *et al.*, 2015, Awas *et al.*, 2016, Yunana and Dahiru, 2015, Awede *et al.*, 2015). Eight stereoisomers of 2,3-dihydrobenzo [b] furan neolignans (gardenifolins A-H (1a-d and 2a-d)) (Tshitenge *et al.*, 2017).



Quercetin- 4', 7-O- dimethylether



Naringenin-7-O- methylether



Kaempferol-7-O- methylether

## Test for identity and purity

**Moisture content:** Air dried coarse powder does not lose more than 7.2% (stem bark) at 105°C.

**Total ash:** not more than 11.1%

**Ash insoluble in acid:** not more than 0.5%

**Water soluble extractive:** not less than 9.0%

**Ethanol soluble extractive (70%):** not less than 7.0%

## Chromatographic fingerprint

### Thin Layer Chromatography

**Preparation:** About 5 g of the powdered stem bark were extracted with ethyl acetate by cold maceration, filtered and the filtrate concentrated to a small volume. A small spot was then applied to the TLC plate for analysis.

**Chromatographic conditions:** Analytical TLC on silica gel G60 F254, 0.25 mm layer in hexane/ethyl acetate (7:3) as the mobile phase.



**Detection:** Visualized in daylight after spraying with anisaldehyde-sulphuric acid (Lane 1) and vanillin-sulphuric acid reagents (Lane 2) (Stahl, 1969), heating to 110°C for 10 mins.

The TLC chromatogram showed three prominent spots with R<sub>f</sub>s of 0.91 (pink), 0.85 (pink) and 0.68 (pink) when sprayed with both anisaldehyde and vanillin.



### High Performance Liquid Chromatography

**Sample preparation:** A sample of about 10 mg of the hydroethanolic extract of *G. ternifolia* stem bark was reconstituted in 3 mL acetonitrile in a 10 mL volumetric flask with sonication for 17 minutes. It was then diluted to volume with the mobile phase under chromatographic conditions. It was centrifuged to obtain a clear test solution which was subsequently filtered through a 0.45 µm filter into an HPLC vial and analyzed.

### Chromatographic system

#### Optimized chromatographic conditions

**Mode:** LC

**Column:** YMC ODS, 4.6 x 150 mm, 5 µm

**Column temperature:** Ambient – 30°C

**Mobile phase:** Acetonitrile: Methanol: Water (60:20:20 v/v/v)

**Elution mode:** Isocratic

**Injection volume:** 20 µL

**Flow rate:** 0.5 mL/minute

**Detection wavelengths:** 230 nm, 254 nm and 278 nm.

#### System Suitability parameters

**Number of peaks:** 230 nm (1), 254 nm (1), 278 nm (1)

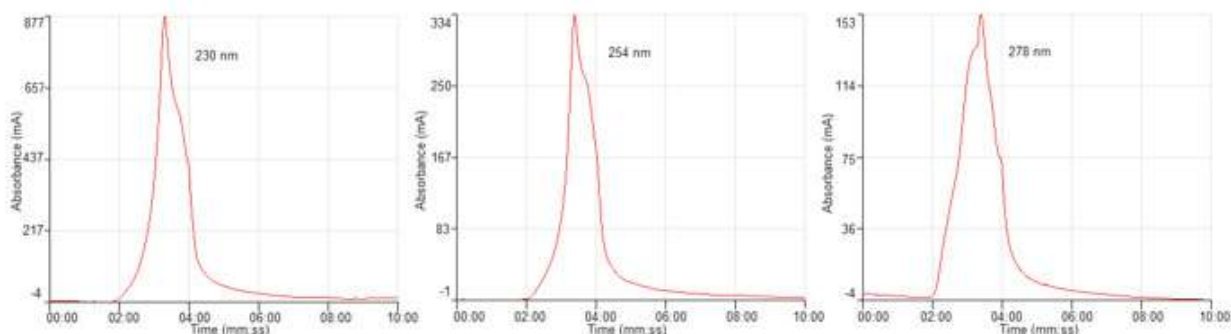
**Retention time (s):** 230 nm (3.18 min), 254 nm (3.23 min), 278 nm (3.23 min)

**Asymmetric factor(s):** 230 nm (0.816), 254 nm (0.830), 278 nm (0.960)

**Tailing factor:** NMT 2.0

**Efficiency:** 230 nm (83.11), 254 nm (94.97), 278 nm (57.56)

**Acceptance criteria:** Sample solution of hydro-ethanolic crude extract of *G. ternifolia* (Stem Bark) conforms to the system suitability parameters.



## FT-IR

A small amount of the dried hydroethanolic extract (70%) was placed on the sample area of the Perkin Elmer UATR Fourier transform infrared (FT-IR) spectrometer and scanned between 4000-400  $\text{cm}^{-1}$  with a resolving power of 4  $\text{cm}^{-1}$  and a cumulative scanning limitation of 24 times. Principal peaks appeared at wave numbers 3282.24, 2926.07 and 1590.99  $\text{cm}^{-1}$

## Macroscopy

Stem bark is brittle, glabrous externally covered with a greyish dusty coating under which appears the green color of the bark. The leaf is spathiculate with acuminate apex. Some are oblong obovate in shape with assymetric acuminate apex. The margin is entire, some times widely wavy and venation reticulate with prominent midrib on the under surface. The upper and lower surface are hairy.

## Microscopy

### Leaf

Upper surface: The epidermal cells of the upper surface are polygonal cells with thick walls, slightly rounded; Trichomes are short, unicellular with a large base, scattered on the epidermis. The stomata are absent. The epidermal cells of the lower surface bear polygonal trichomes and paracytic stomata. Has more unicellular covering trichomes on the lower surface than the upper.

### Transverse Section

The mid rib section is biconvex in shape. The upper section is filled with circular shaped collenchyma cells with thick walls. The main vascular section has two sections. The upper section is distinguished by three layers of xylem, the third of which is divided into sections which contain phloem. The section is an arc of xylem followed by phloem fibres and a distinct layer of parenchyma cells containing calcium oxalate prisms, which surround the vascular system. There is also a group of parenchyma cells at the centre of the vascular bundle, which contain calcium oxalate cluster crystals. The lamina shows rectangular shaped palisade and large parenchyma cells in the mesophyll traversed by vascular bundles with sheaths of calcium oxalate crystals. The lower surface is undulating and large trichomes with thick walls come from both the upper and lower epidermis.

### Powdered plant material

Shows epidermal cells with paracytic stomata and polygonal epidermal cells. Trichomes are of two types, short unicellular with large base and long unicellular. Consists of parenchyma cells with single large calcium

oxalate cluster crystals and those with circular content. Fibres contain circular granules and the xylem vessels are annular.

### Therapeutic actions

Antimalarial, anti-icteric, antioxidant, antibacterial, hepatoprotective, antidiabetic (type 2 diabetes) anticancer, antiprotozoan

### Therapeutic indications

Malaria, jaundice, oxidative stress, bacterial infection, liver disease, diabetes.

### Safety data

LD<sub>50</sub> by oral route was estimated to be beyond 3000 mg/kg. There were no signs of CNS depression/stimulation or autonomic effects at all the doses tested. The hydroalcoholic extract of the leaves of *G. ternifolia* did not significantly affect the macro-anatomical structure of the liver, kidney and lungs. The relative organ weights of the kidney, spleen, thymus and adrenals did not change. The extract did not significantly affect any of the haematological parameters, but caused mild elevations in ALT and GGT. ALP and AST were not affected significantly by the treatment. The extract caused increase in serum proteins including albumin and globulins especially at low doses. Serum bilirubin increased with Gardenia administration due to elevations in unconjugated bilirubin. There were mild elevations in urea, but creatinine was not affected. Pentobarbitone-induced sleeping time was also not affected. There were corticomedullary necrotic lesion and parenchyma cell necrosis in the kidneys.

### Precautions for use

The use of *G. ternifolia* in patients with kidney disease and active ulcer is not advisable. Concurrent use with other non-steroidal antiinflammatory drugs or herbs can be detrimental and should be avoided.

### Adverse effects

Diarrhoea and vomiting.

### Contraindication

Pregnancy, active kidney and peptic ulcer disease.

### Dosage forms

Powder, decoction, infusion, tincture

### Dosage

Decoction: Put about 20 to 30 g of root bark powder in one liter of water and boil for 30 minutes. Filter. Take half a teacup of decoction 2 to 3 times a day.

Infusion: 30 g of dried aerial part in 600 mL of water; 3-4 teacups daily

Tincture: 1:5, 45% ethanol; 5 ml three times daily

### Storage

Powder to be stored in a tightly closed glass bottle in a dry place

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**Botanical name***Guiera senegalensis* J.F Gmel.**Family**

Combretaceae

**Synonyms***Guiera senegalensis* (Lam), *Guiera glandulosa* (Sm).**Common Names**

Guiera (English), Guiera du Sénégal (French)

**Common Local names****Benin:** Fon - Saabara; Dendi - Bomigue**Burkina Faso:** Dioula – Kounguiè; Fulfuldé - Ngéloki; Mooré - Wilinwiga**Côte d'Ivoire:** Malinké-Koubélégelman; Dioula-Fufanikay; Senoufo-Koubélégelman**Gambia:** Diola – Fufanikay; Mandinka - kankanar; Wolof - Nger**Guinea:** Pulaar- Bali niama; Maninka - konguélé**Guinea-Bissau:** Balanta-Biôcê; Mandiak-Bissem antchom; Mankanya-Bitchiante**Mali:** Bambara- N'Kunj□; Dogon- Gorôgou, Guru; Peulh- N'geloki**Niger:** Arabic-Abesh; Hausa-Sabara.; Tamachek-Tuhila**Nigeria:** Hausa-Kurukuru; Igbo-Sabaata; Yoruba-Olofun**Senegal:** Diola-Buhunuk; Wolof-Nger; Pular-Eloko**Togo:** Anufo-Kahangbanyawa; Moba-Kampiembuateng**Description of the plant**

*Guiera* is a shrub with erect small bole, or branched bush at the base about 1-2 (-5) m high, with irregular crown. The whole plant is covered with small black glands. The plant has a characteristic appearance due to its small green, grey or bluish leaves. The stem bark is fibrous, smooth or finely scaly, grey with light brown patches. The young branches are soft and hairy. The leaves are opposite or sub-opposite, ovate, orbicular or elliptical measuring 3-5.5 x 2-3 cm. The leaf-blade has round or mucronate apex, with rounded or subcordate base, downy on both sides, especially on the underside, which appears grey, riddled with black dots. The petiole is pubescent, 2-5 mm long with pinnate nerves, which are not very prominent. It bears 5-6 (-8) pairs of secondary veins connecting to the apex. The inflorescence is spherical, with a diameter of 15 mm and a peduncle 2.0-3.5 cm long. The flowers are creamy white to yellowish, with a calyx riddled with black heads and the corolla with 5 petals, 10 filiform stamens widely exceeding the corolla. It bears linear or fusiform fruits covered with rosy grey silky hair, 3.0-4.5 cm long (Sanogo, 2012).



A and B - *Guiera senegalensis* flower, C – *Guiera senegalensis* whole plant

#### Herbarium specimen number

Benin: 2360 (AP)  
 Burkina Faso: MSLS 849 (CNSF); 121 (OAU)  
 Côte d'Ivoire: CNF 8607  
 Ghana: GH 457/KNUST  
 Mali: 537/DMT  
 Nigeria: KASU/PCG/018  
 Senegal: IFAN 4  
 Togo: TG 00658

#### Habitat and geographical distribution

*G. senegalensis* occurs in savannahs and fallow land, from sea-level up to 1000 m altitude. It grows well on all types of soil but mainly on dry sandy or degraded soils, sometimes in areas which are temporarily flooded. It does not tolerate heavy shading. It colonizes degraded areas, where it can become gregarious and very abundant. The plant is very drought resistant. It is widely distributed in tropical Africa, especially drier areas from Senegal to Sudan (Koumaré, 1968).

#### Plant material of interest

Leaves

#### Other part used

Stems, roots, galls

## Definition of plant material of interest

*Guiera* consist of the dried of fresh leaves of *Guiera senegalensis* J.F Gmel. (Combretaceae).

## Ethnomedical uses

The different parts of *G. senegalensis* are used in traditional medicine for a wide range of diseases hence its name 'cure-all'. A leaf decoction or infusion, sometimes combined with other species, is drunk to treat dysentery, diarrhoea, colic, gastroenteritis, beriberi, rheumatism, hypertension, eczema, epilepsy, leprosy, impotence, venereal diseases, malaria, fever, cough, colds, asthma, bronchitis and tuberculosis. It is also taken as a diuretic, as an antiemetic in small doses and as an emetic in larger doses. Crushed leaves are mixed with tamarind pulp and eaten as a laxative and appetizer. Women take dried pounded leaves in food after childbirth to increase milk flow and as general tonic and blood restorative. A leaf infusion is used to wash new-born babies. Dried leaves are mixed with tobacco and smoked to treat respiratory problems. The powdered leaves are also taken as a snuff to treat headache and sinusitis. Ground leaves, leaf powder or a leaf decoction is applied to wounds to help cicatrization and treat skin problems, including Guinea worm, boils, burns, sore mouth, tumours, syphilitic sores and leprosy (Koumaré, 1968; Kerharo and Adams, 1974; ENDA, 1993; Nacoulma, 1996). A steam bath of the leaves is taken to treat tooth-ache caused by caries. A leaf infusion is also used as a mouth wash for the same purpose. Powdered or crushed leaves are added to milk and taken to treat amoebic dysentery and leprosy. Young leaves are chewed to treat coughs. In Sudan, a leaf infusion is taken for diabetes. Powdered and boiled roots are commonly taken to treat diarrhoea and dysentery, including amoebic dysentery and intestinal worms. A root decoction is also drunk to treat insomnia, pneumonia, tuberculosis, haemorrhoids, poliomyelitis and gonorrhoea. A bark decoction is used to treat colic. A fruit decoction is taken to stop hiccups and to treat rectal prolapse. The powder of the roasted fruit is eaten to treat cough. A decoction of all plant parts is applied locally and taken orally, to treat oedema, while the bark powder is applied as a dressing. Aqueous extract of the powdered plant galls with charcoal are drunk as a strong diuretic in oliguria and anuria, as well as cerebral malaria. The leaves and roots are used to treat malaria, dysentery, diabetes and hypertension. The galls are used in Burkina Faso to increase milk production in cows and to treat fowlpox infection in chickens. The leaves are fed to cows to fatten them, and to increase fertility and milk production (Koumaré, 1968; Kerharo and Adams, 1974).

## Biological and pharmacological activities

The aqueous extracts from different parts of the plant showed antidiarrhoeal and antitussive properties (Koumaré, 1968; ENDA, 1993; Sanogo *et al.*, 1998b). The extracts showed antibacterial activities against *Bacillus subtilis*, *Escherichia coli*, *Corynebacterium*, *Pseudomonas aeruginosa* (Bassene *et al.*, 1995). The methanolic extract of the leaves induced significant inhibition of the growth of *Haemophilis influenza*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes* and *Moraxella catarrhalis* responsible for respiratory infections with minimal inhibitory concentrations (MICs) between 1.9 and 2 µg/mL (Sanogo *et al.*, 1998a). The ethanolic extracts and "guieranone" inhibited the growth of *Cladosporium cucumerium* (Silva and Gomes, 2003). The hydroacetic extract of the leaves of *G. senegalensis* showed a strong *in vitro* antiviral activity on human herpes virus (Lamien, 2005). The stem and leaf decoctions and infusions have antiplasmodial activity on two strains of *Plasmodium falciparum* (Benoit *et al.*, 1996). The chloroform extract of the roots showed activity against *Plasmodium falciparum* (strain D6 and W2) with a 50% inhibitory concentration (IC<sub>50</sub>) < 25 µg/mL. Harmane and tetrahydroharmane isolated from the chloroform extract of the roots showed a high activity with IC<sub>50</sub> < 4 µg/mL (Ancolio *et al.*, 2002). The methanolic extract of the leaves of *G. senegalensis* was found to be active in mice infected with *Plasmodium berghei*, with a moderate analgesic effect (Jigam *et al.*, 2011). Extracts from the leaves and galls of *G. senegalensis* have demonstrated antiinflammatory properties (Koumaré, 1968, Sombié *et al.*, 2011a). The methanolic extract inhibited pain induced by acetic acid in a dose-dependent manner in mice (Olotu *et al.*, 2016). Isolated



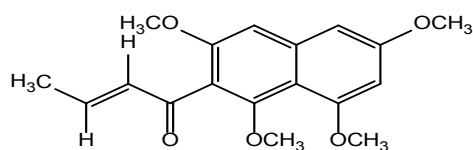
tannins of *G. senegalensis* showed antioxidant activity (Bouchet *et al.*, 1998). Methanolic extracts of leaves and galls demonstrated DPPH scavenging antioxidant activity with  $IC_{50}$  values of 39.12  $\mu\text{g/mL}$  and 19.5  $\mu\text{g/mL}$ , respectively (Kouamé *et al.*, 2009). The dichloromethane extract of the leaves showed a relative antiradical activity (AAR) of 0.60 and the methanolic extract of the galls gave an AAR of 0.53 (Kouamé *et al.*, 2009). In a test of extracts of the leaves of *G. senegalensis* as an antidote against snake venom, a dose of 10 mg provided 80% protection against *Echis carinatus* venom and a strong remission of neurotoxic signs caused by *Naja nigricollis* venom (Abubakar *et al.*, 2000). The gall extracts showed neuroprotection (Sombié *et al.* 2011b). Extracts of leaves and galls of *G. senegalensis* demonstrated antiproliferative activity on three cell lines (U373, PC3 and MCF7) (Kouamé *et al.* 2009). All gall extracts showed cytotoxic activity on breast cancer cells superior to the reference drug etoposide. Decoction of galls showed an antiproliferative effect ( $IC_{50} = 2.1 \pm 0.5 \mu\text{g/mL}$ ), comparable to that of taxol on the same cell line ( $IC_{50} = 1.27 \pm 0.04 \mu\text{g/mL}$ ). Leaf extracts showed no activity on cell proliferation (Kouamé *et al.*, 2009) and guieranone A, a naphthyl butanone isolated from the leaves, was found to exhibit strong cytotoxic activity (Kouete *et al.*, 2012). Extracts of the leaves of the plant showed hypotensive activity *in vivo* (Koumaré, 1968). They also caused vasorelaxant effect *in vitro* on the isolated rabbit aorta ring pre-treated with phenylephrine in the presence and absence of endothelium (Ouedraogo, 2008). The gall extracts showed antidiabetic activity (Sombié, 2012).

### Clinical data

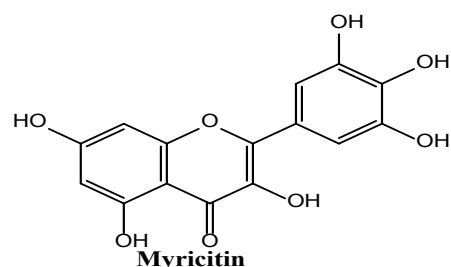
Clinical trials of extracts of the plant in cholera endemic areas of Ouagadougou (Konaté, 1984) and Dakar (Ndour *et al.*, 2006) demonstrated the antidiarrhoeal activity of the leaves of *G. senegalensis*, with promising results. In other studies, a syrup (D2 syrup) of the plant showed promising antitussive activity in children and adults in 62.5% of cases in Mali (Denou, 2008). In Burkina Faso, the syrup showed very high antitussive activity in children aged 6 months to 6 years in 98% of cases.

### Chemical constituents

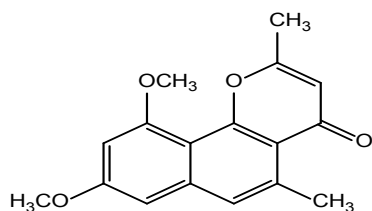
Alkaloids harmane, harmalane, tetrahydroharmine or eleagnine, hyoscyamine and solanine; flavonoids, naphopyrans, tannins and naphthylbutenone (Guieranone A) (Koumaré *et al.*, 1968; Combier *et al.*, 1977; Bucar *et al.*, 1996; Mahmoud and Sami, 1997; Bouchet *et al.*, 2000; Ancolio *et al.*, 2002; Silva and Gomes, 2003; Fiot *et al.*, 2006; Salihu and Usman, 2015); flavonoids quercetin, kaempferol, quercitrin, apigenin, epigallocatechin gallate, rutin, rhamnetin (Lamien, 2005), myricitrin, myricetin-3-rhamnoside, myricetin-3-O- $\beta$ -D glucopyranoside, myricetin-3-O- $\beta$ -D galactopyranoside, myricetin-3-O- $\beta$ -D (6''-O-galloyl)-lucopyranoside, myricetin-3-O- $\alpha$ -L-arabinopyranoside, quercetin, quercitrin, quercetin-3-O- $\alpha$ -L-arabinopyranoside, vitexin, catechin and thilioside (Ficarra *et al.*, 1997; Males *et al.*, 1998).



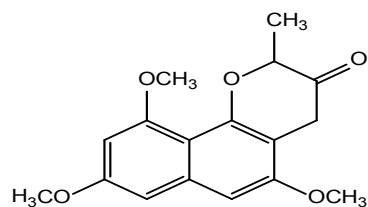
Guieranone A



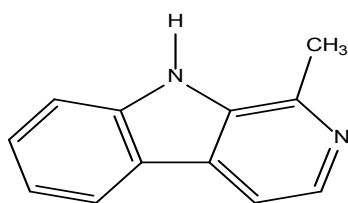
Myricetin



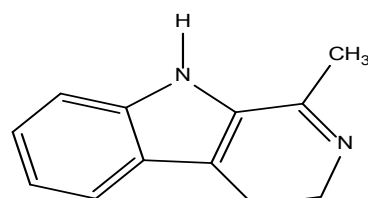
5-methylflavasperone



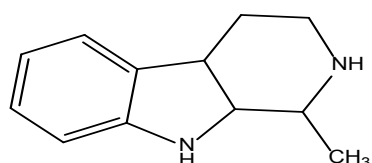
Methyldihydroflavasperone



Harmane



Harmalane



Tetrahydroharmane

### Test for identity and purity

**Moisture content:** air dried coarse powder does not lose more than 6.5% (leaves), 8.0% (stem bark) at 105°C.

**Total ash:** not more than 5.7% (leaves), 10.8% (stem bark)

**Acid insoluble ash:** not more than 0.8% (leaves) and 0.5% (stem bark)

**Water soluble extractive:** not less than 6.0% (leaves), 14.0% (stem bark)

**Ethanol soluble extractive (70%):** not less than 2% (leaves), 16.0% (stem bark)

### Chromatographic fingerprints

**Preparation:** About 5 g of the powdered leaves were extracted with ethyl acetate by cold maceration, filtered and the filtrate concentrated to a small volume. A small spot was then applied to the TLC plate for analysis.

**Chromatographic conditions:** Analytical TLC on silica gel G60 F254, 0.25mm layer in hexane/ethyl acetate (7:3) as the mobile phase.

**Detection:** Visualized in daylight after spraying with anisaldehyde-sulphuric acid (Lane 1) and vanillin-sulphuric acid reagents (Lane 2) (Stahl, 1969), heating to 110°C for 10 mins.

The TLC chromatogram showed two prominent spots with R<sub>f</sub>s of 0.79 (pink) and 0.59 (purple) when sprayed with both anisaldehyde and vanillin. An additional spot each, appeared at R<sub>f</sub> of 0.12.



### High Performance Liquid Chromatography

**Sample preparation:** About 10 mg of the hydroethanolic extract of *G. senegalensis* leaves were reconstituted in 3 mL acetonitrile in a 10 mL volumetric flask with sonication for 17 min. The resulting solution was then diluted to volume with the mobile phase under chromatographic conditions. It was centrifuged to obtain a clear test solution which was subsequently filtered through a 0.45µm filter into an HPLC vial and analyzed.

### Chromatographic system

#### Optimized chromatographic conditions

**Mode:** LC

**Column:** YMC ODS, 4.6 x 150 mm, 5 µm

**Column temperature:** Ambient – 30°C

**Mobile phase:** Acetonitrile: Methanol: Water (60:20:20 v/v/v)

**Elution mode :** Isocratic

**Injection volume :** 20 µL

**Flow rate:** 0.5mL/minute

**Detection wavelengths:** 230 nm, 254 nm and 278 nm.

#### System Suitability parameters

**Number of peaks:** 230 nm (1), 254 nm (1), 278 nm (1)

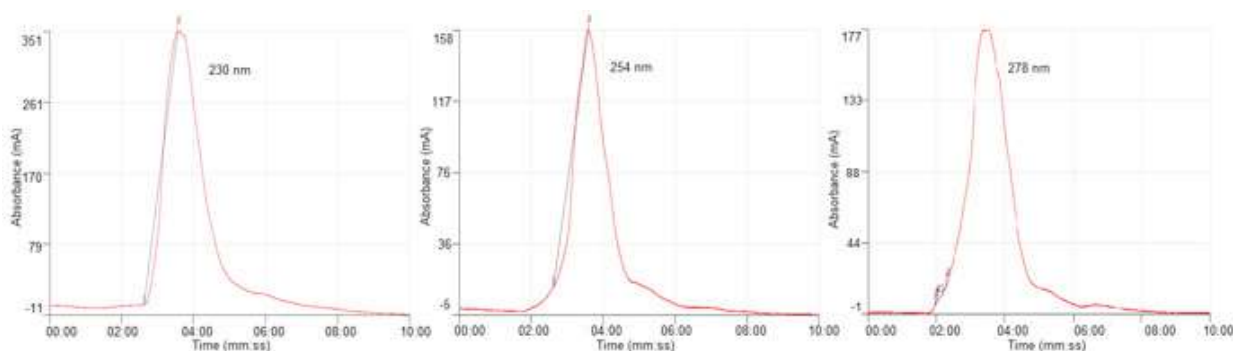
**Retention time (s):** 230 nm (3.35 min), 254 nm (3.35 min), 278 nm (3.35 min)

**Asymmetric factor(s):** 230 nm (1.138), 254 nm (0.944), 278 nm (0.869)

**Tailing factor:** NMT 2.0

**Efficiency:** 230 nm (64.68), 254 nm (86.07), 278 nm (54.07)

**Acceptance criteria:** Sample solution of hydro-ethanolic crude extract of *G. senegalensis* (leaves) conforms to the system suitability parameters.



HPLC chromatogram of the leaves of *Guiera senegalensis*

## FT-IR

A small amount of the dried hydroethanolic extract (70%) was placed on the sample area of the Perkin Elmer UATR Fourier transform infrared (FT-IR) spectrometer. It was scanned between  $4000\text{--}400\text{ cm}^{-1}$  with a resolving power of  $4\text{ cm}^{-1}$  and a cumulative scanning limitation of 24 times. Principal peaks appeared at wavenumbers  $3264.54$ ,  $2926$  and  $1604.80\text{ cm}^{-1}$ .

## Macroscopy

The leaves are dark green and glaucous on the upper surface with black dots on the underside. Leaf shape is oval, lamina smooth with reticulate venation. It has an entire margin and mucronate apex. The petiole is short and hairy and leaf is softly hairy on both surfaces.

## Microscopy

### Leaf surface

Upper epidermal cells have wavy walls, stomata are anomocytic surrounded by three to five subsidiary cells. There are numerous unicellular appressed trichomes. Cells of the veins are rectangular. The lower surface epidermis is covered with trichomes, which are so numerous and woven together making it difficult to see the stomata or epidermal cells. The trichomes are long and curved.

### Transverse Section

Upper section of the midrib is concave. Midrib section has large collenchyma cells. The main vascular bundle follows. Xylem surrounds the phloem. Below the xylem in the lower section of the midrib is a row of groups of phloem cells divided into four sections by xylem vessels. These are bound on the outside by groups of phloem fibres. The lower section of the midrib has large parenchyma and collenchyma interspersed with schizolysigenous glands. There are long uniseriate trichomes arising from both epidermal surfaces. The lamina shows one layer of columnar palisade cells and intermittent vascular bundles with closely packed spongy mesophyll.

## Powdered plant material

### Leaves

Dark green powder; trichomes of various sizes characterise the powder; uniseriate, unicellular, straight, curved trichomes; fragments of epidermal cells with wavy walls; anomocytic stomata; fragments of spongy

mesophyll, palisade and epidermal cells as seen in transverse section; bundles of thick walled fibres; large calcium oxalate cluster crystals; fragments of annular xylem vessels; polygonal epidermal cells and round starch granules with T-shaped hilum. Examination of the gall powder revealed the presence of leaf hairs, epidermal fragments, rectangular and irregular sclerotic cells, sclerenchyma fibres, calcium oxalate crystals and some rare spiral vessels (Abubakar, 1993).

### Therapeutic actions

Antidiarrhoeal, antitussive, antibacterial, antiviral, antiplasmodial, antiinflammatory, analgesic, antivenom, neuroprotective, antiproliferative, hypotensive, vasorelaxant and antidiabetic.

### Therapeutic indications

Diarrhoea, cough, bacterial infections, human herpes, malaria; inflammations, snakebite, cancers, arterial hypertension, diabetes (Sombié, 2012).

### Safety data

LD<sub>50</sub> by oral route was estimated to be above 3000 mg/kg in rats. CNS and ANS were not affected by treatment with the aqueous extract. Subacute studies did not show changes in gross organ morphology of the liver and kidney. Levels of liver transaminases, proteins and bilirubin were not changed by the treatment. Renal function remained normal. Treatment prolonged pentobarbitone sleeping time significantly but histopathological examination of treated organs did not suggest signs of cellular damage. The extract is safe within the recommended doses. It has significant sedative properties.

### Precautions for use

Aqueous extracts may cause hypotension and hypoglycaemia. Guieranone A has cytotoxic effect, hence the plant must not be used in pregnant women. Guiera should not be used together with sedatives as it may cause drowsiness. and impair driving. Caution should be exercised in combination therapy with other sedatives and patients should be advised not to drive or operate machinery.

### Adverse effects

Respiratory depression, hypotension.

### Contraindications

Respiratory distress, hypotension

### Dosage and dosage forms

Decoction, Infusion, tincture

Infusion: 20-30 g of dried plant per liter of water; drink 3-4 cups a day.

Decoction: 30-50 g of dried leaves in 500 mL of water; drink 3-4 cups a day.

Tincture: 1:5, 45% ethanol; 5 ml three times daily

### Storage

Store in tightly closed glass bottles, protected from light.

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**Botanical name**

*Jatropha gossypiifolia* L.

**Family**

Euphorbiaceae

**Synonyms**

*Adenoropium gossypifolium* (L.) Pohl, *Jatropha glandulifera* Roxb, *Jatropha staphysagriifolia* Mill.

**Common Names**

Bellyache bush, black physic nut, wild cassada (English), Faux ricin médicinier bâtard, médicinier rouge (French)

**Common Local names**

**Benin:** Fon – Nyikpotinvovo; Yoruba - Botuje pupa; Dendi - Bukatu nucire

**Burkina Faso:** Dioula – Baga; Mooré - Wan-bin-banguem-daaga

**Côte d'Ivoire:** Baoulé - Aploplo oklouè; Bété - Bataigniniégogoua

**Gambia:** Madinka - Tubabutaboo

**Ghana:** Asante – Kaagya; Fante - Aburokyiraba

**Mali:** Bambara - Sampèrèdjiri

**Nigeria:** Igbo- Ake mbogho; Yoruba – Botuje pupa; Hausa – Binidi Zugu

**Togo:** Mina-Babatidjin; Kabiyè-Fédélaou kissèmou; Tem-Saou kisse mou

**Description of the plant**

*J. gossypiifolia* is a small shrub with dark green or more frequently purplish-red dark leaves. The leaf blade is 16.0–19.0 cm long and 10.0–12.9 cm wide. They are alternate, palmate, and pubescent, with an acuminate apex, cordate base, and serrated margin. It is 3 veined from the base with many lateral veins in each lobe. The blade is membranous, glabrous on both surfaces; lobes obovate or obovate-lanceolate. The flowers are unisexual, purple, and in cymose summits, with the calyx having five petals, which in male flowers may form a petaloid tube. The fruit is capsular, with three furrows, containing a dark seed with black spots (Khyade and Vaikos, 2011; Aworinde *et al.*, 2009; Lisowski, 2008).



A- *Jatropha gossypifolia* L leaves B – fruits , C- *Jatropha gossypifolia* L.

#### Herbarium specimen number

Burkina Faso: 510 (CNSF); 517 bis (Guinko)

Côte d'Ivoire: 7560 (CNF)

Ghana: GH 477/KNUST

Mali: 1688/DMT,

Nigeria: FHI111919

Senegal: IFAN 3794

Togo: TG 12753

#### Habitat and geographical distribution

Native to tropical America and introduced as an ornamental plant, *J. gossypifolia* has become invasive in all plains of savannah areas in West Africa. It is found on the ruderals, fallow fields, and sometimes planted in villages (Arbonnier, 2002; Akoègninou, *et al.* 2006)

#### Plant material of interest

Leaves

#### Other part used

Stems, Seeds; latex

#### Definition of plant material of interest

*Jatropha gossypifolia* consists of the fresh or dried leaves of *J. gossypifolia* L. (Euphorbiaceae)

## Ethnomedical uses

Various medicinal properties for *J. gossypifolia* are reported in traditional medicine. Different parts of the plant, such as leaves, stems, roots, seeds, and latex, are used in different dosage forms ( Félix-Silva *et al.*, 2014). The leaves are used as blood purifier, febrifuge, purgative and stomachic. A decoction is taken to cleanse the blood and to treat venereal diseases, heart problems, diarrhoea, stomach ache and indigestion (Burkill, 1985; Fern, 2018). The leaf sap is applied to the tongues of babies to treat thrush (Burkill, 1985). A poultice of the leaves is used for treating sores, bruising, swellings, inflammations, headaches and piles. An infusion of the leaves is mixed with soft grease and applied to cuts (DeFilipps *et al.*, 2018). The sap has a widespread reputation for as a haemostatic for treating wounds and for skin problems. It is applied externally to treat infected wounds, ulcers, cuts, abrasions, ringworm, eczema, dermatomycosis, scabies and venereal diseases. It is also used against pains, including bee and wasp stings. The fruits and seed are boiled in water and taken as a remedy for stomach ache (Burkill, 1985). The seeds are used as a purgative and to expel internal parasites. An oil obtained from the seeds is a powerful purgative and emetic, with an action similar to that of *Jatropha curcas*. It is taken to expel internal parasites. The oil is used externally as a rubefacient to treat rheumatic conditions and a variety of skin infections, including leprosy, although its use on the skin may also cause an irritating rash. The yellowish-brown pith of old stems is used in Ghana for treating headaches. It is wrapped in a clean cloth and inserted into the nostrils of the patient to cause sneezing. A bark decoction is used as an emmenagogue, and the dried and pulverized root bark is made into poultices and taken internally to expel worms and to treat oedema.

## Biological and pharmacological activities

*J. gossypifolia* has a wide range of pharmacological activities. The aqueous extract of the leaves of the Togolese species showed a significant inhibition of the growth of *Plasmodium falciparum* (Gbeassor *et al.*, 1989). The apolar (dichloromethane) and polar (methanol) extracts of the leaves showed moderate antiplasmodial activity ( $15 \mu\text{g/ml} < 50 \mu\text{g/ml IC}_{50}$ ) against the chloroquine-sensitive 3D7 strain of *Plasmodium falciparum* (Jansen *et al.*, 2010). Aerial extracts administered at a dose of 200 mg/kg/day revealed hepatoprotective activity in carbon tetrachloride-induced liver injury in Wistar albino rats (Panda *et al.*, 2009b). The methanolic extract administered at a dose of 200  $\mu\text{g}/100 \mu\text{l}$  showed *in vitro* antibacterial activity against *Staphylococcus aureus*, *Streptococcus pyogenes* and *Escherichia coli* and a good antifungal activity on *Aspergillus niger*, *Candida albicans*, *Penicillium notatum* and *Saccharomyces cerevisiae* (Purohit and Purohit, 2011). The ethyl acetate and aqueous fractions of the methanolic extract of the aerial parts at a concentration of 1  $\mu\text{g/ml}$  showed inhibitory activity against *Microsporus canis* (MacBae, 1988). The chloroform extract is active against *Salmonella typhi*, *Pseudomonas aeruginosa*, *Bacillus cereus* and *Candida albicans*. The aqueous extract has *in vitro* antibacterial properties against *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cereus*, *Klebsiella aerogenes*, *Proteus vulgaris* and *Candida albicans*, but inactive against *Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus niger* (Dabur *et al.*, 2007). The raw latex has demonstrated *in vitro* antibacterial activity against *Listeria monocytogenes*, *Salmonella tyhimurium*, *Salmonella typhi*, and *Staphylococcus aureus* (Rocha and Dantas, 2009). Chloroform, methanolic and aqueous leaf extracts showed *in vitro* antibacterial activity against *Shigella dysenteriae* (David and Oluyege, 2006). The non-polar extract (petroleum ether) of the leaves, at a concentration of 1 mg/ml, was active against *Pseudomonas aeruginosa*, *Staphylococcus epidermidis* and *Salmonella typhimurium* (Chariandy *et al.*, 1999). The ethyl acetate extract of the leaves (1 mg/ml), was active against *Staphylococcus aureus* (Ravindranath *et al.*, 2003), while the methanolic extract of the leaves, administered at a dose of 500 and 1000 mg/kg, exhibited significant antiinflammatory activity in the carrageenan-induced Wistar rat paw oedema (Bhagat *et al.*, 2011). Oral intake of the methanolic extract of the aerial parts at doses of 100 and 200 mg/kg/day, showed significant antiinflammatory effect on carrageenan-induced paw oedema in mice (Panda *et al.*, 2009a). *In vitro* administration of the aqueous leaf extract at doses of 100 and 200  $\mu\text{g/mL}$ , significantly prevented haemolysis (Nagaharika *et al.*, 2013). A study by Panda *et al.*

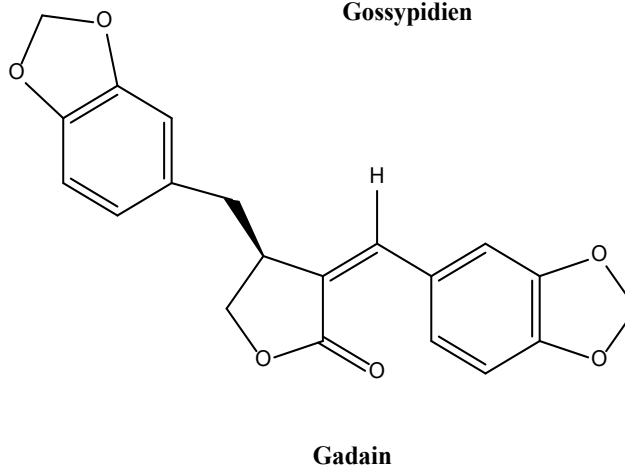
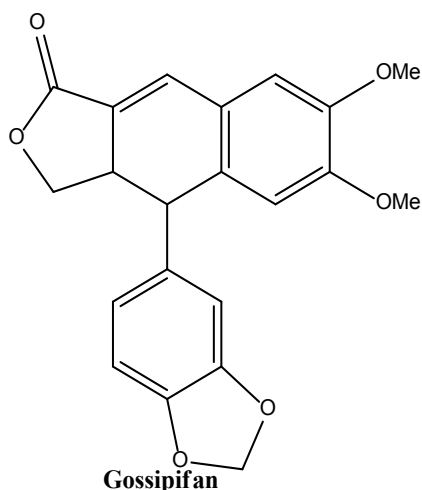
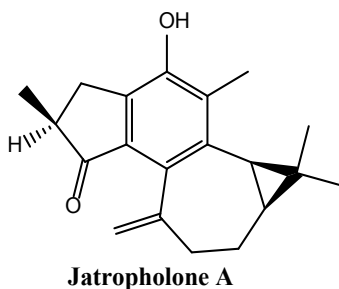
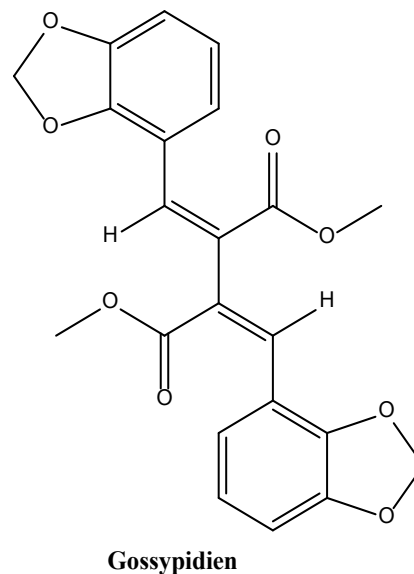
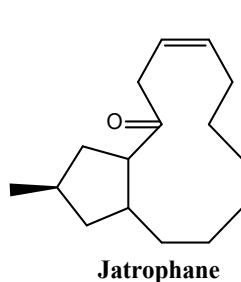
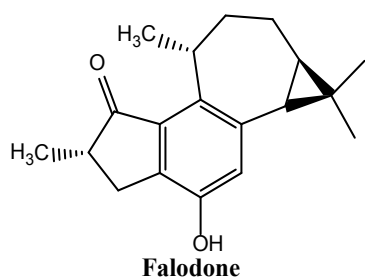
in 2009 found that extracts from the aerial parts have antiinflammatory and analgesic properties in mice (Panda *et al.*, 2009a). The antiinflammatory activity of the bark of *J. gossypiifolia* (methanol and petroleum extracts) has also been demonstrated in carrageenan-induced paw oedema in rats (Purohit and Purohit, 2011). Ethanolic extract of the roots as well as the compound jatrophone (diterpene macrocycle), showed significant *in vitro* inhibitory activity against cells derived from human nasopharyngeal carcinoma (KB) and P-388 lymphocytic leukaemia, and four animal tumour systems' standard *in vivo* (Kupchan *et al.*, 1970). The diterpenes (2 $\alpha$ -hydroxyjatrophone, 2 $\beta$ -hydroxy-5,6-isojatrophone and 2 $\beta$ -hydroxyjatrophone) isolated from the petroleum ether extract of the roots showed *in vivo* and *in vitro* activity on the P-388 lymphocytic leukaemia, as well as *in vitro* activity against nasopharyngeal carcinoma (Taylor *et al.*, 1983) cell lines. Falodone isolated from the roots showed an inhibitory activity against proliferation of the human cancer cell line A-549, with an IC<sub>50</sub> of 120  $\mu$ g/ml (Falodun *et al.*, 2012). The methanolic extract of the fruits and leaves administered at doses of 200 and 400 mg/kg, orally, showed anxiolytic and sedative activity in mice (Apu *et al.*, 2012). Oral administration of ethanolic extract of the aerial parts at doses of 500, 1000 and 2000 mg/kg, also showed significant antispasmodic activity in mice (Silva *et al.*, 2011). The ethanolic extract of the aerial parts of *J. gossypiifolia* administered orally at 125 and 250 mg/kg/day, for 4 weeks, resulted in a reduction in systolic blood pressure in normotensive rats. The ethanolic extract also showed vasorelaxant activity on a mesenteric artery devoid of isolated rat endothelium previously treated with norepinephrine or calcium chloride (Abreu *et al.*, 2003). Genotoxicity of the ethanolic extract of the leaves was more marked than the aqueous extract (Almeida *et al.* 2016). The ethanolic extract of the flowers provoked a sterility effect in rats (Jain *et al.*, 2013).

#### Clinical data

Not available

#### Chemical constituents

Coumarin-lignoids propacin, venkatasin, gossypifan, gossypiline, gossypidien, isogadain, jatrodien, gossypibetilene, tetrahydrogossypibetilene, citlalitrone (Das and Venkataiah, 2001, 1999; Das *et al.*, 2004; Ravindranath *et al.*, 2003), cleomiscosin and 4'-O-demethyl retrochinensin; cyclic heptapeptide cyclogossine A (Horsten *et al.*, 1996); jatropholones A and B, falodone and (4E)-jatrogrossidentadione acetate; lignans, jatrophone, gadain and venkatasin (Ravindranath *et al.*, 2003; Banerjia *et al.*, 1984) and 12-deoxy-16-hydroxy-phorbol.



### Test for identity and purity

**Moisture content:** air dried coarse powder does not lose more than 6.3% (leaves) and 4.7% (stem bark) at 105°C.

**Total ash:** not more than 20% (leaves) of 13.6% (stem bark)

**Acid insoluble ash:** not more than 6.0% (leaves) and 0.1% (stem bark).

**Water soluble extractive:** not less than 8% leaves) and 9.0% (stem bark)

**Ethanol soluble extractive:** not less than 2 % (leaves) of 10.0% (stem bark).

### Chromatographic fingerprint

#### Thin Layer Chromatography

**Preparation:** About 5 g of the powdered leaves were extracted with ethyl acetate by cold maceration, filtered and the filtrate concentrated to a small volume. A small spot was then applied to the TLC plate for analysis.

**Chromatographic conditions:** Analytical TLC on silica gel G60 F254, 0.25 mm layer in hexane/ethyl acetate (7:3) as the mobile phase.

**Detection:** Visualized in daylight after spraying with anisaldehyde-sulphuric acid (Lane 1) and vanillin-sulphuric acid reagents (Lane 2) (Stahl, 1969), heating to 110°C for 10 mins. The TLC chromatogram showed seven prominent spots with R<sub>f</sub>s of 0.85 (pink), 0.78 (mauve), 0.67 (pink), 0.64 (yellow), 0.60 (pink), 0.44 (yellow) and 0.33 (yellow) when sprayed with both anisaldehyde and vanillin reagents.



### High Performance Liquid Chromatography

**Sample preparation:** A sample of about 10 mg of the hydroethanolic extract of *J. gossypiifolia* leaves was reconstituted in 3 mL acetonitrile in a 10 mL volumetric flask with sonication for 17 mins. It was then diluted to volume with the mobile phase under chromatographic conditions. It was centrifuged to obtain a clear test solution which was subsequently filtered through a 0.45 µm filter into an HPLC vial and analyzed.

### Chromatographic system

#### Optimized chromatographic conditions

**Mode:** LC

**Column:** YMC ODS, 4.6 x 150 mm, 5 µm

**Column temperature:** Ambient – 30°C

**Mobile phase:** Acetonitrile: water (60:40 v/v)

**Elution mode :** Isocratic

**Injection volume :** 20 µL

**Flow rate:** 0.5 mL/minute

**Detection wavelengths:** 230 nm, 254 nm and 278 nm.

#### System Suitability parameters

**Number of peaks:** 230 nm (1), 254 nm (1), 278 nm (2)

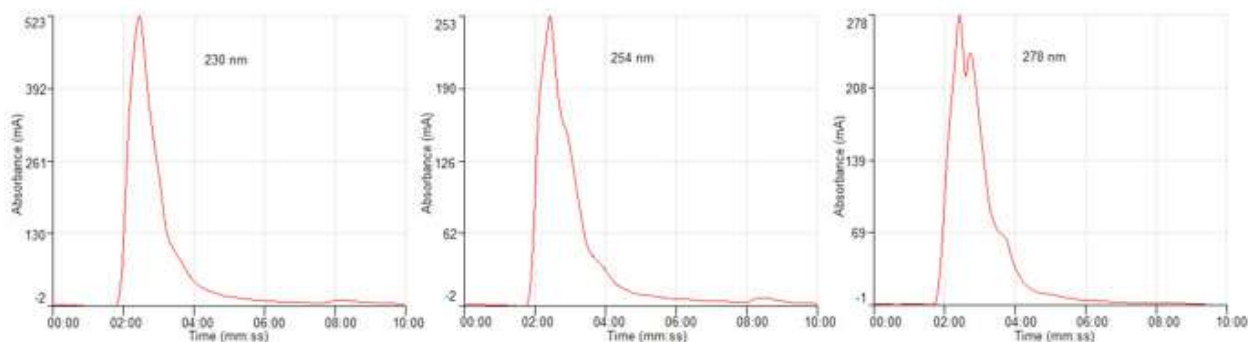
**Retention time (s):** 230 nm (2.27 min), 254 nm (2.25 min), 278 nm (rt1-2.29 min, rt2-2.35 min)

**Asymmetric factor(s):** 230 nm (0.762), 254 nm (1.206), 278 nm (af1-0.989, af2-0.860)

**Tailing factor:** NMT 2.0

**Efficiency:** 230 nm (49.91), 254 nm (40.32), 278 nm (E1-1140.23, E2-1345.02)

**Acceptance criteria:** Sample solution of hydroethanolic crude extract of *J. gossypifolia* L. (leaves) conforms to the system suitability parameters.



### HPLC chromatogram of *Jatropha gossypifolia*

#### FT-IR

A small amount of the dried hydro-ethanolic extract (70%) was placed on the sample area of the Perkin Elmer UATR Fourier Transform infrared (FT-IR) spectrometer and scanned between  $4000\text{--}400\text{ cm}^{-1}$  with a resolving power of  $4\text{ cm}^{-1}$  and a cumulative scanning limitation of 24 times. Principal peaks appeared at wavenumbers  $3263.77$ ,  $2924.20$ ,  $2853.67$  and  $1581.71\text{ cm}^{-1}$ .

#### Macroscopy

Leaves are palmate in shape, three lobed, each lobe carrying a main vein from the base. The venation in the lobes is pinnate. The lobes are lanceolate–obovate in shape with an acuminate apex. The leaf is pubescent cordate at the base with a serrated margin. The blade is membranous, glabrous on both surfaces.

#### Microscopy

Upper surface has paracytic stomata with half-moon shaped subsidiary cells; other epidermal cells are polygonal with anticlinal walls. Cells along the veins are elongated and rectangular. Lower surface has paracytic stomata with half-moon shaped subsidiary cells more numerous than on the upper surface. Other epidermal cells are polygonal with anticlinal walls.

#### Transverse section

Circular thick-walled collenchyma occurs above and below the midrib bundle. The main vascular bundle is collateral arch shaped bundle, with three small ones at the upper part of the vascular system. The phloem is surrounded by the xylem vessels and the vascular system is bound by rows of fibres with calcium oxalate cluster crystals. The lamina has one row of palisade cells and numerous calcium oxalate crystals scattered in the spongy mesophyll.

#### Powdered plant material

The powder shows fragments of upper epidermis with polygonal cells and anomocytic stomata. Fragments of the leaf pedicel with some showing stumps of broken trichomes. Spongy mesophyll with numerous calcium oxalate crystals; bundles of fibres lined with calcium oxalate crystals and annular and spiral xylem vessels.

#### Therapeutic actions

Antimalarial; antidiabetic, hepatoprotective, antibacterial, antifungal, antiinflammatory, analgesic, antihemolytic, anticancer, anxiolytic, sedative, hypotensive and vasorelaxant.

## Therapeutic indications

Malaria, liver disease, inflammation, pain, infections, leukaemia, anxiety, hypertension, stress and diabetes.

## Safety data

LD<sub>50</sub> by oral route was estimated to be above 3000 mg/kg in rats. CNS and ANS were not affected by treatment with the aqueous extract. In the sub-acute toxicity test, 1000 mg/kg of *Jatropha* inhibited growth in the test animals, with the animals dying within a week of drug administration. The LD<sub>50</sub> of the subacute dosing (886 mg/kg) was significantly lower than the LD<sub>50</sub> of acute dosing (3000 mg/kg), suggesting a possible cumulative effect. The hydroalcoholic leaf extract of *J. gossypifolia* (100 – 300 mg/kg) did not significantly affect the macro-anatomical structure of the liver, kidney, heart and lungs. The relative organ weights of liver, kidney, spleen, thymus and adrenals were not changed by the treatment. *Jatropha* (100-300 mg/kg) did not affect RBC count and other RBC indices. However, it mildly elevated WBC count. There were alterations in the proportions of granulocytes and agranulocytes. *Jatropha* appears to enhance neutrophil and MID cell counts whilst depressing lymphocyte numbers. *Jatropha* extract (100-300 mg/kg) did not affect serum proteins or bilirubin. Renal function was not affected. Pentobarbitone-induced sleeping time was enhanced marginally. No changes suggesting cellular damage were seen in the liver and kidney in the histopathological studies. The present results and others indicate that *Jatropha* is a potentially toxic plant and should be used with caution.

## Precautions for use

Although *J. gossypifolia* is considered a potential source of pharmacologically and biotechnologically relevant secondary metabolites, it must be used with extreme care due to the potential cytotoxicity, genotoxicity and/or mutagenicity observed experimentally for both the ethanolic and aqueous leaf extracts and the latex (Almeida *et al.*, 2015; 2016). *Jatropha* must be used with caution in pregnant women.

## Adverse effects

Hypotensive and hypoglycaemic.

## Contraindications

In pregnant women due to its potential cytotoxicity, genotoxicity and / or mutagenicity

## Dosage form

Powder, decoction, infusion, tincture

## Dosage

Decoction: 30 g of dried plant material in 900 mL water; simmer until reduced to 600 mL; 1-3 tablespoonfuls daily

Infusion: 30 g of dried aerial part in 600 mL of water; 3-4 teacups daily

Tincture: 1:5, 45% ethanol; 5 ml three times daily

## Storage

Store in a cool, dry place away from light



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**Botanical name**

*Newbouldia laevis* (P. beauv) seem.

**Family**

Bignoniaceae

**Synonyms**

*Bignonia glandulosa* Schumach. & Thonn.,  
*Newbouldia pentandra* (Hook.) Seem., *Spathodea laevis* P.Beauv., *Spathodea pentandra* Hook.

**Common Names**

African Border Tree, Boundary Tree (English) (Arbonnier, 2004)

**Common Local names**

**Benin:** Fon-Kpatin; Yoruba-Akoko; Dendi-Deebu

**Côte d'Ivoire:** Bete-Gba bui; Guere-Bolu; Fulfulde-Sukunde

**Gambia:** Pulaar – kallih; Manding– kunjumburo; Wolof - jamjam

**Ghana:** Akan - sesemasa; Baule – tonzue; Krachi – bonchu

**Guinea:** Pular-Sukunde; Mandeng-Kunjumborong; Susu-Kinki

**Guinea-Bissau:** Mandyak-Becuape; Mankanya-Boukouf; Creole-Manduco de feticero

**Liberia:** Mano - a lah

**Mali:** Manding- kinkin

**Nigeria:** Hausa – àdùrùkù ; Igbo - egbo; ẹ̀bwo; *Yoruba* - akoko

**Senegal:** Diola-Egompa ; Pular-Kôdomburu ; Wolof-Ngam

**Sierra Leone:** Susu-Kinki; Mende-Pomamagbe; Gola-Zodo

**Togo:** Gbe-Vhe – lifui; Tem – akinale; Yoruba – aboboe

**Description of the plant**

*Newbouldia laevis* is a shrub or tree that grows in West Africa, reaching 7–8 m high in the West (Senegal) to 20 m in the East (Nigeria) and to 2.70 m in girth (Sierra Leone). It is erect with vertically ascending branches, of wooded savanna and deciduous forest. The plant has shiny dark green leaves and bears large terminal purple flowers. The leaves are imparipinnate, opposite verticillate, rachis 15-40 cm long and 3-6 cm wide; toothed, base abruptly asymmetrical, apex acuminate; terminal panicle composed of pink or pinkish white flowers. The corolla is nearly regular, narrowly campanulate, about 6 cm long, glabrous on the outside. The fruit is about 30 cm long, seeds winged at each end. It is often grown as an ornamental and is easily propagated by cuttings (Mshana *et al.*, 2000).



A – *Newbouldia laevis* leaves, B- flowers, C- unripe fruits, D- mature fruit, E – trunk of *N. laevis*.

#### Herbarium specimen number

Côte d'Ivoire: 15762 (CNF)

Ghana: GH491/KNUST

Nigeria: UPFH 119

Senegal: IFAN 1271

Togo: TG 02453

#### Habitat and geographical distribution

The plant is native to tropical Africa and grows in secondary and dry forests, from Guinea Savannahs to dense forests, on moist and well-drained soils. Also found in regenerating forests, woody savannah and deciduous forests (Burkill, 1985). It is distributed in West tropical Africa from Senegal to Cameroon, Gabon, Democratic Republic of Congo and Angola (Arbonnier, 2004).

#### Plant material of interest

Stem bark

#### Other part used

Leaves

#### Definition of plant material of interest

*Newbouldia laevis* consists of the dry or fresh stem bark of *Newbouldia laevis* (P. beauv) Seem. (Bignoniaceae)

## Ethnomedical uses

The stem bark is widely used in traditional medicine in Africa. The bark is analgesic and stomachic. A decoction is used in the treatment of coughs, diarrhoea and dysentery, whilst it is also given to children for treating epilepsy and convulsions (Burkil, 1985). A decoction of the bark, combined with chilli, is used in the treatment of chest pains. The dried bark and young twigs, pounded with *Xylopia* sp, is given as a decoction or infusion to treat uterine colic and dysmenorrhoea (Burkill, 1985). The bark is given in the form of an enema as a treatment for constipation and piles. One or two sniffs of a snuff made from the sun-dried bark, when ground and combined with palm salt ( $K_2CO_3$ ) and the fruits of *Piper guineense*, can be used to treat headache, sinusitis and the severe migraine (Burkill, 1985). Applied externally, the bark is believed to treat a range of skin conditions including septic wounds, abscesses, ulcers, and snake bites. A poultice of the bark is applied to the joints to treat rheumatism. The inner soft bark is put into the ear as a treatment for earache, and a decoction of the leaves is used to treat ophthalmia and conjunctivitis. The leaves are cooked in palm-oil soup and taken by pregnant women to ease delivery and to promote a rich milk supply. The leaf-ash, mixed with salt, is taken as a remedy for chest pains. A decoction of the leaves, combined with those of *Psidium guajava*, is taken for the treatment of diarrhoea and dysentery. The leaves are chewed and applied to snake bites, and then sucked to draw out the venom (Fern, 2014). A decoction of the pounded roots is used in the treatment of intestinal problems, syphilis and as a vermifuge against roundworm (Barwick, 2004). It is applied externally as a poultice to treat aching limbs. Root scrapings combined with chilli, is applied to carious tooth. A decoction of the leaves and root scrapings are used together as a remedy for hernia, or for any form of orchitis. In Nigeria, the bark is chewed and swallowed for stomach pains, diarrhoea and toothache (Lewis and Manony, 1977). The plant has been found to be effective in the treatment of elephantiasis, dysentery, rheumatic swellings, syphilis, constipation, piles and as a vermifuge. It has also been found useful for earache, sore feet, chest pain, epilepsy and convulsion in children (Akunyili, 2000). The leaf, stem and fruits have been used as a febrifuge, wound dressing and stomachic (Iwu, 2000).

## Biological and pharmacological activities

The stem bark extract of *N. laevis* (10-300 mg/kg p.o.), dose-dependently decreased both phases of formalin-induced nociceptive behaviour. It was found to exhibit central and peripheral analgesic properties (Ainooson *et al*, 2009). Similarly methanol extracts of *N. laevis* stem bark was found to exhibit a dose-dependent inhibition of carrageenan-induced oedema in rat hind paw. It also caused a decrease in yeast-induced pyrexia in mice and gave absolute protection against leptazol-induced seizures in mice (Olumayokun *et al*, 1997). The inhibitory effect of *N. laevis* extract on  $\alpha$ -glucosidase was assessed *in vitro* using baker's yeast and rat intestinal  $\alpha$ -glucosidases, while its inhibitory effect on  $\alpha$ -amylase was assayed using rat pancreatic  $\alpha$ -amylase. *N. laevis* extract had significant  $\alpha$ -glucosidase inhibitory activity *in vitro* with  $IC_{50}$  values of 2.2  $\mu$ g/mL and 43.5  $\mu$ g/mL for baker's yeast and rat intestinal  $\alpha$ -glucosidase respectively. The extract also inhibited rat pancreatic  $\alpha$ -amylase activity with  $IC_{50}$  value of 58.7  $\mu$ g/mL. In both diabetic and non-diabetic rats, *N. laevis* extract caused a significant reduction in postprandial blood glucose level after oral sucrose load. *N. laevis* extract exerts its glucose-lowering effect through inhibition of  $\alpha$ -glucosidase and  $\alpha$ -amylase (Kolawole and Akanji, 2013). Naphthoquinones from *N. laevis* stem bark showed antifungal activity against *Cladosporium cucumerinum* and *Candida albicans*, and activity against *Bacillus subtilis* and *Escherichia coli* (Gafner *et al.*, 1996). In another study, methanolic leaf extract of *N. laevis* was evaluated for anticoagulant properties using blood clotting time, bleeding time and thrombin-induced clotting assay. The extract significantly ( $p < 0.05$ ) prolonged blood clotting times from the baseline value of  $11.0 \pm 0.6$  s for the blood sample to  $18.0 \pm 0.7$  s and  $32.0 \pm 1.0$  s at 5 % and 10 % concentrations respectively. The crude extract also exhibited appreciable *in vivo* and *in vitro* anticoagulant potency. High doses of the extract were most significant ( $p < 0.01$ ) in inducing rabbit bleeding, which was prolonged to  $55.8 \pm 1.4$  s and  $73.1 \pm 0.8$  s at 100 and 200 mg/kg respectively compared to the baseline ( $18.0 \pm 0.2$  s) and reference anticoagulant aspirin and heparin (Nwaehujor *et al.*, 2015). The aqueous and ethanol extracts of the leaves of *N. laevis* were tested on isolated

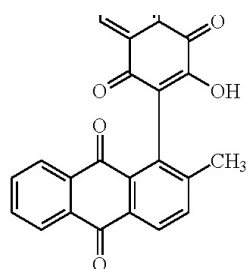
uterine preparations of non-pregnant rats. The extract significantly increased the frequency ( $P < 0.05$ ) of spontaneous contractions without significantly affecting the amplitude. The extracts and acetylcholine were observed to directly stimulate uterine contractions (Bafor and Sanni, 2009).

### Clinical data

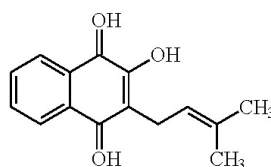
Not available

### Chemical constituents

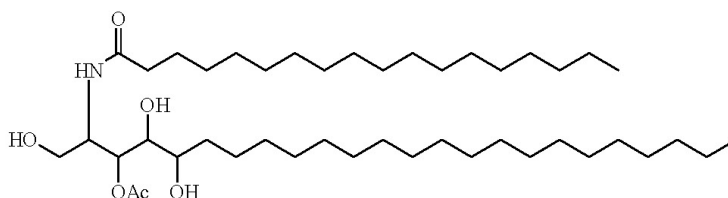
Naphthoquinones (newbouldiaquinone, 2-acetylfuro-1,4-naphthoquinone and 2-hydroxy-3-methoxy-9,10-dioxo-9,10-dihydroanthracene-1-carbaldehyde); apigenin, lapachol,  $\beta$ -sitosterol-3-O- $\beta$ -D-glucopyranoside, oleanolic acid, canthanic acid, newbouldiamide, 2-(4-hydroxyphenyl)-ethyltrioctanoate, chrysoeriol (Kuetze *et al.*, 2007), 6-hydroxydehydroiso- $\alpha$ -lapachone, 7-hydroxydehydroiso- $\alpha$ -lapachone, 5, 7-dihydroxydehydroiso- $\alpha$ -lapachone, 3-hydroxy-5-methoxydehydroiso- $\alpha$ -lapachone ((Eyong *et al.*, 2006; Gafner *et al.*, 1996); furanonaphthoquinones, atraric acid and a benzofuran (Gormann, *et al.*, 2003); pyrazole alkaloids withasomnine, 4'-hydroxy-withasomnine, newbouldine and 4'-hydroxynewbouldine (Adesanya *et al.*, 1994).



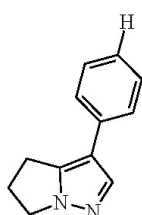
Newbouldiaquinone



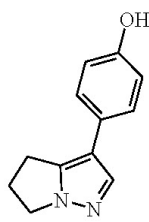
Lapachol



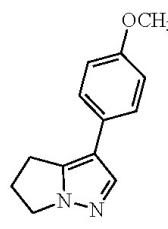
Newbouldiamide



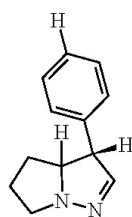
Withasomnine



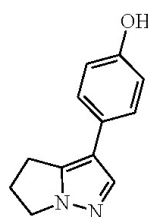
4-hydroxywithasomnine



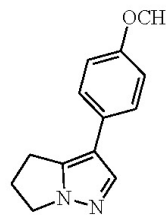
4-methoxywithasomnine



Newbouldine



4-hydroxynewbouldine



4-methoxynewbouldine

## Test for identity and purity

### Identity and purity test

Newbouldia laevis (leaves and stem bark)

**Moisture content:** air dried coarse powder does not lose more than 6.0% (leaves) and 6.2% (stem bark) at 105°C.

**Total ash:** not more than 6.2% (leaves) and 9.8% (stem bark)

**Acid insoluble ash:** not more than 1.7% (leaves) and 0.7% (stem bark)

**Water soluble extractive:** not less than 11.0% (leaves) and 14.0% (stem bark)

**Ethanol soluble extractive (70%):** not less than 7.0% (leaves) and of 2.0% (stem bark)

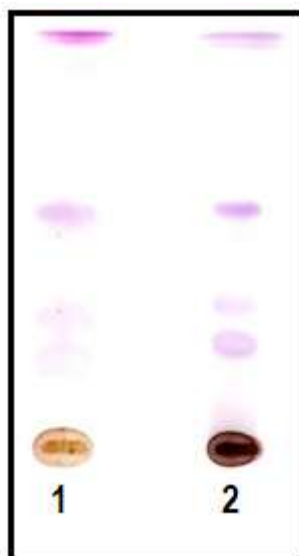
### Chromatographic fingerprint

#### Thin Layer Chromatography

**Preparation:** About 5 g of the powdered stem bark were extracted with ethyl acetate by cold maceration, filtered and the filtrate concentrated to a small volume. A small spot was then applied to the TLC plate for analysis.

**Chromatographic conditions:** Analytical TLC on silica gel G60 F254, 0.25mm layer in hexane/ethyl acetate (7:3) as the mobile phase.

**Detection:** Visualized in daylight after spraying with anisaldehyde-sulphuric acid (Lane 1) and vanillin-sulphuric acid reagents (Lane 2) (Stahl, 1969), heating to 110°C for 10 mins. The TLC chromatogram showed three prominent spots with R<sub>f</sub>s of 0.56 (purple), 0.27 (purple) and 0.25 (purple) when sprayed with both anisaldehyde and vanillin



#### High Performance Liquid Chromatography

**Sample preparation:** About 10 mg of the hydroethanolic extract of *N. laevis* stem bark were reconstituted in 3 mL acetonitrile in a 10 mL volumetric flask with sonication for 17 minutes. The solution was then diluted to volume with the mobile phase under chromatographic conditions. It was centrifuged to obtain a clear test



solution which was subsequently filtered through a 0.45 µm filter into an HPLC vial and analyzed.

### Chromatographic system

#### Optimized chromatographic conditions

**Mode:** LC

**Column:** YMC ODS, 4.6 x 150 mm, 5 µm

**Column temperature:** Ambient – 30°C

**Mobile phase:** Acetonitrile: water (60:40 v/v)

**Elution mode:** Isocratic

**Injection volume:** 20 µL

**Flow rate:** 0.5mL/minute

**Detection wavelengths:** 230 nm, 254 nm and 278 nm.

#### System Suitability parameters

**Number of peaks:** 230 nm (1), 254 nm (1), 278 nm (1)

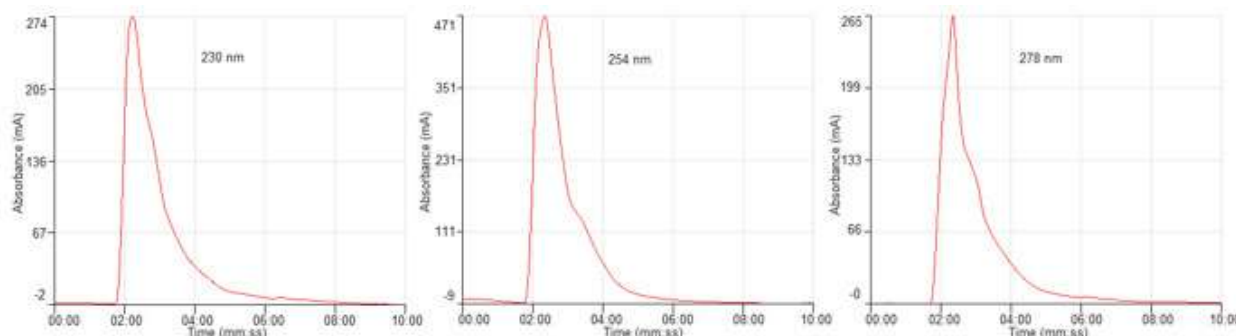
**Retention time (s):** 230 nm (2.20 min), 254 nm (2.12 min), 278 nm (2.20 min)

**Asymmetric factor(s):** 230 nm (0.535), 254 nm (0.845), 278 nm (0.437)

**Tailing factor:** NMT 2.0

**Efficiency:** 230 nm (43.95), 254 nm (36.43), 278 nm (53.29)

**Acceptance criteria:** Sample solution of hydro-ethanolic crude extract of *Newbouldia laevis* (P.Beauv.) Seem. (Stem Bark) conforms to the system suitability parameters.



### HPLC chromatogram of *Newbouldina laevis*

#### FT-IR

A small amount of the dried hydro-ethanolic extract (70%) was placed on the sample area of the Perkin Elmer UATR Fourier Transform infrared (FT-IR) spectrometer and scanned between 4000–400 cm<sup>-1</sup> with a resolving power of 4 cm<sup>-1</sup> and a cumulative scanning limitation of 24 times. Principal peaks appeared at wavenumbers 3318.08, 2924.22, 2853.50 and 1601.60 cm<sup>-1</sup>.

#### Microscopy

##### Stem bark

The transverse section shows rows of cork cells interspersed with rows of sclereids. The first row of cork cells is about fourteen rows in depth. This is followed by a row of groups of large stone cells which are yellowish in colour. A row of cells with brown content follows. Another row of cork cells of about twelve

cells in depth, is followed by a last row of groups of stone cells, deeper yellow in colour than those of the first row. Cortex is made of large groups of stone cells and fibres. The parenchyma cells contain raphids and acircular crystals. Medullary rays are two rows of cells, running from the endodermis to the cambium. Primary phloem has bands of fibres followed by sieve elements. Stone cells are thick-walled and have little lumen.

### **Powdered plant material**

The powder is characterised by abundant groups of fibres attached together, numerous groups of sclerenchyma and many cortical cells filled with circular starch granules; raphids and single needles are scattered.

### **Therapeutic actions**

Analgesic, antiinflammatory, antimicrobial, antidiabetic

### **Therapeutic indications**

Rheumatic pain, chest pains, infections, diabetes.

### **Safety data**

LD<sub>50</sub> by oral route was estimated to be above 3000 mg/kg in rats. There were no signs of CNS depression/stimulation or autonomic effects at the tested doses of 0-1000 mg/kg. The hydroalcoholic extract of the stem bark of *N. laevis* did not significantly affect the macroanatomical structure of the liver, kidney, heart or lungs. The relative organ weights of the liver, kidney, spleen, thymus and adrenals did not change. The extract did not significantly affect any of the haematological parameters. Treatment with the extract decreased AST and ALP. ALT also decreased but only at the highest dose of 1000 mg/kg. GGT was not affected by treatment. *Newbouldia* appears to induce a mild hypoalbuminaemia without affecting serum globulins. *Newbouldia* also decreased both conjugated and unconjugated bilirubin although the effect was not statistically significant. Treatment did not affect creatinine levels but caused mild elevations in urea at all doses. Pentobarbitone-induced sleeping time was enhanced by the treatment. No histopathological changes were seen in liver and kidney of treated animals. *N. laevis* appears to have a safe toxicity profile (Kolawole *et al.*, 2013). The present studies did not show any significant changes in platelets. Mild elevation in WBCs was however noticed. Decreases in liver enzymes even in naïve animals may be a confirmation of its hepatoprotective effect.

### **Precautions for use**

Care should be taken in concomitant use with sedatives.

### **Adverse effects**

None observed

### **Contraindications**

Pregnancy and children under the age of 12 years. Patients with impaired kidney function

### **Dosage form**

Powder, decoction, infusion, tincture

## Dosage

Decoction: 30 g of dried plant material in 900 mL water; simmer until reduced to 600 mL; 1-3 tablespoonfuls daily

Infusion: 30 g of dried aerial part in 600 mL of water; 3-4 teacups daily

Tincture: 1:5, 45% ethanol; 5 ml three times daily

## Storage

Keep in a cool, dry place away from light.

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**Botanical name**

*Olax subscorpioidea* Oliver

**Family**

Olacaceae

**Synonyms**

*Olax chariensis* A. Chev., *Olax durandii* Engl.

**Common names**

Olax, Stink Ant forest (English)

**Common Local names**

**Benin:** Fon-Amitin; Yoruba-Efun; Dendi-Borosonu

**Burkina Faso:** Dioula-Kouassoumbara

**Côte d'Ivoire:** Akan – Samanua; Akye - hacbéchémon zaku; Ando - akanji baka

**Gambia:** Wolof-Toll; Madinka - folah

**Ghana:** Twi - Ahoohenedua

**Guinea:** Konianke-Djèssè ; Kpèléwo-Niabènè ; Maninka-Djèsoli

**Niger:** Gwandara-Gwano kurmi

**Nigeria:** Yoruba - Ewe Ifon; Igbo- Igbulu; Hausa – Gwanonkurmi

**Togo:** Akasselem-Kpahabenté ; Ewe - Emiti

**Description of the plant**

*Olax subscorpioidea* Oliv. is a small tree and often described as a shrub with its roots, stem, leaves and branches reported to be medicinal (Ibrahim *et al.*, 2007). It can grow up to 10 m high. It is usually a shrub with long thin drooping branches. The bark is green with smooth, shallow, but angular and brown longitudinal distinct ridges. It has a light brown slash, which smells of garlic. The leaves are almost without stalk and the flowers are greenish white, and the fruits yellow to orange and globose. The flowers are directly attached to the branches (Mshana *et al.*, 2000).



A – *Olax subscorpioidea* Oliv, B – Stem , C - Flowers, D – fruit

#### Herbarium specimen number

Benin: 2355 (AP)

Burkina Faso: *MCLS 1370 (CNSF) ; 3519 (OAU)*.

Cote d'Ivoire: 15825-CNF

Ghana: GH 551/KNUST

Mali: 1832/DMT

Nigeria: UPFH 120

Senegal: IFAN 3958

Togo: TG 05414

#### Habitat and geographical distribution

*O. subscorpioidea* (Oliv.) is widely distributed in forests across the region from Senegal to Western Cameroon (Kazeem *et al.*, 2015). It is found growing in forests and fringing forest, as well as in savanna regions (Mshana *et al.*, 2000).

#### Plant material of interest

Roots

#### Other parts used

Leaves and stem bark

#### Definition of plant material of interest

Olax consists of the fresh or dried roots of *Olax subscorpioidea* Oliv (Olacaceae)

## Ethnomedical uses

The leaves are used to treat allergic rhinitis, dracontiasis and the roots for jaundice. A decoction of the leaves is taken orally for the treatment of malaria (Bla *et al.*, 2015). Oral administration of a decoction of the powdered roots squeezed into a juice mixed with *Pennisetum glaucum* has been reported to treat intestinal worms in cattle, sheep, goat and dogs (Kone *et al.*, 2004). The root of the plant is used for the treatment of asthma (Sonibare and Gbile, 2008; Fatokun, *et al.*, 2016), intestinal worms and as a chewing stick (Kone *et al.*, 2004). It is also used in the treatment of dermatosis, fever, jaundice, rheumatism, colic, blennorrhoea, syphilis, arthritis and mental illness (Konan *et al.*, 2013). In addition, the root is used as an ingredient in a decoction to treat infantile ailments such as skin infections, screaming, convulsions and malaria (Kayode and Omotoyinbo, 2013; Aworinde and Erinoso, 2015). Leaves and root decoction and infusion are used in the treatment of diabetes (Olabanji *et al.*, 2008; Soladoye *et al.*, 2012). The whole plant, together with *Eleusine indica*, has been used to treat mental illness (Ibrahim *et al.*, 2007). Roots, leaves, stem bark and twigs are used for the treatment of yellow fever, jaundice, guinea worm, toothache, venereal diseases and mental illness (Olowokudejo *et al.*, 2008). The root has been used for treating sickle cell and breast cancer (Gbadamosi, 2015), while the stem bark has been used for epilepsy (Wahab, 2015), convulsion and polio in children (Kayode and Sanni, 2016). The stem bark is also used in the treatment of neurodegenerative diseases (Sonibare and Ayoola, 2015). The roots, leaves, stem bark and twigs are used for yellow fever, jaundice, guinea worm and venereal diseases (Chukwuma *et al.*, 2015). Powdered seeds of *O. subscorpioidea* mixed with *Tetrapleura tetraptera* and soap are used to bath children to protect them from skin and scalp infections. *O. subscorpioidea* is used to treat pains, rheumatoid arthritis, yellow fever, depression, constipation and as a genital stimulant (Kayode and Sanni, 2016).

## Biological and pharmacological activities

Ishola *et al.*, (2015) reported that the dried leaves of *O. subscorpioidea* had antinociceptive effect through interaction with 5-HT<sub>2</sub> (serotonin), dopamine (D<sub>2</sub>) and sensitive potassium ATP channels as well as anti-inflammatory effect, thus confirming its folkloric use in the treatment of painful and inflammatory conditions. Gottardi *et al.*, (2016) reported the antimicrobial activity of *O. subscorpioidea* against *Candida albicans* and *Chlamydia tropicalis*. Ayandele and Adebisi (2007) inferred that the ethanol extract of the leaves of *O. subscorpioidea* is a broad spectrum agent against both Gram positive and Gram negative bacteria as well as some fungi. The extract showed activity against *Escherichia coli*, *Salmonella spp*, *Pseudomonas aeruginosa* and *P. vulgaris* obtained from hospital urine, wound and pharmaceutical industrial water isolates. Methanol/dichloromethane (3:1 V/V) extract of the fruit of *O. subscorpioidea* was investigated *in vivo* for antifungal activity in rat model of disseminated candidiasis. The fungal burden was measured in blood and kidney. The fruit extract had the highest antifungal activity particularly against *Candida albicans* and *Candida tropicalis* with MIC of 0.097 mg/ml and 0.048 mg/ml respectively (Dzoyem *et al.*, 2014). Methanolic extracts of the leaf of *O. subscorpioidea* was tested against clinical isolates of *Aspergillus fumigatus*, *Candida albicans*, *Streptococcus aureus*, *Lactobacillus acidophilus* and *Pseudomonas aeruginosa*. The extract was active only against *Aspergillus fumigatus* with MIC 51.2 mg ml<sup>-1</sup> (Orabueze *et al.*, 2016). In a study conducted by Kazeem *et al.*, (2015), the leaves of *O. subscorpioidea* was shown to possess antidiabetic potential in wistar rats, with the possible mechanism of action being inhibition of pancreatic  $\alpha$ -amylase and the intestinal  $\alpha$ -glucosidase, thereby slowing down the absorption of carbohydrates and preventing hyperglycaemia. The hexane extract showed the greatest activity against  $\alpha$ -amylase (IC<sub>50</sub>:0.72 mg mL<sup>-1</sup>) and  $\alpha$ -glucosidase (IC<sub>50</sub>:0.10 mg mL<sup>-1</sup>) (Kayode and Omotoyinbo, 2013). Ethyl acetate fraction of *O. subscorpioidea* showed activity against chloroquine-resistant and chloroquine-sensitive strains of *Plasmodium falciparum* with IC<sub>50</sub> of 28.16  $\pm$  0.5 ug/ml and 32.47  $\pm$  0.3 ug/ml respectively (Kipre *et al.*, 2015). In other studies, the methanol extract of the roots of *O. subscorpioidea* was found to possess antiulcer properties against ulcers produced by necrotizing agents, ethanol and indomethacin (Ukwe *et al.*, 2010). Santalbic acid derived from the seeds of *O. subscorpioidea* showed significant activity with IC<sub>50</sub> value above 10 ug/ml on MiaPaca-2 (prostate cancer

cell lines) and CCRF-CEM cells (leukaemia cell lines). It also showed significant activity on CEM/ADR5000 cells (leukaemia) and  $IC_{50}$  10.6  $\mu\text{g/ml}$  (Kuate *et al.*, 2011). Aqueous leaf extract of *O. subscorpioidea* was investigated *in vivo* for antiinflammatory and antinociceptive activities using albino rats and Swiss albino mice. At doses between 50–400 mg/kg, the extract significantly reduced acetic acid-induced writhes (68.28%, 50 mg/kg). Duration of paw licking/biting was also reduced by 73.10% at the early phase and by 70.50% at the late phase at a 50mg/kg dose in the formalin test. Reaction latency increased by 79.73% and 92.47% at 150 and 189 min at early and late phases respectively. The extract at 400 mg/kg was comparable to 20 mg/kg diclofenac in inhibiting carrageenan-induced oedema by 73.08% and 80.77% respectively when given 5 hours before the carrageenan was administered. The extract at 400 mg/kg showed similar activity to that of celecoxib (76.50%) in significantly reducing CFA-induced chronic inflammation by 85.30%, 12 days after the CFA was administered. The fruit extract did not inhibit xylene-induced ear oedema (Ishola *et al.*, 2015). Oni and Ogungbite (2015) investigated the insecticidal activity of the powders and oil of the stem bark and leaf of *O. subscorpioideae* against *Sitophilus zeamais*. The stem bark and leaf powder, after 72 hours of application, gave mortalities of  $38.35 \pm 0.67\%$  and  $36.65 \pm 0.67\%$  respectively. The oils from the stem bark and leaf at 10% concentration gave  $61.65 \pm 1.20\%$  and  $45.00 \pm 1.00\%$  mortality respectively.  $LD_{50}$  of plant powders after 72 hours of application was 8.22 for the stem bark and 16.84 for the leaf. The powder and the oils of the stem bark also inhibited the emergence of adult insects.

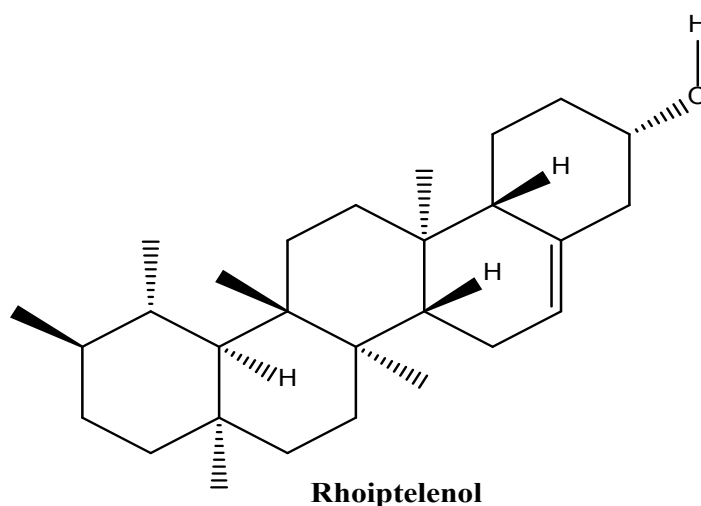
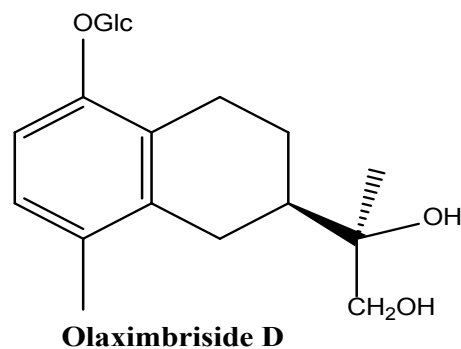
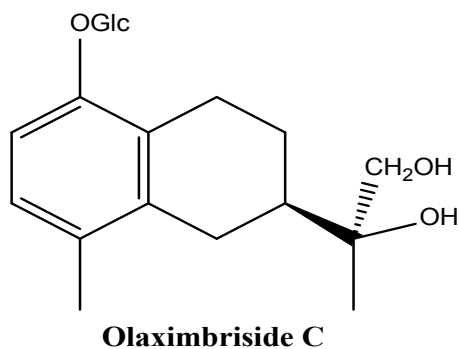
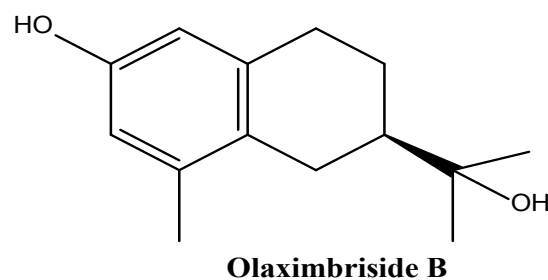
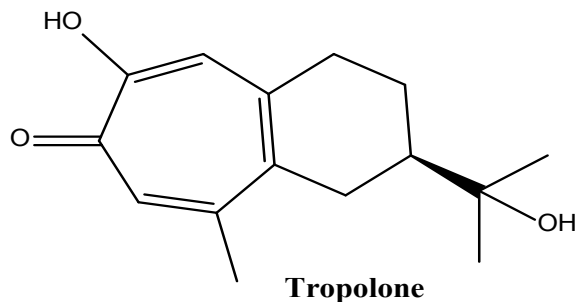
### Clinical data

Not available

### Chemical constituents

There are no records of compounds isolated from *O. subscorpioidea*. However, phytochemical analysis of some species of *Olax* has yielded some compounds. These include rhoiptelenol, glutinol (Sule *et al.*, 2011), olamannoside A–C (Okoye *et al.*, 2015) and olamannoside D–E (Okoye *et al.*, 2016) from *Olax mannii*. Others are olaxoside from *O. andronensis*, *O. glabriflora* and *O. psittacorum*; tropolone, 1,2,3,4-tetrahydronaphthalene derivatives, olaximbricides A–D from *Olax imbricata* (Huong *et al.*, 2019).





### Test for identity and purity

**Moisture content:** Air dried coarse powder does not lose more than 5.7% at 105°C.

**Total ash:** not more than 6.4%

**Acid insoluble ash:** not more than 0.8%

**Water soluble extractive:** not less than 18.0%

**Ethanol soluble extractive (70% v/v):** not less than 9.0%

### Chromatography

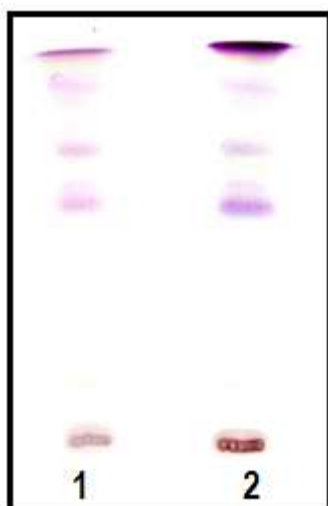
#### *Thin Layer Chromatography*

**Preparation:** About 5 g of the powdered roots were extracted with ethyl acetate by cold maceration, filtered and the filtrate concentrated to a small volume. A small spot was then applied to the TLC plate for analysis.

**Chromatographic conditions:** Analytical TLC on silica gel G60 F254, 0.25mm layer in hexane/ethyl acetate (7:3) as the mobile phase.

**Detection:** Visualized in daylight after spraying with anisaldehyde-sulphuric acid (Lane 1) and vanillin-sulphuric acid reagents (Lane 2) (Stahl, 1969), heating to 110°C for 10 mins.

The TLC chromatogram showed three prominent spots with R<sub>f</sub>s of 0.92 (pink), 0.75 (pink) and 0.60 (pink) when sprayed with anisaldehyde. All three spots however appeared purple in the chromatogram sprayed with vanillin reagent.



### High Performance Liquid Chromatography

**Sample preparation:** About 10 mg of the hydroethanolic extract of *O. subscorpioidea* root were reconstituted in 3 mL acetonitrile in a 10 mL volumetric flask with sonication for 17 minutes. The solution was then diluted to volume with the mobile phase under chromatographic conditions. It was centrifuged to obtain a clear test solution which was subsequently filtered through a 0.45 µm filter into an HPLC vial and analyzed.

### Chromatographic system

#### Optimized chromatographic conditions

**Mode:** LC

**Column:** YMC ODS, 4.6 x 150 mm, 5 µm

**Column temperature:** Ambient -30°C

**Mobile phase:** Acetonitrile: Methanol: Water (60:20:20 v/v/v)

**Elution mode :** Isocratic

**Injection volume :** 20 µL

**Flow rate:** 0.5 mL/minute

**Detection wavelengths:** 230 nm, 254 nm and 278 nm.

#### System Suitability parameters

**Number of peaks:** 230 nm (1), 254 nm (1), 278 nm (1)

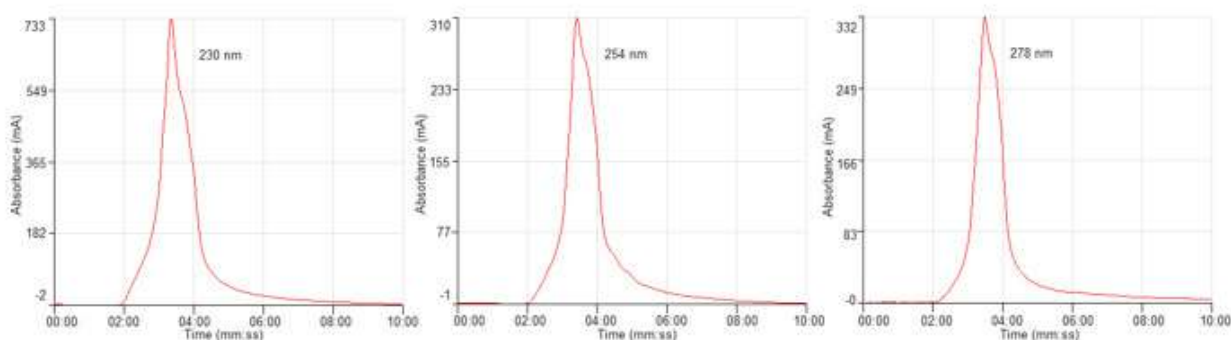
**Retention time (s):** 230 nm (3.21 min), 254 nm (3.25 min), 278 nm (3.29 min)

**Asymmetric factor(s):** 230 nm (0.618), 254 nm (0.803), 278 nm (1.042)

**Tailing factor:** NMT 2.0

**Efficiency:** 230 nm (82.13), 254 nm (91.91), 278 nm (114.98)

**Acceptance criteria:** Sample solution of hydroethanolic crude extract of *O. subscorpioidea* Oliv. (Root) conforms to the system suitability parameters.



## FT-IR

A small amount of the dried hydroethanolic extract (70%) was placed on the sample area of the Perkin Elmer UATR Fourier transform infrared (FT-IR) spectrometer and scanned between 4000-400  $\text{cm}^{-1}$  with a resolving power of 4  $\text{cm}^{-1}$  and a cumulative scanning limitation of 24 times. Principal peaks appeared at wavenumbers 3280.16, 2927.34 and 1597.50  $\text{cm}^{-1}$

## Macroscopy

The leaves are oblong, almost petiolate, with an acute apex. Both surfaces are hairless and the upper surface is darker green than the lower surface. The leaf is leathery in texture and has a characteristic odour. The venation is cross-linked and the main vein is more prominent on the lower surface

## Microscopy

### Leaf

Upper surface has epidermal cells with wavy walls and many cells contain large calcium oxalate cluster crystals. Stomata are absent. Lower surface also has epidermal cells with wavy walls and anomocytic stomata with four to five subsidiary cells.

### Transverse Section

Upper section has several rows of collenchyma cells, which form a half moon shape. This is surrounded by a layer of xylem vessels surrounded, which are also surrounded by phloem and fibres. Large parenchyma cells with irregular walls fill the rest of the midrib surrounding the arc-shaped vascular bundle. The lamina shows a single layer of palisade and spongy mesophyll cells, with no distinct diagnostic characteristics. There are schizogenous glands in the lamina.

## Powdered plant material

Powder (leaf) consists of fragments of upper and lower epidermal cells showing polygonal cells and anomocytic stomata respectively; fibres occur singly and in groups and have acute apex. Fragments of spongy mesophyll and palisade occur with annular xylem vessels and fragments of trichomes.

Powder (root) contains numerous prismatic calcium oxalate crystals. There are characteristic starch granules with marked hilum. There are also xylem vessels of the scalariform and pitted types, bundles of fibres, cork cells and undefined lignified structures

**Therapeutic actions**

Antinociceptive, antidepressant, antiulcer, antiparasitic, anti-infective.

**Therapeutic indications**

Rheumatoid arthritis, depression, malaria, syphilis, ulcer.

**Safety data**

LD<sub>50</sub> by oral route was estimated to be above 3000 mg/kg in rats. There were no signs of CNS depression/stimulation or autonomic effects at doses of 0-1000 mg/kg. In sub-acute studies, the hydroalcoholic extract of *O. subscorpioidea* did not significantly affect the macroanatomical structure of the liver, kidney, heart or lungs. The relative organ weights of liver, kidney, spleen, thymus and adrenals were all not significantly affected by the extract. Olax extract did not induce significant changes in RBC, WBC and platelets. Olax reduced ALT, AST, GGT, ALP at all doses tested. Total serum proteins also decreased. The mild hypoalbuminaemia induced by Olax was dose-dependent, but had no effect on serum bilirubin. Olax had no effect on urea and creatinine levels. Pentobarbitone-induced sleeping time was prolonged by treatment with Olax. No histopathological changes were seen in liver and kidney of treated animals. Olax has a very good safety profile on the liver and kidney. Serum biochemical results seen in the present study is consistent with an earlier finding by Adebayo *et al.*, (2014). The decreases in liver enzymes even in naïve animals may be an indication of its strong hepatoprotective activity. Long term administration may result in mild leucopenia and some mild hematological changes.

**Precautions for use**

Caution should be taken in long-term use

**Adverse effects**

None known

**Contraindication**

In Pregnancy and children under the age of 12.

**Dosage form**

Powders, infusion, decoction, tincture

**Dosage**

Decoction: 30 g of dried plant material in 900 mL water; simmer until reduced to 600 mL; 1-3 tablespoons daily

Infusion: 30 g of dried aerial part in 600 mL of water; 3-4 teacups daily

Tincture: 1:5, 45% ethanol; 5 ml three times daily

**Storage**

Store in a cool, dry place away from light.

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**Botanical name**

*Pavetta owariensis* P. Beauv.

**Family**

Rubiaceae

**Synonyms**

*Pavetta owariensis* var. *owariensis* P. Beauv, *Ixora owariensis* (P.Beauv.) Poir. *Pavetta flaviflora* Hutch & Dalz.

**Common Local names**

**Gambia:** Madinka – Kutufingo

**Ghana:** Akan- Kronkoo

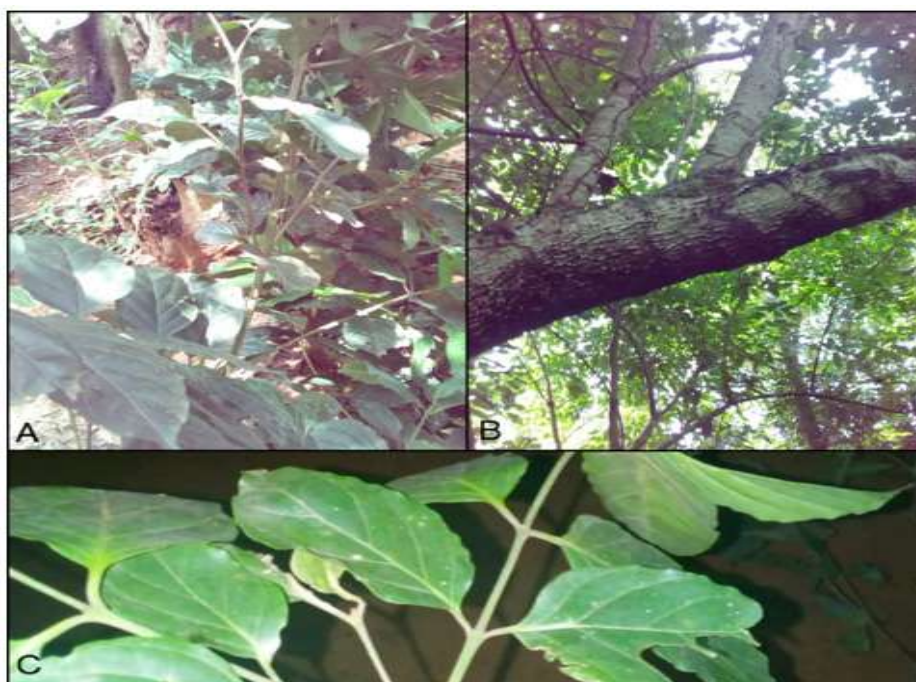
**Guinea:** Bhenhoulaï – Töma; Lagui wulu - Guerzey

**Nigeria:** Edo – akpano; Hausa – Namijim

**Sierra Leone:** Bole-hala; Mende-kunde; Temne-ε mamba

**Description of the plant**

*Pavetta owariensis* is a shrub or forest tree growing up to 7 m tall, bearing young pubescent twigs. The leaves are opposite, petiolated with the leaf blade elliptical to oblanceolate or obovate, 7-22 cm long and 3-9 cm wide. The lamina is penninerve with 6-12 pairs of lateral veins. It bears white flowers, grouped in cymes with corolla 1-1.2 cm long. *P. owariensis* produces rupaceous fruits (Lisowski, 2009).



A - *Pavetta owariensis* P. Beauv. whole plant, B – Tree trunk showing stem bark, C - leaves



**Herbarium specimen number**

**Côte d'Ivoire:** 12932 CNF

**Ghana:** GH 364/KNUST

**Mali:** 0477 (DMT)

**Senegal:** IFAN 426

**Habitat and geographical distribution**

*P. owariensis* occurs in gallery forests, moist and secondary forests, mainly along rivers and clay soil. In Africa, *P. owariensis* is found in countries such as Guinea, Sierra Leone, Nigeria, Côte d'Ivoire, Cameroon and Ghana. In Guinea, its distribution is limited to the forest region (Baldé *et al.*, 1982).

**Plant material of interest**

Stem bark

**Other part used**

Leaves

**Definition of plant material of interest**

*P. owariensis* consists of the fresh or dried stem bark of *Pavetta owariensis* P. Beauv. (Rubiaceae)

**Ethnomedical uses**

The plant is used in Guinean traditional medicine, specifically as anthelmintic against *Ascaris lumbricoides*. The origin of such use could be related to the doctrine of signatures because of the worm-shaped appearance of the stem bark (Baldé *et al.*, 1982). The "white bark" and "red bark" varieties of this plant are used without any distinction by traditional medicine practitioners.

**Biological and pharmacological activities**

*In vivo* activity of a stem bark extract was demonstrated in mice infected with *Schistosoma mansoni*. The reduction of the number of eggs in the liver and intestines, as well as the modulation of bilharzial granuloma in the liver, demonstrated the main schistosomicidal properties of the plant (Baldé *et al.*, 1986). Furthermore, studies on the pronounced *in vitro* antiviral activity of the stem bark extract against herpes simplex and Cocksackie B-2, and the moderate antibacterial effect against *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Neisseria gonorrhoeae* have also been reported (Baldé *et al.*, 1990).

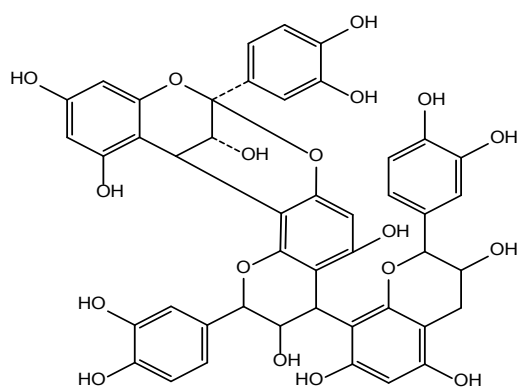
**Clinical data**

Clinical evaluations performed in Guinea and in hospitals indicated a significant antiparasitic effect of the hydroalcoholic extract of the trunk bark against ascariasis (61/68 of recovered patients) and *Schistosoma mansoni* schistosomiasis (108/125 of healed patients). Few side effects have been reported and these have mainly been related to nausea, mild headache, and vomiting (Baldé *et al.*, 1982).

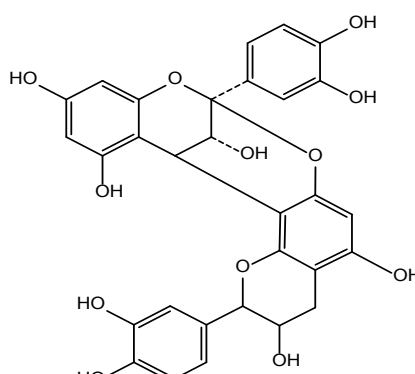
**Chemical constituents**

Catechins, proanthocyanidins (dimers, trimers, tetramers and pentamers), quinic acid esters, fatty acids,

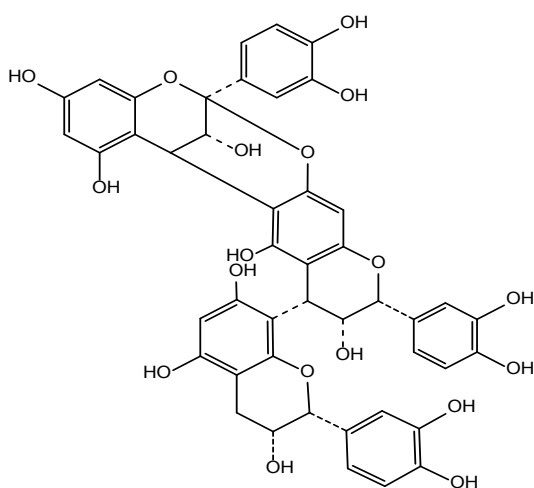
fatty alcohols, ferulic esters (octadecanyl, nonadecanyl, cosanyl, uncossanyl and sterols and cetosteroids (Baldé *et al* 2015, 1995a, 1995b, 1991a, 1991b, 1993); pavetannin A, (+)-catechin, (-)-epicatechin, ent-epicatechin, proanthocyanidin A-2 and proanthocyanidin A-4 (Balde *et al.*, 1991).



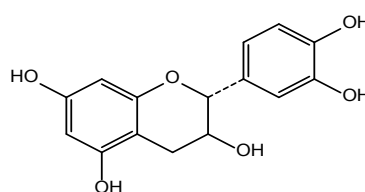
Pavetannin B1



Proanthocyanidin A-2



Pavetannin B



(+)-Catechin

### Test for identity and purity

**Moisture content:** Air dried plant material 10.8%

**Total ash:** not more than 5.06%

**Acid insoluble ash:** not more than 3.00%

**Water soluble extractive:** not less than 10.46%

**Alcohol soluble extractive:** not less than 4.02%

### Chromatographic fingerprint

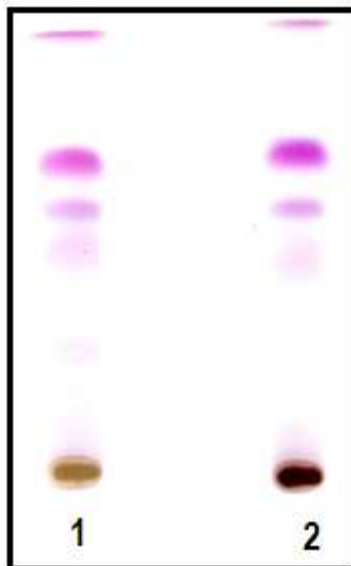
#### Thin Layer Chromatography

**Preparation:** About 5 g of the powdered stem bark were extracted with ethyl acetate by cold maceration, filtered and the filtrate concentrated to a small volume. A small spot was then applied to the TLC plate for analysis.

**Chromatographic conditions:** Analytical TLC on silica gel G60 F254, 0.25 mm layer in hexane/ethyl acetate (7:3) as the mobile phase.

**Detection:** Visualized in daylight after spraying with anisaldehyde-sulphuric acid (Lane 1) and vanillin-sulphuric acid reagents (Lane 2) (Stahl, 1969), heating to 110°C for 10 mins.

The TLC chromatogram showed three prominent spots with R<sub>f</sub>s of 0.69 (pink), 0.59 (pink) and 0.49 (purple) when sprayed with both anisaldehyde and vanillin reagents.



### High Performance Liquid Chromatography

**Sample preparation:** About 10 mg of the hydroethanolic extract of *P. owariensis* stem bark were reconstituted in 3 mL acetonitrile in a 10 mL volumetric flask with sonication for 17 minutes. The solution was then diluted to volume with the mobile phase under chromatographic conditions. It was centrifuged to obtain a clear test solution which was subsequently filtered through a 0.45 µm filter into an HPLC vial and analyzed.

### Chromatographic system

#### Optimized chromatographic conditions

**Mode:** LC

**Column:** YMC ODS, 4.6 x 150 mm, 5 µm

**Column temperature:** Ambient – 30°C

**Mobile phase:** Acetonitrile: Methanol: Water (60:20:20 v/v/v)

**Elution mode :** Isocratic

**Injection volume :** 20 µL

**Flow rate:** 0.5 mL/minute

**Detection wavelengths:** 230 nm, 254 nm and 278 nm.

#### System Suitability parameters

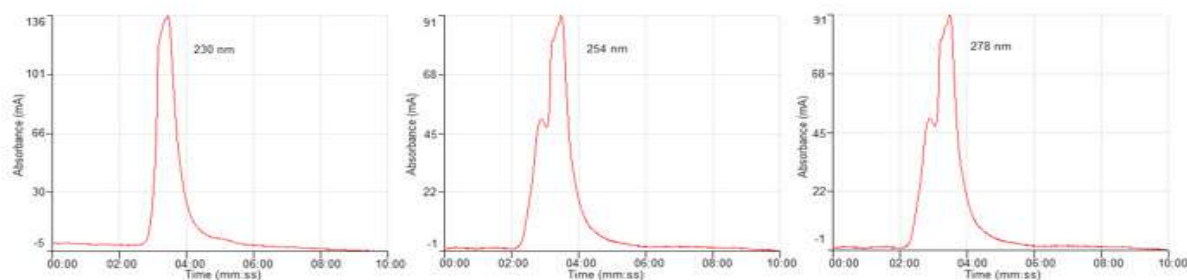
**Number of peaks:** 230 nm (1), 254 nm (2), 278 nm (2)

**Retention time (s):** 230 nm (3.15 min), 254 nm (rt1-2.27 min, rt2- 3.29 min), 278 nm (af1-2.53 min, af2-3.28 min)

**Asymmetric factor(s):** 230 nm (0.981), 254 nm (af1-1.182, af2-0.740), 278 nm (af1-1.426, af2-1.354)

**Tailing factor:** NMT 2.0

**Efficiency:** 230 nm (110.54), 254 nm (E1- 184.27, E2- 276.24), 278 nm (E1-488.12, E2-283.33)



## FT-IR

A small amount of the dried hydroethanolic extract (70%) was placed on the sample area of the Perkin Elmer UATR Fourier transform infrared (FT-IR) spectrometer and scanned between 4000-400  $\text{cm}^{-1}$  with a resolving power of 4  $\text{cm}^{-1}$  and a cumulative scanning limitation of 24 times. Principal peaks appeared at wavenumbers 3341.05, 2926.51, 1687.08 and 1598.74  $\text{cm}^{-1}$ .

## Macroscopy

Opposite leaves, petiolate; leaf blade elliptical to oblanceolate or obovate, 7-22 cm long and 3-9 cm wide, pinnate, with 6-12 pairs of lateral veins.

## Microscopy

Upper surface of the leaf is characterized by epidermal cells with wavy walls and anomocytic stomata with four subsidiary cells, two of which bind the stoma like wings. The remaining two subsidiary cells are parallel to the axis of the guard cells. Numerous anomocytic stomata of same type as the upper surface and unicellular trichomes occur on the lower surface. The cell walls are wavy or sinuous.

## Transverse section

The midrib section is convex at the upper section and the upper epidermis is followed by a clear section of collenchyma cells. The vascular system is V-shaped with the xylem portion coming before the phloem. The vascular system is encompassed by large oval shaped collenchyma, which are large, decreasing in size closer to the epidermis. There are numerous unicellular acutely-tipped trichomes coming from the lower epidermis of the leaf. They occur throughout the leaf on the lower surface only. Large cluster of calcium oxalate crystals are present in the collenchyma, occurring occasionally. They also occur throughout the lamina. The upper epidermis has one row of palisade cells which are rectangular.

## Powdered plant material

The leaf powder is characterized by fragments of the upper surface showing wavy epidermal cells, while the lower surface shows anomocytic stomata; fragments of cells from the veins and groups of fibers and xylem vessels. Numerous fragments of unicellular trichomes, calcium oxalate cluster and needle like crystals and fragments of the leaf lamina showing epidermal cells and palisade in transverse section. The stem bark powder is characterized by stone cells or sclereids, which occur singly or in groups. They are thick walled with very little lumen and are deep yellow in colour and have no distinct shape. Fibres occur singly or in groups and fragments of cork cells with yellowish coloured content. Fragments of parenchymatous cells of ground tissue abound.

**Therapeutic actions**

Schistosomicidal, antiviral, antibacterial.

**Therapeutic indications**

Helminthiasis (intestinal bilharziasis, ascariasis)

**Safety data**

LD<sub>50</sub> by oral route was estimated to be above 3000 mg/kg in rats. There were no signs of CNS depression/stimulation or autonomic effects at all the doses tested up to 1000 mg/kg. In sub-acute studies, no significant changes were noted with highly perfused organs such as the liver, kidney, heart and the lungs. The relative organ to body ratios of spleen, thymus, and adrenals were not significantly affected by the treatment. No significant effects on red blood cells and red blood cell indices were noticed. *Pavetta* extract caused dose-dependent leucopaenia, which was statistically significant at the highest dose of 100 mg/kg. Platelet count was not affected. *Pavetta* at the highest dose of 1000 mg/kg decreased AST and ALP, but did not affect GGT and ALT. *Pavetta* did not affect serum proteins except at the highest dose where there were elevations of both albumin and globulin; bilirubin remained normal. Renal function was not altered. Pentobarbitone-induced sleeping time was prolonged by the treatment. No histopathological changes were seen in the liver and kidney. *Pavetta* is unlikely to cause toxicity.

**Precautions for use**

Must be used with caution in pregnant women. Treatment should not exceed 2 weeks.

**Adverse effects**

Nausea, drowsiness, mild diarrhoea.

**Contraindications**

Not known

**Dosage forms**

Decoction, infusion, tincture

**Dosage**

Decoction: 30 g of dried plant material in 900 mL water; simmer until reduced to 600 mL; 1-3 tablespoonfuls daily

Infusion: 30 g of dried aerial part in 600 mL of water; 3-4 teacups daily

Tincture: 1:5, 45% ethanol; 5 ml three times daily

**Storage**

Keep in a cool dry place, away from light

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**Botanical name***Piliostigma thonningii* (Schumach.) Milne-Redh**Family**

Caesalpiniaceae

**Synonyms***Bauhinia thonningii* Schum and *Piliostigma reticulatum*.**Common Names**

Camel's foot (English), Pied-de-Boeuf, Semellier (French)

**Common Local names****Benin:** Fon-Klon; Yoruba-Abafe ; Dendi-Bakata**Burkina Faso:** Dioula-Gnama baa; Fulfulde-Barkehi; Moré-Barendaoga**Côte d'Ivoire:** Abron-Piti pata; Senoufo-Thiama; Malinké-Niama**Ghana:** Mole-Bage; Twi-Otokotaka; Ewe-Eklo**Guinea :** Peuhl-Barké; Soussou-Yorokoï; Malinké-Poro**Mali :** Bambara- Niama ba; Dogon- Tibisaa; Peulh- Barkere**Senegal:** Diola-Bu rekatod; Peulh-Barkedede; Tanda-Apes.**Togo:** Ewé-Eklo; Mina-Kloè; Yanga-Bany**Description of the plant**

*Piliostigma thonningii* is a bushy shrub growing up to 6 m tall, with a twisted, branchy trunk (von Maydell 1983). The bark is dark brown, fibrous with pink or dark brown patches. The leaves are simple, alternate, bilobed, leathery and pubescent on the abaxial side (Arbonnier, 2000). The male flowers contain 10 stamens, three of which are smaller, while the female flowers are thickly styled with a cap-shaped stigma at the top (Berhaut, 1975). *Piliostigma thonningii* is documented to have three types of flowers: male, female and hermaphrodite in small numbers. The inflorescence is in an axillary or terminal panicle, 10 to 25 cm long (Arbonnier, 2000). The fruits are long indehiscent, flattened brown pods, often twisted and leathery with an average length and width of 15 by 5 cm respectively and a weight of about 22 g. It is dark brown when ripe, woody and covered with a compact ferruginous pubescence. The seeds are numerous, scattered in the pulp. On average, there are 64 seeds per pod and 15,131 seeds per kg (Von Maydell 1983, Ouédraogo 2006b). *P. thonningii* share similar morphological features with *P. reticulatum*. The former differs from *P. reticulatum* by its slightly larger leaves and fruit and the pubescence of all its organs (Aubreville 1950, von Maydell 1983, Arbonnier 2000). The shrub blossoms from May to July and fruits soon after (Malgras, 1992).



A – *Piliostigma thonningii* (Schumach.) Mine-Redh, B – trunk showing stem bark, C – leaves, D- fruits, E - Flowers

#### Herbarium specimen number

Benin: 2356 AP/HNB

Burkina Faso: MSAD 671 (CNSF), Guinko 704 (OAU)

Côte-d'Ivoire: CNF 15937

Ghana: GH 594/KNUST

Mali: 0885/DMT

Nigeria: FHI111915

Senegal: UCAD 4278, IFAN 99

Togo: 0024 TGCl / AK, University of Lome.

#### Habitat and geographical distribution

*Piliostigma thonningii* is widespread in all the Sudano-Guinean savannahs of tropical Africa (Lamessa, 2010), growing on all types of soil. It is an invasive species in fallows, savannahs and degraded forests. In Senegal, for example, it is found in the Sudanian region, but not in the Sahel as it requires more moisture (Kerharo and Adam, 1974). In Mali, it is present in the open savannahs or woodlands in the Sudanian and Sudano-Guinean zones; it colonizes abandoned fallows (Malgras, 1992). It prefers clay soils or stony gravel, but often grows on cultivated soils (Von Maydell, 1983).

#### Plant material of interest

Leaves



### Other part used

Stem bark and roots

### Definition of plant material of interest

*Piliostigma* consists of the fresh or dried leaves of *Piliostigma thonningii* (Schumach.) Milne-Redh (Caesalpiniaceae)

### Ethnomedical uses

*P. thonningii* is a tree with many ethnomedical, medico-religious and medico-magical uses. (Ekoumou, 2003). In Togo, the roots are used as purgative and dewormer. The roots are used together with the leaves as an antidote to snake bite, gonorrhoea and rheumatism. The stem bark is used for colic, diarrhoea, dysentery, smallpox, bronchitis, sore and venereal disease. The stem and leaves are used widely as vaginal enemas after childbirth, and the treatment of ophthalmia and cataract, tooth decay, wounds, ulcers, headache and dizziness. The twigs are often used for chicken pox and the flowers for ophthalmia. The fruits are used for chronic wounds and hence its name in Ewe (eklo or wash). The root bark is employed as an antidote to some plant poisons. Ash from the fruit is used as a substitute for salt. Various parts of *P. thonningii* are used in agriculture and veterinary care. For example, the leaves are used as fodder for livestock. The fruits are also used for smoking hives to attract bee swarms. In the Central African Republic, a decoction of the leaves and stem bark or roots with chilli and salt is drunk to treat influenza and bronchitis (Lamessa, 2010). In Mali, root decoction is used as a purgative and vermifuge, and for treatment of dysentery, malaria and tuberculosis. Roots and leaves decoction are used for wound care. The young leaves are mixed with coagulated blood of beef or mutton and reduced to powder to treat cough in pregnant women. The tender leaves crushed and macerated in water are used in skin diseases including pimples. Infused leaves are used as a febrifuge, or chewed or made into a decoction for the management of vertigo. A decoction of the flowering tops of the plant is instilled in the eye for conjunctivitis, trachoma and cataract or drunk to treat malaria and jaundice. Leafy decoction with mistletoe is used in baths and drinks against onchocerciasis (Malgras, 1992). In the Sahelian zone, young leaves are used in cervical adenitis. The leafy stems of *P. thonningii* and *Ximenia americana* L. (Olacaceae) are used as decoction in filarial itching. Macerated leaves of *P. thonningii* and *Walteria americana* Linn (Sterculiaceae) are used for gingivitis and night blindness. Old wounds are treated with fresh leaves of the plant together with dry branches of *Diospyros mespiliformis* Hochst (Ebenaceae) and roots of *Ampelocessus grantii* Planch (Ampelidaceae) (Ekoumou, 2003). In Cameroon, nacerated leaves are used as a wound dressing. They are also used as an ingredient in the composition of a drug for treating worms (Burkill, 1995). In Senegal, the leaves are reportedly used in the treatment of enteralgia, anuria and epilepsy. Fresh leaf poultice is recommended for ulcers (Kerharo and Adam, 1974).

### Biological and pharmacological activities

Extracts of *P. thonningii* have demonstrated antiviral activity in diseases of viral origin including herpes, influenza and HIV (Bombardelli *et al.*, 1995). Leaf extract has also shown antibacterial activity against *Staphylococcus aureus* (Ibewuiké *et al.*, 1996), *Sarcina lutea* and *Mycobacterium phlei* (Kerharo and Adam 1974; Burkill 1995). Antiinflammatory and antibacterial C-methyl flavonols have been reported from the ethyl acetate fraction of the leaves (Ogundaini, 1999). Similarly methanolic extract of the stem bark showed activity against *Bacillus subtilis*, *Corynebacterium pyogenes*, *Escherichia coli*, *Proteus vulgaris*, *Shigella dysenteriae*, *Staphylococcus aureus* (Fakae *et al.*, 2000). The stem bark and leaf extracts possess antitussive activity in bronchopulmonary diseases (Bombardelli *et al.*, 1994). The ethanolic extract of the stem bark of the plant showed considerable dose-dependent anthelmintic action on *Ascaridia galli* (Asuzu and Onu, 1994). The anthelmintic activity of D-3-O-methylchiroinositol isolated from the stem bark of the

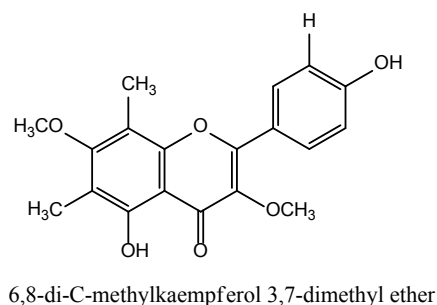
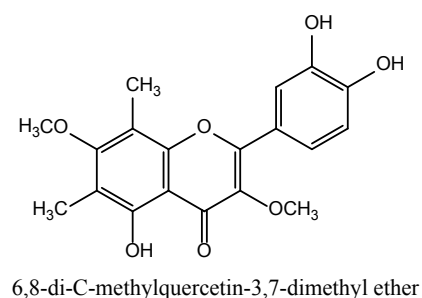
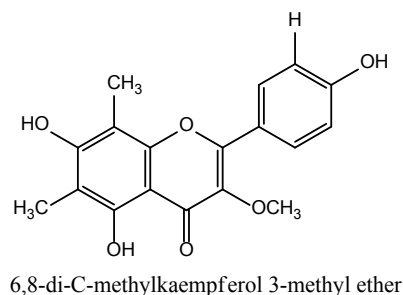
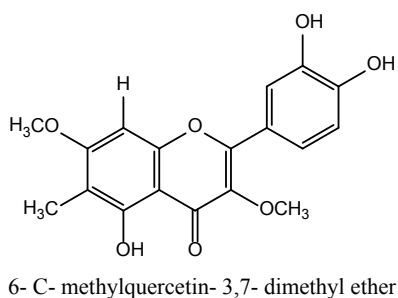
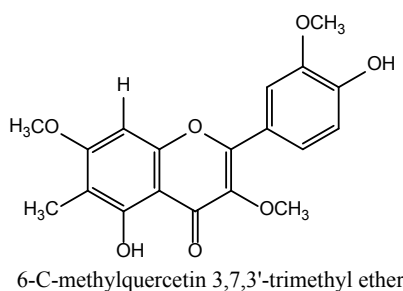
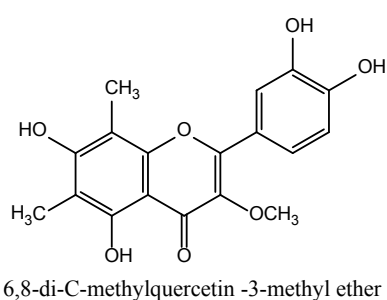
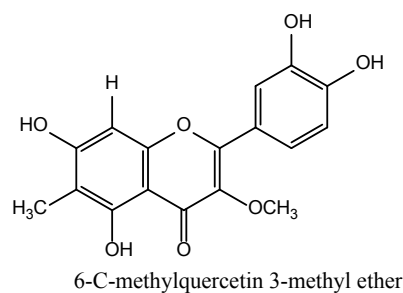
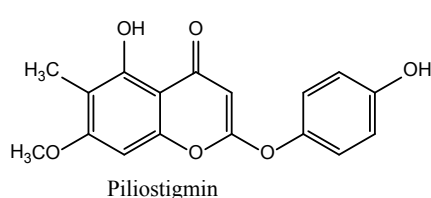
plant has also been demonstrated (Asuzu and Onu, 1993). The proanthocyanidins contained in the plant extract, possess an inhibitory action on glutathione S-transferase of nematode parasites such as *Ascaris* and *Onchocerca* (Fakae *et al.*, 2000). The compounds 2 $\beta$ -methoxy-cloven-9 $\alpha$ -ol and alepterolic acid showed activity against *Trypanosoma brucei brucei* with IC<sub>50</sub> of 7.89 and 3.42  $\mu$ M (Afolayan, *et al.*, 2018).

### Clinical data

No clinical data available

### Chemical constituents

Piliostigmine, C-methylflavonols, quercetin, quercitin (Ibewuiké *et al.*, 1996), quercetol, quercetol-3-glucoside and quercitroside (Bombardelli *et al.*, 1994); stigmastadienol, trans-communic acid, labdane derivatives, lambertianic acid, lambertianol,  $\alpha$ -tocopherol,  $\alpha$ -amyrin, alepterolic acid, anticopalic acid and epicatechin (Snatzke and Wolff, 1989).



**Test for identity and purity**

**Moisture content:** air dried coarse powder does not lose more than 6.2% (leaves) and 7.0% (stem bark) at 105°C.

**Total ash:** not more than 9.8% (leaves) and 11.2% (stem bark)

**Acid insoluble ash:** not more than 1.7% (leaves) and 0.7% (stem bark)

**Water soluble extractive:** not less than 9 % (leaves) and 6.2 % (stem bark)

**Ethanol soluble extractive (70%):** not less than 2.0% (leaves) and 6.2% (stem bark).

**Chromatographic fingerprint**

**Preparation:** About 5 g of the powdered leaves were extracted with ethyl acetate by cold maceration, filtered and the filtrate concentrated to a small volume. A small spot was then applied to the TLC plate for analysis.

**Chromatographic conditions:** Analytical TLC on silica gel G60 F254, 0.25 mm layer in hexane/ethyl acetate (7:3) as the mobile phase.

**Detection:** Visualized in daylight after spraying with anisaldehyde-sulphuric acid (Lane 1) and vanillin-sulphuric acid reagents (Lane 2) (Stahl, 1969), heating to 110°C for 10 mins. The TLC chromatogram showed three prominent spots with R<sub>f</sub>s of 0.89 (yellow), 0.81 (pink) and 0.34 (pink) when sprayed with both anisaldehyde and vanillin. Additionally, two prominent spots appeared with R<sub>f</sub>s of 0.71 (pink) and 0.59 (pink) in the chromatogram sprayed with anisaldehyde. These two spots however appeared purple when sprayed with vanillin.

**High Performance Liquid Chromatography (HPLC)**

**Sample preparation:** About 10 mg of the hydro-ethanolic extract of *P. thonningii* leaves were reconstituted in 3 mL acetonitrile in a 10 mL volumetric flask with sonication for 17 minutes. The solution was then diluted to volume with the mobile phase under chromatographic conditions. It was centrifuged to obtain a clear test solution which was subsequently filtered through a 0.45 µm filter into an HPLC vial and analyzed.

## Chromatographic system

### Optimized chromatographic conditions

**Mode:** LC

**Column:** YMC ODS, 4.6 x 150 mm, 5  $\mu$ m

**Column temperature:** Ambient – 30°C

**Mobile phase:** Acetonitrile: water (60:40 v/v)

**Elution mode :** Isocratic

**Injection volume :** 20  $\mu$ L

**Flow rate:** 0.5 mL/minute

**Detection wavelengths:** 230 nm, 254 nm and 278 nm.

### System Suitability parameters

**Number of peaks:** 230nm (1), 254 nm (3), 278 nm (2)

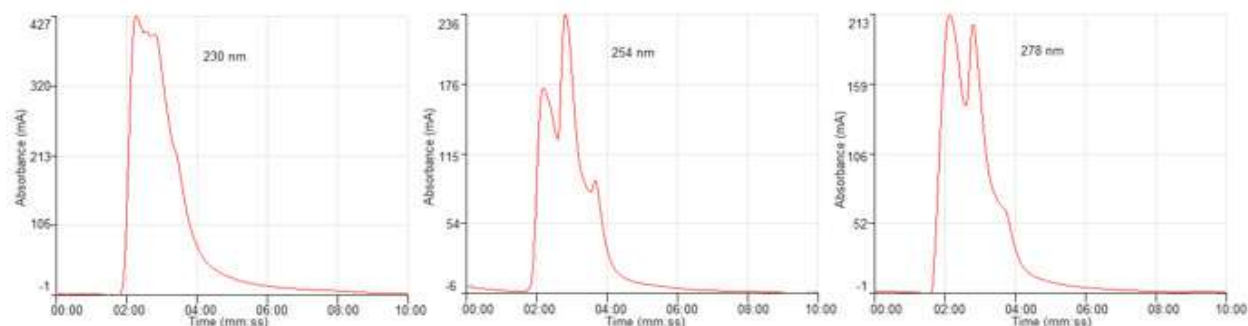
**Retention time (s):** 230 nm (rt1-2.16 min), 254 nm (rt1-2.11 min, rt2-2.48 min, rt3-3.40 min), 278 nm (rt1-2.07 min, rt1-2.47 min)

**Asymmetric factor(s):** 230 nm (af1-1.369), 254 nm (af1-1.452, af2-1.311, af3-1.031), 278 nm (af1-1.464, af2-1.188)

**Tailing factor:** NMT 2.0

**Efficiency:** 230 nm (E1-20.63), 254 nm (E1-154.06, E2-379.60, E3-1448.36), 278 nm (E1-86.02, E2-410.90)

**Acceptance criteria:** Sample solution of hydro-ethanolic crude extract of *P. thonningii* (Schumach.) Milne-Redh. (leaves) conforms with the system suitability parameters.



### HPLC chromatogram of *Piliostigma thonningii*

#### FT-IR

A small amount of the dried hydroethanolic extract (70%) was placed on the sample area of the Perkin Elmer UATR Fourier transform infrared (FT-IR) spectrometer and scanned between 4000-400  $\text{cm}^{-1}$  with a resolving power of 4  $\text{cm}^{-1}$ , and a cumulative scanning limitation of 24 times. Principal peaks appeared at wave numbers 3252.16, 2923.28, 2853.13 and 1602.87  $\text{cm}^{-1}$

#### Macroscopy

The leaf is a dull green colour, two lobed and each lobe is oblanceolate in shape with a mucronate or rounded apex. The leaf has palmate netted principal veins. It is leathery in texture and tough and has a rough surface especially the lower surface.

## Microscopy

### Leaf

Upper leaf surface is made up of polygonal cells with thick cell walls. The lower surface is made up of veins, which are connected in a unique net-like fashion with spaces in between. The spaces have trichomes which are intertwined and form a mesh. Trichomes are long uniseriate, twisted and curved. Stomata are absent.

### Leaf transverse section

The concentric-amphicribal vascular bundle of the midrib occurs in the form of an arc closed towards the upper epidermis. There is a distinct layer of collenchyma cells after the upper epidermis. The lamina shows epidermal cells followed by several layers of parenchyma cells with no distinct palisade. There is a thick layer of cells which contain chloroplasts, forming an undulating layer below the parenchyma cells. Attached to this layer are numerous uniseriate trichomes, some of which are intertwined and form a mesh. There are no stomata on both surfaces.

### Stem bark transverse Section

The transverse section consists of a large layer of cork cells with rectangular shape. The next section is filled with rows of parenchyma with characteristic calcium oxalate rosettes. Occasional sclerenchyma cells occur. This is followed by layers of groups of fibres interspersed with calcium oxalate crystals, prisms and parenchyma cells. More dense layers of fibres with calcium oxalate rosettes and prisms occur.

### Powdered plant material

Consists of numerous curved uniseriate trichomes; sickle shaped trichomes; unicellular straight trichomes; unicellular fibres; spiral xylem vessels; fragments of polygonal epidermal cells with thick walls.

### Therapeutic actions

Antiinfective, wound healing, antiinflammatory, analgesic

### Therapeutic indications

Ulcers, sores, dysentery, diarrhea, herpes and influenza

### Safety data

LD<sub>50</sub> by oral route was above 3000 mg/kg in rats. There were no signs of CNS depression/stimulation or autonomic effects up to a dose of 1000 mg/kg. Sub-acute studies did not show significant changes in the liver, kidney, heart and lungs. The relative organ to body ratios of spleen, thymus, and adrenals were not significantly affected by treatment. Extract did not affect haematological parameters significantly. There were decreases in liver enzymes especially ALT and GGT, but this was not statistically significant. *Piliostigma* extract did not affect serum proteins or serum albumin. Renal function and pentobarbitone-induced sleeping time were also not affected by the treatment. No histopathological changes were observed in the liver and kidney. *P. thonningii* extract did not affect haematological and serum biochemical function and indices significantly. The present findings did not suggest potential toxic effects with the use of this plant.

### Precautions for use

Caution should be taken in long term use.

### Adverse effects

None known

### Dosage forms

Decoction, infusion, tincture

### Dosage

Decoction: 30 g of dried plant material in 900 mL water; simmer until reduced to 600 mL; 1-3 tablespoonfuls daily

Infusion: 30 g of dried aerial part in 600 mL of water; 3-4 teacups daily

Tincture: 1:5, 45% ethanol; 5 ml three times daily

### Storage

Store in a cool, dry place away from light.

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**Botanical name**

*Plumbago zeylanica* L.

**Family**

Plumbaginaceae

**Synonyms**

*Findlaya alba* Bowdich, *Molubda scandens* (L.) Raf., *Plumbagidium scandens* (L.) Spach, *Plumbago scandens* L.

**Common Names**

Leadwort, doctor bush, wild leadwort, wild plumbago (English), dentelaire de Ceylan (French).

**Common Local names**

**Benin:** Fon-Dangblan; Yoruba-Anabiri; Dendi-Sisea

**Burkina Faso:** Moré-Tantaber boèèga

**Côte d'Ivoire:** Agni-Ayéaraklou; Tagouana-Talien koli; Malinké-Sagnia

**Ghana:** Akan-opapohwea; Ga-Aklaatiam baa; Twi-Opapawhea

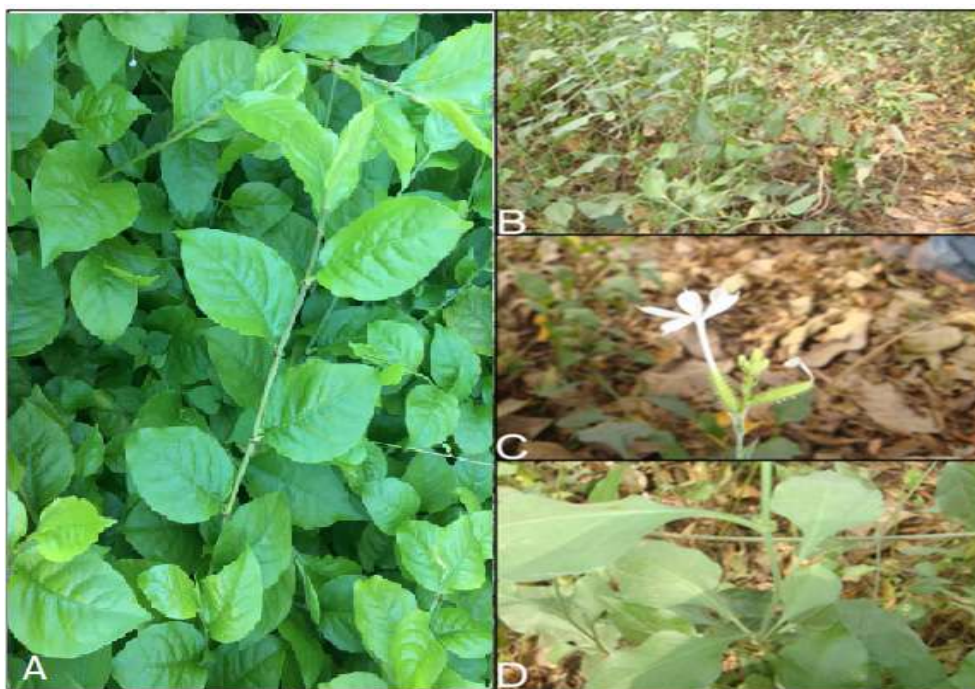
**Nigeria:** Yoruba – Inabiri; Igbo - Onaya ako

**Togo:** Watchi-Lologu; Ewé-Lelemalevi; Mina-Gbomadui

**Description of the plant**

*P. zeylanica* is an evergreen scrambling perennial shrub or herb, 0.3–2.5 m high. The leaf blade is ovate to oblong, 2.5–13 x 1–6 cm with acute apex. The surface is glabrous, with a petiole 2–12 mm long with clasping base. The plant has a semi-woody stem. The flowers are produced along a stalk that is 6–30 cm long, and covered with glandular hairs giving it a sticky coating. The calyx at the base of the flower forms a narrow tube and is also covered in long glandular hairs. The white petals are fused into a tube that is 18–23 mm long and have five lobes that spread widely. The stamens are inserted into the tube formed by the petals. The fruit is a dry capsule, which splits at the base at maturity. The capsules are 4–5 mm long and contains many small dark purple seeds (Wagner *et al.*, 1999). The roots are cylindrical and are irregularly bent having transverse shallow fissures (Bhattacharjee, 1998). Roots are about 30 cm in length, 6 mm in diameter, blackish red in colour, light yellow coloured when fresh, reddish brown when dry, straight unbranched or slightly branched with or without secondary roots, with uniform and smooth texture. It has characteristic odour with acrid and bitter taste. Bark is thin and brown in colour (Burkill, 1985)





A,B,D *Plumbago zeylanica* leaves C. Flowers

#### Herbarium specimen number

Benin: 2356 AP/HNB  
Burkina Faso: 278 (OAU)  
Cote d'Ivoire: CNF 13587  
Ghana: GH 626/KNUST  
Nigeria: UPFH 121  
Senegal: IFAN 59  
Togo: 06874 TG/HNT

#### Habitat and geographical distribution

The plant is widespread in the tropics, throughout West, East, South and tropical Africa. It is found in open, high sunlight, exposed habitats including coastal strand and dry forest.

#### Plant material of interest

Aerial part

#### Other part used

Roots

#### Definition of plant material of interest

*Plumbago zeylanica* consists of the leaves or aerial parts of *Plumbago zeylanicum* L. (Plumbaginaceae)

## Ethnomedical uses

Throughout Africa and Asia, a decoction of the root of *P. zeylanica* is applied externally as a remedy for skin diseases, infections and intestinal worms, leprosy, scabies, ringworm, dermatitis, acne, sores, ulcers of the leg, haemorrhoids and hookworm (Chevallier, 1996). All parts of the plant are used, but the root is considered to have the highest activity. In West Africa, the root or the leaves crushed with lemon juice are used as a counter-irritant and vesicant. The pulped roots or aerial parts are used as an abortifacient. ] In Nigeria, the roots pounded with vegetable oil are applied to rheumatic swellings. In Ethiopia powdered bark, root or leaves are used to treat gonorrhoea, syphilis, tuberculosis, rheumatic pain, swellings and wounds (Mungwini, 2006). In DR Congo and Gabon the pounded root is used to treat itchy skin. In East Africa pounded roots are applied to swollen legs, and in Zambia a root decoction with boiled milk is swallowed to treat inflammation in the mouth, throat and chest. In southern Africa a paste of the root in vinegar, milk and water is used to treat influenza and blackwater fever. *P. zeylanica* root cooked with meat in soup is eaten in Zimbabwe as an aphrodisiac and also as a digestive aid, and a root infusion is taken orally to treat shortness of breath. In Madagascar, the roots are applied as a vesicant, while in Mauritius and Rodrigues a root decoction is used to treat diarrhoea and dyspepsia. A paste of powdered root or the root sap is used for tattooing by different tribes in eastern Africa. The paste or sap causes blisters and the new skin has a darker colour. The long white inflorescence of *P. zeylanica* makes it attractive as an ornamental. Despite the plant being poisonous, it is readily eaten by goats and sheep in West Africa (Mungwini, 2006). In Asia, Plumbago is applied externally as a poultice for the treatment of rheumatism, leprosy, tumours and ringworm (Poosarla *et al.*, 2007; Lewis and Elvin-Lewis, 2003).

## Biological and pharmacological activities

*P. zeylanica* root and leaf extracts have been shown to exhibit a wide spectrum of antimicrobial activity including inhibition of the growth of multi-resistant strains of *E. coli* and Shigella (Sharma and Singh, 2015). Its antimicrobial activity has been attributed to the main compound plumbagin and other secondary metabolites. Plumbagin has shown antibacterial activity against both gram-positive (e.g. *Staphylococcus*, *Streptococcus*, *Pneumococcus* sp.) and gram-negative (e.g. *Salmonella*, *Neisseria*) bacteria. It is also active against certain yeasts and fungi (*Candida*, *Trichophyton*, *Epidermophyton* and *Microsporium* spp.) and protozoa (*Leishmania*). In low concentrations, plumbagin exhibits antimitotic activity comparable to that of colchicine. Plumbagin also has strong antifeedant and moulting inhibiting effects on insects as well as nematocidal and acaricidal activities (Pant *et al.*, 2012). An investigation of the anti-ulcer activity of aqueous root extract of *P. zeylanica* on aspirin and indomethacin-induced acute gastric ulceration in albino rats at doses, 25, 50 and 100 ml/kg found statistically significant ( $p < 0.05$ ) dose-dependent inhibition of aspirin-induced gastric mucosal damage (Sharma and Singh, 2015). The active component plumbagin introduced into hyperlipidaemic rabbits resulted in the reduction of serum cholesterol and LDL-Cholesterol by 53% to 86% and 61% to 91% respectively. Plumbagin prevents the accumulation of cholesterol and triglycerides in liver and aorta (Falang *et al.*, 2012). Oral administration of ethanolic extract of the root (100-200 mg/kg) for 6 weeks in streptozotocin-induced diabetic rats increased hepatic hexokinase activity and decreased hepatic glucose-6 phosphatase, serum acid phosphatase (ACP), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH). Thus the plant exhibited hypoglycaemic and hepatoprotective activity (Zarmouh *et al.*, 2010). Aziz *et al.* (2008), studied the inhibitory effect of plumbagin on growth and invasion of hormones in refractory prostate cancer. The results indicated that *P. Zeylanicum* inhibits the growth and invasion of PCa. Plumbagin inhibits multiple molecular targets including PKCepsilon, a predictive biomarker of PCa aggressiveness and thus considered a novel agent for therapy of hormone-refractory PCa (Mandavkar and Jalapure, 2011). Plumbagin also inhibited T cell proliferation in response to polyclonal mitogen concanavalin A (Con A) by blocking cell cycle progression. Immunosuppressive effects of plumbagin on cytokine levels were seen *in vivo*. Plumbagin completely inhibited Con A induced I kB- $\alpha$  degradation and NF- $\kappa$ B activation. Furthermore, plumbagin prevented graft versus host disease-induced mortality in mice (Checker *et al.*,

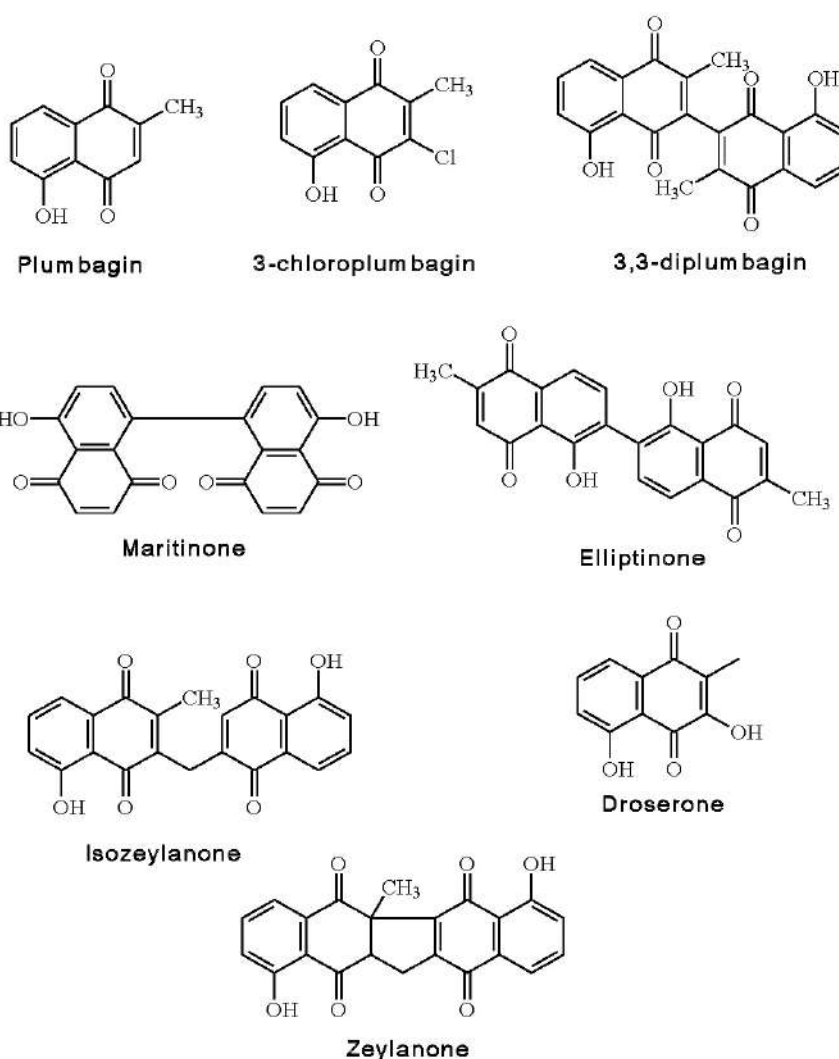
2009). The potential of plumbagin as a novel therapeutic agent for myeloid leukaemia was investigated in NB4 tumour xenograft in NOD/SCID mice. Intraperitoneal injection of plumbagin (2 mg/kg body weight) daily for 3 weeks resulted in a 64.49% reduction of tumour volume compared with the control. There was no overt manifestation of toxicity such as weight loss, tissue damage and behaviour change which appeared in doxorubicin-treated mice (1 mg/kg thrice a week) (Chauhan *et al.*, 2012). Extracts of the plant has also shown antiplasmodial, anticonvulsant and antiinflammatory activities (Simonsen *et al.*, 2001; Vishnukanta and Rana, 2010; Dang *et al.*, 2011)

### Clinical data

Not available

### Chemical constituents

Plumbagin derivatives: 3- chloroplumbagin, 3, 3- biplumbagin, binaphthoquinone, isozeylanone, zeylanone, elliptinone, droserone. isoshinanolone 3'-o-betaglucopyranosyl plumbagic acid and 3'-o-beta- glucopyranosyl plumbagic acid methyl ester, chitranone, maritinone, elliptinone; coumarins such as seselin, methoxyseselin, suberosine, xanthyletin and xanthoxyletin (Lin *et al.*, 2003).



## Test for identity and purity

### *On the leafy stem*

**Moisture content:** Air dried coarse powder does not lose more than 5.8% at 105°C.

**Total ash:** not more than 6.7%

**Acid insoluble ash:** not more than 1.3%

**Water soluble extractive:** not less than 6.0%

**Ethanol soluble extractive (70%v/v):** not less than 2.0%

### *Chromatographic fingerprint*

**Preparation:** About 5 g of the powdered leafy stem were extracted with ethyl acetate by cold maceration, filtered and the filtrate concentrated to a small volume. A small spot was then applied to the TLC plate for analysis.

**Chromatographic conditions:** Analytical TLC on silica gel G60 F254, 0.25 mm layer in hexane/ethyl acetate (7:3) as the mobile phase.

**Detection:** Visualized in daylight after spraying with anisaldehyde-sulphuric acid (Lane 1) and vanillin-sulphuric acid reagents (Lane 2) (Stahl, 1969), heating to 110°C for 10 mins.

The TLC chromatogram showed four prominent spots with R<sub>f</sub>s of 0.78 (light pink), 0.73 (pink), 0.62 (pink) and 0.24 (yellowish brown) in the chromatogram sprayed with anisaldehyde. The first three spots appeared purple when sprayed with vanillin. The spot at R<sub>f</sub> 0.24 appeared yellowish in visible light without spraying (Lane 3).



### *High performance liquid chromatography (HPLC)*

**Sample preparation:** About 10 mg of the hydro-ethanolic extract of *P. zeylanica* aerial part were reconstituted in 3 mL acetonitrile in a 10 mL volumetric flask with sonication for 17 minutes. The solution was then diluted to volume with the mobile phase under chromatographic conditions. It was centrifuged to obtain a clear test solution, which was subsequently filtered through a 0.45 µm filter into an HPLC vial and analyzed.

## Chromatographic system

### Optimized chromatographic conditions

**Mode:** LC

**Column:** YMC ODS, 4.6 x 150 mm, 5 µm

**Column temperature:** Ambient – 30°C

**Mobile phase:** Acetonitrile: water (60:40 v/v)

**Elution mode :** Isocratic

**Injection volume :** 20 µL

**Flow rate:** 0.5 mL/minute

**Detection wavelengths:** 230 nm, 254 nm and 278 nm.

### System Suitability parameters

**Number of peaks:** 230 nm (1), 254 nm (1), 278 nm (3)

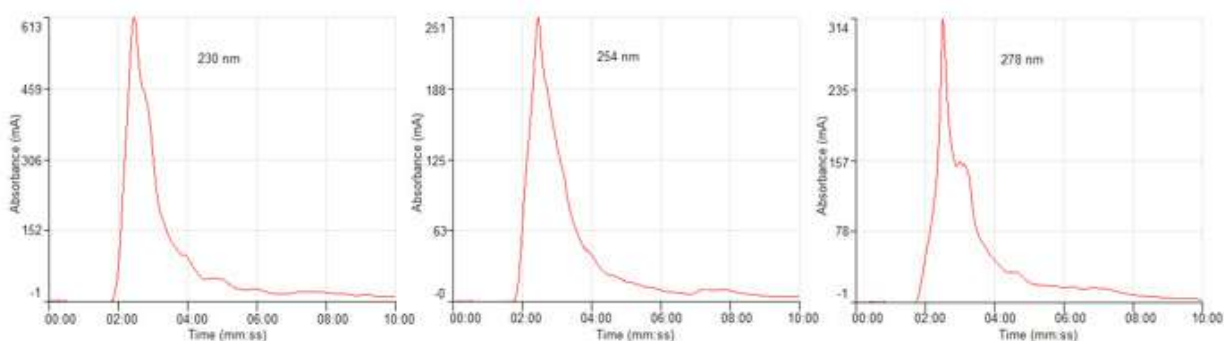
**Retention time (s):** 230 nm (2.27 min), 254 nm (2.27 min), 278 nm (rt1-2.30 min, rt2-2.59 min, rt3- 3.07 min)

**Asymmetric factor(s):** 230 nm (1.125), 254 nm (0.841), 278 nm (af1-0.323, af2-1.407, af3- 1.676)

**Tailing factor:** NMT 2.0

**Efficiency:** 230 nm (60.46), 254 nm (49.39), 278 nm (E1-686.05, E2- 7933.94, E3-6325.84)

**Acceptance criteria:** Sample solution of hydro-ethanolic crude extract of *P. zeylanica* L. (aerial Part) conforms to the system suitability parameters.



## FT-IR

A small amount of the dried hydroethanolic extract (70%) was placed on the sample area of the Perkin Elmer UATR Fourier transform infrared (FT-IR) spectrometer and scanned between 4000-400  $\text{cm}^{-1}$  with a resolving power of 4  $\text{cm}^{-1}$  and a cumulative scanning limitation of 24 times. Principal peaks appeared at wave numbers 3263.79, 2925.12 and 1603.12 (strong)  $\text{cm}^{-1}$ .

## Microscopy

### Leaf

There are anisocytic stomata with three subsidiary cells on both surfaces; epidermal cells are polygonal with slightly curved walls. Four celled head glandular trichomes are scattered all over the surfaces. These are not as numerous on the upper surface as on the lower surface of the leaf.

### Powdered plant material

The powder of the aerial parts is characterised by fragments of the leaf epidermis showing polygonal cells and typical four celled glandular trichomes.

***Fragments of leaf in transverse section.***

There are many bundles of fibres with obtuse ends. Fragments of stem bark showing cork cells. Thin walled large parenchyma cells with palisade cells; annular and spiral xylem vessels are numerous.

**Therapeutic actions**

Antiinfective antimetabolic, antifeedant, nematocidal, acaricidal, antiulcer, antihyperlipidaemic, hypoglycaemic, hepatoprotective (Pant et al., 2012; Zarmouh et al., 2010; Aziz et al. (2008).

**Therapeutic indications**

Skin diseases, intestinal worms, leprosy, scabies, ringworm, dermatitis, acne, leg ulcers, haemorrhoids, hookworm.

**Safety data**

LD<sub>50</sub> by oral route was estimated to be above 3000 mg/kg. There were no signs of CNS depression/stimulation or autonomic effects at doses up to 1000 mg/kg. Sub-acute studies did not show changes in highly perfused organs such as the liver, kidney, heart and lungs. The relative organ to body ratios of spleen, thymus, and adrenals were not significantly affected by treatment. Plumbago extract showed no hematopoietic effects. At the doses used, plumbago did not cause anaemia. It had minimal effects on leucocytes, and inhibited platelets and neutrophils marginally. Plumbago did not elevate or depress liver enzymes. Serum proteins were generally not affected except at high doses. It also did not affect conjugated and unconjugated bilirubin. Plumbago did not alter urea and creatinine. The aerial parts used in this study have a higher safety profile than that of the roots. The slight inhibition of platelets may be clinically significant in coagulation/haemorrhagic disorders.

**Precautions for use**

Must not be used with anticoagulants. Avoid oral intake of the root decoction. Do not expose skin to sunlight after topical use.

**Adverse effects**

Causes skin irritations or burns.

**Dosage forms**

Decoction, poultice, infusion, tincture

**Dosage**

Decoction: 30 g of dried plant material in 900 mL water; simmer until reduced to 600 mL. Apply topically for irrigation of wounds

Poultice: Prepare a paste of root or leaves and use as wound dressing.

Infusion: 30 g of dried aerial part in 600 mL of water; 3-4 teacups daily

Tincture: 1:5, 45% ethanol; 5 ml three times daily

**Storage**

Store in a cool dry place away from light

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**Botanical name**

*Polyalthia longifolia*

**Family**

Annonaceae

**Synonyms**

*Uvaria longifolia*

**Common Names**

Mast tree, Christmas tree, False ashoka tree (English), Arbre à mâtire, Saule Africain (French).

**Common Local names**

**Côte d'Ivoire:** Akan-Bronya dua

**Ghana:** Akan-Bronya dua

**Togo:** Ewé-Ati favi

**Description of the plant**

This is a tall, evergreen, pyramid-like, columnar tree. The main stem is erect, undivided, growing up to 12 m or more. The branches are slender, short, about 1-2 m long bearing glabrous and pendulous leaves. The leaves measuring 7.5-23 by 1.5-3.8 cm is alternate, exstipulate, distichous, mildly aromatic, narrowly lanceolate, tapering to a fine acuminate apex (Katkar *et al.*, 2010). The margin is markedly undulate, pinnately veined, leathery or subcoriaceous, shortly petiolate measuring about 6 mm long. Flowers arise from branches below the leaves and are non-fragrant yellowish to green, in fascicles or shortly pendunculate umbels. The sepals are broad, triangular, the tips reflexed. Stamens are many, cuneate; connective, truncately dilated beyond the cells. Ovaries indefinite; ovules 1-2; style oblong. The ripe fruits are ovoid, 1.8-2 cm long, numerous, stalked, glabrous, 1 seeded; stalk 1.3 cm long, short, glabrous with smooth shining seeds (Yadav *et al.*, 2000; Wallis, 1985)



A, *Polyalthia longifolia* – B, leaves and fruits

#### Herbarium specimen number

Benin: 2335 AP/HNB

Burkina Faso: 850 CNSF

Cote d'Ivoire: 12815 CNF

Ghana: 640 GH /KNUST

Nigeria: UPFH 122

#### Habitat and geographical distribution

The plant thrives in mixed forests, at an altitude of up to 400 m and widely cultivated as an ornamental. It is globally distributed; Asia, China, India, Sri Lanka and also in many tropical countries.

#### Plant material of interest

Leaves

#### Other part used

Stem bark

#### Definition of plant material of interest

*P. longifolia* consists of the fresh or dried leaves of *Polyalthia longifolia* (Sonn.) Thwaites

#### Ethnomedical uses

Almost all parts of the plant are used in Indian traditional medicine for the treatment of various diseases. *P. longifolia* stem bark is used to treat pyrexia (Krishnamurthi, 1969), rheumatism, menorrhagia, scorpion sting

and diabetes (Savithamma *et al.*, 2011). Decoction of the bark is widely used in India for treating mouth ulcer (Pradhan *et al.*, 2011). In India, the Eastern Ghats culture uses the stem bark in combination with *Sesamum indicum* and *Piper nigrum* seeds to treat bone fractures. Similarly, another mixture of *Mimosa intsia* root bark, *Tridax procumbens* leaves and stem bark of *P. longifolia* is applied to fractures, and bandaged daily till they are healed (Sunnetha *et al.*, 2011). The bark is bitter, acrid, cooling and febrifuge. It is also believed to be effective in treating skin diseases, hypertension and helminthiasis. The stem bark is also used in the treatment of digestive, circulatory and urinary disorders, constipation and fever (Katkar, *et al.*, 2010). Traditional medicine practitioners of India, use the stem bark extract orally for indigestion (Sugumaran *et al.*, 2010), diarrhoea and dysentery (Vanila, *et al.*, 2008).

### Biological and pharmacological activities

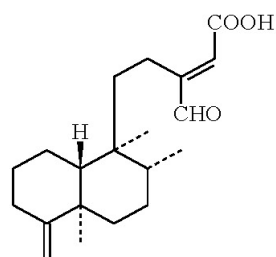
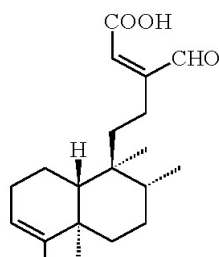
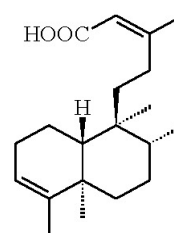
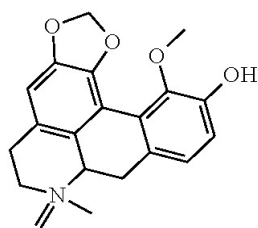
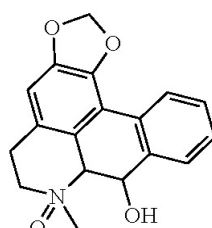
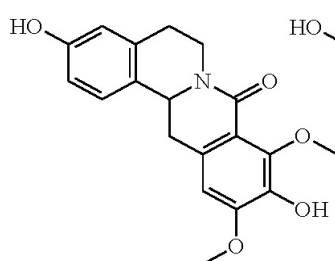
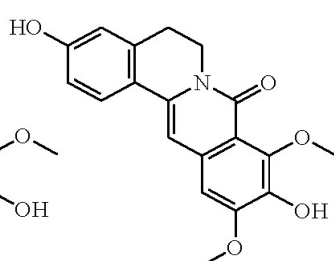
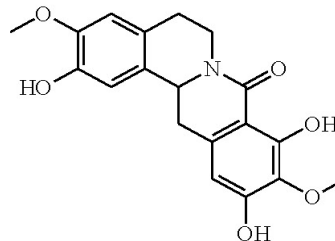
The petroleum ether extract of the stem bark of *P. longifolia* and its diterpenes were found to exhibit antimicrobial activity against a number of kanamycin resistant fungal strains; *Aspergillus fumigatus*, *Saccharomyces caulbequence*, *S. cerevaceae*, *Candida albicans* and *Hensila californica* (Rashid *et al.*, 1996). In another report, the leaf extract of *P. longifolia* inhibited cell proliferation of various human cancer cell lines. It showed maximum activity against colon cancer cells SW-620 with IC<sub>50</sub> value of 6.1 µg/mL, and also induced apoptosis in human leukaemic HL-60 cells through the mitochondrial-dependent pathway in HL-60 cells (Verma, *et al.*, 2008). Similarly, ethanol extract of the stem bark of *P. longifolia* showed concentration-dependent cytotoxicity in Ehrlich's ascites carcinoma (EAC) and Dalton's ascites lymphoma (DLA) cells with IC<sub>50</sub> values of 45.77 and 52.52 µg/mL, respectively. *P. longifolia* extract, at a dose of 100 mg/kg, significantly enhanced mean survival time (MST) and marginally improved haematological parameters when compared to control mice (Manjula, *et al.*, 2010). Methanol extract of the leaves of *P. longifolia* was also found to possess significant antiinflammatory, hepatoprotective and antioxidant activities (Tanna, *et al.*, 2009; Manjula, *et al.*, 2010). Ethanolic and aqueous extracts of the leaves of *P. longifolia* at doses of 200 and 300 mg/kg respectively, decreased gastric content, total acidity, ulcer index, and increased pH of gastric pylorus ligation ulcer model, in a dose-dependent manner. Four new clerodane diterpenes isolated from the stem bark of *P. longifolia*: cleroda-3-ene pyrrole-15,16-dione, cleroda-3-ene, pyrrolidine-15,16-dione, cleroda-3,13(14)E-diene-15,16-diamide, and cleroda-3-ene-15,16-diamide, showed antiplasmodial effects, with IC<sub>50</sub> ranging from 4.5 to 213.8 µM (Annan *et al.*, 2015). Ethanol and chloroform extracts of the leaves of *P. longifolia* showed α-amylase and α-glucosidase enzyme inhibitory activity. The effect was further highlighted by the protection of the extracts against streptozotocin-induced type 1 diabetes mellitus in rats (Sivashanmugam and Chatterjee, 2013). A defatted extract of *P. longifolia* root showed significant ability to reduce blood pressure. It caused 22% and 47% fall in mean arterial blood pressure in rats at doses of 3 mg/kg and 30 mg/kg respectively (Saleem *et al.*, 2005). Annan *et al.*, (2013) demonstrated the antipyretic activity of *P. longifolia* extracts in Wistar rats against lipopolysaccharide (LPS)-induced pyrexia. Methanol extracts of the leaves, stem bark and root of the plant were tested for their antipyretic activities at doses of 30, 100 and 300 mg kg<sup>-1</sup> body weight using LPS-induced antipyretic activity model. All extracts showed significant (p < 0.001) dose-dependent antipyretic activity. At 300 mg/kg, all extracts exhibited activities higher than that of acetylsalicylic acid (aspirin), which had a percentage inhibition of pyrexia of 86%. The root extract was the most active with a percentage inhibition of 127.5%, followed by the leaf extract (123.0%) and the stem bark extract (99.2%). In another study, the ethanolic extract of *P. longifolia* stem bark showed good activity against the multidrug-resistant, K1 strain of *P. falciparum* by the parasite lactate dehydrogenase (pLDH) assay and a good antiplasmodial activity (IC<sub>50</sub> = 22.04). Clerodane diterpenes were found to be responsible for the observed activity (Gbedema *et al.*, 2015).

**Clinical data**

None available

**Chemical constituents**

Diterpenoid alkaloids, tannins and mucilage (Phadnis, *et al.*, 1988); O-methylbulbocapnine-*N*-oxide, polyfothine, *N*-methylnandigerine-*N*-oxide, oliveroline-*N*-oxide, 8-oxopolyalthiane, 16-oxo-5 (10), 13-halimadien-15-oic acid, 16-Oxo-3, 13-clerodadien-15-oic acid, 16-hydroxycleroda-3, 13-dien-16, 15-olide; aporphine and azafluorene alkaloids, proanthocyanidins,  $\beta$ -sitosterol, leukocyanidin, clerodane, ent-helimane diterpenoids; alkaloids polylongine (5-hydroxy-6-methoxy-1-methyl-4-azafluoren-9-ol), (+)-O-methylbulbocapnine- $\beta$ -*N*-oxide, (+)-O-methylbulbocapnine- $\alpha$ -*N*-oxide, (+)-*N*-methylnandigerine- $\beta$ -*N*-oxide (Wu *et al.*, 1990; Wu, 1989), pendulamine A, pendulamine B, penduline, kolavenic acid, isoursuline, liriodenine, noroliveroline, oliveroline- $\beta$ -*N*-oxide, darienine, polyfothine, isocodine (Wu *et al.*, 1990), cleroda-3-ene pyrrole-15,16-dione, cleroda-3-ene, pyrrolidine-15,16-dione, cleroda-3,13(14)*E*-diene-15,16-diamide, and cleroda-3-ene-15,16-diamide (Annan *et al.*, 2015).

**13-halimandien-15-oic acid****Polyalthialdoic acid****Kolavenic acid****O-methylbulbocapnine-*N*-oxide****Oliveroline-*N*-oxide****Pendulamine A****N-pendulamine B****8-oxopolyalthiane**

### Test for identity and purity

**Moisture content:** Air dried a coarse powder does not lose more than 6.0% at 105°C.

**Total ash:** not more than 7.1%

**Acid insoluble ash:** not more than 1.2%

**Water soluble extractive:** not less than 11.0%

**Ethanol soluble extractive (70%):** not less than 6.0%.

### Chromatographic fingerprint

#### *Thin Layer Chromatography*

**Preparation:** About 5 g of the powdered leaves were extracted with ethyl acetate by cold maceration, filtered and the filtrate concentrated to a small volume. A small spot was then applied to the TLC plate for analysis.

**Chromatographic conditions:** Analytical TLC on silica gel G60 F254, 0.25 mm layer in hexane/ethyl acetate (7:3) as the mobile phase.

**Detection:** Visualized in daylight after spraying with anisaldehyde-sulphuric acid (Lane 1) and vanillin-sulphuric acid reagents (Lane 2) (Stahl, 1969), heating to 110°C for 10 mins. The TLC chromatogram showed one prominent spot with R<sub>f</sub> of 0.96 (pink) when sprayed with both anisaldehyde and vanillin. An additional spot each, appeared with R<sub>f</sub> of 0.68 corresponding with colours of purple and blue in the chromatogram sprayed with anisaldehyde and vanillin respectively.



#### *High Performance Liquid Chromatography*

**Sample preparation:** About 10 mg of the hydro-ethanolic extract of *P. longifolia* leaves were reconstituted in 3 mL acetonitrile in a 10 mL volumetric flask with sonication for 17 minutes. The solution was then diluted to volume with the mobile phase under chromatographic conditions. It was centrifuged to obtain a clear test solution which was subsequently filtered through a 0.45 µm filter into an HPLC vial and analyzed

## Chromatographic system

### Optimized chromatographic conditions

**Mode:** LC

**Column:** YMC ODS, 4.6 x 150 mm, 5  $\mu$ m

**Column temperature:** Ambient -30°C

**Mobile phase:** Acetonitrile: water (60:40 v/v)

**Elution mode :** Isocratic

**Injection volume :** 20  $\mu$ L

**Flow rate:** 0.5 mL/minute

**Detection wavelengths:** 230 nm, 254 nm and 278 nm.

### System Suitability parameters

**Number of peaks:** 230 nm (1), 254 nm (1), 278 nm (2)

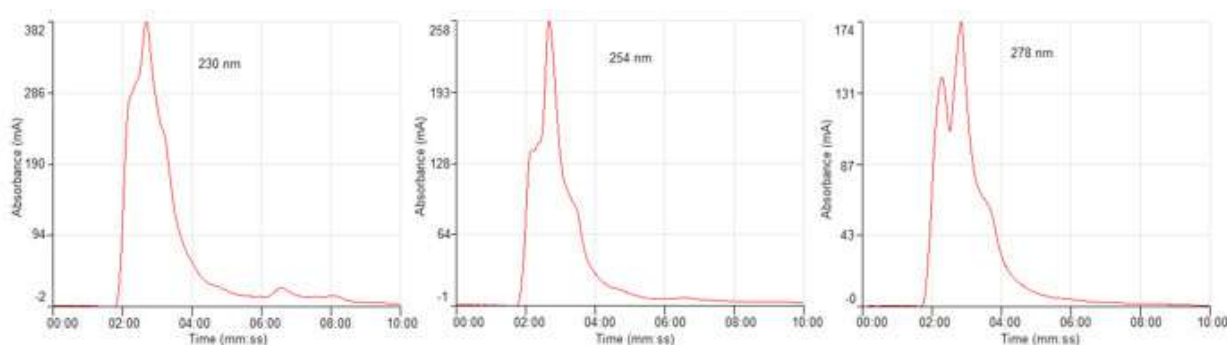
**Retention time (s):** 230 nm (2.41 min), 254 nm (2.40 min), 278 nm (rt1-2.17 min, rt2-2.50 min)

**Asymmetric factor(s):** 230 nm (0.869), 254 nm (1.116), 278 nm (af1-1.545, af2-1.011)

**Tailing factor:** NMT 2.0

**Efficiency:** 230 nm (437.11), 254 nm (394.25), 278 nm (E1-168.01, E2-407.38)

**Acceptance criteria:** Sample solution of hydro-ethanolic crude extract of *P. longifolia* (Sonn.) Hook.f. & Thomon (Leaves) conforms to the system suitability parameters.



## FT-IR

A small amount of the dried hydroethanolic extract (70%) was placed on the sample area of the Perkin Elmer UATR Fourier transform infrared (FT-IR) spectrometer and scanned between 4000-400  $\text{cm}^{-1}$  with a resolving power of 4  $\text{cm}^{-1}$  and a cumulative scanning limitation of 24 times. Principal peaks appeared at wave numbers 3263.25, 2927.59 and 1595.09  $\text{cm}^{-1}$ .

## Microscopy

### Leaf

Lower surface has anomocytic stomata with five subsidiary cells and polygonal epidermal cells. Unicellular trichomes occur occasionally. Upper surface has polygonal epidermal cells with occasional uniseriate trichomes up to six celled.

**Transverse section**

The midrib section is composed of distinct collenchyma cells in the upper region after the upper epidermis. The main vascular section is composed of three distinct vascular bundles each comprising groups of xylem followed by phloem. The three bundles are encompassed by a sheath of fibres below and above forming a continuous layer up to the start of the lamina. The lower section of the midrib is filled with large parenchyma cells. Schizogenous glands occur in this region. The lamina has epidermal cells which are almost square in shape and rectangular shaped palisade cells. Annular xylem vessels traverse the lamina. Schizogenous glands occur in the lamina below the palisade layer.

**Powdered plant material**

Powder consists of fragments of upper epidermal cells showing polygonal cells and lower epidermis showing anomocytic stomata; groups of fibres attached together; fragments of warty unicellular trichomes; annular xylem vessels; tracheids; secretory cells with content; fragments of spongy mesophyll and palisade cells.

**Therapeutic actions**

Antiplasmodial, antiparasitic, antipyretic, hepatoprotective, antiinfective

**Therapeutic indications**

Malaria, pyrexia, infections

**Safety data**

The LD<sub>50</sub> by oral route was above 3000 mg/kg in rats. There were no signs of CNS depression/stimulation or autonomic effects up to a dose of 1000 mg/kg. In subacute studies, organ morphology examination did not show changes suggestive of liver, kidney, heart or lung impairment. The relative organ to body ratios of spleen, thymus, and adrenals were not significantly affected by treatment. RBC count and RBC indices were not altered with Polyalthia treatment. At a dose of about 300 mg/kg, Polyalthia induced leukocytosis, mostly in the agranulocytes. The increased leucocytes, especially the agranulocytes (lymphocytes) corroborates very well with its immunostimulatory effects demonstrated in another study (Doshi and Devidas Une, 2015). Neutrophils were inhibited. Polyalthia suppresses serum AST significantly at all doses. At high dose (1000 mg/kg), Polyalthia suppressed ALT, ALP and GGT, but did not affect serum proteins or serum haemoglobin. Renal function was not affected. Pentobarbitone-induced sleeping time was not affected and histopathological studies did not show any damage to liver or kidney cells. Previous studies by Chanda *et al.* (2012) shows its apparent safety during acute intoxication. Similarly, an 8-week study on the seed flour in rats also gave a good toxicity profile, similar to what was observed in this study. Polyalthia's ability to reduce serum liver enzymes even in naïve animals may be an indication of its hepatoprotective effect.

**Precautions for use**

Do not exceed the prescribed dose. Avoid concomitant administration with conventional medicines.

**Adverse effects**

None documented

**Dosage forms**

Decoction, infusion, tincture

## Dosage

Decoction: 30 g of dried plant material in 900 mL water; simmer until reduced to 600 mL; 1-3 tablespoonfuls daily

Infusion: 30 g of dried aerial part in 600 mL of water; 3-4 teacups daily

Tincture: 1:5, 45% ethanol; 5 ml three times daily

## Storage

Store in a cool dry place away away from light

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**Botanical name**

*Sansevieria liberica* Gérôme & Labroy

**Family**

Agavaceae

**Synonyms**

*Dracaena liberica*

**Common Names**

Bowstring hemp, leopard lily (English), Sansévière, chanvre d'Afrique, lis léopard, herbe à perruque (French) (Burkill, 1995; Herper, 1968).

**Common Local names**

**Benin:** Fon-kponyan; Yoruba-Oja, Ikonko, Ojakoriko; Dendi-Gunubi

**Burkina Faso:** Dioula-Dogoba; Moré-Kaantoabga

**Ghana:** Mole-Kaantoagba, Pennde; Twi-Twiton, nyinankyi, tutukekrema

**Guinea:** Maninka-Komba

**Mali:** Bambara-N'gokoba; Malinké-Koukouba; Dogon- Polo togu

**Niger:** Hausa-Kaba kara; Zarma-Koro Kongu

**Nigeria:** Yoruba-Oja-Ikooko, Igbo-Ebube aje, Hausa-Mooda

**Senegal:** Basari-Anofingéo ; Pulaar- Iacoli ; Manding-Ndolé bua

**Sierra Leone:** Bulom- nomoli, Sherbro; Gola- nomoni, Kissi-nomoliyo

**Togo:** Ewé-Yobo; Mina-Yodobou; Watchi-Yodobo

**Description of the plant**

*S. liberica* is an erect rhizomatous herb bearing several stiff leaves with red and elliptical borders, growing from the thick underground rhizome. The round branched rhizomes measure 19 mm (on average). Leaves occur as a rosette (1-6) without petiole. The leaf blade is oblanceolate measuring about 45-105 cm in length and 5-12.5 in width. They are transverse with dark and green bands and red and white lines mark the margins. The leaves taper at both ends and have a thick fibrous texture. Flowers are cream, arranged in small clusters, and borne on a spike. The flowers are fragrant and soon drop. Fruits are slightly 3 lobed, round, red or orange. The flowers are white and bear on interrupted common stems. The fruits are reddish, almost round, about 1.25 cm long. Each fruit contains a seed (Baker, 1915). The plant is often confused with *Sansevieria senegambica* Baker, which is distributed from Senegal to Côte d'Ivoire and has smaller leaves and flowers with the leaves not distinctly banded (Newton, 2001).



A – *Sansevieria liberica* hort.ex Gerome & Laby, B – Flowers, C – Fruits

#### Herbarium specimen number

**Bénin:** 2337 AP/HNB

**Burkina Faso :** BUR-173 (CNSF), 6046 (OAU)

**Cote d’ivoire:** 10665 CNF

**Ghana:** GH 675/KNUST

**Mali:** 2240 / DMT

**Nigeria:** FHI111922

**Senegal:** IFAN 2402

**Togo:** 09465 – Université de Lomé

#### Habitat and geographical distribution

*Sansevieria liberica* is usually found in shady areas near streams and rocky outcrops. The plant is common in savanna regions and grasslands. It is distributed from Sierra Leone to Nigeria and Central Africa; it is found in Ethiopia, Kenya and Tanzania. It grows in shady places, by streams, on rocky outcrops and on termite mounds in grassland and forest. It is propagated by leaf tip cuttings (Newton, 2001).

#### Plant material of interest

Leaf

## Other part used

Rhizomes

## Definition of plant material of interest

*Sansevieria* consists of fresh or dried leaves of *Sansevieria liberica* Gerome and Labroy

## Ethnomedical uses

A decoction of the root is used in traditional medicine as a tonic and a remedy for coughs and haemorrhoids. In Nigeria, the leaves and roots of the plant are used in the treatment of abdominal pain, asthma, diarrhoea, skin diseases (eczema), gonorrhoea, haemorrhoids, hypertension, sexual weakness, snake bites and wounds (Gill, 1992; Adeyemi *et al.*, 2009). Fermented rhizomes are eaten to treat malaria. The root decoction is used as a treatment for convulsions. In Ghana, the roots are used for abortion and childbirth. In Togo, the rhizome macerated in palm wine, is used in the treatment of hypertrophy of the prostate. Juice expressed from the leaves or a decoction of the leaves is drunk for the treatment of gonorrhoea, earache and toothache. Leaf sap is applied topically to ulcers and sores. The plant is considered fetish in traditional medicine, and hence grown on graves and shrines. It is widely grown as an ornamental (Neuwinger, 1996).

## Biological and pharmacological activities

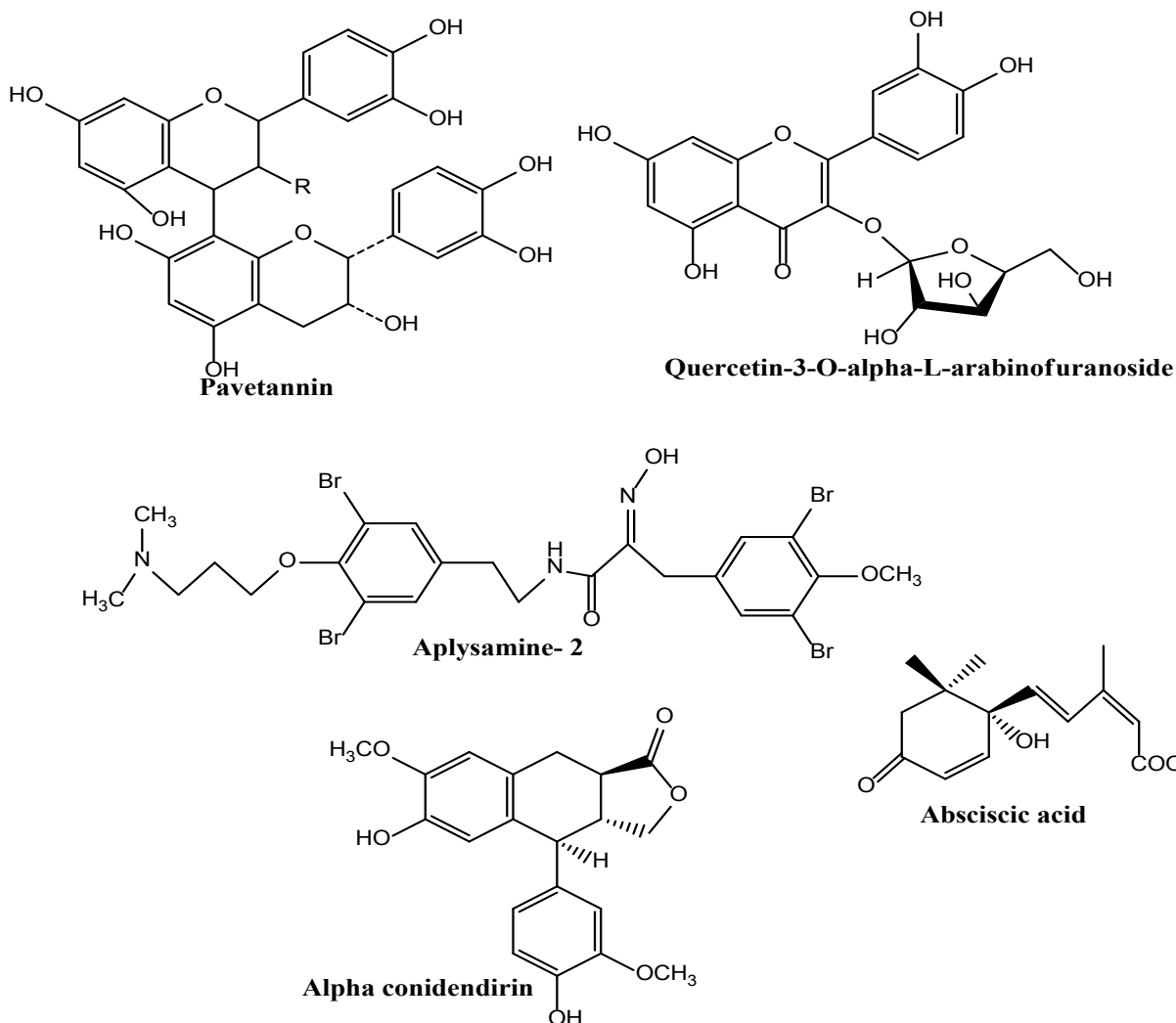
The aqueous extracts of the rhizomes of the plant showed hypoglycaemic, hypolipidaemic, antianaemic, immunomodulatory, oculo-protective, hepatoprotective and nephroprotective activity in diabetic Wistar rat (Ikewuchi and Ikewuchi, 2011). Freeze-dried fresh leaf juice and its organic fractions were anti-inflammatory on rat paw oedema (Ezea *et al.*, 2017). Hydroalcoholic extracts of *S. liberica* rhizomes showed anticarcinogenic effect *in vitro* and *in vivo* (Akindele *et al.*, 2015). Ikewuchi, (2012) showed that aqueous extracts of *S. liberica* leaves protect Wistar rats against carbon tetrachloride-induced liver injury. The aqueous extracts and fractions of the plant (rhizome) was found to have no significant effect on cytochrome p450 (CYP) activity, whereas extracts and fractions obtained with organic solvents, had the ability to inhibit CYP activity at high doses (Akindele, 2016). The total ethanolic extracts of *S. liberica* rhizomes exert antimicrobial and antifungal effect *in vitro* and are cytotoxic to Vero cells (Agassounon *et al.*, 2001). *In vitro* antitrypanosomal, antileishmanial and antiplasmodial activities of the plant have been reported (Bero *et al.*, 2009; Bero *et al.*, 2011). The antidiarrhoeal (Adeyemi *et al.*, 2009), CNS depressant, anticonvulsant (Adeyemi *et al.*, 2007; Umukoro and Ashorobi, 2008), analgesic (Umukoro and Ashorobi, 2008) and anti-inflammatory (Chinasa *et al.*, 2011) activities of extracts of the plant have also been reported.

## Clinical data

None documented

## Chemical constituents

Pavetanin, palyamine-2, abscisic acid,  $\alpha$ -conidendirin and Quercetin-3-O- $\alpha$ -L-arabinofuranoside.



### Test for identity and purity

**Moisture content:** Air dried coarse leaf powder does not lose more than 7.7% at 105°C.

**Total ash:** not more than 17.70%

**Acid insoluble ash:** not more than 1.20%

**Water soluble extractive:** not less than 15.0%

**Ethanol soluble extractive (70%):** not less than 2.0%

### Chromatographic fingerprint

#### Thin Layer Chromatography

**Preparation:** About 5 g of the powdered leaves were extracted with ethyl acetate by cold maceration, filtered and the filtrate concentrated to a small volume. A small spot was then applied to the TLC plate for analysis.

**Chromatographic conditions:** Analytical TLC on silica gel G60 F254, 0.25 mm layer in hexane/ethyl acetate (7:3) as the mobile phase.

**Detection:** Visualized in daylight after spraying with anisaldehyde-sulphuric acid (Lane 1) and vanillin-sulphuric acid reagents (Lane 2) (Stahl, 1969), heating to 110°C for 10 mins.

**Results:** The TLC chromatogram showed one prominent spot with R<sub>f</sub> of 0.62 (purple) when sprayed with both anisaldehyde and vanillin. Two additional spots appeared with R<sub>f</sub>s of 0.53 (pink) and 0.21 (light pink) in the chromatogram sprayed with anisaldehyde. The spot at R<sub>f</sub> 0.53 appeared yellow when sprayed with vanillin.



### High Performance Liquid Chromatography

**Sample preparation:** About 10 mg of the hydroethanolic extract of *S. liberica* leaves were reconstituted in 3 mL acetonitrile in a 10 mL volumetric flask with sonication for 17 minutes. The solution was then diluted to volume with the mobile phase under chromatographic conditions. It was centrifuged to obtain a clear test solution which was subsequently filtered through a 0.45 µm filter into an HPLC vial and analyzed

### Chromatographic system

#### Optimized chromatographic conditions

**Mode:** LC

**Column:** YMC ODS, 4.6 x 150 mm, 5 µm

**Column temperature:** Ambient – 30°C

**Mobile phase:** Acetonitrile: Methanol: Water (60:20:20 v/v/v)

**Elution mode :** Isocratic

**Injection volume:** 20 µL

**Flow rate:** 0.5 mL/minute

**Detection wavelengths:** 230 nm, 254 nm and 278 nm.

#### System Suitability parameters

**Number of peaks:** 230 nm (1), 254 nm (1), 278 nm (1)

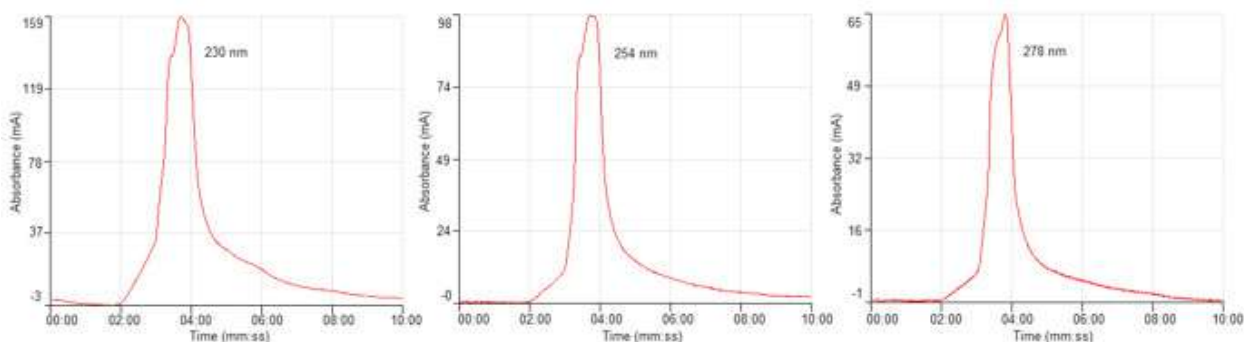
**Retention time (s):** 230 nm (3.42 min), 254 nm (3.44 min), 278 nm (3.48 min)

**Asymmetric factor(s):** 230 nm (0.801), 254 nm (1.318), 278 nm (1.017)

**Tailing factor:** NMT 2.0

**Efficiency:** 230 nm (106.81), 254 nm (132.94), 278 nm (185.38)

**Acceptance criteria:** Sample solution of hydro-ethanolic crude extract of *Sansevieria liberica* hort. ex Gérôme & Laby (Leaves) conforms to the system suitability parameters.



## FT-IR

A small amount of the dried hydroethanolic extract (70%) was placed on the sample area of the Perkin Elmer UATR Fourier transform infrared (FT-IR) spectrometer and scanned between 4000-400  $\text{cm}^{-1}$  with a resolving power of 4  $\text{cm}^{-1}$  and a cumulative scanning limitation of 24 times. Principal peaks appeared at wavenumbers 3266.07, 2922.51 and 1557.93 (very strong) and 1403.03  $\text{cm}^{-1}$ .

## Macroscopy

Round, underground rhizome, about 19 mm in diameter, with several rigid leaves, with red, elliptical borders from the rhizome. Leaves are erect, long lanceolate, variously and irregularly mottled or horizontally streaked, light green or white in colour, with margins marked by red and white lines; about 60 cm long and 10 cm wide

## Microscopy

### Leaf

Epidermal cells of the upper surface are polygonal, mostly hexagonal, elongated and arranged like the honey comb of bees. Stomata are anomocytic with two cells parallel to the axis of the stoma, elongated along the same axis. Stomata and epidermal cells of the lower surface are similar to that of the upper surface.

### Transverse section

Upper and lower epidermal surfaces have clear cuticles and one row of epidermal cells. There are no palisade cells. There are collateral vascular bundles in a row at the middle of the lamina running through to the tips of the leaf. There is a row of smaller vascular bundles below the epidermis of both surfaces. Each vascular bundle is bound at the base by phloem fibres. These are smaller than the central bundles and run through to the tips of the leaf. The main leaf tissue is made up of large parenchyma cells. There are no trichomes.

## Powdered plant material

Fragments of epidermis of both surfaces showing abundant polygonal cells and anomocytic stomata, which are characteristic. Bundles of long fibres are present along with annular xylem vessels and fragments of large parenchyma cells.

## Therapeutic actions

Antimicrobial, healing, antitumour, antitussive, antidiabetic, antihæmorrhoidal, hepatoprotective, antiinflammatory, hypolipidaemic, antianaemic, immunomodulatory.

**Therapeutic indications**

Microbial infections, diabetes, dyslipidaemia, otitis, wound, prostatic hypertrophy, fatigue

**Safety data**

LD<sub>50</sub> by oral route was above 3000 mg/kg in rats. There were no signs of CNS depression/stimulation or autonomic effects up to a dose of 1000 mg/kg. Leaves of *S. liberica* had insignificant effects on macroanatomical structures of the liver, kidney, lungs, and heart. It did not significantly increase relative organ weight. Sansevieria extract did not affect RBC count, and haemoglobin content of the cell on the hematopoietic system. The extract at doses above 300 mg/kg stimulated leukocytosis, probably an indication of its immunomodulatory effects. The effect was particularly pronounced on agranulocytes, which showed significant decline. It also stimulated platelet count. The extract had minimal effect on liver enzymes. It depressed ALT at the highest dose of 1000 mg/kg. Sansevieria extract had positive effect on plasma proteins. At doses below 300 mg/kg, it appeared to increase serum proteins. The extract did not significantly affect bilirubin. Urea and creatinine levels did not change significantly and therefore did not affect renal function. Pentobarbitone-induced sleeping time was prolonged by the treatment. No histopathological changes were seen in the liver and kidney. Generally, no evidence of toxicity was observed, as reported by Achi and Ohaeri, (2012). The effect on liver enzymes may be a reflection of its much researched hepatoprotective effects. It is neuroactive with hypnosedative effects.

**Precautions for use**

Pregnancy, hypotension

**Adverse effects**

Sedation

**Contraindication**

Caution should be taken during concurrent use with alcohol and other CNS depressants.

**Dosage forms**

Decoction, infusion, poultice, tincture

**Dosage**

Decoction: 30 g of dried plant material in 900 mL water; simmer until reduced to 600 mL; 1-3 tablespoonfuls daily

Infusion: 30 g of dried aerial part in 600 mL of water; 3-4 teacups daily

Tincture: 1:5, 45% ethanol; 5 ml three times daily

**Storage**

Keep in a cool dry place



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**Botanical name**

*Strophanthus gratus* (Wall. & Hook.) Baill.

**Family**

Apocynaceae

**Synonyms**

*Strophanthus perrotii* A. Chev., *Roupellia grata* Hook

**Common Names**

Climbing Oleander, Cream Fruit, poison arrow vine, Rose Allamanda (English), *Strophanthus glabre* du Gabon (French).

**Common Local names**

**Benin:** Dendi-Dimbelou; Yoruba -Lagba omodè, Isharo.

**Côte d'Ivoire:** *Akye*- Kalanmeni; *Baule*- m-moropo; *Kyama*- siniabié salo

**Ghana:** Twi- Omaatwa, Omaatwanini, eguro-eguro

**Liberia:** Mano-Konen

**Nigeria:** Yoruba- Sagere; Igbo-Osisi kanguru ; Hausa- Kwan-Kwani

**Sierra Leone:** Mende- gohõndo

**Togo:** Ewé-Amagan; Ewé-Ahadati.

**Description of the plant**

*Strophanthus gratus* is a woody vine or shrub growing up to 25 m and exudes a clear or translucent latex. The stem grows up to a diameter of 10 cm with dark brown twigs and scattered white lenticels in older plants (Beentje, 2006). The elliptic or oval elliptic leaves are opposite with the leathery lamina measuring 12 to 15 cm long and 4-6 cm wide, wedge-shaped, pointed and sharp acuminate tip. Each leaf has a rounded base with entire margins and bears 7 to 9 lateral veins fairly spreading and arching, ascending to the summit and looping at 3 mm from the margin; there are no nerves between the lateral veins, which gives the leaf a special stamp with a lighter green underside. It bears short petioles measuring 3 to 10 mm (Hendrian, 2001; Burkill, 1985). Flowers are purplish-pink, 6-7 cm long, wide tube, spreading top 5 to 6 cm wide, with 5 oval lobes without ribboned appendages at the top. Few to many flowers on a terminal dichasial cyme inflorescence. Flowers are bisexual, regular, 5-merous, fragrant; pedicel 4–13 mm long; sepals free, unequal, obovate or broadly obovate, 7–18 mm long, emarginate, rounded or apiculate; corolla tube 25–45 mm long, widening at 33–55% of its length into a cylindrical upper part. Fruit consists of 2 ellipsoid follicles, 2 siliques opposite end to end, 2.5 cm long and 2 to 3 cm wide gradually acuminate. Seeds spindle-shaped, 10 mm long, topped with a 3-6 cm long ridge with fine white bristles 3-4 cm long (Beentje, 2006).



A and B – *Strophanthus gratus* flower, C – Fruit, D – *Strophanthus gratus* whole plant

#### Herbarium specimen number

Benin: 2343 AP/HNB  
 Burkina Faso: CNSF-850  
 Côte d'Ivoire: 10939 CNF  
 Ghana: GH 675/KNUST  
 Senegal: IFAN 795

#### Habitat and geographical distribution

The plant is indigenous to West and Central Africa, from Senegal to Congo. It is cultivated for its medical and poisonous applications in Nigeria, Cameroon and Gabon. The plant thrives mainly in primary and secondary moist forest, often at forest margins or on river banks, from sea-level up to 650 m altitude (Beentje, 2006).

#### Plant material of interest

Leaves and roots

#### Other part used

Stem, latex, seeds

### Definition of plant material of interest

*Strophanthus* consists of the roots and leaves of *Strophanthus gratus* (Wall. & Hook.) Bail.

### Ethnomedical uses

In Sierra Leone, the leaves are used alone or in combination with other plants for the management of gonorrhoea (Dalziel, 1937). Decoction of the leaves and twigs is ingested orally in Cote d'Ivoire for neonatal conjunctivitis (Kerharo and Bouquet, 1950) and also for fever (Burkill, 1985). In Ghana, sap from the leaves is applied to ulcers (Kerharo and Bouquet, 1950) with the leaf poultice applied to guinea-worm sores (Irvine, 1961). The leaves again find application in Sierra Leone as an antidote to poison by the black-headed cobra (*Naja nigricollis*) (Irvine, 1961). Sap from the fresh bark is mixed with that of *Parquetina nigrescens* (Periplocaceae) to produce an arrow-poison in Congo. The root-bark is used as an antidote to food poisoning (Kerharo and Bouquet, 1950). Root and stem bark decoctions are used as expectorant and also for the management of syphilis (Ainslie, 1937). The seeds are used as a poultice to effectively treat rheumatoid arthritis. Sap, wood, fruit and seeds are used in preparations of arrow poisons. The juice of fresh barks in combination with *Omphalogonus calophyllus* is used in a similar manner. The seeds are rich in strophanthine and its aqueous extract is a violent poison. An antidote to this poison is the external application of the bark powder of *Erythrophleum guineense* and also the sap of *Alstonia congensis* (De Wild) internally. The plant is used in the treatment of certain heart conditions (Burkill, 1985). In a number of West African countries, a leaf paste is put onto sores, including guinea worm sores. In Nigeria a leaf infusion is taken to treat constipation, and is rubbed on the body to treat fever. A root decoction is said to be an aphrodisiac. In West Africa, the plant is used as a good luck charm (Beentje, 2006).

### Biological and pharmacological activities

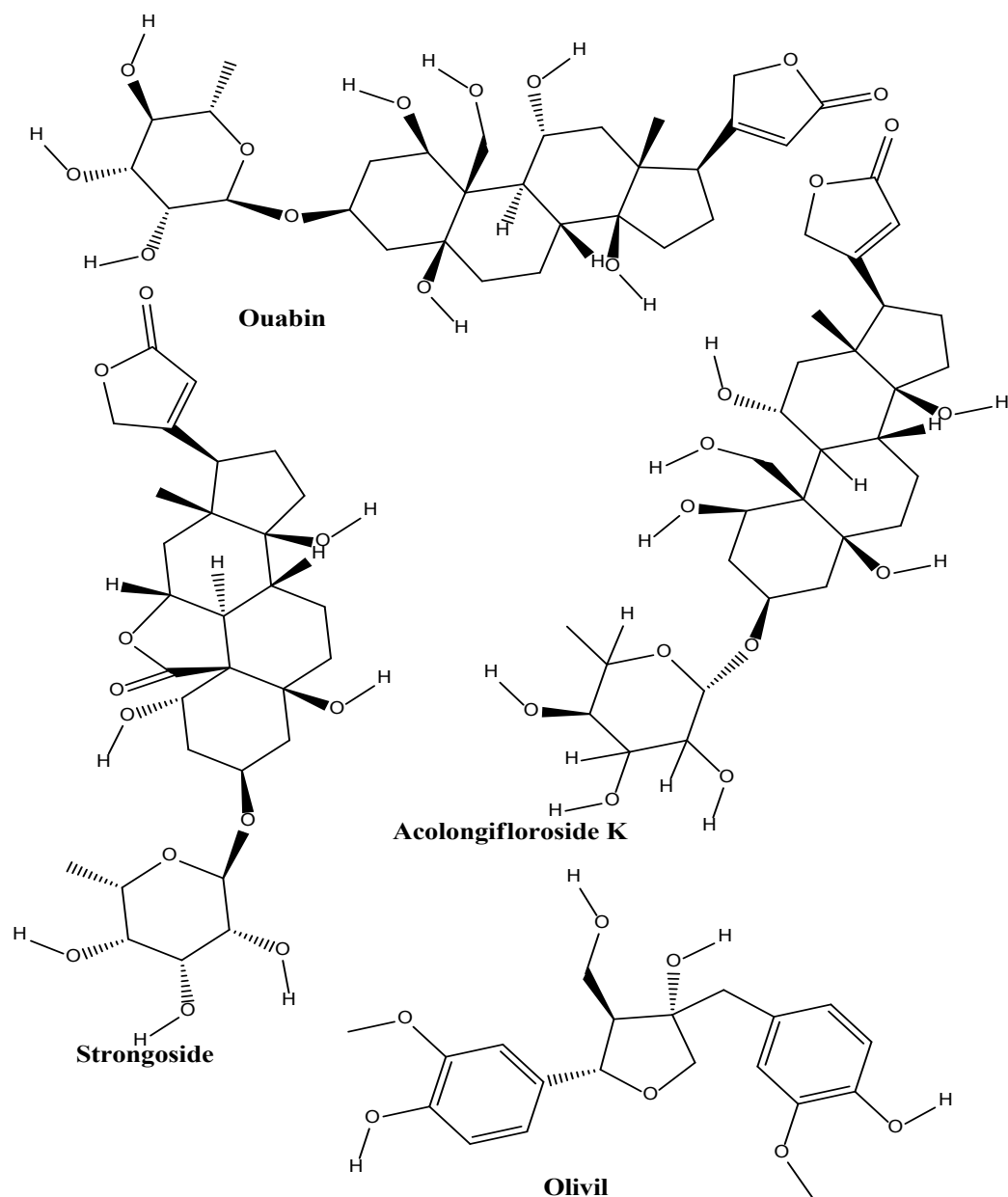
Like all cardiac glycosides, ouabain from *S. gratus* showed direct cardiotoxic action on the myocardium, resulting in an increase in the force of contraction. However, ouabain was found to have rapid onset of action, but of short duration with little risk of accumulation (Jäger *et al.*, 1965). In another study, ouabain induced programme cell death in androgen-independent human prostate cancer cell lines *in vitro* (McConkey *et al.*, 2000). Aqueous leaf extracts of *S. gratus* exhibited the ability to prolong clotting time for blood treated with a standard dose of the venom of the viper (*Echis carinatus*), in a dose-dependent manner. The venom caused rapid intra-arterial blood clotting leading to death (Houghton and Skari, 1994).

### Clinical data

None documented

### Chemical constituents

Cardiac glycosides (cardenolides): ouabagenin, acolongifloroside K, strogoside, sarnovide, sarmentosides, strophanthine; lignans: pinosresinol, 8-hydroxypinosresinol and olivil (Cowan *et al.*, 2001; Burkill, 1985).



## Chromatographic fingerprint

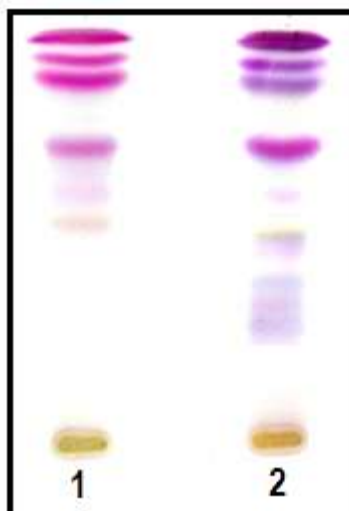
### Thin Layer Chromatography

**Preparation:** About 5 g of the powdered roots were extracted with ethyl acetate by cold maceration, filtered and the filtrate concentrated to a small volume. A small spot was then applied to the TLC plate for analysis.

**Chromatographic conditions:** Analytical TLC on silica gel G60 F254, 0.25mm layer in hexane/ethyl acetate (7:3) as the mobile phase.

**Detection:** Visualized in daylight after spraying with anisaldehyde-sulphuric acid (Lane 1) and vanillin-sulphuric acid reagents (Lane 2) (Stahl, 1969), heating to 110°C for 10 mins.

The TLC chromatogram showed one prominent spot with R<sub>f</sub> of 0.71 (purple) when sprayed with both anisaldehyde and vanillin. Four additional spots, appeared with R<sub>f</sub>s of 0.91 (pink), 0.87 (pink), 0.62 (pink) and 0.53 (pink) in the chromatogram sprayed with anisaldehyde. The spots at R<sub>f</sub>s 0.91 and 0.87 appeared purple when sprayed with vanillin.



### Test for identity and purity

**Moisture content:** Air dried powdered material does not lose more than 5.2% (leafy stem) and 10.6% (roots) at 105°C.

**Total ash:** not more than 8.6% (leafy stem) and 20.5% (roots)

**Acid insoluble ash:** not more than 0.20% (leafy stem) and 3.06 (roots)

**Water soluble extractive:** not less than 11.0% (leafy stem) and roots 18.45% (roots)

**Ethanol soluble extractive (70%):** not less than 1.0% (leafy stem) and 8.89% (roots)

### High Performance Liquid Chromatography

**Sample preparation:** About 10 mg of the hydroethanolic extract of *S. gratus* root were reconstituted in 3 mL acetonitrile in a 10 mL volumetric flask with sonication for 17 minutes. The solution was then diluted to volume with the mobile phase under chromatographic conditions. It was centrifuged to obtain a clear test solution which was subsequently filtered through a 0.45 µm filter into an HPLC vial and analyzed

### Chromatographic system

#### Optimized chromatographic conditions

**Mode :** LC

**Column:** YMC ODS, 4.6 x 150 mm, 5 µm

**Column temperature:** Ambient -30°C

**Mobile phase:** Acetonitrile: Methanol: Water (60:20:20 v/v/v)

**Elution mode :** Isocratic

**Injection volume :** 20 µL

**Flow rate:** 0.5 mL/minute

**Detection wavelengths:** 230 nm, 254 nm and 278 nm.

#### System Suitability parameters

**Number of peaks:** 230 nm (1), 254 nm (2), 278 nm (1)

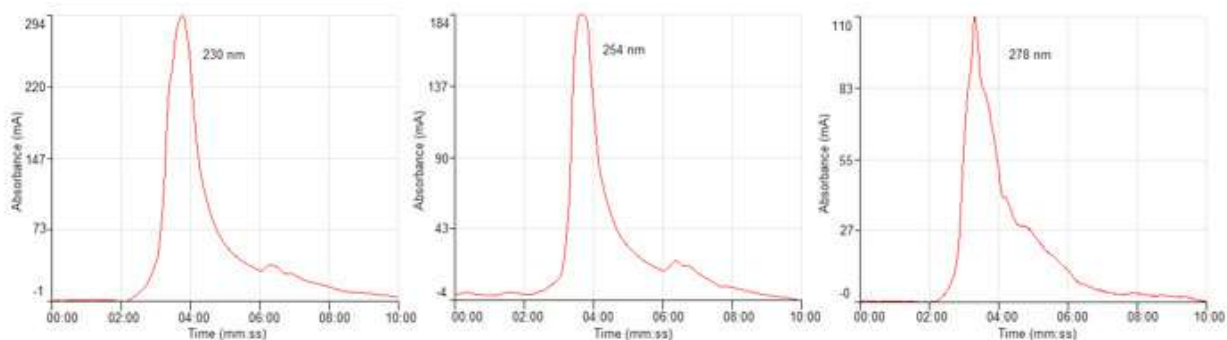
**Retention time (s):** 230 nm (3.47 min), 254 nm (rt1-3.36 min, rt2- 6.21 min), 278 nm (3.18 min)

**Asymmetric factor(s):** 230 nm (0.687), 254 nm (af1-0.848, af2-1.392), 278 nm (1.203)

**Tailing factor:** NMT 2.0

**Efficiency:** 230 nm (97.11), 254 nm (E1-143.10, E2- 3269.63), 278 nm (119.70)

**Acceptance criteria:** Sample solution of hydroethanolic crude extract of *S. gratus* Franch. (Root) conforms to the system suitability parameters.



## FT-IR

A small amount of the dried hydroethanolic extract (70%) was placed on the sample area of the Perkin Elmer UATR Fourier Transform Infrared (FT-IR) spectrometer and scanned between  $4000\text{--}400\text{ cm}^{-1}$  with a resolving power of  $4\text{ cm}^{-1}$  and a cumulative scanning limitation of 24 times. Principal peaks appeared at wavenumbers 3280.32, 2934.57, 1557.89, 1401.48  $\text{cm}^{-1}$ .

## Microscopy

Transverse section revealed layers of cork cells; followed by the phelloderm, which consists of parenchyma cells, groups of sclereids, fibres and large cluster of calcium oxalate crystals scattered throughout the parenchyma. The phloem region shows cells with medullary rays running through the cambium. The medullary rays run through the xylem area to the pith. Medullary rays are up to three cells.

## Powdered plant material

Powder is characterised by fragments of cork cells in the transverse section and surface view; sclereids occur in groups and singly and are yellowish in colour. Bundles of fibres; pitted xylem vessels; parenchyma with clusters of calcium oxalate crystals.

## Therapeutic actions

Antipyretic, wound healing, antimicrobial, analgesic.

## Therapeutic indications

Fever, ulcers, asthenia, gonorrhoea, eye disorders, rheumatoid arthritis, cardiac disorders.

## Safety data

$LD_{50}$  by oral route was estimated to be beyond 3000 mg/kg in rats. CNS and autonomic systems were not affected at doses of 0-1000 mg/kg. No significant changes on the gross anatomy occurred in the liver, kidney, heart or the lungs. The relative organ to body ratios of spleen, thymus, and adrenals did not change. Treatment had insignificant effects on red blood cells, white blood cells and platelets. Its effects on serum liver enzymes were minimal. However ALP was elevated at all doses although not statistically significant.



It did not affect serum proteins at low doses, but at high doses, there were elevations in albumin and globulin. Strophanthus did not affect direct and indirect bilirubin. Pentobarbitone-induced sleeping time was prolonged by the treatment. It induced significant renal necrosis. Strophanthus decreased serum creatinine dose-dependently although not statistically significant. It caused statistically significant elevations in serum urea. It also altered the urea/creatinine ratio. *S. gratus* extract, though rich in cardiac glycosides shows very safe apparent acute intoxication profile. The seeds have higher cardiac glycoside content and it is used as an arrow poison for hunting. The hydroalcoholic extract may be nephrotoxic. It has the potential to cause cardiotoxicity as well. Its toxicity can be affected by the serum electrolytes level particularly serum potassium levels of the individual.

### Precautions for use

Known kidney disease

### Adverse effects

Nephrotoxicity, sedation

### Contraindications

Not to be combined with stimulant laxatives. Avoid drinking water for a few minutes, after oral ingestion.

### Dosage form

Decoction, body bath, infusion, tincture

### Dosage

Decoction: 30 g of dried plant material in 900 mL water; simmer until reduced to 600 mL; 1-3 tablespoonfuls daily

Infusion: 30 g of dried aerial part in 600 mL of water; 3-4 teacups daily

Tincture: 1:5, 45% ethanol; 5 ml three times daily

### Storage

Keep in a cool dry place away from light

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**Botanical name***Terminalia macroptera* Guill. & Perr.**Family**

Combretaceae

**Synonyms**

*Myrobalanus macroptera* (Guill. & Perr.) Kuntze; *Terminalia chevalieri* Diels, *Terminalia adamanensis* Engl. & Diels, *Terminalia claessensii* De Wild, *Terminalia dawei* Rolfe, *Terminalia suberosa* auct.

**Common Names**

Badamier du Senegal (French).

**Common Local names****Benin:** Fon-Pavu; Yoruba-Ori odo; Dendi-Bèro**Burkina Faso:** Moré-Kodpooko; Dioula-Woloba; Fulfuldé-Bodévi**Côte d'Ivoire:** Senufo - mango figué; Mossi-Kondré**Gambia:** Diola-bujingkabo ; Pulaar-bodehi ; Mandinka-wolo**Ghana:** Dagaari- kawtri; Dagbani - korli-nyao; Moore - kwodaga**Guinea:** Pulaar- bodévi ; Maninka- woro ba ; Mandinka - hóló-fóro**Guinea-Bissau:** Balanta-fadi ; Crioulo- macête ; Mandyak- braqui**Mali:** Bambara- Wolomuso, wolofira; Dogon- Badjoukokô; Peulh- Bdévi**Niger:** Germa-Farka hanga; Fulfulde – bodévi; Hausa- bauché bochy**Nigeria:** Hausa-Bayankada; Yoruba-orin idi òdan; Fulfulde- ßoodi**Senegal:** Mandinka -uolossa wolo; Diola Flup-busalaba; Manding-wolo ba.**Togo:** Batonnu- béro; Somba- mukindimu; Tem – soria dau**Description of the plant**

*Terminalia macroptera* is a shrub or a tree with an open, spreading crown; it usually grows from 4 - 10 metres tall, occasionally reaching a height of 20 metres (Fern, 2018). It has a short bole that is rarely straight, often twisted and low-branched, up to 100 cm in diameter. The stem bark surface is deeply fissured, brown to black with a fibrous, brown to orange inner bark. The crown is open with spreading branches. The twigs are glabrous, grey-brown to purplish black, becoming quickly corky. The spirally arranged simple leaves have petioles measuring 0-2 cm long with no stipules. The leaf blade is elliptic to obovate measuring 15-37 cm × 6-17 cm, wedge-shaped at base, rounded to obtuse or shortly acuminate at apex. The leaf texture is leathery, glabrous, pinnately veined with 12-25 pairs of lateral veins. Flowers occur as inflorescence, an axillary spike 8-22 cm long, glabrous or shortly hairy. Flowers are bisexual or staminate bearing creamy white, fragrant; glabrous and 5-lobed calyx. The corolla is absent; stamens 10, 3.5-4.5 mm long, exerted; ovary inferior, 1-celled. Fruit is oblong to ellipsoid winged nut, (4-) 8-10 (-13) cm × 2.5-4 (-8) cm long. It is reddish-brown, indehiscent and 1-seeded (Sanogo, 2013).



A – *Terminalia macroptera* Guill & Perr, B- flowers, C- fruits and D- leaves

#### Herbarium specimen number

Bénin: 2357 (AP)  
 Burkina Faso: MSAD 692 (CNSF) Thiombiano & al. 926 (OAU)  
 Côte d'Ivoire: CNF 6138  
 Ghana: GH 698 /KNUST  
 Mali: 3752/DMT (DMT)  
 Senegal: IFAN 367  
 Togo: TG 00731

#### Habitat and geographical distribution

*Terminalia macroptera* Guill. & Perr. (Combretaceae) is a tree that grows in Western Africa from Senegal to Cameroon, eastward to western Ethiopia and Uganda, and southward to north-eastern DR Congo; occasionally as far as Sudan. The tree is mostly found in Guinean and Sudanese-Guinean savannahs, preferably in moist areas and clayey ground (Burkill, 1985; Arbonnier, 2009). The plant is also scattered in open woodland; wooded grassland with tall grass-cover on black cotton soil or on rocky slopes; hills and plateau; lateritic pans and the margins of flooded plains; at elevations from 160 - 1,400 metres (Fern, 2018).

#### Plant material of interest

Stem bark

### Other part used

Roots, leaves

### Definition of plant material of interest

*Terminalia* consists of the dried stem bark of *Terminalia macroptera* Guill. & Perr.

### Ethnomedical uses

Various parts of the plant are commonly used in traditional medicine against a wide range of diseases. The leaf decoction is taken orally and as a bath in the treatment of fever, liver diseases (jaundice and hepatitis), syphilis, hypertension, tuberculosis, intestinal pain (enteralgia) and gastritis (Malgras, 1992). The decoction is also used as an eyewash to treat conjunctivitis and orally for diarrhoea and dysentery (Pham, 2011). Ash from the leaves is mixed with fat and applied topically twice a day for one week against inflammation. Decoction of the roots is used orally in the treatment of liver diseases (Adjanooun et al., 1981). It is also used for fever, jaundice, gonorrhoea, urinary disorders, rectal prolapse and as a diuretic (Malgras, 1992; Pham et al., 2011). The juice is used as a local application for wound healing and conjunctivitis (Kerharo and Adam, 1974). Macerated root is used orally against coughing and applied topically as a haemostatic agent. The powder is applied locally to treat wounds. Powder from the root bark, mixed with shea butter, is used to treat ear infections. The decoction is used in baths three to four times daily against skin diseases of children, and twice a day as a mouthwash (Pham, et al., 2011). The root bark powder is used to treat vaginal infections and also added to porridge or drink to treat female infertility (Pham et al., 2011). Macerated root is used for urinary retention, diarrhoea, skin diseases and gastric ulcer (Nadembega et al., 2011).

Decoction of the stem bark is taken orally against body pains, malaria, syphilis, liver disease, erectile dysfunction and asthenia (Kerharo and Adam 1974; Traoré et al., 2013). It is also used as a mouthwash against tooth decay and gingivitis. The decoction mixed with porridge is consumed three times a day to treat hepatitis. Powdered stem bark is used topically as a wound healing agent (Malgras, 1992). The powder, mixed with salt, is used orally or with food to treat female infertility (Pham et al., 2011). A decoction with stem bark of *Anogeissus leiocarpa* and root bark of *Strophanthus sarmentosus* (3-4 tablespoonful of the mixture) is taken orally twice daily for five days against hepatitis (Pham et al., 2011). Ash from the inner stem bark is mixed with *Lannea acida* oil to treat boils (Inngjerdingen et al., 2004). The fruits mixed with fruits of *Ficus* sur Forssk., and the inner root bark of *Balanites aegyptiaca* are applied locally to treat snake bites (Inngjerdingen et al., 2004)

### Biological and pharmacological activities

Extracts of all plant parts have been investigated for a number of pharmacological activities. A 70% ethanolic extract of the roots showed antiparasitic activity on strains of *Trypanosoma brucei*, *Trypanosoma cruzi* and *Leishmania infantum*. The IC<sub>50</sub> was 2.7 µg/mL on *Trypanosoma brucei*, 20.2 µg/mL on *Trypanosoma cruzi* and 20.2 µg/mL on *Leishmania infantum* (Traoré et al., 2014). The methanolic extract of the leaves showed antibacterial and antifungal activity on two strains of bacteria (*Proteus mirabilis* CIP588104 and *Staphylococcus aureus* ATCC 25923) and two strains of fungi (*Rhizopus nigricans* ATCC 622713, *Red Mucor* ATCC 24905) (Tchacondo et al., 2012). Silva et al. (2012) showed the anti-*Helicobacter pylori* activity of extracts (80% ethanolic extract and hexane, diethyl ether, ethyl acetate, water fractions) of the leaves. The best activity was obtained from the aqueous fraction with a MIC of 100 µg/mL. The same extracts exhibited antibacterial activity on strains of *Neisseria gonorrhoeae* with MICs between 25-400 µg/mL. The 50% ethanolic extract of the leaves, stem and root barks showed antifungal activity on twenty (20) strains of pathogenic fungi with minimal inhibitory concentrations (MICs) ranging between 0.25-4 mg/mL (Batawila et al., 2005). The ethanolic extract of the roots showed antiviral activity on Herpes virus type 1 (HSV-1)

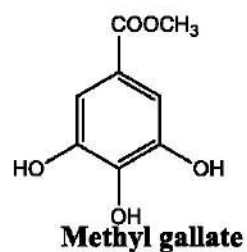
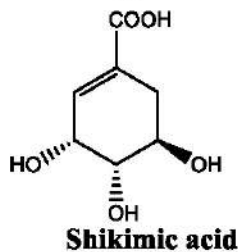
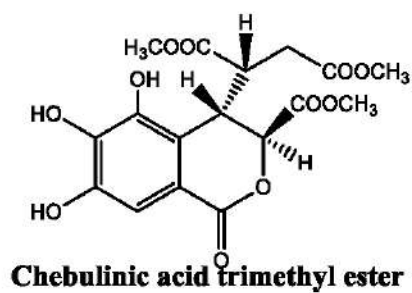
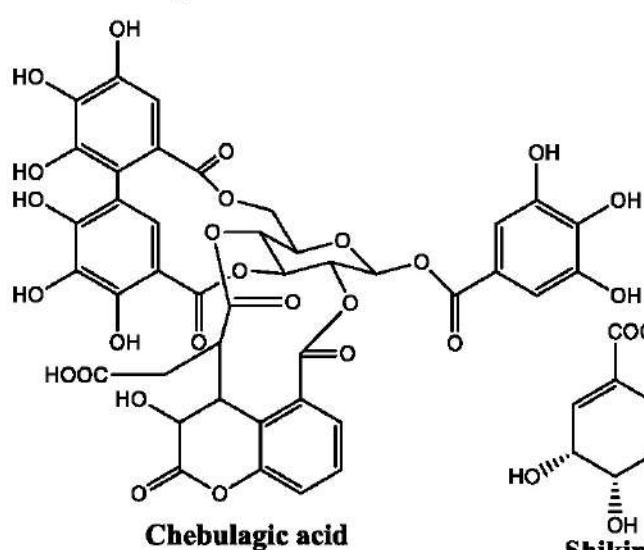
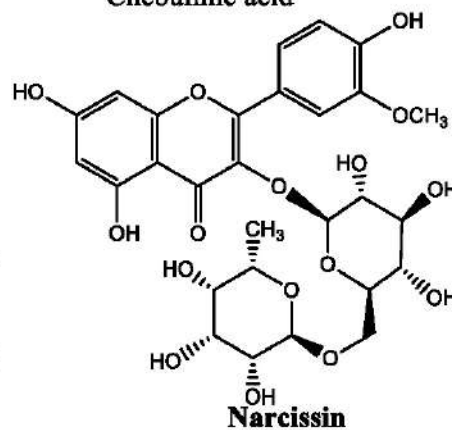
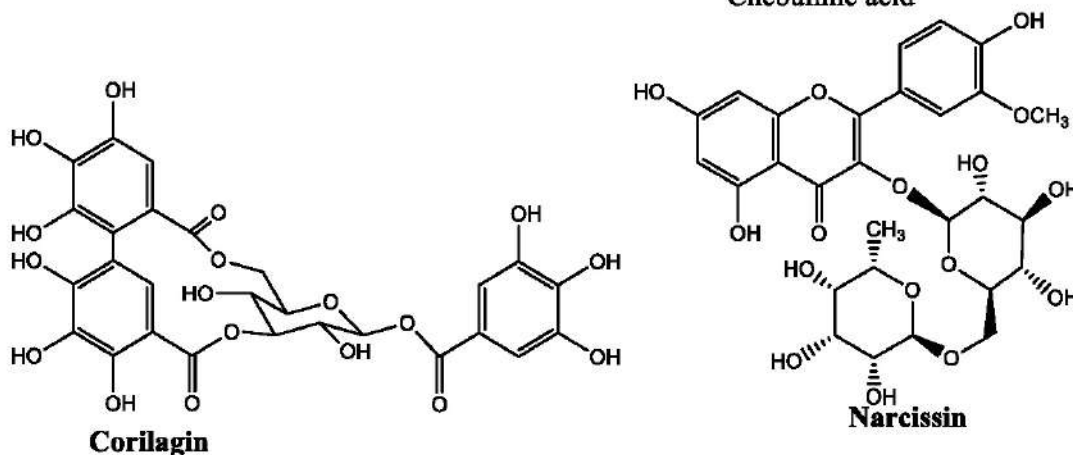
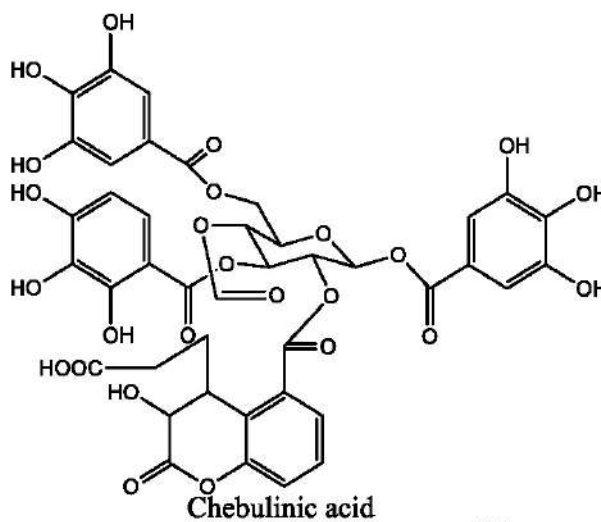
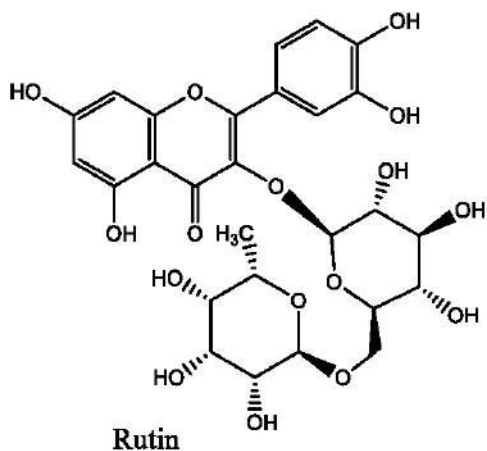
with 98% inhibition (Silva *et al.*, 1997a). The same extract showed antibacterial activity on *Vibrio cholera*, *Streptococcus faecalis*, *Campylobacter*, *Shigella dysenteriae* and antifungal activity on *Candida albicans* (Silva *et al.*, 1996, Silva *et al.*, 1997b). Methanolic extract of the leaves demonstrated *in vitro* haemolytic activity (Tchacondo *et al.*, 2012). Pectic polysaccharides from *T. macroptera* have been found to have immunomodulatory and complement fixing properties (Zou *et al.*, 2014a, 2014b, 2015). Interestingly, such activities were present in preparations made in the same way by traditional healers (Zou *et al.*, 2014a). The methanol crude extract had high radical scavenging activity ( $6.2 \pm 0.4 \mu\text{g/mL}$ ) and showed moderate inhibition ( $52 \pm 5 \mu\text{g/mL}$ ) of xanthine oxidase, an enzyme involved in production of superoxide radical anion. Xanthine oxidase inhibition is of medicinal importance in the treatment of gout. Isolated compounds corilagin and chebulagic acid were very good radical scavengers, with  $\text{IC}_{50}$  values less than half of the positive control quercetin. Rutin and chebulagic acid inhibited xanthine oxidase. They were, however, less active than quercetin (positive control). Shikimic acid was inactive (Pham *et al.*, 2011). Toxicity towards brine shrimp was low ( $\text{LD}_{50} > 100 \mu\text{g/mL}$  for all extracts;  $> 200 \mu\text{M}$  for all pure compounds). The crude methanol extract had considerable activity as a 15-lipoxygenase inhibitor ( $\text{IC}_{50} 27.9 \pm 1.5 \mu\text{g/mL}$ ), comparable to the positive control quercetin and an  $\alpha$ -glucosidase inhibitor ( $\text{IC}_{50} 0.47 \pm 0.03 \mu\text{g/mL}$ ) acarbose. Thus, *T. macroptera* is a rich source of bioactive compounds with good antioxidant, radical scavenging activity and as an  $\alpha$ -glucosidase inhibitor. Polysaccharides from the plant are reported to possess antiinflammatory properties *in vitro*. The compounds 2,3-O-(S)-hexahydroxydiphenoyl-D-glucose and punicalcortin C isolated from the barks showed anti-cancer activity on 5637 cells (primary carcinoma of the human bladder) (Conrad *et al.*, 2001). Extracts and polysaccharides isolated from leaves, stem and root bark showed immunomodulatory activity by inhibiting haemolysis of sheep cells sensitized by human antibodies (Zou *et al.*, 2014b; Zou *et al.*, 2015). The aqueous bark extract administered by gavage in mice at a dose of 200 mg/kg body weight showed antidiarrhoeal activity by decreasing the average frequency of liquid stools.

### Clinical data

None documented

### Chemical constituents

Gallic acid, punicalagin, terflavin A, terchebulin, ellagic acid, 3,38-di-O-methylellagic acid 3,4,38,48-tetra-O-methylellagic acid (Silva *et al.*, 2000; Silva *et al.*, 2012); chlorogenic acid, quercetin (Prista *et al.* 1962); orientin, isoorientin (Nongonierma *et al.*, 1987) vitexin, isovitexin (Nongonierma *et al.*, 1988; Kone *et al.*, 2012); terminolic acid; 23-galloylarjunolic acid and its glucosyl ester and glucosides of 24-deoxysericoside and chebuloside II (Nongonierma *et al.*, 1988), rutin, narcissin, corilagin, chebulagic acid, chebulinic acid and chebulic acid trimethyl ester, methyl gallate and shikimic acid.



### Test for identity and purity

**Moisture content:** Air dried coarse powder does not lose more than 4.2% (leaves) and 6.5% (stem bark) at 105°C.

**Total ash:** not more than 10.7% (leaves) and 26.3% (stem bark)

**Acid insoluble ash:** not more than 1.3% (leaves) and 0.8% (stem bark)

**Water soluble extractive:** not less than 13.0% (leaves) and 11.0% (stem bark)

**Ethanol soluble extractive (70%):** not less than 6.0% (leaves) and 14.0% (stem bark)

### Chromatographic fingerprint

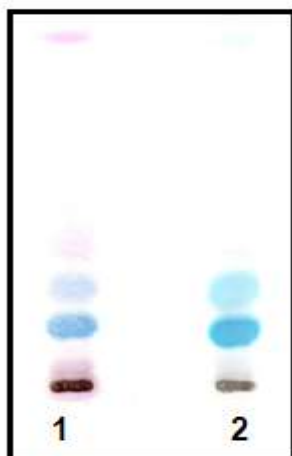
#### *Thin Layer Chromatography*

**Preparation:** About 5 g of the powdered stem bark were extracted with ethyl acetate by cold maceration, filtered and the filtrate concentrated to a small volume. A small spot was then applied to the TLC plate for analysis.

**Chromatographic conditions:** Analytical TLC on silica gel G60 F254, 0.25 mm layer in hexane/ethyl acetate (7:3) as the mobile phase.

**Detection:** Visualized in daylight after spraying with anisaldehyde-sulphuric acid (Lane 1) and vanillin-sulphuric acid reagents (Lane 2) (Stahl, 1969), heating to 110°C for 10 mins.

The TLC chromatogram showed two prominent spots with R<sub>f</sub>s of 0.29 (sky blue) and 0.16 (sky blue) when sprayed with both anisaldehyde and vanillin reagents.



#### *High Performance Liquid Chromatography*

**Sample preparation:** About 10 mg of the hydroethanolic extract of *Terminalia macroptera* stem bark were reconstituted in 3 mL acetonitrile in a 10 mL volumetric flask with sonication for 17 minutes. The resulting solution was then diluted to volume with the mobile phase under chromatographic conditions. It was centrifuged to obtain a clear test solution which was subsequently filtered through a 0.45 µm filter into an HPLC vial and analyzed



## Chromatographic system

### Optimized chromatographic conditions

**Mode:** LC

**Column:** YMC ODS, 4.6 x 150 mm, 5  $\mu$ m

**Column temperature:** Ambient – 30°C

**Mobile phase:** Acetonitrile: water (60:40 v/v)

**Elution mode :** Isocratic

**Injection volume :** 20  $\mu$ L

**Flow rate:** 0.5 mL/minute

**Detection wavelengths:** 230 nm, 254 nm and 278 nm.

### System Suitability parameters

**Number of peaks:** 230 nm (1), 254 nm (1), 278 nm (1)

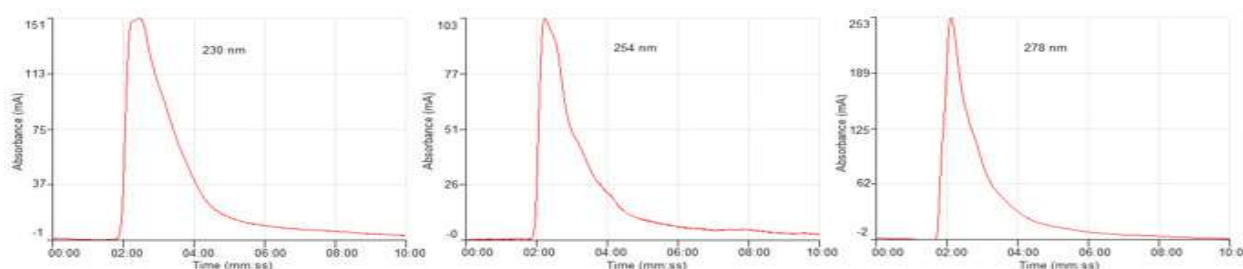
**Retention time (s):** 230nm (2.27min), 254 nm (2.14 min), 278 nm (2.07 min)

**Asymmetric factor(s):** 230 nm (0.964), 254 nm (0.643), 278 nm (0.573)

**Tailing factor:** NMT 2.0

**Efficiency:** 230 nm (20.51), 254 nm (42.71), 278 nm (49.87)

**Acceptance criteria:** Sample solution of hydroethanolic crude extract of *Terminalia macroptera* Guill. & Perr. (Stem bark) conforms to the system suitability parameters.



### HPLC chromatogram of *Terminalia macroptera*

#### FT-IR

A small amount of the dried hydro-ethanolic extract (70%) was placed on the sample area of the Perkin Elmer UATR Fourier transform infrared (FT-IR) spectrometer and scanned between 4000-400  $\text{cm}^{-1}$  with a resolving power of 4  $\text{cm}^{-1}$  and a cumulative scanning limitation of 24 times. Principal peaks appeared at wavenumbers 3240.45, 2922.98, 1709.08, 1603.21  $\text{cm}^{-1}$ .

#### Macroscopy

The stem bark surface is deeply fissured, brown to black with a fibrous, brown to orange inner bark.

#### Microscopy

##### *Transverse section*

Transverse section shows three layers of cork cells; the first layer about eight cells in depth with yellowish content; second cork layer of about six cells in depth with brown content; third layer of cork of about nine cells in depth. Cork is followed by a row of groups of thick walled fibres surrounded at the base by large

cluster crystals of calcium oxalate. Secondary phloem consists of layers of phloem, fibres and sheaths of calcium oxalate crystals; interspaced by two to three celled rows of medullary rays up to the cambium.

### **Powdered plant material**

Powder is characterised by numerous calcium oxalate rosettes; fragments of cork cells in both surface and transverse view; fibres with sheaths of calcium oxalate rosettes and groups of sclerieds.

### **Therapeutic actions**

Antiplasmodial, antibacterial, antifungal, antioxidant, antidiabetic, antidiarrhoeal immunomodulatory

### **Therapeutic indications**

Malaria, diarrhoea, dysentery, conjunctivitis, infectious diseases, oxidative stress, immunodeficiency, diabetes

### **Safety data**

LD<sub>50</sub> by oral route was above 3000 mg/kg in rats. There were no signs of CNS depression/stimulation or autonomic effects up to a dose of 1000 mg/kg. No significant change in the gross anatomy occurred in the liver, kidney, heart and the lungs. The relative organ to body weight ratios of spleen, thymus, and adrenals were not affected. *Terminalia macroptera* extract enhances RBC count, haemoglobin concentration as well as the pack cell volume. Lymphocyte count increased dose-dependently. This resulted in mild increase in WBC at all doses of the extract used. Neutrophils and MID cells also decreased with Terminalia extract treatment. Platelet count was unaffected. Terminalia caused decreases in ALP at all doses. It did not significantly affect ALT, and GGT but caused an increase in AST. *T. macroptera* extract did not affect serum proteins significantly. The extract did not significantly affect bilirubin and renal function. Pentobarbitone-induced sleeping time was potentiated. Histopathological studies did not show evidence of cellular damage to the liver and kidney. The extract appears to stimulate the production of red cells and could be a potential haematinic. Reduction in enzyme levels could provoke drug interactions.

### **Precautions for use**

Should not be administered with conventional drugs

### **Adverse effects**

None

### **Contraindication**

Sedatives

### **Dosage forms**

Decoction, infusion, tincture

### **Dosage**

Decoction: 30 g of dried plant material in 900 mL water; simmer until reduced to 600 mL; 1-3 tablespoonfuls daily

Infusion: 30 g of dried aerial part in 600 mL of water; 3-4 teacups daily

Tincture: 1:5 in 50% alcohol 5m L three times daily

## Storage

Store in cool dry place

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from *Terminalia macroptera* root bark, stem bark and leaves. *Molecules*, 19, 7440–7458.

### Botanical name

*Thevetia peruviana* (Pers.) K. Sehum

### Family

Apocynaceae

### Synonyms

*Cerbera penruviana* Pers. or *T. neriifolia* Juss. ex A.DC

### Common Names

Milk bush; exile oil plant, yellow oleander, be still tree, digoxin, lucky nut, Nerium oleander, yellow bells (English), Laurier rose-jaune, Laurier jaune des Indes, Chapeau de Napoléon (French).

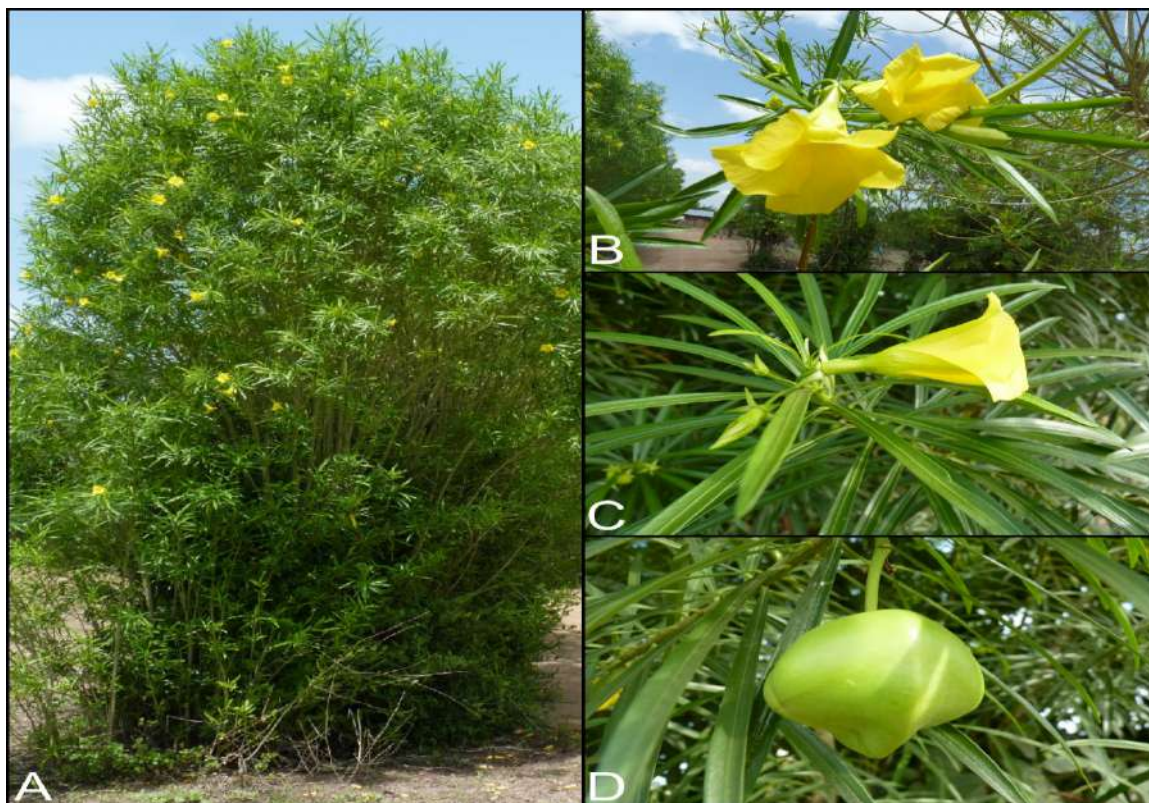
### Common Local names

**Côte d'Ivoire** : Attié-Achiko

**Ghana**: Ga-Kpoteo; Asante-Nyereme nyereme

### Description of the plant

*Thevetia peruviana* is a shrub 2-4 (6) m high, with the stem branched at the base with an open crown. The leaves are clustered at the end of branches. Leaves alternate or spirally arranged, glabrous, almost sessile, linear lamina 9-18 × 0.6-1 cm at base and base attenuate to tip, petiole not always distinct, 1-3 mm long. Only the rib, midrib is visible. Inflorescence, cyme of 2-4 flowers, arranged at the ends of the branches; yellow or orange-yellow corolla flower in tube with 5 lobes twisted to rounded or cornered apices, 5-7 cm long. Fruit drupe smooth, wider than loin, a little bilobed, slightly winged or keeled at the top, 4 × 4-4.5 cm, yellow when ripe and black when it remains long on the tree. It is 2-4-seeded within the stony endocarp. Seeds obovoid, 2 cm × 1.5 cm, flattened. The bark is smooth, grey and exudes a white latex when slashed (Schmelzer, 2006)



A – *Thevetia neriifolia* Juss. Ex A.DC, B and C – flowers, D – Fruit

#### Herbarium specimen number

Benin: AP 2344

Burkina Faso: BUR-080 (CNSF), Ouédraogo, H. 31 (OAU)

Côte d'Ivoire : 267 CNF

Ghana: GH712/KNUST

Senegal: IFAN UD 3

#### Habitat and geographical distribution

*Thevetia peruviana* can be found in pastures, in savanna and on the banks of watercourses. Species introduced and planted in villages, agglomerations and, around schools. It is native to tropical America and is widely cultivated throughout the tropics as an ornamental. It spreads from Senegal to Cameroon in tropical Africa (Zibbu and Batra, 2011).

#### Plant material of interest

Leaves

#### Other part used

Latex, fruit, root, bark, seed

#### Definition of plant material of interest

It consists of the dried leaves of *Thevetia peruviana* (Pers.) K. Sehum

## Ethnomedical uses

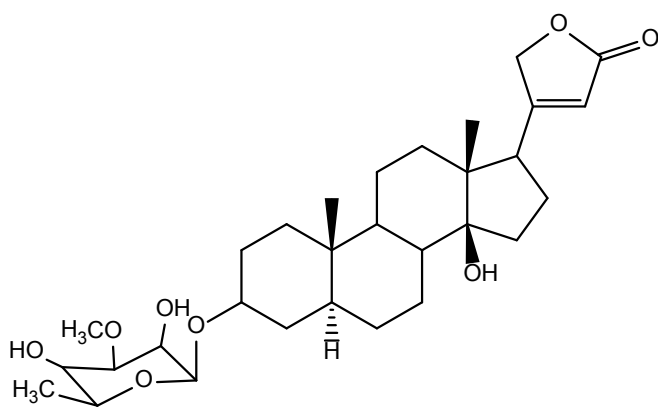
Most parts of *Thevetia peruviana*, including the latex, are highly toxic; the seeds the most toxic. Despite its toxicity, the plant is used for its medicinal properties throughout the tropics. It is used in the treatment of malaria, headaches, colds, skin infections, wounds, stomach aches, measles, amenorrhoea and haemorrhoids (Klotoe, 2015). The bark is a purgative and used against intermittent fevers. A decoction of the bark and roots is used for the treatment of amenorrhoea. A combination of bark and seed is used in the treatment of fever. The seed oil treats skin infections, scabies, purges, dropsy, and rheumatism. The latex treats scabies and leprosy. The root is used in the treatment of snake bites. A decoction of the bark or leaves is taken orally as a laxative and as an emetic, and is known to be effective in treating intermittent fevers. In Senegal, oral decoction of the leaves and bark is used in the treatment of amenorrhoea. In Mali, the latex is applied to soften corns and calluses. In Côte d'Ivoire and Benin, leaf sap is used as eye and nasal drops to relief violent headaches. A drop of the decoction in the nostrils is used to revive a fainting person and to treat colds. Kenya's Luos use water containing crushed leaves to treat colds. The seeds can be used as a purgative. Seed oil is used externally in India to treat skin infections. In Benin and Uganda, root infusion is used to treat snake bites, and in Ghana, a decoction of leaves is taken to treat jaundice, fever, and as a purgative against intestinal worms. The bark and seeds are used as rat poison, and also for criminal purposes. In southern Africa and Cameroon, seeds are used as arrow poison, while in India and Sri Lanka, seeds are used to commit suicide or homicide. Other reports suggests the use of the seeds as abortifacient. The seeds act as contact poison; when reduced to a slurry with a soap solution, they are used as an insecticide. After purification, the initially toxic seed oil is safe for consumption (Schmelzer, 2006).

## Biological and pharmacological activities

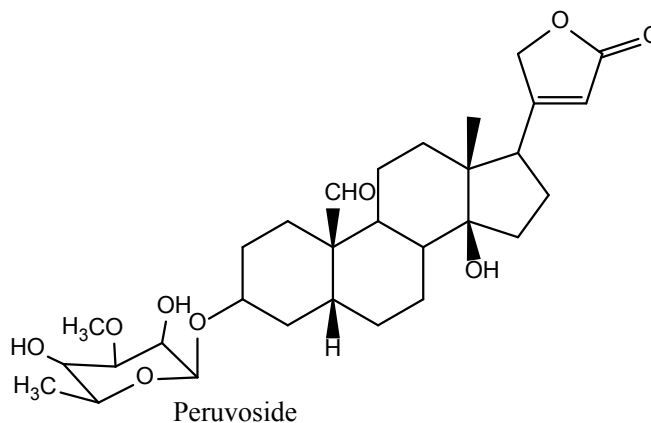
Ethanol extracts of *Thevetia peruviana* showed antimicrobial activity *in vitro* against *Escherichia coli*, *Streptococcus lactis*, *Alcaligenes faecalis*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and fungal species of *Fusariumoxysporum*, *Alternariahelianthii*, *Curvularialunata*, *Aspergillus* and *Penicillium*. In other studies, the methanol extract of the stem was found to inhibit spermatogenesis in rats (Rajbhar and Kumar, 2014). The fresh flowers of *T. peruviana* was shown to exhibit biphasic antiinflammatory activities. The seed oil demonstrated termicidal properties. The *in vivo* antidiabetic activity of *T. peruviana* bark was performed in streptozotocin-induced diabetic rats. The plant showed significant activity in a dose-dependent manner (Gogoi and Bhuyan, 2014). Methanol extracts of *T. peruviana* fruit showed antitumour activity against Ehrlich's ascites carcinoma (EAC) cell line in Swiss albino mice (Haldar *et al.*, 2015).

## Chemical constituents

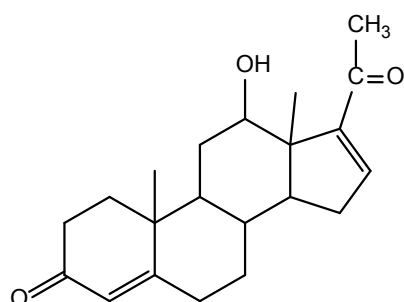
Cardiac glycosides of cardenolide (thévetine A& B); nerifoline, cerberine (2'-O-acetylneriifolin), peruvoside (cannogenin-théviolate), ruvoside (cannogénol -évoriolate), digitoxigenin, thevetoxin, theveridoside and perubosidic acid (perusitine) (Rajhans *et al.*, 2019).



3-beta-O-(alpha-L-acofriosyl)-14-beta-hydroxy  
5-alpha-carda-20:22-enolide



Peruvoside



12-beta hydroxy-4,16-pregnadien-3,20-dione

## Clinical data

Large-scale clinical trials have shown that all forms of heart failure can be successfully treated with peruvoside in approximately 85% of patients. However, peruvoside is no longer used in Western medicine because of dosage difficulties, the narrow therapeutic index, and the low bioavailability due to rapid dissociation. The thevethin mixture have actually been used clinically in cases of cardiac failure, although its effective dose is quite close to its toxic dose. The use of peruvoside for the treatment of herpes has been patented (Schmelzer, 2006).

## Test for identity and purity

**Moisture content:** Air dried coarse powder does not lose more than 7.7% (leaves), 5.3% (stem) and 5.0% (root) at 105°C.

**Total ash:** not more than 10.2% (leaves), 3.9% (stem) and 14.2% (root)

**Acid insoluble ash:** not more than 0.8% (leaves), 0.7% (stem) and 10.3% (root)

**Water soluble extractive:** not less than 14.0% (leaves), 11.0% (stem) and 12% (root).

**Ethanol soluble extractive (70%):** not less than 1.0% (leaves) and 4.0% (stem) and 3.0% (root) at 105°C.

## Chromatographic fingerprint

### Thin Layer Chromatography

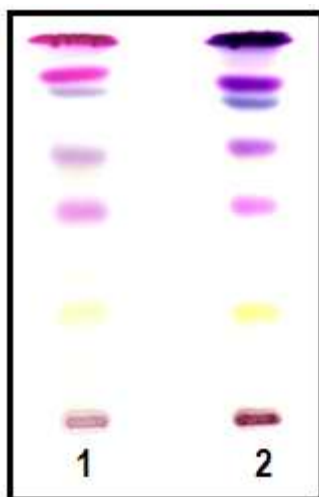
**Preparation:** About 5 g of the powdered leaves were extracted with ethyl acetate by cold maceration, filtered and the filtrate concentrated to a small volume. A small spot was then applied to the TLC plate for analysis.



**Chromatographic conditions:** Analytical TLC on silica gel G60 F254, 0.25 mm layer in hexane/ethyl acetate (7:3) as the mobile phase.

**Detection:** Visualized in daylight after spraying with anisaldehyde-sulphuric acid (Lane 1) and vanillin-sulphuric acid reagents (Lane 2) (Stahl, 1969), heating to 110°C for 10 mins.

The TLC chromatogram showed four prominent spots with R<sub>f</sub>s of 0.86 (blue), 0.70 (purple), 0.55 (pink) and 0.29 (yellow) when sprayed with both anisaldehyde and vanillin. An additional spot at R<sub>f</sub> of 0.90 appeared in each chromatogram with colours pink and violet when sprayed with anisaldehyde and vanillin respectively.



### High Performance Liquid Chromatography

**Sample preparation:** About 10 mg of the hydroethanolic extract of *Thevetia neriifolia* leaves were reconstituted in 3 mL acetonitrile in a 10 mL volumetric flask with sonication for 17 minutes. The solution was then diluted to volume with the mobile phase under chromatographic conditions. It was centrifuged to obtain a clear test solution which was subsequently filtered through a 0.45 µm filter into an HPLC vial and analyzed

### Chromatographic system

#### Optimized chromatographic conditions

**Mode:** LC

**Column:** YMC ODS, 4.6 x 150 mm, 5 µm

**Column temperature:** Ambient – 30°C

**Mobile phase:** Acetonitrile: Methanol: Water (60:20:20 v/v/v)

**Elution mode :** Isocratic

**Injection volume :** 20 µL

**Flow rate:** 0.5 mL/minute

**Detection wavelengths:** 230 nm, 254 nm and 278 nm.

#### System Suitability parameters

**Number of peaks:** 230 nm (1), 254 nm (1), 278 nm (1)

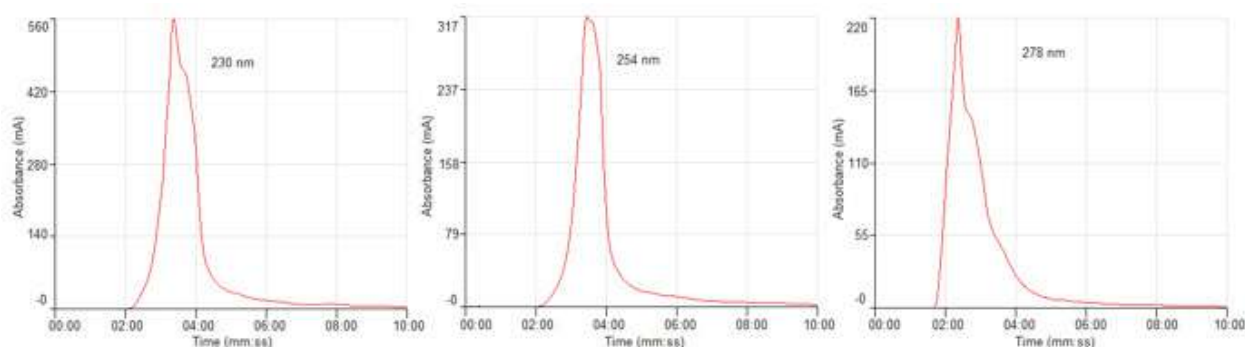
**Retention time (s):** 230 nm (3.22 min), 254 nm (3.26 min), 278 nm (2.21 min)

**Asymmetric factor(s):** 230 nm (1.040), 254 nm (0.828), 278 nm (0.984)

**Tailing factor:** NMT 2.0

**Efficiency:** 230 nm (75.05), 254 nm (113.98), 278 nm (48.14)

**Acceptance criteria:** Sample solution of hydro-ethanolic crude extract of *Thevetia neriifolia* Juss. ex A.DC. (Leaves) conforms to the system suitability parameters.



## FT-IR

A small amount of the dried hydroethanolic extract (70%) was placed on the sample area of the Perkin Elmer UATR Fourier Transform Infrared (FT-IR) spectrometer and scanned between  $4000\text{--}400\text{ cm}^{-1}$  with a resolving power of  $4\text{ cm}^{-1}$  and a cumulative scanning limitation of 24 times. Principal peaks appeared at wavenumbers  $3308.10$ ,  $2924.68$  and  $1601.33\text{ cm}^{-1}$

## Microscopy

Lower surface has numerous anomocytic stomata with four subsidiary cells and other epidermal cells have slightly wavy walls. Two of the subsidiary cells characteristically bind the two axis of the stomata. Upper surface has no stomata and consists of polygonal epidermal cells.

## Transverse section

Transverse section has a central ark shaped bicollateral vascular bundle at the midrib section. The leaf is dorsiventral. There are no trichomes. The upper surface cuticle and epidermal cells are followed by collenchyma at the mid rib section; parenchyma; vascular bundle; collenchyma and the lower surface epidermal cells and cuticle. There is one layer of columnar palisade cells at the upper surface. A layer of vascular bundles comes immediately after the palisade row and calcium oxalate cluster crystals are scattered throughout the mesophyll in parenchyma cells.

## Powdered plant material

Consists of fragments of polygonal cells of the upper epidermal surface with anomocytic stomata and epidermal cells of lower surface. Bundles and fragments of unicellular fibres, septate fibres; fragments of lamina showing epidermal cells with palisade; annular vessels; spongy mesophyll and tracheids occur.

## Therapeutic actions

Anti-HIV, antibacterial, antifungal, emetic, purgative, febrifuge, laxative, cardiotoxic.

### Therapeutic indications

Intermittent fevers, amenorrhoea, skin infections, scabies, leprosy, psoriasis, purges, dropsy, antidote to snake bites, malaria, headaches, colds, skin infections, wounds, stomach aches, measles, haemorrhoids, heart failure (Klotoe 2015; Schmelzer, 2006).

### Safety data

LD<sub>50</sub> by oral route was above 3000 mg/kg in rats. There were no signs of CNS depression/stimulation or autonomic effects at doses up to 1000 mg/kg. Organ morphology of the liver, kidney, heart and lungs were not altered in subacute studies. The relative organ to body ratios of spleen, thymus, and adrenals were not affected by treatment. It did not affect RBC, HB, HCT, MCV, MCH and MCHC, it caused a reduction in white blood cells at all doses albeit insignificant. The reduction was due mostly to the effect on neutrophils. There was elevation of albumin levels at the highest dose and hence total proteins increased. Creatinine was not affected but urea was elevated at the highest dose. Urea creatinine ratio was also elevated. Pentobarbitone-induced sleeping time was prolonged by the treatment. There was evidence of necrotic lesions in the kidneys at the high dose of 1000 mg/kg. All parts of the plant contain cardiac glycosides. The kernel may be more toxic than the leaves. Ingestion is associated with nausea, vomiting, abdominal pain, diarrhoea, dysrhythmias, and hyperkalemia in humans. Clinical severity of symptoms correlates with serum potassium levels. There is a high possibility of affecting renal function at high doses.

### Precautions for use

Thevetia is a violent poison, and must only be used under medical supervision. The recommended dose should not be exceeded in view of the narrow therapeutic index of the cardiac glycosides.

Precautions must be taken in all medical applications, especially for internal use, because of its narrow therapeutic index.

### Adverse effects

Laxative effect and purgatives. Bitter glucoside, thévetine is a tetanizing tonic. It acts quickly on the heart muscles. It also gives emo-cathartic side effects.

### Contraindications

Do not combine with stimulant laxatives, and must not be used in pregnancy, breastfeeding, heart disease, elderly people and children

### Dosage forms

Decoction, Leaf juice, infusion, tincture

### Dosage

Decoction: 30 g of dried plant material in 900 mL water; simmer until reduced to 600 mL; 1-3 tablespoonfuls daily

Leaf juice: Apply latex topically to affected parts

Infusion: 30 g of dried aerial part in 600 mL of water; 3-4 teacups daily

Tincture: 1:5, 45% ethanol; 5 ml three times daily

### Storage

Store in a cool, dry place away from light.

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**Botanical name**

*Vismia guineensis* (L.) Choisy

**Family**

Hypericaceae

**Synonyms**

*Psorospermum guineense* (L) Hochr, *Hypericum guineense* L., *Vismia laurentii polyandra* Hochr., *Vismia leonensis* Hook.f.

**Common Names**

Isabelle sweet wood (English), bois doux isabelle (French)

**Common Local names**

**Benin:** Fon-Amlanmi; Yoruba- Okpa aro; Dendi- Cimbala desihin

**Côte d'Ivoire:** Akan-Titinondra; Abe-Uombéhiapi; Akye-Nguamo

**Ghana:** Akan- σκσsoa-nimmaa; Ga-Kpoteo; Gbe-Vhe- agboti, gboti

**Guinea:** Pular –djon; Maninka- djon; Soussou-Siné

**Liberia:** Kru-ge ahn; Mano-lolo mia

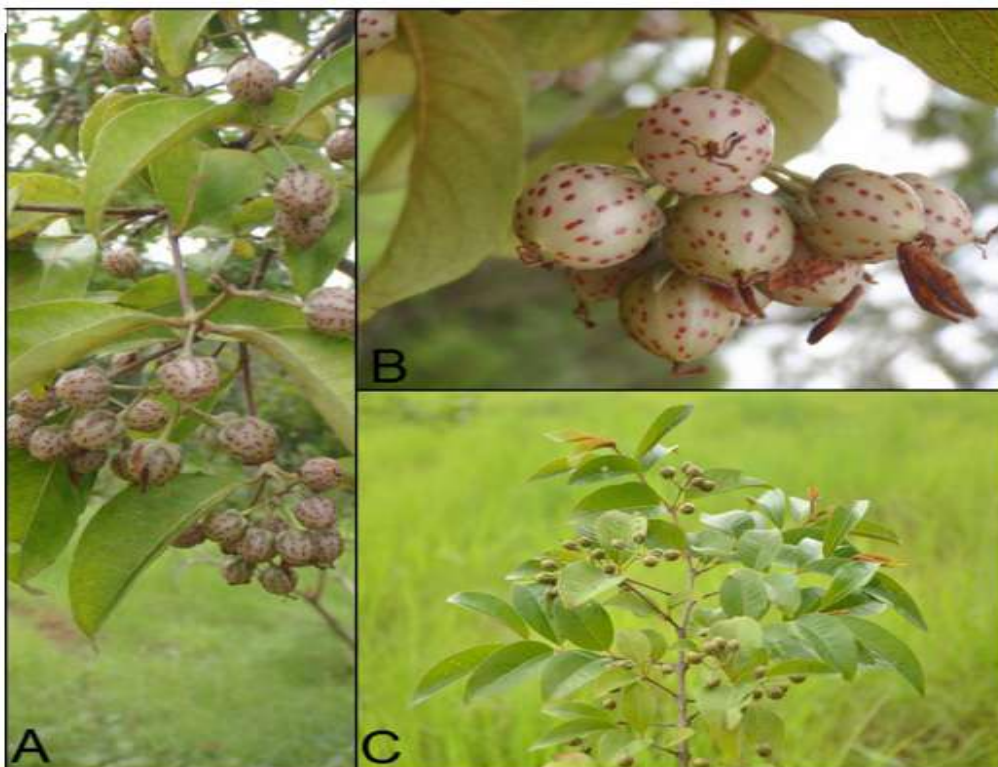
**Nigeria:** Edo-ovitue; Hausa- kiska wali; Igbo-oke oturu

**Sierra Leone:** Gola-Duma; Mende-Mbeli; Kisi-Cholompombo

**Togo:** Ana-lponyi

**Description of the plant**

It is a shrub or small tree growing up to 15 m high. The leaves are opposite, petiolate with the leaf blade ovate to ovate-elliptical, 7-12 cm long and 4-5 cm wide, cuneate at base, acute acuminate at the apex. Stellate hairs on the leaves and black glands below are visible. It has pinnate venation, with 9-11 pairs of lateral veins (Burkill, 1985). The flower is a hermaphrodite, actinomorphic, pedicellate and grouped into subumbelliform cymes, pedunculated, opposite; sepals of approx. 4 mm long. The corolla is yellowish green in colour with petals 1 cm long, pubescent inside. The stamens are numerous and grouped into 5 phalanges. The fruit is an ovoid bacciform of length 5 mm long (Lisowski, 2008). On cutting the bark exudes a reddish or yellowish resinous gum.



A and B– *Vismia guineensis* (L.) Choisy fruit and leaves, C – *Vismia guineensis* (L.) Choisy.

#### Herbarium specimen number

Burkina Faso: 1664bis OAU

Côte d'Ivoire: CNF 584

Guinea: 63 HK554 CRVPM-Dubréka

Ghana: GH 743/KNUST

Mali: 2650 DMT

Senegal : IFAN 1927

Togo: 03953 TG/HNT

#### Habitat and geographical distribution

*Vismia guineensis* grows in savannahs, forest edges, and secondary forests in the western part of the Guineo-Congolese region. The plant abundant in Cameroon and Liberia (Burkill, 1994)

#### Plant material of interest

Stem bark

#### Other part used

Leaves, whole plant

#### Definition of plant material of interest

*Vismia guineensis* consists of dry or fresh bark of *Vismia guineensis* (L.) Choisy

## Ethnomedical uses

All parts of the plant are widely used in traditional medicine for the treatment of malaria and inflammatory conditions in Guinea (Traore *et al.*, 2013). The yellow resin from the stem bark is used in Senegal for dermatitis, leprosy, herpes, scabies and eczema. Decoction of the root is used internally and externally (Kerharo and Adam, 1974) for skin diseases. In Mali, the plant is used in an ointment, containing 1% of the root bark extract, petrolatum ether or shea butter for the treatment of skin problems in women (Politi *et al.*, 2004). The Mende of Sierra Leone utilizes the haemostatic and cicatrisant actions of the plant in circumcision, by allowing the resin from cut young stems to drip onto the freshly made wound. In Cote d'Ivoire, sap expressed from the young leaves is used in baths for infants to treat jaundice. A preparation of the young leaves macerated in palm wine, is taken by adults for the same condition (jaundice). Alternatively some tribes in Côte d'Ivoire, rub the young leaves made into pellets with very little water on the body to treat jaundice. In Liberia the leaf buds are believed to have anodynal effect. Inhaling the vapour of the crushed leaf buds in cupped hands, helps to relieve vertigo, hence its Mano name, 'small pain-killer'. It is also an ordeal plant (Burkill, 1985).

## Biological and pharmacological activities

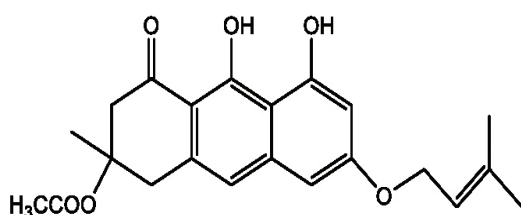
The ethyl acetate extract of the leaves and root bark of *V. guineensis* showed pronounced antiprotozoal activity against *Trypanosoma brucei* and *Trypanosoma cruzi* with  $IC_{50}$  values of 6.80  $\mu\text{g/ml}$  and 2.05  $\mu\text{g/ml}$  respectively (Traoré *et al.*, 2014a). The chloroform extract of the stem bark also showed considerable antiplasmodial activity on a *Plasmodium falciparum* Pf-K1 strain ( $IC_{50} = 1.94 \mu\text{g/ml}$ ) (Traoré *et al.*, 2014b). The methanolic extract of young leaves of three closely related species viz *V. baccifera*, *V. jefensis*, and *V. macrophylla* showed remarkable cytotoxicity against 3 different human breast cancer cell lines (MCF-7), the central nervous system (H-460), and lungs (SF-268). Many of the constituents of *Vismia* are generally described as cytotoxic, insect repellent and have shown various antiprotozoal activities. Vismione H showed antiplasmodial activity with an  $IC_{50}$  of 0.088  $\mu\text{g/ml}$ ; vismione D was active against *T. brucei* rhodesiense and *T. cruzi* ( $IC_{50} < 10 \mu\text{g/ml}$ ) and *Plasmodium falciparum* K1 ( $IC_{50} 1.0 \mu\text{g/ml}$ ), but also cytotoxic with respect to human L6 cells ( $IC_{50} = 4.1 \mu\text{g/ml}$ ) (François *et al.*, 1999; Mbwambo *et al.*, 2004). Emodin from *V. guineense* showed strong antileishmanial activity ( $IC_{50} = 2.0 \mu\text{g/ml}$ ) (Mbwambo *et al.*, 2004). Components isolated from *V. laurentii*, vismiaquinone A and tirucalla-7,24-dien-3-one were found to have considerable antiplasmodial activity with an  $IC_{50}$  of 1.42  $\mu\text{M}$  and 1.18  $\mu\text{M}$  respectively. The activity was 4 to 7 times greater than that of quinone (Hussain *et al.*, 2012).

## Clinical data

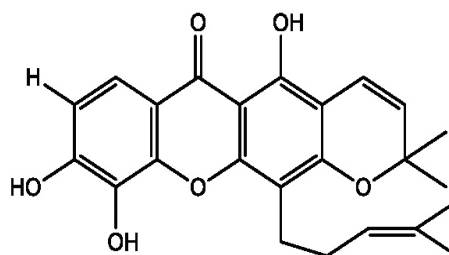
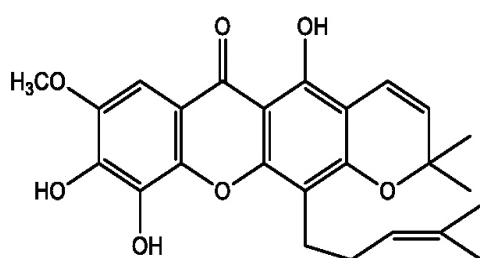
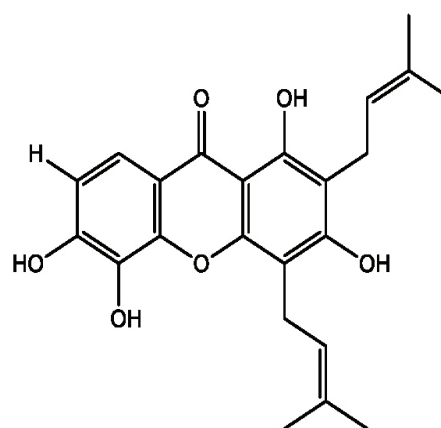
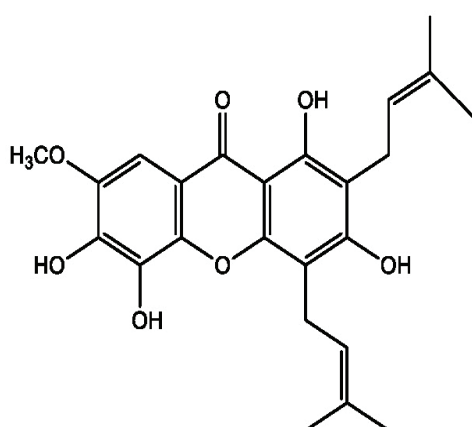
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## Chemical constituents

Ferruginin C, ferruginins A and B, vismin, harunganin; orientine, vismione B, vismione D, vismione F, vismione H, vismione L, vismione M, 7-geranylemodin, vismione G, acetylvismione D, deacetylvismione A, deacetylvismione H, 3-(Acetyloxy)-7-(3,7-dimethyl-2,6-octadienyl)-3,4-dihydro-6,8,9-trihydroxy-3-methylanthracenone, bianthrone A1, bianthrone A3a (Politi *et al.*, 2004); vismiaquinone B, laurentiquinone A, B and C, betulinic acid, lupeol, stigmasta 3 to 4-one (Tala *et al.*, 2013), geranyloxyemodine, geranyloxyemodine anthrone, madagascin anthrone, 3 - [(3,7-dimethyl-2,6-octadienyl) oxy]-1,8-dihydroxy-6-methyl-anthracenone, 5,9,10-trihydroxy-8-methoxy-2,2-dimethyl-12- (3-methyl-2-butenyl) -2H, 6H-pyrano [3,2-b] xanthen-6-one, xanthone V1a, 1,3,5,6-tetrahydroxy-7-methoxy-2, 4-bis (3-methyl-2-butenyl) -xanthone, and 5,9,10-tris (acetyloxy) -2,2-dimethyl-12- (3-methyl-2-butenyl) -2H 6H-pyrano [3,2-b] xanthen-6-one (Botta *et al.*, 1986; François *et al.*, 1999)



vismione H

1,5,6-trihydroxy-6',6'-dimethyl-2-H-pyrano  
(2',3':3,2)-4-(3,3-dimethylprop-2-enyl)xanthone1,5,6-trihydroxy-7-methoxy-6',6'-dimethyl-2-H-pyrano  
(2',3':3,2)-4-(3,3-dimethylprop-2-enyl)xanthone1,5,6-trihydroxy-2,4-di (3,3-dimethyl prop-2 enyl)  
xanthone1,5,6-trihydroxy-7-methoxy-2,4-di(3,3-dimethyl  
prop-2-enyl) xanthone

### Test for identity and purity

**Moisture content:** air dried coarse powder does not lose more than 8.8% (leaves) and 4.3% (stem) at 105°C.

**Total ash:** not more than 8.7% (leaves) and 5.3% (stem)

**Acid insoluble ash:** not more than 0.3% (leaves) and 0.2% (Stem)

**Water soluble extractive:** not less than 20.0% (leaves) and 5.0% (stem)

**Ethanol soluble extractive (70%):** not less than 9.0% (leaves) and 2.0% (Stem)



## Chromatographic fingerprint

### *Thin Layer Chromatography*

**Preparation:** About 5 g of the powdered stem bark were extracted with ethyl acetate by cold maceration, filtered and the filtrate concentrated to a small volume. A small spot was then applied to the TLC plate for analysis.

**Chromatographic conditions:** Analytical TLC on silica gel G60 F254, 0.25 mm layer in hexane/ethyl acetate (7:3) as the mobile phase.

**Detection:** Visualized in daylight after spraying with anisaldehyde-sulphuric acid (Lane 1) and vanillin-sulphuric acid reagents (Lane 2) (Stahl, 1969), heating to 110°C for 10 mins.

The TLC chromatogram showed four prominent spots with R<sub>f</sub>s of 0.95 (yellow), 0.89 (pink), 0.83 (pink) and 0.76 (peach) when sprayed with both anisaldehyde and vanillin. An additional spot appeared with R<sub>f</sub> of 0.45 (pink) in the chromatogram sprayed with anisaldehyde.



### *High Performance Liquid Chromatography*

**Sample preparation:** About 10 mg of the hydro-ethanolic extract of *V. guineensis* stem bark were reconstituted in 3 mL acetonitrile in a 10 mL volumetric flask with sonication for 17 minutes. The solution was then diluted to volume with the mobile phase under chromatographic conditions. It was centrifuged to obtain a clear test solution, which was subsequently filtered through a 0.45 µm filter into an HPLC vial and analyzed

## Chromatographic system

### Optimized chromatographic conditions

**Mode:** LC

**Column:** YMC ODS, 4.6 x 150 mm, 5 µm

**Column temperature:** Ambient – 30°C

**Mobile phase:** Acetonitrile: Methanol: Water (60:20:20 v/v/v)

**Elution mode :** Isocratic

**Injection volume :** 20  $\mu$ L

**Flow rate:** 0.5mL/minute

**Detection wavelengths:** 230 nm, 254 nm and 278 nm.

### System Suitability parameters

**Number of peaks:** 230 nm (1), 254 nm (1), 278 nm (1)

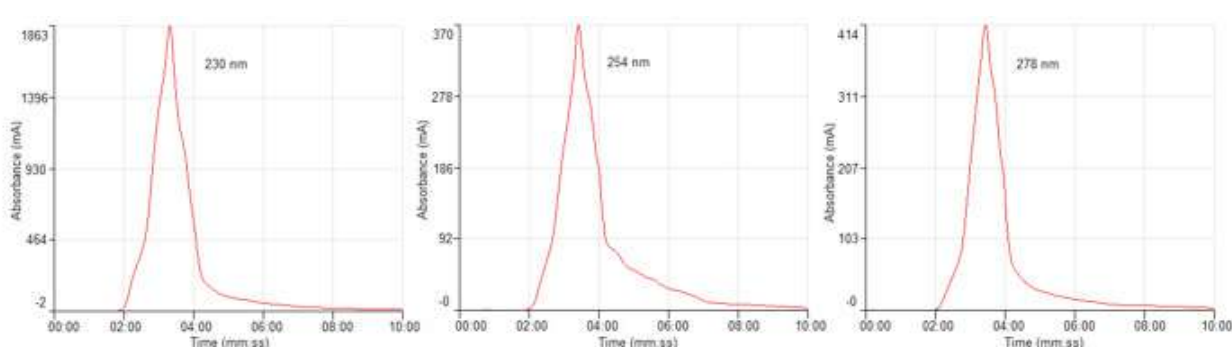
**Retention time (s):** 230 nm (3.19 min), 254 nm (3.25 min), 278 nm (3.26 min)

**Asymmetric factor(s):** 230 nm (0.799), 254 nm (0.925), 278 nm (0.864)

**Tailing factor:** NMT 2.0

**Efficiency:** 230 nm (70.83), 254 nm (78.71), 278 nm (86.07)

**Acceptance criteria:** Sample solution of hydroethanolic crude extract of *V. guineensis* (L.) Choisy (Stem Bark) conforms to the system suitability parameters.



HPLC chromatogram of *Vismia guineense* stem bark

### FT-IR

A small amount of the dried hydroethanolic extract (70%) was placed on the sample area of the Perkin Elmer UATR Fourier Transform Infrared (FT-IR) spectrometer and scanned between 4000-400  $\text{cm}^{-1}$  with a resolving power of 4  $\text{cm}^{-1}$  and a cumulative scanning limitation of 24 times. Principal peaks appeared at wavenumbers 3227.97, 2922.48 and 1603.13  $\text{cm}^{-1}$ .

### Microscopy

#### Transverse Section

Transverse section shows very thick layer of cork followed by several layers of cork cells in irregular rows, under which are layers of parenchyma cells with dark coloured content, followed by a layer of several rows of rectangular squashed cells. The cells with dark coloured content followed by rows of squashed cells, are repeated four times. A large layer of large irregular shaped parenchyma follows. The last segment is a layer where the medullary rays start. Medullary rays, which consist of rectangular, elongated cells run through the ground tissue, made up of parenchyma cells, with many cells containing yellowish material and some calcium oxalate scattered. Medullary rays are up to six rows in width.

#### Powdered plant material

There are many fragments of rectangular medullary ray cells in groups and singly; large parenchyma with coloured substance; polygonal shaped cork cells; fibres with sheaths of calcium oxalate prisms.

**Therapeutic actions**

Antimalarial, antipyretic, antimicrobial

**Therapeutic indications**

Malaria, fever, scabies, dermatitis,

**Safety data**

LD<sub>50</sub> by oral route was estimated to be beyond 3000 mg/kg in rats. There were no signs of CNS depression/stimulation or autonomic effects up to the highest dose of 1000 mg/kg. In sub-acute toxicity studies, organ morphology of the liver, kidney, heart and lungs were not altered. The relative organ to body ratios of spleen, thymus, and adrenals were not significantly affected by treatment. *Vismia guineensis* treatment did not generally affect the haematopoietic system but WBC function was slightly inhibited. The inhibition was seen in MID cells. Treatment reduced ALT, ALP, AST although not statistically significant. There was no significant effect on serum proteins at low doses, but at high doses elevation in globulins was observed. Bilirubin levels did not change, and renal function remained intact. Pentobarbitone-induced sleeping time was prolonged by the treatment. There was evidence of necrotic lesions in the kidneys at the high dose of 1000 mg/kg. The plant is rich in anthraquinones with the potential to evoke diarrhoea.

**Precautions for use**

Care should be taken when administering to the elderly and patients susceptible to dehydration. High doses may induce necrosis in kidney. *Vismia* must be used with caution in pregnant women and children.

**Adverse effects**

May cause diarrhoea in some people

**Contraindications**

Laxatives

**Dosage form**

Decoction, infusion, tincture

**Dosage**

Decoction: 200 g of the bark powder in a litre and a half of water; take three tablespoonful morning and evening until healing.

Infusion: 30 g of dried aerial part in 600 mL of water; 3-4 teacups daily

Tincture: 1:5, 45% ethanol; 5 ml three times daily

**Storage**

Store in a cool, dry place

## References

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**Botanical name**

*Vitex doniana* Sweet

**Family**

Verbenaceae

**Synonyms**

*Vitex cienkowskii* Kotschy & Peyr., *Vitex cuneata* Thonn., *Vitex pachyphylla* Baker

**Common Names**

Black plum, West African plum (English), Prunier noir, koro (French) (Ky, 2008).

**Common Local names**

**Benin:** Fon-Gountin, Fontin; Dendi-Boyi; Yoruba-Osha koro, Ori, Oyi, Oyi.

**Burkina Faso:** Bambara- Koto koro; Bissa-Koum kounda; Moré-Aâdga.

**Côte d'Ivoire:** Baoulé-N'gbri; Dioula-Koro.

**Ghana:** Ewe-Foyiti; Fante-Afua; Twi-Afetewa, Abiswa

**Guinea:** Malinké-Kodo m'ba ; Pular-Boumé; Soussou-Koukoui.

**Mali:** Bambara- Korofin, Koroba; Dogon- Mólô; Malinké-Kutundimon

**Niger:** Djerma/Zarma-Bôye; Peuhl-Galbihi.

**Senegal:** Diola- Egompa; Mandingue-Sokoro; Wolof-- Ool.

**Togo:** Ana – Ori; Ewé – Fonyiti; Chokossi – Kotobaka.

**Description of the plant**

*Vitex doniana*, is a medium-sized deciduous tree, 8-18 m high, with a heavy rounded crown and a bole clear of branches up to 5 m. The pale brown bark is rough with thin vertical fissures. The glabrous compound leaves are oppositely arranged, usually with 5 leaflets on stalk. The leaflets are ovate, obovate-elliptical with an entire margin. The apex of the leaves is emarginate or rounded with a cuneate base. The upper surface of the leaves is dark green, leathery with stellate hairs. The abaxial surface is pale green. The flower petals are white with the exception of the largest lobe, which is purple, in dense opposite and axillary cyme. The greenish immature drupe-like fruit is oblong which turns purple or purplish-black on ripening with a starchy black pulp. Each fruit contains one hard, conical seed (Orwa *et al.*, 2009).



Whole plant of *V. doniana* (A), Flowers (B) Leaves (C) Unripe fruits (D).

#### Herbarium specimen number

Bénin: 2345 AP/HNB

Burkina Faso: BUR-642 (CNSF), 1333 (OUA)

Cote d'ivoire: 178 CNF

Ghana: GH 781/KNUST

Mali: 0614 / DMT

Senegal: IFAN AM 2295

Togo: 09271 Université of Lomé

#### Habitat and geographical distribution

*V. doniana* occur mostly in savannah regions, including Ghana and the dense forest of Sudan and Guinea. A deciduous forest tree of coastal woodland, riverine and swampy forest areas requiring high water table (Orwa *et al.*, 2009). It is widespread from Senegal to Cameroon to East Africa. It is also found in Comoros.

#### Plant material of interest

Leaves

#### Other part used

Stem bark

#### Definition of plant material of interest

Vitex consists of the fresh or dried leaves of *Vitex doniana*

## Ethnomedical uses

Various parts of *V. doniana* are used in traditional medicine for the treatment of a variety of diseases. A decoction of the leaves is used in the treatment of stomach and rheumatic pains, inflammatory disorders, diarrhoea and dysentery (Ezekwesili *et al.*, 2012). The expressed juice from the young immature leaves is used in ocular disorders (Orwa *et al.*, 2009). A decoction of the dried leaves and stem bark is used in the management of dizziness, while a stem bark decoction is administered for gastroenteritis (Bolanle *et al.*, 2014; Kiliani, 2006). The stem bark is employed in the management of epilepsy and leprosy. Also, a stem bark decoction is taken to improve fertility (Kilani, 2006). The root bark is administered for the management of back ache and gonorrhoea (Orwa *et al.*, 2009). The roots and leaves are used for nausea, colic and epilepsy. The fruit is also used to improve fertility and to treat anaemia, jaundice, leprosy and dysentery (Mohammed *et al.*, 2016, Orwa *et al.*, 2009). In Togo the plant is used in the treatment of chronic skin wounds, infections, diarrhoea and diabetes (Amegbor *et al.*, 2012).

## Biological and pharmacological activities

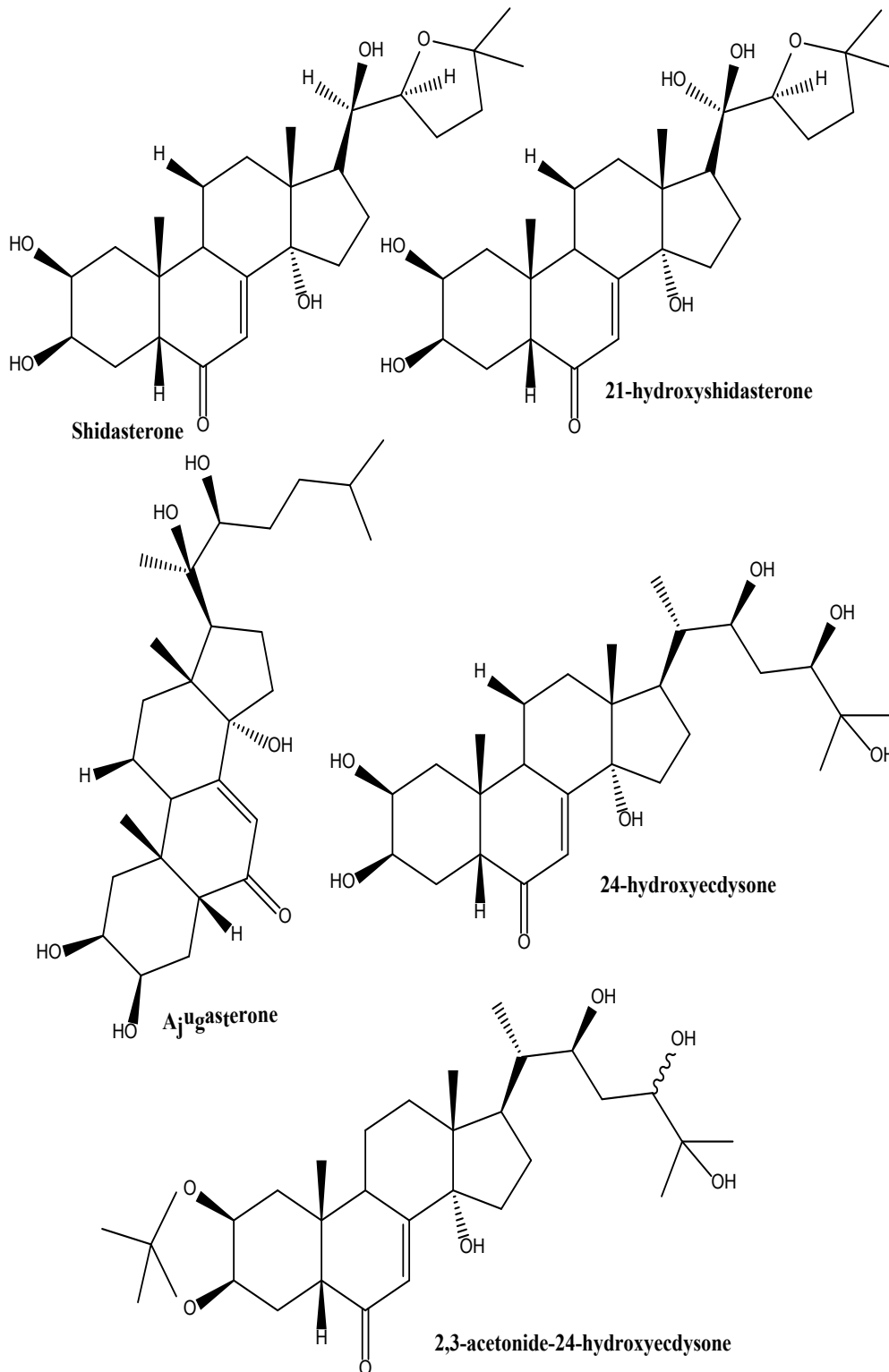
The ethanolic fruit extract of *V. doniana* demonstrated concentration-dependent inhibition of both acetylcholine and histamine-induced contractions. The extract in the same study repressed gastric peristalsis in mice fed with charcoal meal and protected them from castor oil-induced diarrhoea (Suleiman and Yusuf, 2008). The root extract exhibited antiviral activity by inhibiting the replication of HIV-1 *in vitro*. Concomitant administration of the extract and antiretroviral drugs, did not show any interference in activity of the latter (Suleiman and Yusuf, 2008). Neuwinger (2000) reported the antitrypanosomal activity of the stem bark extract against *Trypanosoma brucei*. The extract exhibited dose-dependent antiplasmodial activity against *Plasmodium falciparum* (Mudi, 2011). The antioxidant activities of the leaf, stem and root have been reported (Bolanle *et al.*, 2014). Aqueous extracts of the root bark, leaves and stem bark exhibited hepatoprotective effect by significantly lowering liver enzyme biomarkers in rats, which were initially increased by the administration of CCl<sub>4</sub> (Bolanle *et al.* 2014). Phytoecdysteroids isolated from the methanol stem bark significantly ( $P \leq 0.05$ ) demonstrated antiinflammatory activity at 100 mg/kg dose on rat paw oedema induced by carrageenan in Sprague Dawley rats (Ochieng *et al.*, 2012). Aqueous leaf extract showed antidiabetic activity in Wistar albino rats by causing considerable decrease in blood sugar levels from 492.8 to 84.5 mg/dl (Ezekwesili *et al.*, 2012). Kilani (2006) demonstrated the antibacterial activity of the methanol stem bark extract against clinical strains of *Salmonella typhi*, *Shigella dysenteriae* and *Escherichia coli*. Extracts of fruits showed transient reduction in reproductive functioning in female olive baboons (*Papio hamadryas anubis*). The presence of progestogen-like compounds in the fruit has been suggested as probable cause of fertility reduction (Higham *et al.*, 2007).

## Clinical data

None documented

## Chemical constituents

Triterpenoids 1 $\alpha$ , 3 $\beta$ -dihydroxybauer-7-en-28 oic acid, 2 $\beta$ , 3 $\beta$ , 19 $\alpha$ , 24-tetrahydroxy-23-norus-12-en-28-oic acid and (3 $\beta$ , 5 $\alpha$ , 7 $\beta$ )-3,7-dihydroxy-4, 4, 14-trimethyl-11, 15-dioxochol-8-en-24-oic acid are listed in the leaves (Mohammed *et al.*, 2016); 20-hydroxyecdysone (Tijjani *et al.* 2017), 11 $\beta$ -hydroxy-20-deoxyshidasterone, 21-hydroxyshidasterone, 2, 3-acetonide-24-hydroxyecdysone, ajugasterone, shidasterone, 24-hydroxyecdysone, 11 $\beta$ , 24-dihydroxyecdysone (Ochieng *et al.*, 2013), quercetin and myricetin (Mohammed *et al.*, 2017).



### Test for identity and purity

**Moisture content:** air dried coarse powder does not lose more than 6.3% (leaves) and 5.2% (stem bark) at 105°C.

**Total ash:** not more than 8.3% (leaves) and 5.4% (stem bark)

**Acid insoluble ash:** not more than 1.0% (leaves) and 0.8% (stem bark)

**Water soluble extractive:** not less than 11% (leaves) and 5% (stem bark)



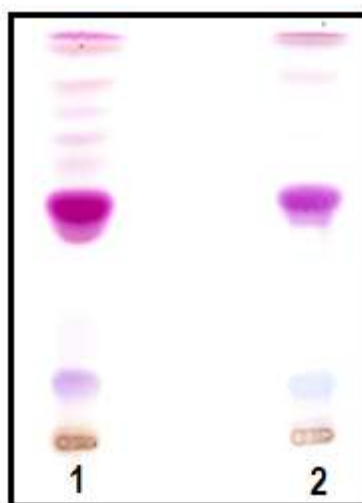
**Ethanol soluble extractive (70%):** not less than 4% (leaves) and 5% (stem bark)

### Chromatographic fingerprint

#### *Thin Layer Chromatography*

**Preparation:** About 5 g of the powdered leaves were extracted with ethyl acetate by cold maceration, filtered and the filtrate concentrated to a small volume. A small spot was then applied to the TLC plate for analysis.

**Chromatographic conditions:** Analytical TLC on silica gel G60 F254, 0.25 mm layer in hexane/ethyl acetate (7:3) as the mobile phase.



**Detection:** Visualized in daylight after spraying with anisaldehyde-sulphuric acid (Lane 1) and vanillin-sulphuric acid reagents (Lane 2) (Stahl, 1969), heating to 110°C for 10 mins.

The TLC chromatogram showed one prominent spot with Rf of 0.88 (pink) when sprayed with both anisaldehyde and vanillin. Five additional spots, appeared with Rfs of 0.82 (pink), 0.75 (pink), 0.67 (pink), 0.56 (purple) and 0.14 (mauve) in the chromatogram sprayed with anisaldehyde. The spots at Rfs of 0.56 and 0.14 appeared violet and light blue respectively when sprayed with vanillin.

#### *High Performance Liquid Chromatography*

**Sample preparation:** About 10 mg of the hydroethanolic extract of *Vitex doniana* leaves were reconstituted in 3 mL acetonitrile in a 10 mL volumetric flask with sonication for 17 minutes. The solution was then diluted to volume with the mobile phase under chromatographic conditions. It was centrifuged to obtain a clear test solution which was subsequently filtered through a 0.45 µm filter into an HPLC vial and analyzed

### Chromatographic system

#### Optimized chromatographic conditions

**Mode:** LC

**Column:** YMC ODS, 4.6 x 150 mm, 5 µm

**Column temperature:** Ambient – 30°C

**Mobile phase:** Acetonitrile: Methanol: Water (60:20:20 v/v/v)

**Elution mode :** Isocratic

**Injection volume :** 20  $\mu$ L

**Flow rate:** 0.5mL/minute

**Detection wavelengths:** 230 nm, 254 nm and 278 nm.

### System Suitability parameters

**Number of peaks:** 230 nm (1), 254 nm (1), 278 nm (2)

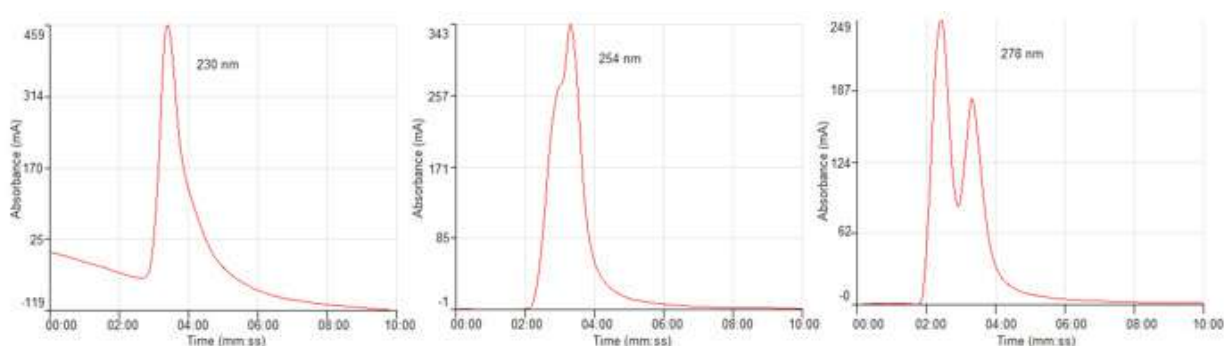
**Retention time (s):** 230 nm (2.13 min), 254 nm (3.18 min), 278 nm (rt1-2.26 min, rt2-3.19 min)

**Asymmetric factor(s):** 230 nm (1.172), 254 nm (0.993), 278 nm (af1-1.253, af2-1.030)

**Tailing factor:** NMT 2.0

**Efficiency:** 230 nm (102.49), 254 nm (63.48), 278 nm (E1-123.40, E2- 274.00)

**Acceptance criteria:** Sample solution of hydro-ethanolic crude extract of *V. doniana* Sweet (Leaves) conforms to the system suitability parameters.



### HPLC chromatogram of *VITEX donian*

#### FT-IR

A small amount of the dried hydroethanolic extract (70%) was placed on the sample area of the Perkin Elmer UATR Fourier Transform Infrared (FT-IR) spectrometer and scanned between 4000-400  $\text{cm}^{-1}$  with a resolving power of 4  $\text{cm}^{-1}$  and a cumulative scanning limitation of 24 times. Principal peaks appeared at wavenumbers 3278.78, 2925 and 1599.28  $\text{cm}^{-1}$ .

#### Macroscopy

Finely cracked and fibrous bark, grey to light brown, with a yellowish edge, quickly becoming brown; twig grey-brown, more or less pubescent, becoming glabrous. Opposite, digitate compound leaves, with 5 obovate to elliptical leaflets, 7-15 (-18) x 5-9 (-10) cm, the central leaflets being larger than the lateral ones; laminae glabrous or more or less pubescent below, leathery, with full edge, obtuse apex or wedge, sometimes obscurely acuminate, wedge-shaped or attenuated: pubescent glabrous petiole, 7-15 cm long; petiolule 0.5-2.5 cm long; pinnate venation, with 9-12 pairs of protruding secondary ribs connecting towards the apex, erased parallel nerves. Fruit, globose and glabrous drupe, about 2.5 cm long, surrounded at the base of the persistent calyx and enlarged in a cup, blackish at maturity, enclosing a hard core embedded in a thin pulp.

#### Microscopy

The entire leaf surface is covered with stellate and dendritic trichomes with a high trichome density on the

abaxial surface. Very few anomocytic stomata are seen on the abaxial but absent from the adaxial surface. A number of secretory cavities are found on both leaf surfaces. Small sized, straight walled epidermal cells can be found on both surfaces.

### Transverse section

Has an almost flat dorsal surface with a bulge on the ventral or abaxial surface. Stellate trichomes abound on the lower surface. There is a single row of epidermal cells covered with a very thin cuticle. The vascular bundle is surrounded by a sclerenchymatous sheath.

### Fruit

Characterized by numerous reticulate elements; large parenchyma cells; diagnostic annular xylem vessels that are pear shaped and fragments of seed coat with polygonal cells with thick walls.

### Powder microscopy of the leaf

Leaf fragments containing few isolated stomata can be seen. A number of stellate dendritic trichomes and their fragments are scattered about. Epidermal cell fragments and few xylem vessels can also be seen

### Therapeutic actions

Healing, antidiarrhoeal, antimicrobial, antidiabetic, ophthalmic, analgesic, antioxidant

### Therapeutic indications

Microbial infections, diarrhoea, oxidative stress, wound, pain, rheumatism, diabetes.

### Safety data

The LD<sub>50</sub> by oral route was above 3000 mg/kg in rats. There were no signs of CNS depression/stimulation or autonomic effects up to the highest dose of 1000 mg/kg. In subacute toxicity studies, gross anatomy of the liver, kidney, heart and lungs did not change. The relative organ to body ratios of spleen, thymus and adrenals were not altered significantly by the treatment. At all doses (100-1000 mg/kg), Vitex extract enhanced RBC count, haemoglobin concentration as well as the pack cell volume. It did not affect MCH, MCHC and MVC. Lymphocyte count also increased at all doses. This resulted in mild increase in WBC especially in low doses of the extract used. Neutrophils decreased significantly at all doses but MID cells were not affected. Platelet count was also not affected. The extract had no effect on AST, ALP, GGT, but reduced ALT. Serum proteins and bilirubin were not altered by the treatment. The extract did not affect serum urea and creatinine and hence renal function. Pentobarbitone-induced sleeping time was prolonged marginally at the highest dose of 1000 mg/kg. Histopathology did not reveal damage to the kidneys and liver. Vitex appears to have a beneficial effect on haematological parameters. It significantly increased the number of red blood cells as well as their haemoglobin content and the pack cell volume. It is known to be immunostimulatory, and appears to increase lymphocytes and decrease neutrophils count. It did not affect platelet count. Vitex did not increase liver function enzymes, serum proteins as well as bilirubin levels. Renal function was also not affected significantly. However high doses of the root bark extract may cause mild necrosis in the liver and kidney (Abdulrahman *et al.*, 2007).

### Precautions for use

Should be used with care in children and pregnant women as well as in patients on other CNS medications.

**Adverse effects**

None known

**Dosage form**

Decoction, infusion, tincture

**Dosage**

Decoction: 30 g of dried plant material in 900 mL water; simmer until reduced to 600 mL; 1-3 tablespoonfuls daily

Infusion: 30 g of dried aerial part in 600 mL of water; 3-4 teacups daily

Tincture: 1:5, 45% ethanol; 5 ml three times daily

**Storage**

Keep in a cool, dry place.

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**Botanical name**

*Ximenia americana* L.

**Family**

Ximeniaceae

**Synonyms**

*Ximenia exarmata* F.Muell., *Heymassoli inermis* Aubl., *Heymassoli spinosa* Aubl.,  
*Ximenia inermis* L.

**Common Names**

Wild olive, wild lime; tallow nut; seaside plum, spiny plum, mountain plum; false sandalwood (English), citronnier, de mer; prune épine; prune de mer; prune bord de mer; prunellier chimène (French).

**Common Local names**

**Benin:** Bariba-Gamororou; Peulh- Golohi ; Yoruba-Igo.

**Burkina Faso:** Dioula-Minigoli; Fulfulde-Tchabouli; Moré-Lèega.

**Côte d'Ivoire:** Baoulé-Assoukrou; Bambara-Nongbé; Dioula-Nomnoundo

**Ghana:** Twi-Kwaemm, Samanankaa; Mole-lenga

**Guinea:** Malinké/Dioula-Tonkain, Sènè, Doungué, Gouani; Peuhl-Tybbhulé.

**Mali:** Bambara-Ntonké; Peulh- Ntonké

**Niger:** Gwandara-Tsada; Haoussa-Mararuwu; Germa-Lulay

**Nigeria:** Babur-Shamzura; Hausa-Tsada; Kanuri-Daad'um.

**Senegal:** Bambara-Tonga; Diola-Bu ripina; Socé- Tôko..

**Togo:** Ewé- Kotadiabli; Moba-Wongag; Yanga-Léang

**Description of the plant**

*Ximenia americana* is a shrub or small tree growing up to 4 to 5 m tall. The leaves are simple and alternate lanceolate to elliptical of variable thickness (semi-succulent to thin), obtuse or emarginate. Petioles are short, slender, growing up to 6 mm long. When crushed, young leaves smell of bitter almonds. The diameter of the trunk rarely exceeds 10 cm; the bark is dark brown to pale grey, smooth to scaly. The lax branch, generally divergent, is rounded or conical. There are purple-red branches. The species is remarkable for its stiff, straight, very sharp spines, with a spine usually found in the axils of each leaf. The inflorescences are small umbelliform racemes. The fragrant white, yellow-green or pink flowers occur in branched inflorescences borne on short peduncule. The fruits are globose to ellipsoid drupes about 3 cm long, 2.5 cm thick, glabrous, greenish from its youth, becoming yellowish (or, rarely, orange-red) at maturity, containing juicy pulp and one seed. The seed is woody, yellow growing up to 1.5 cm long, 1.2 cm thick with a fragile shell (Orwa et al., 2009)



A and B– *Ximenia americana* fruit and leaves, C – *Ximenia americana* L.

#### Herbarium specimen number

Benin: 2359 AP/HNB

Burkina Faso: MSAD 873 (CNSF), Guinko 121 (OAU)

Côte d'Ivoire: 18127 CNF

Ghana: GH 889/KNUST

Mali: 0764 / DMT

Senegal: IFAN 78

Togo: 05428 TG/HNT

#### Habitat and geographical distribution

*X. americana* occurs in all dry African savannahs, coastal sands, shoreline thickets in contact with tides and gallery forests. It grows in the undergrowth of the Sudan dry forest, especially on clay soils and also on the banks of rivers. It is common in the Guinean Sudano savannahs, from Senegal to the Central African Republic via Cameroon and Chad. The species is also widespread in the savannahs of Latin America, Australia and New Guinea.

#### Plant material of interest

Roots

#### Other part used

Barks and leaves

## Definition of plant material of interest

*Ximenia* consists of the roots of *Ximenia americana* L.

## Ethnomedical uses

The plant is well-known throughout the savannah of dry tropical Africa for its medicinal benefits. All parts are used in traditional medicine. A decoction of leafy twigs flavoured with lemon juice is prescribed in some cases of gonorrhoea. A macerate of the root of *Commiphora africana* and *X. americana* is used in the prevention or mitigation of heart problems. Powdered *X. americana* root powder is indicated for the treatment of gangrene. The root decoction is used for the treatment of gastric ulcer, and the pulverized roots are used against rheumatism (Fern, 2018). The leafy stems of *X. americana* are used for the treatment of angina pectoris, helminthiasis, fever, jaundice, yellow fever, pleurisy, stomachache and migraine. The root decoction treats dysentery, haemorrhoids, fever, leprosy, sleeping sickness, constipation, poisoning, mental diseases, oedema, schistosomiasis, shigellosis, amenorrhoea, jaundice, gangrene, albuminuria and female sterility. The fruits are edible. The seed contains an oil used in cooking (Arbonnier, 2002). The fruit is useful in treating chronic constipation. When eaten in large quantities it acts as a vermifuge. The skin of the fruit (epicarp) is astringent (Fern, 2018).

## Biological and pharmacological activities

The stem bark of *X. americana* is documented to show antipyretic activity comparable to lysine acetylsalicylate on the beer yeast induced hyperthermia rat. Crude extracts of *X. americana* show antimicrobial and antifungal activities. The methanol extract of the leaves inhibited the growth of *Neisseria gonorrhoea*, *Candida albicans* and *Cryptococcus neoformans* (Geyid *et al.*, 2005). Similarly the root and leaf aqueous and methanolic extracts of *X. americana* inhibited the growth of *Staphylococcus aureus* and *Klebsiella pneumoniae*, but *Salmonella typhi* and *Escherichia coli* were not affected (Omer and Elnima, 2003). The methanol stem bark extract showed antiviral effect against measles virus *in vitro* by the plaque reduction neutralization assay (Parker *et al.*, 2007). Methanol stem bark extract demonstrated *in vitro* antitrypanosomal activity against *Trypanosoma congolense* (Maikai *et al.*, 2008). The aqueous extract of the stem bark showed analgesic properties at doses of 10 to 100 mg/kg, comparable to phenylbutazone (Soro *et al.*, 2009). Similarly, antinociceptive effect of the methanol leaf extract was demonstrated by its inhibition of abdominal writhes induced by acetic acid at doses of 200, 400 and 600 mg/kg *i.p.* In the formalin test, the administration of 200, 400 and 600 mg/kg *i.p.* of the extract had no effect in the first phase (0 to 5 min), but produced a dose-dependent analgesic effect in the second phase (15 to 40 min) (Siddaiah *et al.*, 2009). Soro *et al.*, (2009) demonstrated the antipyretic effect of the aqueous stem bark extract in beer yeast induced hyperthermia in rats. Activity of the extract was comparable to the reference drug lysine acetylsalicylate (aspegic). Other studies found different solvent extracts of *X. americana* leaves show considerable antioxidant and antiinflammatory activities (Shettar *et al.*, 2015). *In vivo* antitumour activity was determined in CC531 colorectal rat model with significant anticancer activity following oral administration. In another study, sesquiterpenes, isolated from the stem bark of *X. americana* did not inhibit the growth of human leukaemia, human colon and human breast cancer cell lines (Monte *et al.*, 2012). Antiulcerogenic activity of *X. americana* aqueous stem bark extract (100, 200 and 400 mg/kg) was demonstrated by inhibition of the gastric lesions induced by ethanol, acidified ethanol and indomethacin. The extract reduced gastric contents and acidity but did not alter the production of gastric mucus. The activity was mediated in part by –SH and NO groups (Aragão *et al.*, 2018).

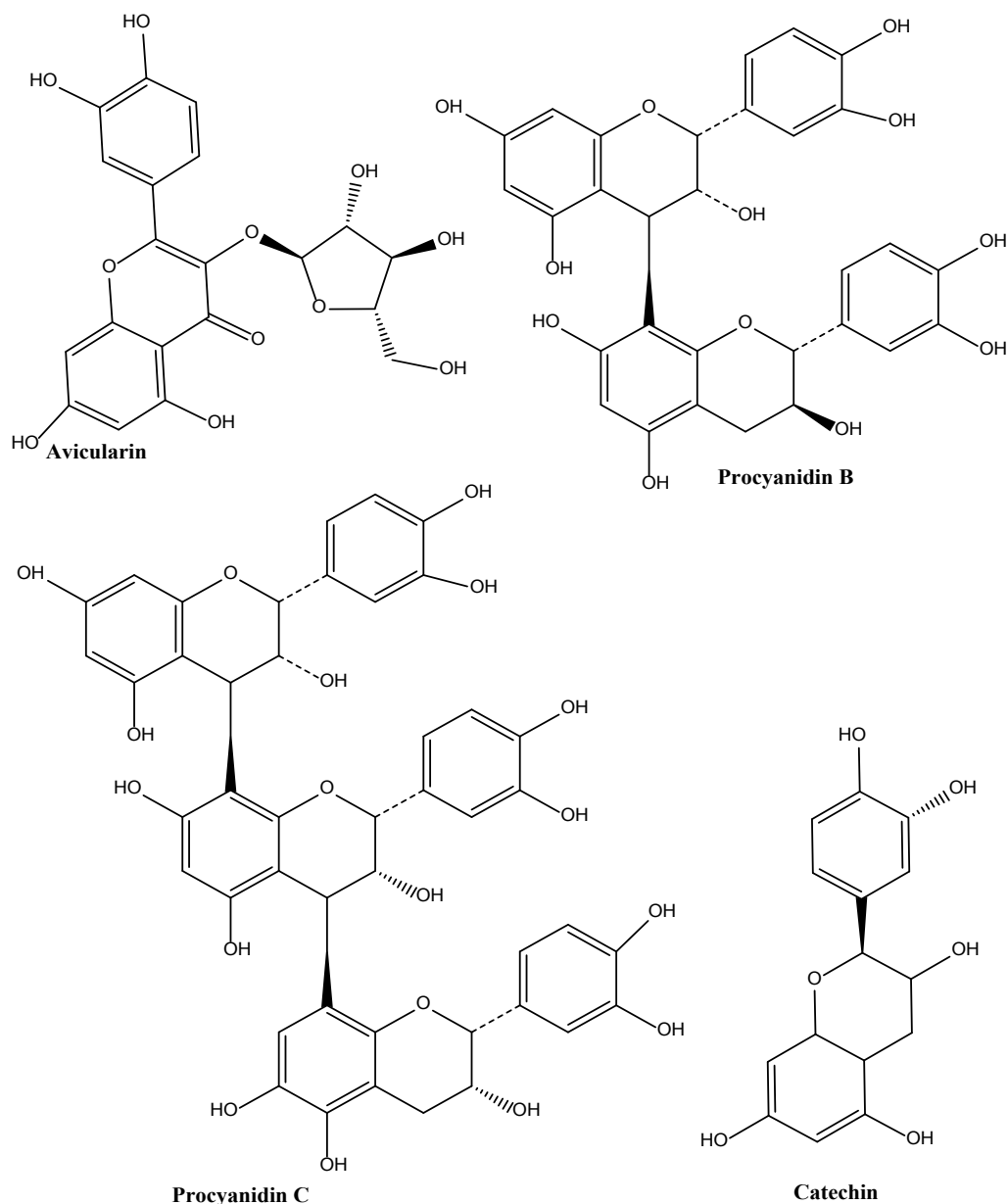
## Clinical data

Non documented



## Chemical constituents

3-Methyl-1-oxoisochroman-8-carboxylic acid, ergosta-4,6,8,22-tetraen-3-one (Abdalla *et al.*, 2013), cyanogenic glycoside sambunigrin, gallic acid,  $\beta$ -glucogallin, 1,6-digalloyl- $\beta$ -glucopyranose; quercetin, quercitrin, avicularin, quercetin-3-O- $\beta$ -xylopyranoside, quercetin-3-O-(6"-galloyl)- $\beta$ -glucopyranoside, kaempferol-3-O-(6"-galloyl)- $\beta$ -glucopyranoside, 3-olean-12-enyl palmitate (Fatope *et al.*, 2000),  $\beta$ -sitosterol, stearic and *trans*-4-octadecenoic acids.



## Test for identity and purity

**Moisture content:** air dried coarse root powder does not lose more than 10.2% moisture at 105°C.

**Total ash:** not more than 15.3%<sup>w/w</sup> (roots)

**Acid insoluble ash:** not more than 0.5 (leaves) and 6.3% (root)

**Water-soluble extractive:** not less than 20% (leaves) and 9.0% (root)

**Ethanol (70%) soluble extractive:** not less than 21% (leaves) and 7.0% (root).

## Chromatographic fingerprint

### *Thin Layer Chromatography*

**Preparation:** About 5 g of the powdered roots were extracted with ethyl acetate by cold maceration, filtered and the filtrate concentrated to a small volume. A small spot was then applied to the TLC plate for analysis.

**Chromatographic conditions:** Analytical TLC on silica gel G60 F254, 0.25 mm layer in hexane/ethyl acetate (7:3) as the mobile phase

**Detection:** Visualized in daylight after spraying with anisaldehyde-sulphuric acid (Lane 1) and vanillin-sulphuric acid reagents (Lane 2) (Stahl, 1969), heating to 110°C for 10 mins.

The TLC chromatogram showed two prominent spots with R<sub>f</sub>s of 0.87 (purple) and 0.73 (purple) when sprayed with both anisaldehyde and vanillin. An additional spot appeared at R<sub>f</sub> of 0.65 (purple) in the chromatogram sprayed with anisaldehyde.



### *High Performance Liquid Chromatography*

**Sample preparation:** About 10 mg of the hydroethanolic extract of *X. Americana* roots were reconstituted in 3 mL acetonitrile in a 10 mL volumetric flask with sonication for 17 minutes. The solution was then diluted to volume with the mobile phase under chromatographic conditions. It was centrifuged to obtain a clear test solution which was subsequently filtered through a 0.45 µm filter into an HPLC vial and analyzed

### Chromatographic system

#### Optimized chromatographic conditions

**Mode:** LC

**Column:** YMC ODS, 4.6 x 150 mm, 5 µm

**Column temperature:** Ambient – 30°C

**Mobile phase:** Acetonitrile: Methanol: Water (60:20:20 v/v/v)

**Elution mode :** Isocratic

**Injection volume :** 20 µL

**Flow rate:** 0.5 mL/minute

**Detection wavelengths:** 230 nm, 254 nm and 278 nm.

## System Suitability parameters

**Number of peaks:** 230 nm (1), 254 nm (2), 278 nm (1)

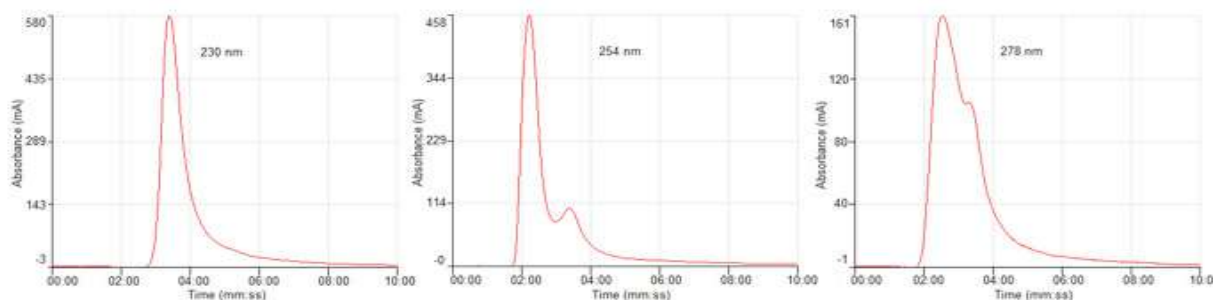
**Retention time(s):** 230 nm (3.23 min), 254 nm (af1-2.31 min, af2-3.18 min), 278 nm (3.20 min)

**Asymmetric factor(s):** 230 nm (0.546), 254 nm (af1-1.357, af2-1.661), 278 nm (1.162)

**Tailing factor:** NMT 2.0

**Efficiency:** 230 nm (161.46), 254 nm (E1-76.11, E2-415.12), 278 nm (94.7)

**Acceptance criteria:** Sample solution of hydro-ethanolic crude extract of *X. americana* L. roots conforms to the system suitability parameters.



## FT-IR

A small amount of the dried hydroethanolic extract (70%) was placed on the sample area of the Perkin Elmer UATR Fourier Transform Infrared (FT-IR) spectrometer and scanned between 4000-400  $\text{cm}^{-1}$  with a resolving power of 4  $\text{cm}^{-1}$  and a cumulative scanning limitation of 24 times. Principal peaks appeared at wavenumbers 3222.29, 2924.62 and 1603.53  $\text{cm}^{-1}$ .

## Microscopy

Lower epidermis of the leaf consists of polygonal cells with thick walls, and upper epidermis shows anomocytic stomata with subsidiary cells up to five in number and other epidermal cells which are polygonal and have slightly wavy walls.

### ***Transverse section of leaf***

There is a clear section of collenchyma with thick walls after the upper epidermis incurring into the vascular system. The vascular system follows with the phloem coming before the xylem section, forming an arc around the collenchyma. A layer of phloem fibres, which are circular in shape and have numerous calcium oxalate prisms, surrounds the xylem. At the connection between the midrib and the lamina is a small circle of phloem cells surrounded by a circle of parenchyma cells containing calcium oxalate prisms. Scattered in the collenchyma are numerous calcium oxalate prisms. The lamina has one row of palisade cells, which are columnar. Small vascular bundles traverse the lamina and spongy mesophyll are tightly packed with little intercellular spaces.

### ***Transverse section of root***

The transverse section of the root shows a large layer of cork cells followed by a section of parenchyma cells, which have calcium oxalate crystals. The cambium is made up of parenchyma cells forming a distinct demarcation. After the cambium, the xylem section shows large xylem vessels and is traversed by medullary rays.

**Powdered plant material***Root Powder*

Consists of fragments of fibres in bundles and singly, large sieve elements and large reticulate xylem vessels. Fragments of cork cells in both surface view and transverse view occur. There are groups of fibres with calcium oxalate crystals.

*Leaf Powder*

Consists of many fragments of the leaf showing polygonal cells of upper and anomocytic stomata of the lower surface; fibres and veins.

**Therapeutic actions**

Antipyretic, antimicrobial, antiparasitic, antinociceptive, antitumour, antiulcerogenic.

**Therapeutic indications**

Gonorrhoea, gangrene, rheumatism, parasitic diseases, dysentery.

**Safety data**

The LD<sub>50</sub> by oral route was estimated to be above 3000 mg/kg. There were no signs of CNS depression/stimulation or autonomic effects up to the highest dose of 1000 mg/kg. In sub-acute toxicity studies, gross anatomy of the liver, kidney, heart and lungs were not altered. The relative organ to body ratios of spleen, thymus, and adrenals were not significantly affected by treatment. It had no significant effects on RBC, WBC and platelets. Treatment increased AST although statistically insignificant. Generally, treatment had inhibitory effects on serum proteins. Globulins and albumin decreased although not statistically significant. Bilirubin was normal. Serum urea was lowered significantly but not creatinine. It also decreased the urea to creatinine ratio. Although Ximania showed high LD<sub>50</sub>, the extract may be toxic. It generally has insignificant effects on the hematopoietic system. Its toxicity is on serum proteins and liver enzymes. There was an increase in AST but not ALT.

**Precautions for use**

Caution must be exercised in active liver and kidney diseases.

**Adverse effects**

Restlessness

**Dosage forms**

Decoction, infusion, tincture

**Dosage**

Decoction: 30 g of dried plant material in 900 mL water; simmer until reduced to 600 mL; 1-3 tablespoonfuls daily

Infusion: 30 g of dried aerial part in 600 mL of water; 3-4 teacups daily

Tincture: 1:5, 45% ethanol; 5 ml three times daily

### Storage

Store in a cool dry place away from light

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