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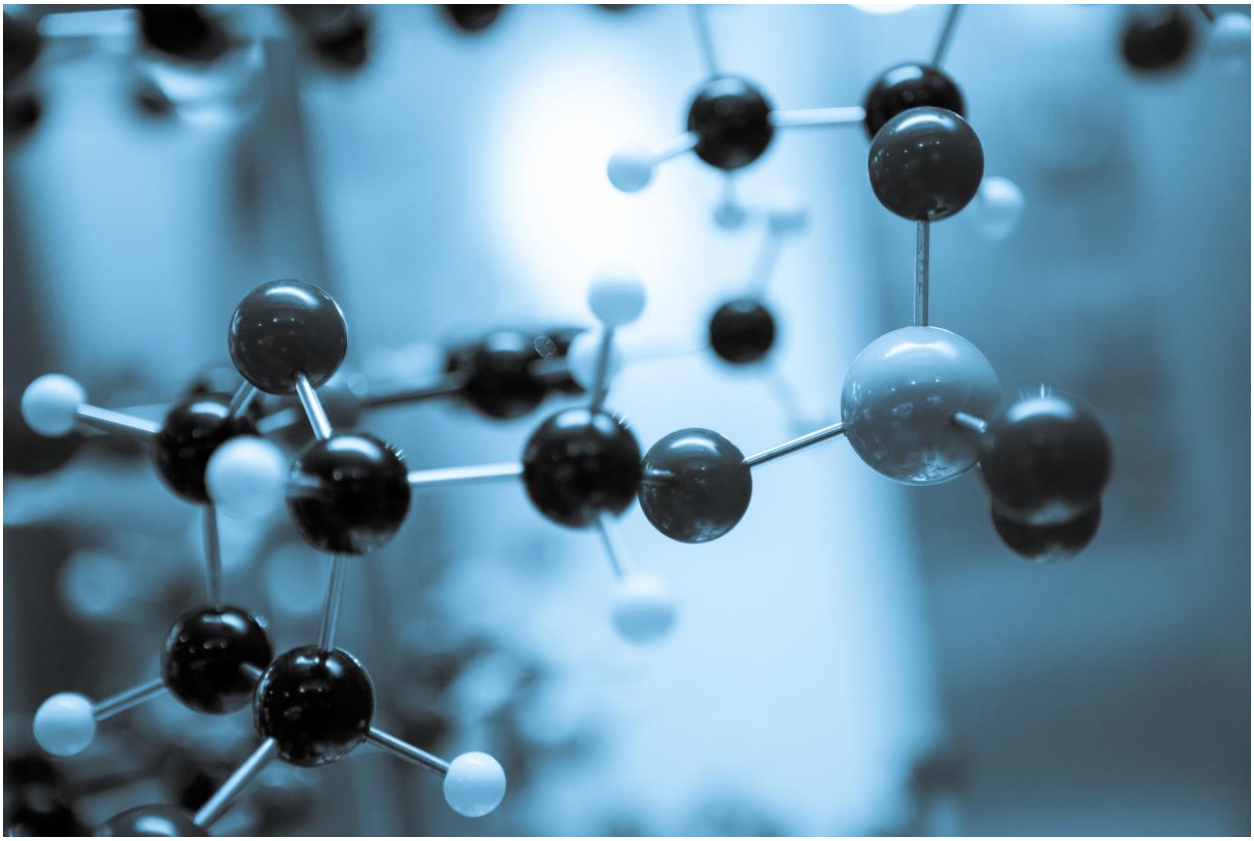

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Review Article

OLAX SUBSCORPIOIDEA OLIV. (OLACACEAE): AN ETHNOMEDICINAL AND PHARMACOLOGICAL REVIEW

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Abstract

Background: *Olax subscorpioidea* Oliv. (Olacaceae) is a woody shrub that is widely distributed in Africa. It has trado-medicinal importance and is used in the treatment of asthma, cancer, convulsion, diabetes, intestinal worm infections, jaundice, mental illnesses, neurodegenerative disorders, sexually transmitted infections, swellings and rheumatism, and yellow fever.

Aims: To review available literature on the phytochemistry, ethnobotany, pharmacology and toxicity of *Olax subscorpioidea* Oliv.

Method: Published findings were searched in online databases such as Web of Science, Scopus, Pubmed, Google Scholar and other relevant sources, and the data were sorted by relevance. Combinations of keywords used in the search include *Olax subscorpioidea*, Olacaceae, Olax, Ewe Ifon, and African medicinal plants.

Results: The presence of alkaloids, anthraquinones, cardiac glycosides, flavonoids, phenolic compounds, proanthocyanidins, saponins, tannins and triterpenes has been reported from *O. subscorpioidea*. Several secondary metabolites have been identified, importantly the cytotoxic santalbic acid from the seeds. Bioactivity studies on this plant demonstrated its medicinal potential mainly as an analgesic, anthelmintic, anti-arthritic, antidepressant, antihyperglycaemic, anti-inflammatory, antioxidant, antimalarial and antimicrobial agent. Oral acute toxicity of the leaf extracts in rats appears to be negligible.

Conclusion: Published literature available to date on *O. subscorpioidea* Oliv. provides some preliminary scientific basis for the ethnomedicinal uses of this plant. However, some ethnomedicinal uses have not been scientifically validated yet, and similarly, only a limited amount of information is available on properly isolated and identified phytochemicals from this plant that link to its bioactivities.

INTRODUCTION

Olax subscorpioidea is a shrub or tree which belongs to the family Olacaceae. The family Olacaceae comprises about 30 genera and about 200 species (Rogers, 2006). The genus *Olax* has more than 50 species of evergreen trees or shrubs. The name *Olax* has its origin in a Latin word which means odorous or malodorous due to the unpleasant smell of some parts of *Olax* plants, including the roots and stem barks of *O. subscorpioidea*. It can grow to a height of about 10 metres. The branches are slender, long and often hang downwards. The fruit is green while unripe but the ripe one is yellow. The roots and cut stem bark have garlic smell.

It is commonly called by several names by the local people, but the common name in English is Stink Ant forest (West African Herbal Pharmacopoiea, 2020). In Nigeria, the Yoruba people of Southwestern Nigeria call it “Ewe Ifon,” it is known as “Aziza” in Nsukka, “Ukpakon” among Esan people in Edo (Victoria et al., 2010), “Gwaanon kurmii” in Hausa, “Osaja or Igbulu” in Igboland and “Ocheja” among the Igalas (Odoma et al., 2015). The Fon or Dahomey people of Benin Republic know it as “Amitin”; Dioula people of Burkina Faso call it “Kouassoumbara”; it is known as “Samanua” (Akan), “hacbéchémon zaku” (Akye) and “akanji baka” (Ando) in Côte d’Ivoire; “Ahoohenedua” (Twi) in Ghana; “Gwano kurmi” (Gwandara) in Nigeria/Niger (West African Herbal Pharmacopoiea, 2020).

GEOGRAPHICAL DISTRIBUTION

O. subscorpioidea grows in most of the West and Central African countries (Figure 1). It can be found in Senegal, Nigeria (Ayandele & Adebiyi, 2007), Cameroon, Côte d’Ivoire, and the Democratic Republic of the Congo (Kazeem et al., 2015).

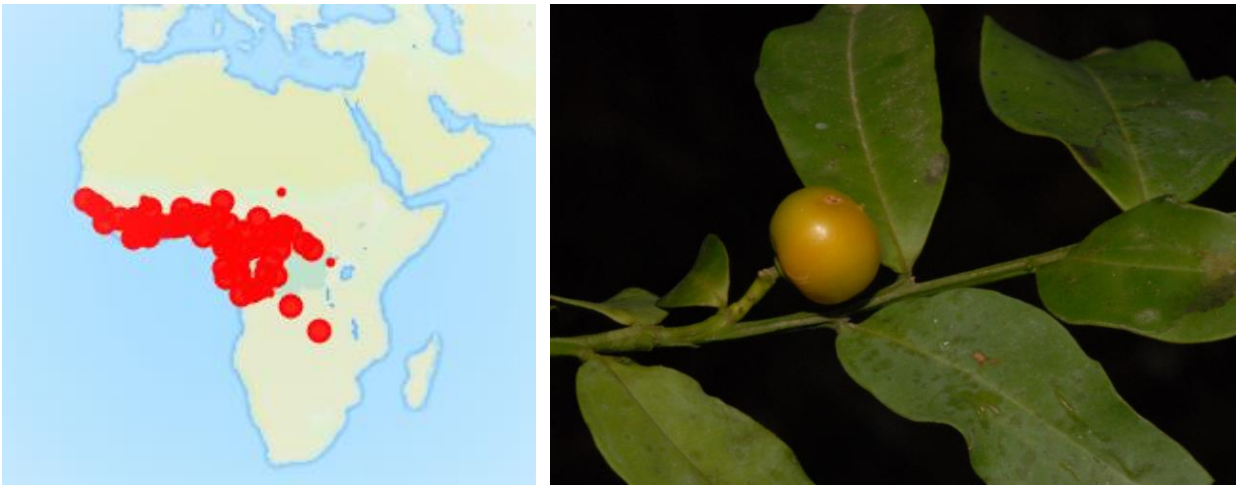


Figure 1. Geographical distribution (African Plant Database, 2022) and appearance of *O. subscorpioidea* Oliv. (Brunken et al., 2008)

SCIENTIFIC CLASSIFICATION

The scientific classification of *Olax subscorpioidea* Oliv. is shown below (The Plant List, 2013).

Kingdom:	Plantae
Clade:	Angioperms
Order:	Santalales
Family:	Olacaceae
Genus:	*Olax*
Species:	*Olax subscorpioidea* Oliv.
Synonyms:	*Olax chariensis* A. Chev, *Olax durandii* Engl., *Olax laurentii* (De Wild.) Engl., *Olax schlechteri* Engl., and *Ptychopetalum laurentii* De Wild.

ETHNOMEDICINAL USES

O. subscorpioidea maintains a rich medicinal usefulness among the Yoruba people in South-western Nigeria (Ajao et al., 2022) (Table 1). Historically, *O. subscorpioidea* (whole plant) is a part of medicinal recipes used for healing in most West and Central African countries (Table 1). This plant has also found its usefulness in foods (Kuete et al., 2011; Dzoyem et al., 2014). The oral decoction of the leaves of *O. subscorpioidea* has been traditionally used in the treatment of convulsion and mental illnesses (Nazifi et al., 2015), constipation, diabetes mellitus (Olabanji et al., 2014), Guinea worm, jaundice and venereal diseases such as infections of *Neisseria gonorrhea* (Okoli et al., 2007; Chukwuma et al., 2015), oedema, pains and swellings (Okoli et al., 2007; Chukwuma et al., 2015; Odoma et al., 2015). The root is used for the treatment of asthma (Sonibare & Gbile, 2008; Fatokun et al., 2016;), cancer (Soladoye et al., 2010), constipation and reduction of fat in pregnancy (Okoli et al., 2007), diabetes mellitus (Soladoye et al., 2012), rheumatoid arthritis (Ogunmefu & Gbile, 2012) and typhoid fever (Fadimu et al., 2014). The decoction of the roots with other plants such as *Alafia barteri* leaf, *Calliandra portoricensis* root, *Clausena anisata* stem bark; *Triclisia subcordata* leaf*, Xylopia aethiopica, Tephrosia vogelii* stem bark, *Anthocleista djalonensis* root, *Macaranga barteri* stem bark and potash is used in the management of breast cancer among Abeokuta people in Ogun State, Nigeria (Gbadamosi & Erinoso, 2016). The stem bark is used in treating infectious diseases (Ayandele & Adebiyi, 2007). The root is also used in Côte d'Ivoire to treat intestinal worm and malaria (Koné et al., 2012; Kipre et al., 2015). Stem and root barks have been reported to be used in treating jaundice, malaria and sexually transmitted infection in Ekiti State, Nigeria (Kayode, 2015).

The Gwandara tribe (Nigeria) use the whole plant to treat mental illnesses (Ibrahim et al., 2007). The root, leaf and stem bark are traded in Lagos (Nigeria) as a remedy for guinea worms, jaundice, mental disorder, toothache, venereal diseases, and yellow fever (Olowokudejo et al., 2008). In Ibadan (Nigeria), the seeds of *O. subscorpioidea* mixed with other plants such as *Xylopia aethiopica* and *Tetrapleura tetraptera* then with traditional black soap are used in the treatment of scalp infections in children. Aqueous decoction of the roots together with other recipes is used in treating abscess (Aworinde & Erinoso, 2015). A survey of the indigenous recipe used in the management of haemorrhoids in six southwestern states in Nigeria included *O. subscorpioidea* (Soladoye et al., 2010). The root decoction is used in Ferkessedougou and Tiassale (Cote d'Ivoire) for the management of anaemia (Koné et al., 2012). An ethnobotanical survey revealed that *O. subscorpioidea* root is used in Bauchi Local Government, Bauchi State (Nigeria) as an aphrodisiac (Sabo et al., 2018).

Table 1. Traditional uses of *Olax subscorpioidea* Oliv. Among different people groups in Africa.

Region/Tribe	Local Name	Plant Part	Traditional Uses	Sources
Yoruba and Esan, Nigeria	Ifon, Ukpakon	Leaf	Yellow fever, pains, oedema	(Okoli et al., 2007; Ajao et al., 2022)
Ekiti, Nigeria	Ifon	Leaf Root stem	*Neisseria gonorrhoea* infections, jaundice, constipation, worm infections	(Okoli et al., 2007, Kayode, 2015)
South-western Nigeria	Ifon	Leaf, root	Diabetes mellitus	(Soladoye et al., 2012; Olabanji et al., 2014)
Yoruba, Nigeria	Ewe Ifon	Leaf	Convulsion in children	(Oyedapo et al., 1997)
Ogun, Nigeria	Ifon	Root	Cancer, breast cancer	(Soladoye et al., 2010; Gbadamosi & Erinoso, 2016; Popoola et al., 2016)
Gwandara, Nigeria	Gwano kurmi	Whole plant	Mental illness	(Ibrahim et al., 2007)
Lagos, Nigeria	Ifon	The root, leaf, stem bark	Jaundice, yellow fever, toothache, mental disorder, guinea worms, venereal diseases	(Olowokudejo et al., 2008)
Ferkessedougou and Tiassale, Cote d'Ivoire	–	Root decoction	Anaemia	(Koné et al., 2012)
Ibadan, Nigeria	Ifon	Root	Abscess in children	(Aworinde & Erinoso, 2015)
South-western Nigeria	Ifon	Root	Haemorrhoids	(Soladoye et al., 2010)
Igala, North Central Nigeria	Ocheja	Leaf	Swellings and pains	(Odoma et al., 2015)
Esanland, Nigeria	Ukpakon	Root	Reduction of fat in pregnancy, constipation	(Okoli et al., 2007)
Akan, Akye & Ando, Cote d'Ivoire	Samanua, hacbéchémon zaku, and akanji baka	–	Intestinal worm and malaria	(Koné et al., 2012)
South-western Nigeria	Ifon	Root	Asthma	(Sonibare & Gbile, 2008; Fatokun et al., 2016)
Bauchi, Nigeria	Gwaanon kurmii	Root	Aphrodisiac	(Sabo et al., 2018)
South-western Nigeria	Ifon tutu	Stem bark	Rheumatism	(Ogunmefu & Gbile, 2012)
Ibadan, Nigeria	Igi Ifon	Stem bark	Neurodegenerative diseases	(Sonibare & Ayoola, 2015)

PHYTOCHEMISTRY

Most of the phytochemical studies carried out on this plant to date is qualitative preliminary phytochemical screening without isolation and identification of individual phytochemicals. The methanol extract of *O. subscorpioidea* roots was found to contain alkaloids, anthraquinones, flavonoids, glycosides, proanthocyanidins, saponins, steroids, tannins and terpenes (Victoria et al., 2010; Gbadamosi et al., 2017) (Table 2). A similar preliminary phytochemical screening of the stem bark revealed the presence of alkaloids, flavonoids and steroids in both aqueous and ethanol extracts. The aqueous extract also contained saponins and tannins (Ayandele & Adebiyi, 2007). Nazifi et al. (2015) reported the presence of alkaloids, carbohydrate, cardiac glycosides, flavonoids, saponins and tannins in the methanol leaf extract of *O. subscorpioidea* while Odoma et al. (2015) reported their presence in the aqueous, *n*-butanol, ethyl acetate and *n*-hexane fractions obtained from methanol extract. A methanol seed extract was reported to contain alkaloids, anthraquinones, flavonoids, phenols, tannins and triterpenes (Fankam et al., 2011).

Some compounds reported from the leaf and seed of *O. subscorpioidea* are shown in Figure 3. The first compound reported from this plant is santalbic acid *(trans*-II -octadecen-9-ynoic acid). Santalbic acid (also known as ximenynic acid) was isolated from the seed of *O. subscorpioidea* and it was evaluated against *Artemia salina* for general toxicity (Cantrell et al., 2003). Santalbic acid was previously isolated and characterized from the kernels of *Santalum acuminatum* which showed antimicrobial activity (Jones et al., 1995). Other compounds revealed by HPLC analysis of the *n*-butanol leaf extract of *O. subscorpioidea* include caffeic acid, quercetin, morin and rutin (Adeoluwa et al., 2019). The GC-MS analysis of the *n*-hexane leaf extract indicated the presence of *n*-hexadecanoic acid (palmitic acid), 7,10,13-hexadecatrienoic acid and methyl ester, hentriacontane, 9,17-octadecadienal (Z)-, 9,12-octadecadienoic acid (*Z,Z*)-, squalene, nonacosane, octadecanoic acid (Oladipupo et al., 2018). No compounds have been isolated from the root and stem bark of this plant (Agbabiaka & Adebayo, 2021).

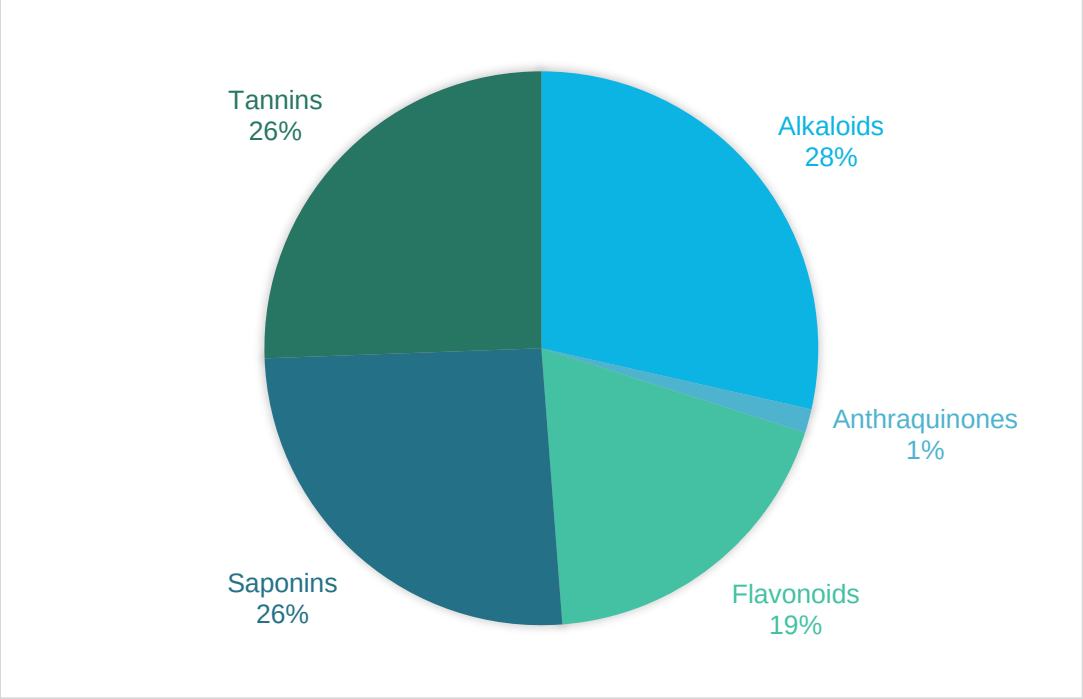
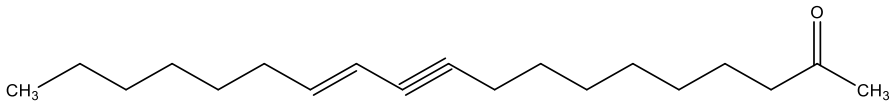


Figure 2. Phytochemical constituents of *O. subscorpioidea* Oliv. (Gbadamosi et al., 2017).

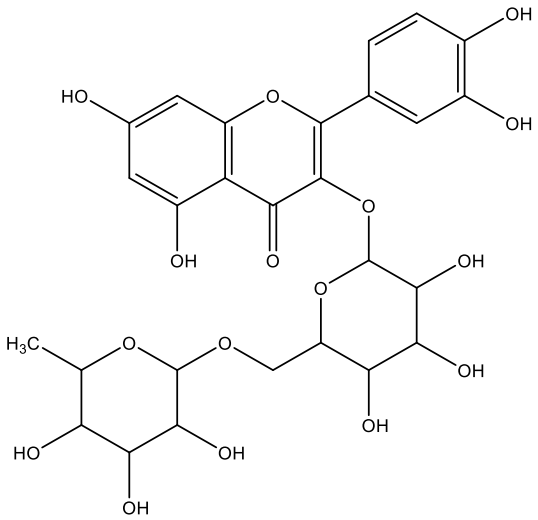
Table 2. Compounds identified from *O. subscorpioidea* Oliv.

Compounds	Method of Identification	Plant Parts	Solvents of Extraction	Biological Activities	Source
Santalbic acid	Flash Chromatography, prep-HPLC	Seed	Methanol	Brine shrimp toxicity	(Cantrell et al., 2003)
Quercetin	HPLC analysis	Leaf	*n*-Butanol	nd	(Adeoluwa et al., 2019)
Morin	HPLC analysis	Leaf	*n*-Butanol	nd	(Adeoluwa et al., 2019)
Rutin	HPLC analysis	Leaf	*n*-Butanol	nd	(Adeoluwa et al., 2019)
Caffeic acid	HPLC analysis	Leaf	*n*-Butanol	nd	(Adeoluwa et al., 2019)
n-Hexadecanoic acid (palmitic acid)	GC-MS analysis	Leaf	*n*-Hexane	nd	(Oladipupo et al., 2018)
7,10,13-Hexadecatrienoic acid and methyl ester	GC-MS analysis	Leaf	*n*-Hexane	nd	(Oladipupo et al., 2018)
Hentriacontane	GC-MS analysis	Leaf	*n*-Hexane	nd	(Oladipupo et al., 2018)
9,17 Octadecadienal (Z)-, 9,12 Octadecadienoic acid (Z,Z)-	GC-MS analysis	Leaf	*n*-Hexane	Nd	(Oladipupo et al., 2018)
Squalene	GC-MS analysis	Leaf	*n*-Hexane	nd	(Oladipupo et al., 2018)
Nonacosane	GC-MS analysis	Leaf	*n*-Hexane	nd	(Oladipupo et al., 2018)
Octadecanoic acid	GC-MS analysis	Leaf	*n*-Hexane	nd	(Oladipupo et al., 2018)

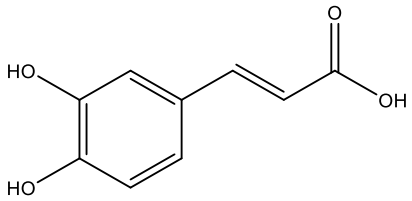
nd: activity not determined



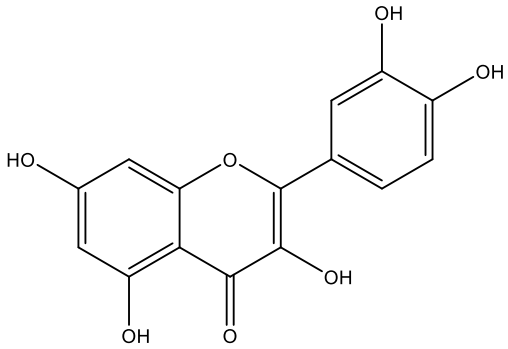
Santalbic acid*



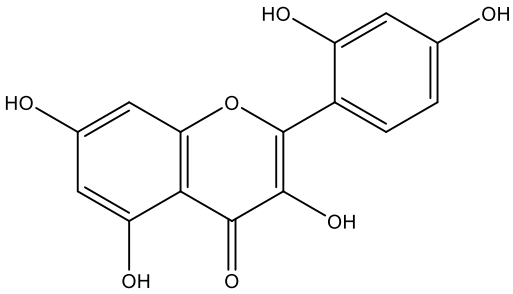
Rutin‡



Caffeic acid‡



Quercetin‡



Morin‡

Figure 3. Compounds identified from *O. subscorpioidea* Oliv. (*: from seed; ‡: from leaf).

BIOACTIVITIES LINKING ETHNOPHARMACOLOGICAL PROPERTIES

Many scientific studies have provided preliminary evidence for some of the ethnobotanical information on *O. subscorpioidea* and suggested that this plant could be a good source of new drug candidates (Table 3). Some of the pharmacological activities that have been established in the literature include anti-arthritics, anticonvulsive, antidepressant, antidiabetic, anti-inflammatory, antimicrobial, antinociceptive, antioxidant, antiproliferative, anti-ulcer, cytotoxic, and hepatoprotective effects, among others (Agbabiaka & Adebayo, 2021; Ahmad et al., 2021).

Anticancer activity

The root, seed and leaf extracts of *O. subscorpioidea* have been investigated for their anticancer potentials. Popoola et al. (2020) demonstrated chemotherapeutic potential of *O. subscorpioidea* root through its DNA-damaging effect and ability to ameliorate cisplatin-induced oxidative stress in an animal model. Using indices such as free-radical-scavenging, protection against oxidative stress, and lipid peroxidation, antimitotic effect and DNA damage, the study demonstrated possible chemopreventive and antiproliferative potentials of the extracts. A methanol extract of *O. subscorpioidea* root demonstrated potent Nrf-2-inducing, antioxidant and anti-inflammatory effects in cell-based models as a way to further establish its mechanisms of anticancer activity (Popoola et al., 2021). The leaf extracts were shown to induce intrinsic apoptosis via mitochondrial membrane permeability transition (Adegbite et al., 2015), while a seed extract displayed significant activity against multidrug resistance in CEM/ADR5000 cells (IC_{50}: 10.65 μg/mL) (Kuete et al., 2011).

Cytotoxicity and Genotoxicity

Brine shrimp lethality assay (BSLA) and *Allium cepa* root were used to assess the general toxicity and genotoxicity of the leaf and stem extracts of *O. subscorpioidea*. The leaf extract had IC_{50} of 10.7 µg/mL against brine shrimp (*Artemia salina*) nauplii, and 47.03 and 60.16 µg/mL against *Allium cepa* root after 24 and 48 h of exposure, respectively. The stem extract had IC_{50} of 45.2 µg/mL against brine shrimp *(Artemia salina)* nauplii, and 71.87 and 81.93 µg/mL against *Allium cepa* root after 24 and 48 h of exposure (Oladipupo et al., 2019).

Anticonvulsant activity

Pentylenetetrazole-induced and strychnine-induced seizure models in rats as well as maximal electroshock test in chicks were used to evaluate the anticonvulsant effect of the methanol leaf extract of *O. subscorpioidea* at different concentrations. At a dose of 200 mg/kg, 70% protection against maximal electroshock-induced seizure in chicks and 50% protection against strychnine-induced seizure in rats were observed (Nazifi et al., 2015). The leaf extract showed mild sedative property and it ameliorated symptoms of seizures using the pentylenetetrazole-, picrotoxin- and strychnine-induced convulsion models (Adeoluwa et al., 2016).

Antinociceptive activity

An ethanol extract of the leaf exhibited significant analgesic effect in both chemical (acetic acid-induced abdominal writhing and formalin paw-licking tests) and thermal (hot plate kept at 55±0.5°C) nociception models in mice in a dose-dependent manner (Adeoluwa et al., 2014). Ishola et al. (2015) estimated that the antinociceptive effect of the aqueous leave extract of O. *subscorpioidea* is mediated via serotonin ($5\text{-}HT_2$) and dopaminergic (D_2) pathways and sensitive potassium ATP channels. The root extract also displayed significant analgesic effect by inhibiting formalin-induced pain and anti-inflammatory effect by inhibiting xylene-induced ear oedema (Popoola et al., 2016). The analgesic activity of the leaf extract was established using acetic acid writhing induction, hot plate tests and formalin pain induction models in mice. The results showed that leaf extracts reduced induced pains significantly (Odoma et al., 2015). The study by Odoma et al. (2017) suggested that the mechanisms of analgesic activity might involve serotonin, opioid and nitric oxide-l-arginine pathways.

Anti-arthritic activity

In order to provide scientific basis for the popular use of this plant as an anti-arthritic remedy, the root extract of *O. subscorpioidea* was evaluated in an induced arthritis rat model using the chicken type II-complete Freund's adjuvant (CFA) (Ezeani et al., 2019). The aqueous and ethanol extracts of the roots showed significant anti-arthritic potential when compared to the positive reference drug indomethacin.

Table 3. Biological activities of *O. subscorpioidea* Oliv.

Activity	Plant part	Solvent of Extraction	Type of Experiment	Results	Sources
Anticancer	Seed, root	Methanol	*In vitro*	Seed: IC_{50}: 10.65 μg/mL against CEM/ADR5000 cells	(Kuete et al., 2011; Popoola et al., 2020)
Cytotoxicity	Seed, leaf, stem bark	Methanol	*In vitro* Brine shrimp lethality assay (BSLA)	Leaf IC_{50}: 10.7 µg/mL; Stem IC_{50}: 45.2 µg/mL; Seed IC_{50}: 44.8 µg/mL	(Cantrell et al., 2003; Oladipupo et al., 2019)
Antidepressant	Leaf	Ethanol	*In vivo*	Active	(Adeoluwa et al., 2016)
Antiulcer	Root	Methanol	*In vivo*	Active	(Victoria et al., 2010)
Anti-arthritis	Root	Aqueous, ethanol	*In vivo*	Active	(Ezeani et al., 2019)
Analgesic	Leaf, root	Ethanol	*In vivo*	Active	(Adeoluwa et al., 2014; Ishola et al., 2015; Odoma et al., 2015, 2017)
Antimicrobial	Root, seed	Ethanol, methanol	*In vitro*	MIC: 5–40 mg/mL (bacteria) MIC: 0.048–0.097 mg/mL (*Candida* sp)	(Ayandele & Adebiyi, 2007; Fankam et al., 2011; Dzoyem et al., 2014)
Antioxidant & anti-inflammatory	Leaf	Hydro-ethanolic, methanol, butanol, aqueous	*In vivo*	Active	(Konan et al., 2015; Odoma et al., 2015; Odoma et al., 2020)
Anthelmintic	Root	90% ethanol	*In vitro*	Active	(Koné et al., 2012)
Anticonvulsant	Leaf	Methanol	*In vivo*	Active	(Nazifi et al., 2015)
Hepatoprotective	Leaf	Hydro-ethanolic, ethanol	*In vivo*	Active	(Konan et al., 2015; Okoro et al., 2021)
Anti-hyperglycaemic	Leaf	n-hexane, ethyl acetate, acetone	*In vitro &* *In vivo*	n-hexane IC_{50}: 0.72 mg/mL (α-amylase); 0.10 mg/mL (α-glucosidase)	(Kazeem et al., 2015)
Hypolipidemic	Root	Ethanol	*In vivo*	Active	(Gbadamosi et al., 2017)

Antioxidant and anti-inflammatory activities

Konan et al. (2015) reported the *in vivo* antioxidant properties of the hydro-ethanolic leaf extract in experimental animals. Rats were injected intraperitoneally with hydro-ethanolic leaf extract *of O. subscorpioidea* at 25 mg/kg and 100 mg/kg body weight for 7 days and CCl_4 on the 7th day. The animals were sacrificed on the 8th day. Serum sample was collected and then analysed for antioxidant activity using DPPH free radical scavenging assay. Pretreatment of animals with hydro-ethanolic extracts of *O. subscorpioidea* was observed to significantly enhance serum antioxidant potential compared with CCl_4 treated group (Konan et al., 2015). Odoma et al. (2015) also reported anti-inflammatory potential of the methanol leaf extract using the carrageenan-induced hind paw oedema model in rats. In a 2020 study, using *n*-butanol and aqueous leaf fractions of *O. subscorpioidea,* a significant reduction in the concentrations of pro-inflammatory cytokines IL-1, VEGF & EGF, and a significant increase in the concentrations of anti-inflammatory cytokines IL-5, IL-6 & IFN-γ in the carrageenan-induced hind paws exudates in rats were observed. This study suggested that the plant's extracts inhibit pro-inflammatory cytokines IL-1, VEGF & EGF; and/or enhance the production of anti-inflammatory cytokines IL-5, IL-6 & IFN-γ (Odoma et al., 2020). Aqueous leaf extract was reported to have inhibited carrageenan-induced oedema by more than 70% (Ishola et al., 2015)

Antimicrobial activity

O. subscorpioidea root (ethanol extract) showed activities against Gram-negative bacteria, Gram-positive bacteria and some fungal species, with zones of inhibition ranging from 7.2 to 21.5 mm, and MICs ranging from 5 to 40 mg/mL (Ayandele & Adebiyi, 2007). Fankam et al. (2011) also reported antibacterial effect of the methanol seed extract against some strains of gram-negative bacteria, including MDR strains. *O. subscorpioidea* fruit extract also exhibited significant inhibitory activity against *Candida albicans* and *Candida tropicalis* (MIC, 0.097 mg/mL & 0.048 mg/mL, respectively). Oral pre-treatment of experimental rats with *O. subscorpioidea* fruit extract also displayed promising antifungal activity by reducing *Candida albicans* cell loads (cfu/mL) in rat blood (Dzoyem et al., 2014). An *in vitro* antimicrobial study against clinical oral isolates which include fungal (*Aspergillus fumigatus* and *Candida albicans*) and bacterial (*Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus species* and *Lactobacillus acidophilus*) strains was conducted using methanol stem bark extract of *O. subscorpioidea.* A more significant growth inhibition was observed for *Aspergillus fumigatus* (MIC: 51.2 mg/mL; zone of inhibition: 20–26.5 mm) (Orabueze, Amudalat, 2016).

Anthelmintic activity

A single oral dose of 400 mg/mL of *O. subscorpioidea* root extract was able to achieve total worm burden reduction of 60.2% and female worm burden reduction of 84.5% in *Schistosoma mansoni* infected mice (Koné et al., 2012).

Anti-ulcer activity

A methanol extract of *O. subscorpioidea* root produced dose-dependent anti-ulcer effect in indomethacin- and ethanol-induced ulcer models in experimental animals. A more significant reduction of the ulcers was observed in the ethanol model than the indomethacin model (Victoria et al., 2010).

Antidepressant activity

The antidepressant potential of *O. subscorpioidea* ethanol leaf extract was evaluated using the forced swimming, tail suspension, yohimbine-induced lethality and reserpine-induced depression models. The extract exhibited dose-dependent decrease in the immobility time in forced swimming test and in tail suspension test, and reduced diarrhoea in the reserpine-induced depression test (Adeoluwa et al., 2015). Adeoluwa et al. (2019) reported the involvement of monoaminergic pathways in the depression inhibitory effect of the butanol leaf extract of *O. subscorpioidea* in rats.

Antisickling activity

O. subscorpioidea together with other 27 plants formed a recipe for the management of sickle cell anaemia among indigenous people in Ibadan, Nigeria. An *in vitro* antisickling effect of this recipe was investigated using the method which involves inhibiting sodium metabisulphite-induced sickling of red blood cells collected from patients without symptoms of sickle cell disease. The result showed 63.4% inhibition of sickling of HbSS red blood cells (Egunyomi et al., 2009).

Hepatoprotective activity

A study by Konan et al. (2015) induced hepatotoxicity in rats using carbon tetrachloride (CCl_4), manifested with increase in the concentration of serum total bilirubin, GOT, GGT and ALP, as well as reduction in the serum activities of albumin, α_1-globulin and total protein. Pre-treatment with the hydro-ethanolic leaf extract of *O. subscorpioidea* was reported to decrease enzyme activities and total bilirubin levels. Histopathological examinations of the liver cells also revealed no significant cellular damage, as evidence of hepatoprotective properties. Similar results were reported for the ethanol extracts of *O. subscorpioidea* leaf and stem bark in CCl_4-induced hepatic injury in rats (Okoro et al., 2021).

Antihyperglycaemic activity

The *in vitro* effect of the *n*-hexane, ethyl acetate and acetone extracts of the leaf of *O. subscorpioidea* on diabetic-related α-amylase and α-glucosidase activities was examined. With *n*-hexane extract displaying highest inhibition on the enzymes (IC_{50}: 0.72 mg/mL for α-amylase; and 0.10 mg/mL for α-glucosidase), it was further studied in an *in vivo* model by administering it orally to starch-loaded rats. The rats' blood glucose level was monitored for 2 h. A significant reduction in the sugar levels of the rats was noted (Kazeem et al., 2015).

Hypolipidaemic activity

The lipid lowering potential (hypolipidaemic) of the ethanol root extract of *O. subscorpioidea* was studied in rats in order to provide scientific support for its use in the management of obesity in traditional medicine. A significant decrease was noted in total cholesterols (TC), triglycerides (TG) and low density lipoprotein (LDL) of extract-treated animal groups (Gbadamosi et al., 2017).

Antimalarial activity

The effect of *O. subscorpioidea* on the improvement of chloroquine efficacy was investigated by Kipre et al. (2015). When examined alone, the ethyl acetate leaf extract had IC_{50} of 32.47±0.31µg/mL and 28.16±0.5µg/mL against chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum,* respectively. However, addition of *O. subscorpioidea* at a concentration of 12 µg/mL to chloroquine against *Plasmodium falciparum* resistant strain FCB1 significantly reduced its IC_{50} to 43.5±1.5 nM from 105.05±2.87 nM (Kipre et al., 2015).

TOXICITY

An oral median lethal dose (LD_{50}) of >5,000 mg/kg was obtained for aqueous and butanol leaf fractions of O. *subscorpioidea* in mice and rats. However, a decreased value (2,154 mg/kg) was obtained for hexane fraction in mice and 3,808 mg/kg in rats (Odoma et al., 2015). The result also showed that the leaf extract induced some alterations on liver and kidney functions biomarkers (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, urea and creatinine, total bilirubin, total albumin) and haematopoietic parameters (white blood cells, % lymphocyte, % neutrophils, haemoglobin levels), but had no deleterious anatomical alterations on rats' liver and kidney tissues (Abiodun et al., 2014).

ETHNOMEDICINAL USES YET TO BE CONFIRMED BY SCIENTIFIC REPORT

Although the folklore claims of *O. subscorpioidea* have been largely established in the literature by both *in vivo* and *in vitro* experiments, evidence has not been provided for some of the traditional uses. In an ethnobotanical survey by Sabo et al. (2018), traditional users mentioned *O. subscorpioidea* root as a recipe in the management of sexual problems. It has also been reported that the plant is used in the management of asthma (Sonibare & Gbile, 2008) and haemorrhoids (Soladoye et al., 2010). There is scanty information in the literature linking *O. subscorpioidea* directly to aphrodisiac effect, asthma, constipation, haemorrhoids, hyperbilirubinaemia (jaundice) and viral infections (yellow fever) (Table 1).

CONCLUSIONS

O. subscorpioidea Oliv. is a key ingredient in Western-African traditional medicinal practices, with many ethnomedicinal uses, including as an aphrodisiac and in the management of asthma, cancer, constipation, convulsions, diabetes, haemorrhoids, jaundice, neurodegenerative disorders, rheumatism, sexually transmitted diseases (STDs), swelling and pains, worm infections in children, and yellow fever. Several of these popular uses have been confirmed by the scientific literature. However, its roles as an aphrodisiac and in the management of a few other conditions which include asthma, haemorrhoids and viral infection

are yet to be reported. Similarly, even though several studies confirmed anticancer potentials of the root extracts of *O. subscorpioidea,* with other studies suggesting the mechanisms of its chemopreventive and chemotherapeutic actions, its anticancer bioactive molecules have not been characterised. There is also a need for thorough phytochemical investigation of various extracts of several plant parts, especially the bioactive compounds responsible for the bioactivities of this plant.

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AUTHORS CONTRIBUTION

(Y.A.A.) (B.B.S.) (A.A.F.) (L.N.) (S.D.S.) Conceptualization, Methodology, Formal analysis, Investigation, Writing, Review & Editing.

Conflict of Interests

Authors declare no conflicts of interests.

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Original Article

PHYTOCHEMICAL FINGERPRINT AND BIOLOGICAL ACTIVITIES OF THREE MALAYSIAN *FICUS DELTOIDEA* CULTIVARS.

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Abstract

Background
Ficus deltoidea (Moraceae), is a Malay Traditional Medicine locally known as Mas Cotek. Three varieties (*angustifolia, deltoidea* and *kunslerii*) has been indictincty used.

Aims
We here aim to better understand their chemistry and bioactivities to inform future scientific and agronomic research.

Methods
We extracted and analyzed (HPTLC and HPLC-UV) samples from these varieties. The *in vitro* screening included the scavenging of DPPH and NO radicals, activity upon tyrosinase and cytotoxicity against three human prostate cancer cells (PC3, DU145 and LNCaP) using the sulforhodamine B proliferation assay and the MTT mitochondrial viability assay.

Results
We show that vitexin, orientin and isoorientin may act as intraspecific and interorgan phytomarkers The biological activities of the extracts point out to the antioxidant value of extracts from the *deltoidea* and *kunslerii* varieties whilst the inhibition of tyrosinase is only present in the roots extract of the var. *deltoidea* which is also endowed with cytotoxic activity against prostate cancer cells.

Conclusion
We suggest that the three Malaysian *Ficus deltoidea* botanical varieties (*angustifolia*, *deltoidea* and *kunslerii*) can be considered chemovars. The most active extract was from the roots of var. *deltoidea* that shows antioxidant, antimelanogenic and cytotoxic potential.

INTRODUCTION

Ficus deltoidea, known as Mas Cotek in Malaysia and as Malaysian Mistletoe Fig internationally, is one of the species of fig tree from the Moraceae family, which is a traditional medicinal herb and has been widely used in postpartum medication among the Malays for a long time (Bunawan et al., 2014). The functions of this herb are thought to be capable of detoxifying the body, reducing cholesterol, restoring energy, improving blood circulation, repairing blood flow and the problems associated with blood flow (Huda Farhana et al., 2007). Thus, *Ficus deltoidea* also has been indicated in the treatments of wounds, rheumatism and sores. In addition, it has been applied to treat disorders related with the menstrual cycle, diabetic leucorrhoea, high blood pressure and gout (Burkill and Haniff, 1930). Its fruits are traditionally used to relieve toothache, cold and headache by means of chewing. Apart from this, its formulated products such as capsules, tea, and tonic tea are distributed throughout Malaysia. The herbal juices made from *Ficus deltoidea* are often used to improve health and beauty (Ramamurthy et al., 2014).

Figure 1. *Ficus deltoidea* Jack (Credits: Forest and Kim Starr, under CC-BY-3.0).

Ficus deltoidea is attracting many researchers' attention and some active compounds have already been isolated and determined from its leaves and figs. There are three major groups of constituents which are phenolic compounds (catechins, flavones, naringin, vitexin, isovitexin), tannins (ellagic acid and gallic acid) and phenylpropenes (caffeic acid, coumaric acid and ferulic acid (Ramamurthy et al., 2014). Apart from this, some researchers have now also isolated and studied active compounds contributing to its floral fragrances (aliphatic groups and terpenoids including terpenes, triterpenes and sesquiterpenes) and fruits of *Ficus deltoidea* which are rich in volatile compounds deriving from the shikimic acid pathway (Grison-Pig´e et al., 2002). According to this research, it has been found that these constituents are related to many antioxidant, anti-inflammatory, antibacterial, anti-diabetic, anti-nociceptive, anti-ulcer, anti-hypertensive and multiple cancer preventives as well as other activities (Bunawan et al., 2014).

Like other botanicals with a wide range of medicinal properties, much research and experiments have been conducted to explore its potential therapeutic values especially in anti-diabetic activity, anti-nociceptive activity, antioxidant and anticancer related properties (anti-melanogenic effects and anti- Human leukemic HL-60 cell line properties) (Norrizah et al., 2012). To elaborate and clarify the effectiveness and mechanisms of action of *Ficus deltoidea*, *in vitro* (cell, non-cell) studies, *in vivo* studies, research in animal

subjects been conducted, but also sophisticated analytical techniques (Lip et al., 2009; Omar et al., 2011) have been applied in research to determine the structures of related compounds. However, compared to other studies, the anti-melanogenic effects of this plant species have received little scientific research and little success. One recent study (Oh et al., 2011) was carried out to evaluate the anti-melanogenic activity of *Ficus deltoidea* by testing its extract with cultured B16F1 melanoma cells. In addition, α-MSH-induced melanin synthesis (α-MSH) assay, intracellular tyrosinase activity assay and the expression of microphthalmia-associated transcription factors (MITFs) assay were also carried out by the authors. According to the writers, they have drawn the conclusion that the abilities of *Ficus deltoidea*, down-regulation of cellular melanogenic components including tyrosinase, MITF and α-MSH, show that this plant can be a promising whitening cosmetic active ingredient. However, the related information about this plant still seems to be very limited. Moreover, although researchers have pointed out the mechanisms of antioxidant of *Ficus deltoidea* are concerned with its major compounds- flavonoids and tannins in many cases, the chemical variations of different varieties of *Ficus deltoidea* as well as their bioactivities are seldom evaluated or studied (Dzolin et al., 2010). Therefore, further studies in these aspects on *Ficus deltoidea* can help researchers to understand its potential therapeutic values better.

This species presents a high regional variability with 2 subspecies, 13 varieties and 4 forms of the species have been recognized, the vast majority of them present in Malaysian forests: var. *bilobata*, var. *angustifolia*, var. *kunstleri*, var. *intermedia*, var. *motleyana*, var. *deltoidea*, var. *kinabaluensis* and var. *trengganuensis*. Local collectors of *F. deltoidea* identify them mainly based on the leaf and fruit morphology (Berg, 2003; Fatihah et al., 2012). Recently, the genetic basis for such variability have been studied and points towards a low intraspecific genetic diversity of *F. deltoidea* population in Malaysia (Zimisuhara et al., 2015).

We here aim to study the diversity in chemical constituents of *Ficus deltoidea* cultivars, by developing a HPTLC method to fingerprint these plant materials when extracted by different solvents (hexane extract, chloroform extract, water extract and ethyl acetate extract). The identity of characteristic compounds is achieved by the combination of HPTLC and HPLC-UV chromatography. Some basic biochemical properties such as scavenging against $DPPH^{\cdot}$ and $NO^{\cdot}$ radicals, inhibition of tyrosinase enzyme and cytotoxicity are also evaluated.

MATERIALS AND METHODS

Plant material

Plant materials used in this research were collected from Kedah, Malaysia over the period of September 2013 to February 2014. All the plant materials were authenticated by Mr Husnui Hanani Solb at Universiti Putra Malaysia, Malaysia. The vouchers were deposited at the Institute of Bioproduct Development, Universiti Teknologi Malaysia, Malaysia.

Table 1: Botanical characteristics of the *Ficus deltoidea* varieties.

Cultivars	Part	Code	Voucher
***Ficus deltoidea* var. *angustifolia* (Miq.) Corner**	Aerial	FDAa	SK 2309/13
Ficus deltoidea* Jack var. *deltoidea	Aerial	FDDa	SK 2310/13
Ficus deltoidea* Jack var. *deltoidea	Root	FDDr	SK 2311/13
***Ficus deltoidea* var. *kunsleri* (King) Corner**	Aerial	FDKa	SK 2312/13

Standards and other reagents

1,1-diphenyl-2-picrylhydrazyl, rutin, kaempferol, caffeic acid, quercetin, vitexin, *L*-tyrosine (reagent grade, ≥ 98%), and dimethyl sulfoxide were purchased from Sigma-Aldrich (Sigma-Aldrich, USA). Acetic acid (Fisher Scientific) and formic acid (for synthesis ≥97%, Alfa Aesar) were used. 0.5 g of diphenylboric acid aminoethyl ester was weighed and dissolved in 100 ml ethyl acetate (≥99.9 %, Fisher Scientific) as the natural product reagent for HPTLC. 2.5 g of polyethylene glycol-4000 (laboratory reagent) was weighed and dissolved in 50 ml absolute ethanol as the PEG reagent for HPTLC.

Preparation of plant extracts

50 grams of five plant samples were transferred into five Erlenmeyer flasks and 500 ml methanol (≥99.9% for HPLC, Sigma-Aldrich) was added in each of the flasks. Each flask was covered with aluminum foil and placed in a magnetic stirrer (KMO 2 basic, labortechnik, IKA). The samples were extracted with methanol for 24 hours. Then the solvent in each flask was collected and a fresh solvent was added to the plant samples. This procedure was repeated three times. Each extract was transferred into a round-bottom flask and the solvent was evaporated completely by a rotary evaporator (Laborota 4003 control, Heidolph). The dried plant samples were dissolved in 10% methanol (100 ml), and sonication bath (Grant Instruments Ltd) was applied to dissolve the plant samples in the solvent. Each 10% methanol extract was subjected to liquid-liquid partition with 100 ml hexane (≥99.9% for HPLC, Sigma-Aldrich) for 3 times and the hexane fraction was collected. The rest of the methanol extract was concentrated using a rotary evaporator to about 90 ml, and deionized water (Milli-Q water system, Millipore, Bedford, USA) was used to top-up the volume to 100 ml. Then the aqueous extract was sequentially partitioned with equal volume of chloroform (≥99.9% for HPLC, Sigma-Aldrich) and ethyl acetate (≥99.9% for HPLC, Sigma-Aldrich). In each partition step, the procedure was repeated 3 times. All of the chloroform fractions, aqueous fractions and ethyl acetate extracts were collected. Each fraction was transferred to a round-bottom flask. Then the solvents of each fraction (hexane, chloroform and ethyl acetate) were completely evaporated by a rotary evaporator whereas a freeze dryer was applied to eliminate the water in aqueous fractions. All the fractions were stored in a fridge (-20 ℃) until needed.

Thin Layer Chromatography (TLC) analyses

The TLC analysis was performed on 10×20 silica gel 60 F254 TLC plate, aluminum sheet Analytical Chromatography, (Merck, Germany) for identifying flavonoids compounds in different plant ethyl acetate extracts. Approximately 10 µL of each sample (10 mg/ml) was applied on each TLC plate along with reference standards (1 mg/ml). All these samples were spotted on the TLC plate, 1 cm from the bottom of the plate, and then developed in a TLC chamber (24 cm×24 cm) saturated with the developing solvent system (ethyl acetate: acetic acid: formic acid: water (100:11:11:26) (Sherma and Fried, 1996; Shafaei et l., 2012). After the development, the natural product reagent and polyethylene glycol (PEG) 4000 were sprayed on each plate to enhance the fluorescence and viewed under UV light at white light, 254 nm and 365 nm by HPTLC (Camag, TLC Visualizer).

High-Performance LIquid Chromatography (HPLC) analyses

For HPLC analysis, extracts were mixed with methanol at 10 mg/ml and dispersed in an ultrasonic bath. A volume of 5 ml of each sample was filtered through a 0.45µm filter before analysis. The rest were evaporated to dry and stored in a freezer. The filtered samples were injected (50 µL). The HPLC system was a Perkin Elmer series 200 EP Diode Array Detector combined with series 200 pump, Flexar LC autosampler and TotalChrom software (Perkin Elmer Company, USA), with an Agilent Aqua-C18 column (250mm×4.6mm i.d., 5µm) was used for the extract analysis. All samples were eluted with a mobile phase consisting of formic acid solution (A, 0.1 % v/v) and acetonitrile (B, ≥99.9% for HPLC, Fisher Scientific) using a linear gradient program (0%-25% B in 0–55 min, 25%-30% B in 55-65 min, 30%-40% B in 65-75 min, 40%-100% B in 75-77 min, 100% B in 77-79 min). The flow rate was 1.0 mL/min and the detector wavelength was 365nm. Each standard was dissolved in 1 mg/mL methanol and 10 µL injected.

1,1-Diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging assay

This assay measures the free radical scavenging potential of each crude extract. The method described by Sharma & Bhat (2009) and modified by Prieto et al. (2012) was used. Briefly, 100 µL of a 0.1 mM DPPH ethanolic solution was added to 100 µL of each diluted extract or reference standard antioxidant in 96-microwell plates. After 30 min of incubation in the darkness at room temperature the absorbance was measured at 517 nm against a blank.

% radical scavenging activity = [1- [(Abs Sample + DPPH) – (Abs Sample Blank)] / (Abs Control)] x 100

Nitric oxide scavenging assay

The experimental protocol is based on the Griess reaction as previously described (Sreejayan, 1997). A volume of 200µl of sodium nitroprusside (5mM) and 50µl of sample are mixed in microtiter plates (96 wells). At 1-hour intervals, pipet 50µl supernatant onto a second plate, add 50µl of Griess reagent (1% sulphanilamide, 0.1%). This was then incubated again at room temperature for an additional 15 minutes. The absorbance was read at 540 nm and the percentage of NO inhibition and total NO remaining in solution was calculated using a calibration curve built up with Sodium Nitrite.

% NO scavenging = [(conc. of control - conc. of extract)/ conc. of control)] x 100.

Cell Lines

The following tumour cell lines were used: the PC-3 cell line (ATCC Number: CRL-1435™) was kindly provided by Dr Cyrill Bussy (Centre for Drug Delivery Research, UCL School of Pharmacy, UK), the DU145 cell line (ATCC Number: HTB-81™) was purchased from Sigma Aldrich UK, and was obtained from the American Type Culture Collection (ATCC) and the LNCaP clone FGC cell line (ATCC Number: CRL-1740™) was purchased from Sigma Aldrich UK, and was obtained from the American Type Culture Collection (ATCC). All cell lines are adherent cells that tend to grow as monolayer and are classified as Biosafety Level 1. cells.

Cell Culture Protocols

Both PC-3 and LNCaP cell lines were grown in a cell culture flask (Nunc, UK), surface area 75cm^2 and maintained in RPMI-1640 (Roswell Park Memorial Institute medium) (Lonza, BE12-702F) containing L-glutamine. The media was supplemented with 10% of heat-inactivated fetal bovine serum (FBS) (Gibco, UK) and 1% penicillin-streptomycin antibiotics containing 10000 Units/ml of penicillin and 10000 µg/ml streptomycin (Gibco, UK) to prevent bacterial growth. DU145 cell line was grown in a cell culture flask (Nunc), surface area 75cm^2 and maintained in EMEM (Eagle's Minimum Essential Medium) (Sigma, M4655) containing Earle's salt, L-glutamine and Sodium bicarbonate in an incubator (NuAir Inc.) with humidified air of 5% CO_2 and atmosphere at 37ºC (Freshney, 2005). The media was supplemented with 10% of heat-inactivated fetal bovine serum (Gibco, UK) and 1% penicillin-streptomycin antibiotics containing 10000 Units/ml of penicillin and 10000 µg/ml streptomycin (Gibco, UK) to prevent bacterial growth. All cells were maintained at 37ºC in a humidified atmosphere of 5% CO_2. The prepared media was used to grow and seed the cells in a 96-well plate for cellular based assays and for plant extracts as well as fractions dilution.

Tyrosinase inhibitory activity

Quantitative inhibition was assessed according to the method described by Masuda et al. (2005). 2.5 mM L-tyrosine in buffer and the sample in DMSO are mixed in a total volume of 160 µL, to which 40 µL of mushroom tyrosinase (46 units/mL) is added. After 20 minutes the absorbance was measured for each well at 475 nm, then the % of Tyrosinase inhibition calculated against the control.

$$\%Tyrosinase\ activity = \frac{Absorbance\ (sample) - Absorbance\ (blank)}{Absorbance\ (vehicle\ control) - Absorbance\ (blank)} \times 100$$

Sulforhodamine B assay (SRB) proliferation assay

The assay was performed independently in triplicate according to a previously described by Vichai & Kirtikara (2006). The cells were seeded at density of 10,000 cells/well in a 96-well plate (Thermo Scientific) and left overnight at 37°C to adhere. Afterwards, cells were treated with a serial dilution of the plant extracts (200, 100, 50, 25, 12.5, 6.25 µg/mL) at several time points. Upon completion of the incubation period, the cells were fixed with trichloroacetic acid solution for 1 h at 4°C. After washing with water, cellular protein was stained with SRB solution and left at room temperature for 1 h, followed by washing the plate four times with 1% acetic acid and flicking to remove unbound dye. Then, Tris base buffer solution was added to each well and the absorbance was measured at 510 nm. Cell growth was calculated using the following equation:

$$\%Cell\ growth = \frac{Absorbance\ (sample) - Absorbance\ (blank)}{Absorbance\ (vehicle\ control) - Absorbance\ (blank)} \times 100$$

Mitochondrial viability Assay (MTT)

The MTT assay was performed independently in triplicate according to the previously described method by Mosmann (1983). Briefly, 10 µL of the MTT solution (5 mg/ml dissolved in PBS) was added into all wells after the incubation period and then further incubated for 4 hours. After 4 hours in a humidified atmosphere at 37°C, both the cell media and the MTT solution were removed from the wells and 200µl of DMSO was added in each well in order to allow dissolution of the purple MTT-formazan crystals. The absorbance (optical density, OD) was measured at a wavelength of 570 nm and reference wavelength 630 nm with a microplate reader (Tecan Infinite® M200). The relative difference to control was determined by the following equation:

$$Relative\ difference\ to\ control = \frac{OD\ (sample)\ -\ OD\ (Blank)}{OD\ (Control)\ -\ OD(Blank}$$

Statistics

The results present in this study were repeated three times (twice for the negative results). Data analysis was performed by Excel 2013 (Microsoft office, USA) and Graphpad prism version 6.0 (San Diego, USA). The results are given as Mean ± SD.

RESULTS AND DISCUSSION

Yield of extracts

The yields of hexane extracts, chloroform extracts, water extracts and ethyl acetate extract of the three varieties of *F. deltoidea* were calculated and are presented in Table 2. Water soluble matter predominates in all extracts. The yield of the ethyl acetate soluble matter clearly differentiates *kunsleri* variety clearly from *angustifolia* and *deltoidea* varieties.

Table 2. Yield of the *Ficus deltoidea* chemovars

Variety	Fractions	Yield (%)
FDAa	Hexane	0.27
	Chloroform	0.96
	Aqueous	13.18
	Hexane	0.22
FDDa	Chloroform	0.65
	Aqueous	13.10
	Ethyl Acetate	1.95
FDDr	Hexane	0.24
	Chloroform	0.25
	Aqueous	6.92
	Ethyl Acetate	1.79
FDKa	Hexane	0.58
	Chloroform	0.60
	Aqueous	7.70
	Ethyl Acetate	4.30

HPTLC fingerprint of Ficus deltoidea varieties

A targeted chromatographic fingerprint using HPTLC was performed (Figure 2). The results of the HPTLC analyses (shown in Figure 2 and summarised in Table 3) clearly point out that these Malaysian *Ficus deltoidea* varieties are chemovars: orientin in the aerial parts can differentiate *kunslerii* (+) from the other two (-). Isoorientin differentiates *angustifolia* (-) from *deltoidea* (+). Vitexin also qualitatively differentiates *angustifolia* (+) from *deltoidea* (-). Moreover, the presence of vitexin and orienting differentiates the aerial parts from the roots of *deltoidea* variety.

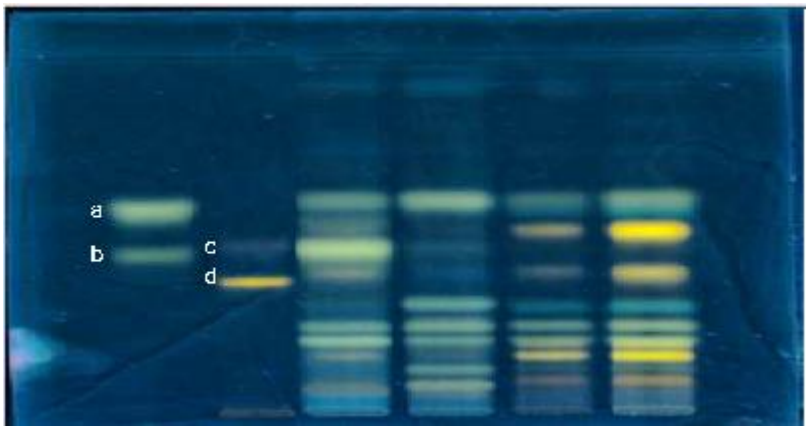


Figure 2. HPTLC fingerprint of extracts of Malaysian *Ficus deltoidea* varieties. Lane 1: Isovitexin (a) and vitexin (b); Lane 2: Isoorientin (c) and orientin (d).

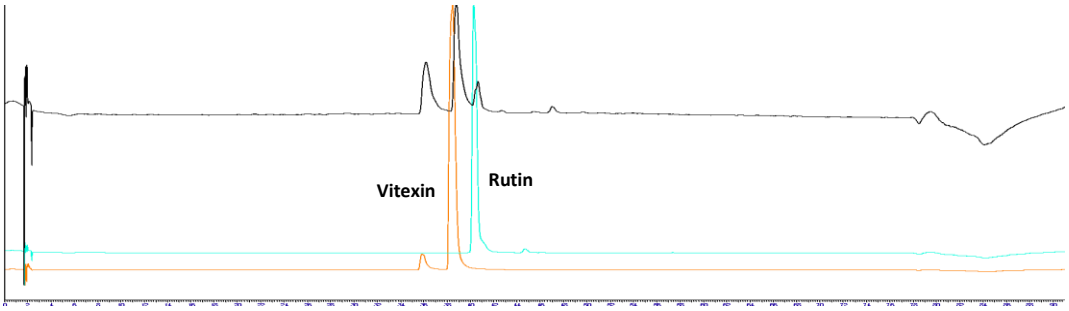
Table 3. Presence of phytochemicals according HPTLC analyses.

	FDAa	FDDa	FDDr	FDKa
Isovitexin	+	+	+	+
Vitexin	+	+	-	-
Orientin	-	-	+	+
Isoorientin	-	+	+	+

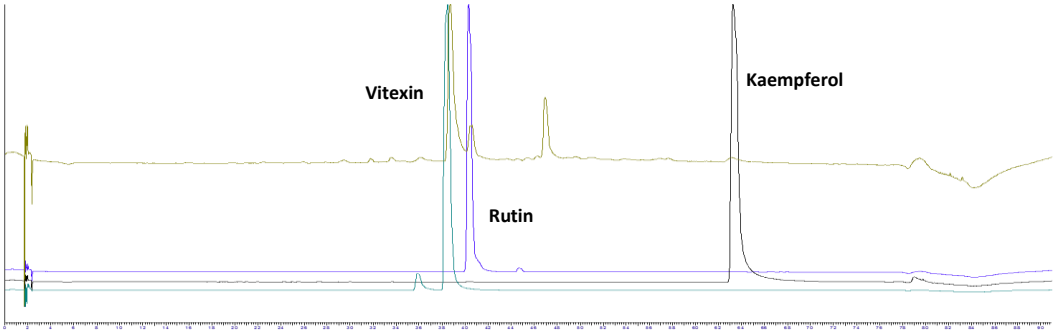
HPLC fingerprint of Ficus deltoidea varieties

The HPLC analyses suggested the presence of both vitexin and small traces of rutin in all samples, although with some small Rf and colour variations. We decided to corroborate these findings with HPLC-UV. Figure 3 shows the presence of these phytochemicals in all varieties as well as suggesting that traces of kaempferol are also present in var. *deltoidea*.

(A) *Ficus deltoidea* var. *angustifolia* aerial parts.



(B) *Ficus deltoidea* var. *deltoidea* aerial parts.



(C) *Ficus deltoidea* var. *kunsleri* aerial parts.

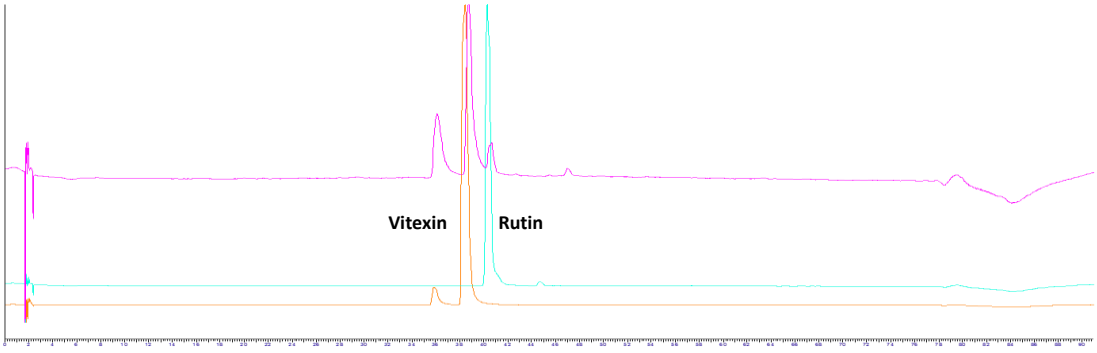


Figure 3. HPLC of ethyl acetate extracts (upper trace) overlapped with standards (lower traces).

Antioxidant activity of the plant extracts

The free radical scavenging activity of the active extracts of the *Ficus deltoidea* varieties are presented in Table 5. The hexane and chloroform extracts were inactive a below 100 µg/ml. The *angustifolia* variety is the less active. The *kunsleri* variety is as antioxidant as *deltoidea* variety and the later concentrates its water-soluble antioxidants in the roots. None of the extract were active against the nitric oxide radical (data not shown). We used Caffeic acid as reference (EC_{50}=1.7 µg/ml).

Table 5. DPPH radical scavenging activity (EC50, µg/ml) of *Ficus deltoidea* extracts.

	FDAa	FDDa	FDDr	FDKa
Ethyl acetate extract	52±1	15±2	14±1	11±2
Water extract	>100	72±3	16±2	25±3

Reference: Caffeic acid (EC50=1.7 µg/ml).

Tyrosinase inhibition assay

The inhibition of tyrosinase inhibition activity by each extract are presented in Table 6. Only the root extract of the *deltoidea* variety (IC50, 124±8 µg/ml) is significantly most active than the rest. The hexane and chloroform extracts were inactive a below 100 µg/ml. We used kojic acid as reference (IC50=1.0 µg/ml).

Table 6: Tyrosinase inhibition of *Ficus deltoidea* extracts.

	FDAa	FDDa	FDDr	FDKa
Water extract	> 200	> 200	124±8	(-)
Ethyl acetate extract	(-)	> 500	> 500	(-)

(-) Interference of the extract; Reference: kojic acid (IC50=1.0 µg/ml).

Cytotoxic activity of the plant extracts

The cytotoxic activities of *F. deltoidea* var *angustifolia* and var. *deltoidea* were previously reported (Hanafi et al., 2017). The authors favoured the study of the aerial parts but pointed out to the roots of the later as a potentially interesting organ to follow up. In table 7 we show the results of different fractions of increasing polarity Overall, it follows the same trend as the aerial parts of the plant. The correlation between Inhibitory concentration 50% (IC50) in the MTT assay and Growth Inhibition 50% (GI50) in the SRB assay may indicate a cytotoxic mechanism mediated by inhibition of mitochondrial viability.

Table 7. Cytotoxicity of *Ficus deltoidea* var. *deltoidea* root extracts (µg/ml) against a panel of human prostate cancer cells (MTT and SRB assays at 72 hours).

Treatment	Extract	IC50 - MTT		GI50 - SRB		
		DU145	**LNCaP**	**PC3**	**DU145**	**LNCaP**
FDAr (µg/ml)	*n*-Hexane	>200	175±12	>200	>200	175±9
	Chloroform	28±5	26±2	34±3	30±5	29±2
	Aqueous	>200	48±3	>200	>200	51±6

Reference drug: Paclitaxel (IC_{50} ≤ 0.01 µM for all lines).

DISCUSSION

The results from HPTLC fingerprints indicated that all tested *Ficus deltoidea* samples significantly differed from each other in their phytochemical constituents. The condition used allows the establishment of a simple, rapid and effective method for the accurate identification of some flavonoids in the selected Malaysian *Ficus* varieties. We believe they may be reproduced in normal TLC plates as long as the analyst operates with the necessary manual skills.

The results mutually support that these botanical varieties here studied are also chemical varieties (chemovars) and that there is an evident interorgan chemical metabolic differentiation in the case of *F. deltoidea* var. *deltoidea*. HPTLC (and perhaps the careful use of TLC).

Orientin in the aerial parts is specific for var. *kunslerii* whilst isoorientin differentiates var. *angustifolia* from var. *deltoidea*. Vitexin also qualitatively differentiates var. *angustifolia* from var. *deltoidea*. Moreover, the presence of vitexin and orienting differentiates the aerial parts from the roots of the *deltoidea* variety (Figure 4). These results support the chemotaxonomical value of C-glycoflavones in the genus *Ficus* (Kuijt & Hansen, 2015). Rutin and kaempferol traces also show in the HPLC analyses.

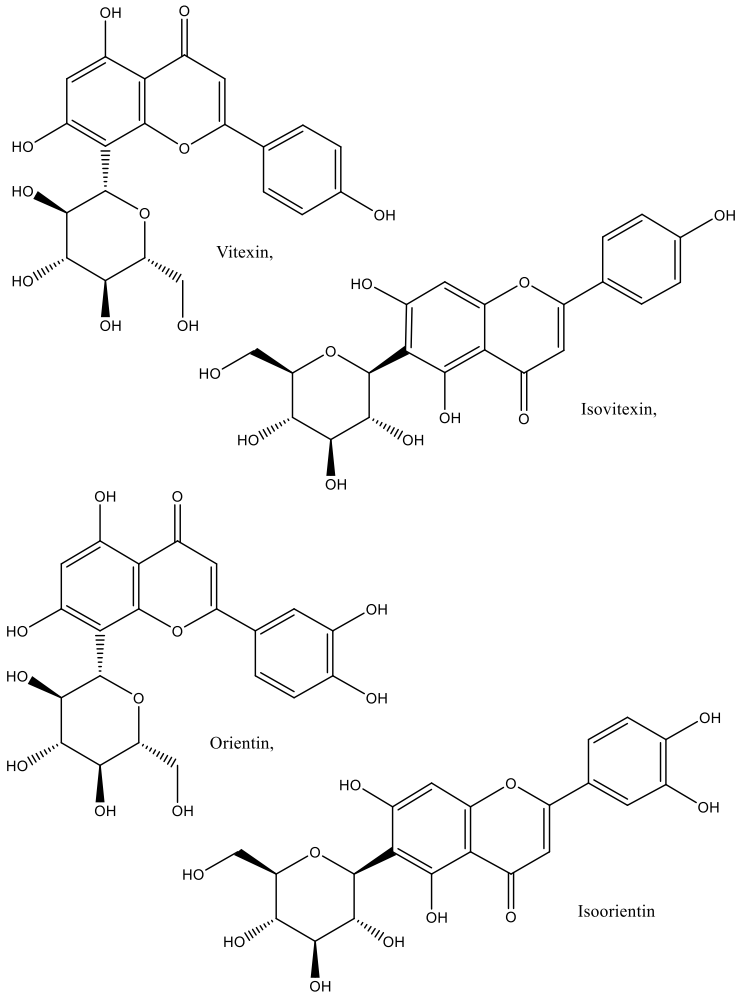


Figure 4. Vitexin, Isovitexin, Orientin, and Isoorientin.

The water extracts and ethyl acetate extracts of almost all these plants showed high antioxidant activity, while only *Ficus deltoidea* roots may contain some tyrosinase inhibitors. The compounds related to these activities, antioxidant activity and tyrosinase inhibition activity, from these plants on melanogenesis as well as melanoma cells may need to be further researched in future.

The three prostate cancer cell lines present different characteristics: LNCaP cells have androgen receptors that are functional enabling them to be androgen sensitive and these cells also secrete prostate-specific antigen (PSA). Both PC3 and DU145 cells are androgen independent but PC3 cells are highly invasive with strong metastatic potential as compared to DU145. The ethyl acetate extract of roots of *F. deltoidea* var. *deltoidea* only shown similar IC_{50} for all of them in both proliferation and viability assays, suggesting a hormone-independent mechanism of action targeting the mitochondria. This is in line with our previous report (Hanafi et al., 2017) were a the active plant extracts of two farmed varieties (var. angustifolia and var. deltoidea) induced apoptosis in PC3 cells via the intrinsic pathway, as evidenced by a significant activation of caspases 3 and 7and their ability to affect the gene expression of proteins such as Bcl-2, and Smac/DIABLO. Smac/DIABLO is a novel mitochondria-derived pro-apoptotic protein that plays an important role in sensitizing tumor cells to die by apoptosis (Du et al., 2000; Verhagen et al., 2000).

CONCLUSION

We here achieve to analytically differentiate three Malaysian *Ficus deltoidea* botanical varieties / cultivars (*angustifolia*, *deltoidea* and *kunslerii*) and suggest that they are also chemovars. Their biological activities point out to the antioxidant value of extracts from the *deltoidea* and *kunslerii* varieties whilst the inhibition of tyrosinase is only present in the roots extract of the var. *deltoidea* which is also endowed with cytotoxic activity against prostate cancer cells.

Conflicts of Interest

The authors declare no personal or financial conflict of interest related to this work.

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Authors contribution

(MMMH) Investigation; (O.S.O.A.) Investigation; (L.G.) Investigation; (H.Y.) Resources, Funding; (JMP) Conceptualization, Writing, Supervision.

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Mini-Review

NUTRITIVE AND MEDICINAL VALUE OF *GONGRONEMA LATIFOLIUM* BENTH. (ASCLEPIADACEAE).

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Keywords

Ethnopharmacology

Hypoglycaemic

Hypolipidemic

Cytotoxic

Antioxidant

Antimicrobial

Abstract

Background

The tropical rainforest plant *Gongronema latifolium* Benth. (Asclepiadaceae) is popular for its nutritive and medicinal value across many African nations. It is commonly used as a vegetable in soups, salad or as a spice in other food preparations. The rich phytochemistry of this plant may explain its ethnopharmacological uses in diabetes, malaria, hepatitis, stomachache, anorexia and cough.

Aims

To cover details about the origin, botanical features, ethnopharmacological uses, indigenous rights, phytochemical profile and pharmacological properties of *G. latifolium*.

Methods

PubMed and Google Scholar databases were searched for the name "*Gongronema latifolium*".

Results & Conclusion

This short review tried to establish the ethnomedical importance of *G. latifolium*. It is enriched with varities of flavonoids, saponins, alkaloids and steroidal phytochemicals which exhibit prominent pharmacological actions such as hypoglycaemic, hypolipidemic, cytotoxic, antioxidant, and antimicrobial *in-vitro* and *in-vivo*. One of the bioactive compounds, iloneoside, showed potent antileukemic activity. It should be evaluated against other cancer cell lines. Lastly, further research is required to understand the true potential of this African plant.

Keywords: *Gongronema latifolium*, Ethnopharmacological, Phytochemistry, Ileonoside, cancer

INTRODUCTION

The rich floral diversity of the tropical rainforests is blessed with an enormous amount of natural plant products known for their high dietary benefits and medicinal value (Dalziel et al.1937). One such plant is *Gongronema latifolium* Benth. of the family Asclepiadaceae, formerly known as *Marsdenia latifolia* (Okafor 1975). *G. latifolium* is grown locally in West Africa and addressed with different names such as “Utasi” by the Ibibios, Quas and Efiks ethnic groups; “Utazi” by the Igbos and “Arokeke” by the Yorubas (Hutchinson 1973; Edim et al. 2012). The popularity of *G. latifolium* further extends to Ghana and Senegal where it is known as “Akan-Asante aborode” and “Server gasule” respectively (Hutchinson 1973). This edible, highly nutritious plant has a sharp, bitter and slightly sweet characteristic taste when consumed fresh. Moreover, the plant has green leaves, yellow colored flowers and produces white latex on the incision (Balogun et al. 2016).

The leaves of *G. latifolium* are rich in fats, proteins, vitamins, minerals and many essential amino acids collectively contributing to its high nutritional value (Eleyinmi 2007). “Utazi” is commonly used as a vegetable in soup and salad preparations or as a spice in dried powdered form (Dalziel et al.1937; Okafor 1975 and Morebise et al. 2002). Medicinally, the sliced plant is boiled with lime juice or infused in water for at least three days to produce liquor, which is taken as a purgative against intestinal worms and for colic and stomach pain (Okafor 1975; Onike 2010). The main aim of this mini-review is to provide a detailed description of the origin and geographical distribution, botanical characteristics, ethnopharmacological use, phytochemical profile, and pharmacological properties of *G. latifolium.*

ORIGIN AND GEOGRAPHICAL DISTRIBUTION

G. latifolium plant originates from the West of Africa. It is grown widely throughout the tropical and sub-tropical countries such as Nigeria, Guinea-Bissau, Western Cameroon, Ghana, Senegal, Côte d'Ivoire, and Sierra Leone, and can be propagated easily using seed or stem cuttings. It is also found in America, Northern and Southeastern Asia. *G. latifolium* is present in the wild African forest and is also cultivated in family farms due to its medicinal and nutritional importance (Nelson 1965; Okafor 1975; Agbo et al. 2005; Owu et al. 2012).

BOTANICAL CHARACTERISTICS

G. latifolium is a climbing perennial shrub capable of twining around vertical support, as well it can grow horizontally on the ground up to 5 metres long. The soft woody stem produces adventitious roots in contact with soil (Osuagwu et al. 2013). The stem of the plant is hollow, soft, and hairy in texture and contains white latex which is released on incision or injury. The base of the stem is hard and woody to provide rigid support. It has simple, opposite, decussate, and occasionally whorled green leaves with an entire margin and long petiole (Osuagwu et al. 2013). The leaf blade is broadly ovate to almost circular with a deep cordate base and an acuminate apex (Balogun et al. 2016).

The flowers of *G. latifolium* are small, fragrant, bisexual, star-shaped (actinomorphic) and pale yellow in color with axillary cymes type of inflorescence (Osuagwu et al. 2013). The calyx lobes are elliptical to rounded shaped and hairy at apex. The corolla is long, tubular and campanulate at the apex; the corona has five fleshy and creamy lobes with a brown base (Hutchinson and Dalziel, 1931). Anthers are erect with membranous apical appendages. There are two pollinia per pollinarium; the ovary is superior (Balogun et al. 2016; Osuagwu et al. 2013 and Mosango 2022). In Nigeria, *G. latifolium* plant flowers in July and August annually (Mosango 2022).

The fruit of *G. latifolium* is green initially and turns dark brown to black on maturity. It is a dehiscent seed pod called a follicle which is oblong-lanceolate (Osuagwu et al. 2013). At maturity, the fruit splits open lengthwise releasing flat seeds which are attached to a white silky tuft (pappus) which aids dispersal for pollination (Balogun et al. 2016; Osuagwu et al. 2013). The seeds are small, comma-shaped about 0.5 cm in length (Osuagwu et al. 2013).

The plant, when grown from stem cuttings, matures in 12 months. It usually requires a hot climate of 32º to 37.5º C. Flowers are pollinated by insects due to their attractive color and fragrance. Fruits develop very slowly and often the mature old fruits meet the new flowers on the plant. The seeds of *G. latifolium* germinate in 1 to 2 weeks at 27º C with a 67% germination rate (Osuagwu et al. 2013).

As per recent anatomical characterisation by Aderiran et al. (2022), *G. latifolium* microscopically shows anomocytic stomata, rosette-shaped calcium oxalate crystal and non-glandular, uniseriate multicellular trichome. Moreover, the stomatal number and index were found to be 8.25 ± 0.52 and 17.60 ± 0.95, respectively.

ETHNOPHARMACOLOGICAL USES

The medicinal properties of all parts of *G. latifolium* have been exploited by different ethnic groups for different ethnomedical indications (Table 1). *G. latifolium* leaves are used traditionally by the Ikales of Ondo State of Nigeria to treat malaria, nausea, and anorexia (Morebise and Fafunso 1998; Morebise et al. 2006). As per the reports by Owu et al. (2012) and Mosango et al. (2022), some communities in West Africa use *G. latifolium* in the treatment of cough, intestinal worms, dysentery, dyspepsia, and malaria. Moreover, the people of Sierra Leone use stems of *G. latifolium* to prepare an infusion or decoction with lime juice which is consumed orally to treat colic and stomach pain (Oliver-Bever 1986). The utility of *G. latifolium* is different in Senegal and Ghana, where the leaves are rubbed topically on body joints of children to help them walk while the boiled extract of the fruit is used as a laxative (Mosango 2022). Edet et al. (2011) describe the use of leaf extract by Efik and Quas tribes belonging to the Cross River state of Nigeria to treat diabetes, malaria, hypertension, and constipation.

Table 1: Ethnomedicinal uses of different parts of *G. latifolium* plant

Part of the Plant	Ethnomedicinal uses	Method of extraction	References
Leaf	Dysentery, antihelmintic, catarrh, congested chest, running nose, cough, viral hepatitis, bilharzias, malaria, hypertension, diabetes, asthma, constipation, nausea, and anorexia	Maceration/Chewing	(Oliver-Bever 1986; Essien et al. 2007; Juliani et al. 2009; Edet et al. 2011; Owu et al. 2012; Chioma 2014; Mosango 2022; Ihesie 2022)
Root	Root Sickle cell anemia, relieve wheezing associated with asthma	Decoction	(Balogun et al. 2016)
Stem	Purgative, hypertension and diabetes	Decoction	(Farombi, 2003)
Fruit	Laxative, stomachache, malaria	Chewing	(Osuagwu et al. 2013)
Latex	Dental caries	Incision & collection	(Osuagwu et al. 2013)

It is widely used for the treatment of cough in Nigeria (Essien et al., 2007). Additionally, fresh leaves are chewed by asthmatic patients to relieve wheezing while oral cold macerated preparation of roots of *G. latifolium* is prescribed for the treatment of asthma (Essien et al. 2007; Mosango 2022). A few communities in Africa also use this plant in the treatment of viral hepatitis, bilharzia, and other microbial infections (Mosango 2022). One of the famous polyherbal preparations for hepatitis and malaria is a decoction of *G. latifolium*, *Mormodica charantia* or *Veronica amygdalina* and *Ocimum gratissimum* given to help cleanse the liver (Ihesie 2022). The extract of *G. latifolium* is consumed widely across Nigeria for the maintenance of blood glucose level (diabetes) and as a cleansing purge by Muslims during Ramadan, respectively (Juliani et al. 2009; Chioma 2014). Fruits of *G. latifolium* are consumed orally with or without seeds for stomachache, malaria and as a laxative (Osuagwu et al. 2013). The leaves are also added to foods such as soups, porridges, and popular Ibo stews such as the *Nkwobi* (cow leg pepper soup) and *Isi ewu* (Goat head pepper soup). These leaves a bitter taste impart, sweet aroma and stimulate the appetite (Adelaja and Fasidi 2009; Osuagwu et al. 2013).

PHYTOCHEMISTRY OF *GONGRONEMA LATIFOLIUM*

The ethnomedicinal and nutritional value of *G. latifolium* in the African communities attracted many phytochemists to investigate the composition of this herb. There is a wealth of studies on the distribution and occurrence of major classes of secondary metabolites in different parts of the plant summarized in Table 2. The dried leaves of *G. latifolium* contain a high concentration of saponins (18.11%), tannins (16.23%), cyanides (14.32%), flavonoids (11.13%) and phenols (11.11%) with scarce quantity of alkaloids (0.12%) (Offor et al., 2015). Another phytochemical investigation on fresh leaves by Osuagwu et al. (2013) reported high alkaloid content (10%) in comparison to the dried sample. Egbung et al. (2011) also observed higher concentration of flavonoids, alkaloids, hydrogen cyanide and tannins in root extract of *G. latifolium* than stem.

Table 2: Presence of major classes of phytoconstituents in the different plant parts of *G. latifolium*.

	Leaves	Root	Fruit	Stem
Alkaloids	X	X	X	X
Anthraquinones	X			
Cardiac glycosides	X			
Coumarins	X			
Cyanogenic glycoside	X	X	X	X
Essential oil	X			
Fats and oil	X			
Flavonoids	X	X	X	X
Glycosides	X	X		
Iridoids	X			
Organic acids	X			
Oxalate	X			
Resins	X			
Saponins	X	X	X	X
Steroids	X			
Tannins	X	X	X	X
Terpenoids	X			

(Ekundayo 1980; Schneider et al. 1993; Antai et al. 2009; Aka et al., 2011; Egbung et al. 2011; Osuagwu et al. 2013; Enemor et al. 2014; Ezekwe et al. 2014; Offor and Uchenwoke 2015; Gyebi et al. 2017; Ugada and Ibiam 2014 and Beschel et al. 2020).

The active principle(s) of this plant is not fully established although Iwu et al. (1998) reported flavones and sterols as the most likely active constituents. The claim was strengthened when Morebise and Fafunso et al. (1998) examined the antimicrobial activity of a methanolic extract containing saponins and flavonoids. The presence of tannins (polyphenolic compounds) in the leaves was also confirmed by Eze and Nwanguma (2013), who proposed the potential of *G. latifolium* extract as a food preservative. The results by Osuagwu et al. (2013) showed that the fruits of *G. latifolium* are more potent than leaves as believed by the local tribes due to a higher concentration of alkaloids, saponins and phenols. A recent comparative phytochemical analysis showed that *G. latifolium* leaf extract contains higher quantity of alkaloids, glycosides, saponins, tannins and reducing sugars than bitter leaf, African basil and African black pepper, respectively (Mgbeje et al. 2019).

More detailed phytochemical investigations isolated a number of secondary metabolites suumarised in Table 3. Of note, the 80% methanolic extract of *G. latifolium* dried leaves by Gyebi et al. (2017) revealed the presence of iloneoside (Figure 1), a new ditigloylated pregnane glycoside with potent antileukemic activity

Table 3. Isolated and Identified compound in *G. latifolium*.

CLASS Subclass	Compound	Reference
PHENOLICS		
Flavonoids		
	Rutin	Beschel et al. (2020)
	Kaempferol	Beschel et al. (2020)
Coumarins		
	Scopoline	Beschel et al. (2020)
	Esculetin	Beschel et al. (2020)
TERPENES		
	14-Methyl-8-hexadecenol	Ugadu and Ibiam (2014)
	Ester-9-octadecanoic acid	Ugadu and Ibiam (2014)
Iridoids		
	Ebuloside	Beschel et al. (2020)
	Valerenic acid	Beschel et al. (2020)
Triterpenes and Steroids		
	3-β-Acetate lup-20(29)-en-3-ol	Ugadu and Ibiam (2014)
	Acetate-19-cyclolanost-24-en-3-ol	Ugadu and Ibiam (2014)
	Cholestane-3-5,-dichloro-6-nitro-(3β, 5α, 6β)	Morah and Inaku (2021).
	Oleic acid	Ugadu and Ibiam (2014)
	β-Sitosterol	Schneider et al. (1993)
	Lupenyl cinnamate	Schneider et al. (1993)
	Lupenyl acetate	Schneider et al. (1993)
	Lupeol	Schneider et al. (1993)
	Lycopene	Morah and Inaku (2021).
Saponins	Oleananesaponin	Beschel et al. (2020)
	Timosaponin B II	Beschel et al. (2020)
	Metasaponin 1 and 2	Beschel et al. (2020)
Pregnane glycosides	Ileoneoside	Gyebi et al. (2017)
	Marsectohexol	Schneider et al. (1993)
	Ajugoside	Schneider et al. (1993)
	Marsdenin derivative 1	Schneider et al. (1993)
	Marsdenin derivative 2	Schneider et al. (1993)
Cardiac glycosides		
	digoxigenin	Morah and Inaku (2021)
Essential Oil		
	aromadendrene hydrate (9.8%)	Chioma et al. (2014).
	linalool (19.5%)	Chioma et al. (2014).
	(E)-phytol (15.3%)	Chioma et al. (2014).

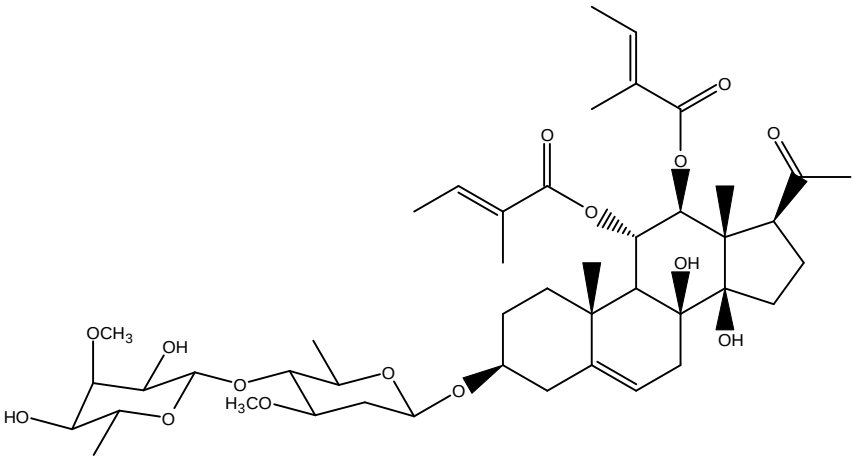

Figure 1: Structure of iloneoside

The investigation on primary metabolites -carried out on leaves by Offor and Uchenwoke (2015) and Mensah et al. (2008) to determine the nutritive composition of *G. latifolium*- showed high amounts of carbohydrates (38.55%) and proteins (33.60%) followed by moisture content (11.13%), ash content (9.11%), crude fibre (4.22%) and fat (3.41%). Eleyinmi (2007) reported almost similar composition along with the presence of significant amounts of leucine, valine, phenylalanine, aspartic acid, glutamic acid and glycine amino acids and minerals such as potassium, iron, magnesium, manganese, sodium, calcium, copper and zinc (Offor and Uchenwoke 2015). Moreover, Enemor et al. (2014) concluded the presence of vitamin A, C, E and B_3 in the leaves of *G. latifolium.*

PHARMACOLOGICAL PROPERTIES OF *GONGRONEMA LATIFOLIUM*

G. latifolium exhibits multiple pharmacological actions due to the presence of a diverse class of phytochemicals. The exact mechanism of action behind each pharmacological response is not known. However, the high ethnopharmacological importance of *G. latifolium* continues to attract researchers for various *in-vitro* and *in-vivo* evaluations of this herb.

Hypoglycemic activity

Akah et al. (2011) reported a significant antidiabetic effect of intraperitoneal administration of the aqueous and methanolic extract of *G. latifolium* in alloxan-induced diabetic rats. However, the potency of methanolic extract was highest with a LD_{50} value of 900mg/kg. Adebajo et al. (2012) investigated *in-vitro* insulin stimulating activity using INS-1 cells and *in-vivo* hypoglycemic activity in glucose-loaded rats of methanolic extract of *G. latifolium* roots and stems. The *in-vitro* and *in-vivo* test results showed significant antihyperglycemic activity similar to glibenclamide drug confirming insulin as an unreported mechanism of action of the plant. An experiment by Ezekwe et al. (2014) strengthens the fact that an intact pancreas is required for the hypoglycemic action of *G. latifolium* which follows similar mechanism of action like sulfonylureas. Studies by Saidu and Okorocha (2013) and Udo et al. (2013) also reported the *in-vivo* hypoglycemic activity of ethanolic and aqueous extracts in rats. The flavonoids of *G. latifolium* are believed to be responsible for the hypoglycemic effect (Ezekwe et al., 2014; Saidu and Okorocha, 2013).

Several *in-vivo* investigations on flavonoid rich *G. latifolium* extract showed management of blood sugar levels via fetuin-A and tumour necrosis factor-alpha, inhibition of inflammatory cytokines with redox imbalance and increasing levels of insulin, respectively (Ajiboye et al. 2022; Ojo et al. 2020 and Oyinloye 2022). However, marsectohexol, a pregnane phytochemical isolated from *G. latifolium* leaf showed much potent in-vitro α-amylase inhibition with IC_{50} = 3.712 µg/mL than reference inhibitor acarbose (IC_{50}= 15.418 µg/mL). As per molecular docking analysis, marsectohexol exhibited the highest binding affinity (-8.8 kcal/mol) to human pancreatic α-amylase than acarbose (-8.1 kcal/mol). Hence, this pregnane active compound may be responsible for antihyperglycaemic effects (Ogunyemi et al., 2020). While another *in-silico* study identified eleven compounds (mainly flavonoids) such as apigenin, baicalin, chicoric acid, genistein, gallocatechin, quercetin, kaempferol, naringenin, luteonin, robinetin, and rosmaric acid which

formed stable complexes with antidiabetic protein targets along with moderate toxicity and drug-drug interaction and good G.I absorption (Ajiboye et al., 2022).

Anticancer activity

Emeka et al. (2015) reported potent *in-vitro* cytotoxic activity of a dichloromethane leaf extract of *G. latifolium* against A-549 human lung carcinoma and MCF-7 breast cancer cell lines with IC_{50} of 9.57µg/mL and 6.51µg/mL, respectively. Moreover, a recent update by Gyebi et al. (2017) discovered iloneoside from methanolic leaf extract of *G. latifolium*, which was found to be a potent inhibitor of human leukaemia cell line *in-vitro*. They further strengthen their claim using molecular docking analysis where iloneoside could accommodate within the hot spots of anti-apoptotic protein Bcl-2. An *in-vivo* investigation of aqueous extract of *G. latifolium* on tumour necrosis factor-α and transforming growth factor-β was carried out in rabbits. The extract exhibited significant inhibition of both cytokines with the greatest inhibition at a dose of 400 mg/kg (Rowaiye et al. 2021).

Antioxidant activity

Experiments on streptozotocin-induced diabetic rats showed a significant increase in superoxide dismutase, glutathione reductase, glutathione peroxidase and glucose-6-phosphate dehydrogenase activities while a decrease in lipid peroxidation, suggesting the antioxidant action of aqueous and ethanolic extract of *G. latifolium*, respectively (Ugochukwu and Babady, 2002) and Ugochukwu et al., 2003). *In-vitro* free radical scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) was also reported by Emeka et al. (2015).

A similar result was achieved by Adegbenro et al. (2021) who used a blanched and unblanched *G. latifolium* supplemented diet on fat-induced hyperlipidemic rats. Interestingly, the group receiving blanched *G. latifolium* showed better antioxidant activity than the animals receiving unblanched leaves due to the higher content of flavonoids. Another *in-vivo* investigation on male Wistar rats exhibited the antioxidant potential of ethanolic extract of *G. latifolium* by reducing liver, kidney and heart malondialdehyde levels and increase in antioxidant enzyme levels (Analike et al. 2022). Okeke et al. (2022) reported the radioprotective potential of ethanolic extracts of *G. latifolium* against radiation-induced oxidative stress in Wistar albino rats due to the presence of antioxidants like flavonoids and polyphenols which can scavenge free radicals and regulate endogenous enzymes.

Antimicrobial activity

As per the in-vitro antibacterial evaluation carried out by Eleyinmi (2007), the methanolic extract showed activity against *S. enteritidis*, *S. cholerasius* ser *typhimurium, L. monocytogenes* and *P. aeruginosa* while the aqueous extracts were active only against *E. coli* and *P. aeruginosa.* According to Nwinyi et al. (2008) the ethanolic leaf extract was nearly 4 times more potent than the aqueous leaf extract of *G. latifolium* against *E. coli* and *S. aureus.* Dose-dependent inhibition of *Staphylococcus aureus, S. pneumonia, E. coli, P. mirabilis* and *P. aeruginosa* by ethanolic leaf extract was also reported by Omodamiro and Ekeleme (2013).

However, this disagrees with recent works which suggest poor activity against *S. aureus* and *E. coli* and no statistical difference in antibacterial activity between aqueous and ethanolic extracts, respectively (Akani et al. 2020; Ndubueze et al. 2020). An *in-vivo* study exploring the anti-malarial activity of *G. latifolium* extract against *P. berghei* infected mice showed chemo-suppressive and prophylactic effects, but the standard drug chloroquine performed way better (Orumwensodia and Uadia 2022). Adenayo et al. (2022) investigated the anti-plasmodial activity of pregnane glycosides previously isolated from *G. latifolium*. Iloneoside showed significant activity *in-vitro* and was able to potentiate the activity of chloroquine by 3200% against drug resistant strain of *P. falciparum* at dose higher than 0.625 µg/mL. As per *in-silico* analysis, iloneoside bonded with similar binding pattern and tendency to the selected P*f*proteins as chloroquine, suggesting similar mechanism of action.

Anti-inflammatory activity

Morebise et al. (2002) tested the *in-vivo* anti-inflammatory activity of aqueous extract of *G. latifolium* in rats. The extract successfully inhibited carrageenan-induced rat paw oedema, carrageen-induced leucocyte migration in the animal and dye leakage induced by intraperitoneal injection of acetic acid. Another *in-vivo* study of methanolic leaf extract by Morebise et al. (2005) reported the inhibition of nystatin-induced rat paw oedema and stabilization of erythrocyte membrane subjected to lysis by heat and hypotonic solution.

Immunomodulatory effect

The *in-vivo* immunostimulatory effect of the methanolic extract in Wistar albino rats was reported by Simeon et al. (2014). They observed a significant increase in interferon-γ, delayed type hypersensitivity, primary and secondary antibody titer along with non-significant increase in tumour necrosis factor-α and interleukin-2. As per the *in-vivo* experiment by Akpan and Effiong (2015), administration of *G. latifolium* leaves in streptozotocin-induced diabetic rats resulted in a decrease in the level of CD_4^+ cell count, WBC, platelets, monocyte, neutrophil and a significant increase in RBC, hemoglobin and lymphocyte count as compared to the diabetic control.

Hypolipidemic activity

The ethanolic root extract, when administered in diabetic rats, showed a reduction in serum glucose, triacylglycerol, total cholesterol, and very low-density lipoprotein with an increase in high-density lipoprotein. However, no significant change was seen in serum low-density lipoprotein (Robert et al. 2013). This is in agreement with two recent in-vivo investigations in rats, which recorded an improvement in lipid profile and increase in activity of antioxidant enzymes reducing metabolic and cardiovascular risks (Uchendu et al. 2021; Beschel et al. 2019). Furthermore, Adebayo et al. (2022) investigated the cardiovascular effects of marsdenin derivative isolated from *G. latifolium* in albino mice. The results revealed hypolipidemic effects along with reduction in heart and plasma creatine kinase activities and heart Calcium-Magnesium-ATPase activity. Hence, marsdenin derivative may not predispose subjects to atherosclerosis but may cause problems due to interference with cardiac muscle contraction and relaxation at high doses.

Haematological effects

Aqueous leaf extract given to female albino rats showed a decrease in mean haemoglobin concentration, packed cell volume, platelet count, total white blood cell count, mean bleeding and clotting time (Oguwike et al. 2013).

Renal effects

Onuoha and Chinaka (2013) and Ndodim et al. (2014) reported a reduction in urea and creatinine levels in rats induced with carbon tetrachloride and chloroquine respectively, on the administration of aqueous leaf extract of *G. latifolium*. However, this is in disagreement with a recent *in-vivo* study in rats where ethanolic extract recorded an increase in serum urea and a decrease in serum triglycerides and creatinine levels suggesting mild renal disturbances/injury (Sulaiman et al. 2022).

Effects on the nervous system

In-vitro analysis on alkaloids isolated from *G. latifolium* revealed concentration-dependent inhibition of acetylcholinesterase, butyrylcholinesterase and monoamine oxidase with IC_{50} 87.39 µg/ml, 118.65 µg/ml and 61.37 µg/ml respectively. Moreover, GC-FID analysis showed the abundance of choline in the extract (Nwanna et al. 2019). Interestingly, an *in-silico* analysis of flavonoids isolated from *G. latifolium* showed inhibition of leucine-rich repeat kinase 2, glycogen kinase 3β and mitogen-activated protein kinase 14 with moderate pIC_{50} values. All three protein kinases are associated with the pathogenesis of Alzheimer's and Parkinson's disease. Additionally, flavonoids such as catechin, gallocatechin, butein and isorhamnetin exhibited drug-likeness characteristics with low drug-drug interaction and high GI absorption (Oyinloye et al. 2021). An *in-vivo* experiment in Swiss albino mice by Ujong et al. (2022) reported that ethanolic extract of *G. latifolium* improved visio-spatial learning and cognitive memory in mice.

Effects on the reproductive system

An animal study of the ethanolic extract of *G. latifolium* on male Wistar rats exhibited an increase in serum concentration of testosterone, follicle stimulating hormone and luteinizing hormone at doses 100 mg/kg and 200 mg/kg body weight whereas, decreased in progesterone levels. Surprisingly, at a higher dose (200 mg/kg body weight) slightly greater progesterone levels (15 mg/dl) were recorded as compared to the level 13 mg/dl at a dosage of 100 mg/kg body weight, while the normal group showed 19 mg/dl. Moreover, the authors did not attempt to discuss this (Dasofunjo et al. 2020).

Another *in-vivo* investigation on male wistar albino rat showed significant increase in serum concentrations of male reproductive hormones and aphrodisiac effects (Effiong et al. 2022). Effects of methanolic extract of *G. latifolium* on lactating Wistar albino rats showed a dose-dependent increase in serum prolactin level

and milk production whereas, oxytocin levels remain unchanged (Ogbonna et al. 2022). This may be due to the presence of saponins and flavonoids which have phytoestrogenic effects (Wina et al. 2005; Das et al. 2012; Di Gioia and Petropoulos 2019).

Hepatoprotective activity

Omodale et al. (2017) investigated the hepatoprotective potential of aqueous root extract of *Gongronema latifolium* against paracetamol-induced hepatotoxicity in adult albino rats. The results of this study showed a dose-dependent decrease in the serum liver enzymes. However, at high doses and prolonged usage, alcoholic extract of *G. latifolium* may cause hepatotoxicity as per two in-vivo studies (Al-Hindi et al. 2019; Omodamiro et al. 2021). Moreover, aqueous leaf extract in lactating dams exhibited increase in liver and pancreatic weight index with slight inflammation cells but no hepatotoxicity (Katchy et al. 2020). Lastly, another in-vivo experiment confirms hepatoprotective effect of *G. latifolium* by decreasing activities of alanine aminotransferase, aspartate aminotransferase, creatine kinase and lactate dehydrogenase enzymes along with absence of pathological lesion at 15% inclusion of the herb (Adeyemi-Doro et al. 2021).

CONCLUSION

The research studies highlighted in this essay tried to demonstratethe ethnomedical importance of *G. latifolium*. The plant, *G. latifolium* truly requires further research on isolation and characterization of the pharmacologically active compound(s), along with a complete understanding of the mechanism of action at the molecular level, sustainability, variation among botanical species and safety profile in man It would be interesting to find out is iloneoside and other pregnane glycosides fulfils their anticancer potential in future investigations.

Conflicts of Interest

The authors declare no personal or financial conflict of interest related to this work.

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