





Phytochemical, Pharmacological, Phyto-cosmeceutical, Toxicity, and *In silico* Toxicological Evaluations of *Vernonia amygdalina* Delile – A Review

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Abstract: *Vernonia amygdalina* Delile, which is in the Asteraceae family, is used as food and medicine all over the world, especially in Africa and Asia. This review reports the phytochemical, ethnopharmacological, phyto-cosmeceutical, and toxicological potentials of the plant. Recent scientific exploration of the plant has mainly focused on both its nutritional potential and ethnopharmacological properties. The antioxidant, anti-inflammatory, antidiabetic, hepatoprotective, hematological, anti-plasmodial, antimicrobial, anticancer, neurological, cosmeceutical, and other pharmacological values of *V. amygdalina* continue to be extensively explored. Many empirical studies of the therapeutic potential of the plant have attributed the ethnomedicinal properties of the plant to its phytochemical constituents, which include glycosides, saponins, tannins, terpenoids, etc. Compounds obtained from the leaves, root, stem, and flowers, which include vemoniosides, vernoamiosides, vernoniomyosides, vernoniosides, vernolide, vernodalol, vernodalol, vernomenin, vernomygdin, vernodalinol, epivernodalol, vernolepin, coumarins, luteolin, edotides, etc. have been identified as bioactive constituents responsible for numerous pharmacological activities of the plant. In addition, the toxicological evaluation of the plant revealed that it is safe for consumption at relatively high concentrations.

Keywords: Medicinal plant; nutraceuticals; antidiabetic; antioxidant; vemoniosides; bioactives

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1. INTRODUCTION

Medicinal plants form the fulcrum of folk medicine due to their numerous economic, pharmacological, nutritional, and tremendous health benefits to humans. The utilization and relevance of medicinal plants, particularly in the drug development and food industries, cannot be overemphasized. Their medicinal and pharmacological properties have been

attributed to the presence of phytoconstituents, which are typically bioactive compounds capable of producing definite physiological action in humans (1). Since ancient practice, medicinal plants have been adopted ethnobotanically for medicine, food, clothing, hunting, and, in some religious ceremonies, even though their primary use has been for health care (2). Different parts of medicinal plants have been used to cure specific ailments.

Recently, a gradual renewal of interest in the use of medicinal plants in developing countries was rekindled because herbal medicines are reported to be safe and have fewer adverse side effects when carefully prepared in comparison with conventional drugs (1, 2). Among the edible plants whose parts are highly valued in ethnomedicine is *V. amygdalina* (Figure 1).

V. amygdalina, a tree or shrub of the family Asteraceae and genus *Vernonia*, is well known in the tropics, particularly in Africa and Asia. The perennial plant is characterized by the bitter taste of the sap from the leaf, and this has been widely explored for its ethnobotanical applications in traditional medicine (3, 4). The plant has been found to be rich in minerals, especially phosphorus, calcium, potassium, magnesium, zinc, iron, and vitamins A, C, and E. Scientific and pharmacological studies have revealed the antihyperglycemic action of the roots of *V. amygdalina* (5). In addition, there is an increasing dependence on the use of this medicinal plant, as bioactives are prepared as nutraceuticals and chemotherapeutics for conventional applications (2). In addition, findings have suggested that the plant is a reservoir for potent phytochemicals of pharmaceutical importance (6). The overall therapeutic properties of plants are often based on the phytochemical constituents of the plant (7, 8).

V. amygdalina extracts are traditionally used as an antidiabetic, anti-helminthic, antimalarial, laxative, digestive tonic, appetizer, and febrifuge agents (4, 9). In some African countries, including Ethiopia, *V. amygdalina* is among the medicinally significant plants used against malaria, helminth infections, gastrointestinal disorders, and fever. This species is also used to promote wound healing and treat microbial infections (9). The main bioactive constituents of the leaves were reported as sesquiterpene lactones. Some of them include

vernonioside A1, vernonioside A2, vernonioside B1, vernonioside B2, vernodalin, vernolepin, vernomygdin, vernodalol, and vernodalinol (10).



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Figure 1: Photograph of *V. amygdalina*.

2. BOTANICAL OVERVIEW

V. amygdalina of the family Asteraceae or Compositae is an African and Asia renowned shrub or tree with valuable medicinal principles. The plant, otherwise called bitter leaf, is well known in countries that include Cameroon, Nigeria, Egypt, Uganda, Tanzania, and others where it thrives around water paths, forest zones, and home plantations (11). In Africa, the rough brown bark plant often reaches a height of 7 m as the semioblong leaves attain an average size of 10 x 4 cm. The plant produces off-white to cream-colored clusters of flowers that are axillary and terminal with sweet smell (11). The taxonomic classification of *V. amygdalina* is presented in Table 1.

Table 1: Taxonomical classification of *V. amygdalina*.

Classification	Taxonomy
Kingdom	Plantae
Clade	Angiospermae
Order	Asterales
Family	Asteraceae
Genus	<i>Vernonia</i>
Species	<i>V. amygdalina</i>

3. ETHN BOTANICAL AND MEDICINAL USES

In Africa, *V. amygdalina* is adopted for various ethnobotanical and medicinal purposes on account of economic reasons (12). During childbirth, the leaf aqueous extract orally administered is used to induce overdue labor as a result of its capability to stimulate uterine motility (11). Parasites such as ringworms are expelled by applying leaf extract, which is also used to control various infections when taken orally (11). In addition, leaf decoction has been used to manage coughs and colds as well as enhance milking in lactating women. The leaf is notable for its culinary purpose as well. As a staple

vegetable, it is often used for the preparation of special soup and stew in Africa. The soup prepared from the leaf is consumed as both food and medicine (13). *V. amygdalina* contains both major and trace elements that are responsible for some of the observed pharmacological properties (14).

Stem and root bark are used as chewing sticks in some parts of Africa to serve as cleansing agents and antimicrobial agents in oral applications. Antihelminthic, antimalarial, and anti-tumorigenic properties have been properly reported for extracts from this plant. Other studies have demonstrated hypoglycaemic and hypolipidaemic effects of leaf

extract in experimental animals (4). Since ancient times, medicinal plants such as *V. amygdalina* have been widely used in traditional medicine for the prevention and curation of various disease conditions due to their potency, affordability, and ready availability (15).

V. amygdalina has also been used in human and veterinary medicine against several pathological infections (16). In the ancient world, ill chimpanzees were known to suck the stalk of the plant to derive some health remedies while enhancing their body fitness, lost strength, or appetite. Stalk juice is also known to reduce indigestion or bowel looseness in animals. Likewise, birds fed with the leaf extract reportedly indicated reduced mortality compared to those that were not fed with the leaf extract (11). Virtually all parts of the plant possess bioactives of pharmacological relevance in the management of various disease conditions (4, 17).

4. PHYTOCHEMISTRY

A plethora of bioactive phytochemicals have been identified in various parts of *V. amygdalina*. Several studies have reported the isolation and characterization of some bioactive compounds, such as flavonoids, saponins, alkaloids, tannins, phenolics, terpenes, steroidal glycosides, triterpenoids, and several types of sesquiterpene lactones (18-22). Some of the isolated and characterized bioactive compounds are shown (Figure 2). Sesquiterpene lactones, including vernodalol, vernolepin, vernodalinol, vernomygdn, hydroxyvernolide, and vernolide, have been identified in the plant (21, 23-25). In addition, various vernoniosides and steroidal glucosides have also been identified as major types of compounds in plants (26, 20). In fact, *V. amygdalina* is well known for its bitter taste due to the presence of vernoniosides A1, A2, A3, and A4. However, vernoniosides B1, B2, and B3, which were equally present, are not characterized by any bitter taste (27). Recently, stigmastane-type steroid saponins, including vernoniamyosides A-D, vernonioside B, and vernoamyoside D, were reportedly in *V. amygdalina* (28). Vernoamyosides A-D and

stigmastane-type steroid saponins have also been characterized as compounds isolated from the leaves of *V. amygdalina* (20).

Other steroidal saponins have been identified in the plant, with flavonoids, tannins, saponins, and triterpenoids in the plant reported to possess antioxidant and hypolipidaemic effects (10, 29-31). Igile et al. (1994) (32) accounted for the presence of the flavonoids; luteolin, luteolin 7-O- β -glucuronide, and luteolin 7-O- β -glucoside in the leaves of *V. amygdalina*. Other researchers have also affirmed the presence of flavonoids like luteolin, etc, in the plant (33-35).

Other phytochemicals present in the leaves of *V. amygdalina* include terpenes, coumarins, phenolic acids, lignans, xanthenes, phytate, alkaloids, saponins, tannins, oxalate, cardiac glycosides, and anthraquinones without cyanogenic glycosides (34, 36-39). Isorhamnetin, a flavonoid and tricosane, a hydrocarbon, were obtained in flower extracts of *V. amygdalina* (9, 40). Likewise, the presence of bioactive peptides called edotides in the leaves of *V. amygdalina* has also been reported (12, 41). Recently, compounds (Figure 2) that included 4 α -hydroxy-n-pentadecanoic acid, 10-geranylanyl-O- β -D-xyloside, 11 α -hydroxyus-5,12-dien-28-oic acid-3 α ,25-olide, 1-heneicosenol-O- β -D-glucopyranoside, glucuronolactone, and vernoniaolideglucoside were reportedly isolated from the stem bark of the plant (42,43). The phytochemicals of the plant are reportedly responsible for corrosion inhibition when applied to metals (44) and as antifungal agents (45) on surfaces. Among other phytochemicals, saponins and glycosides in the leaf are responsible for bitterness (46). Phenolic compounds identified in the plant can be grouped into flavonoids, tannins, and caffeoyl quinic acid (47). Other compounds, including phytol, stigmastadienol, α -tocopherol, and decanamide, have also been detected in the plant (48). Tannins, glycosides, and saponins have been obtained as the primary phytochemicals from the root and stem bark extracts of *V. amygdalina* (45). A summary of the phytochemicals present in *V. amygdalina* is iterated (Table 2).

Table 2: Phytochemical constituents of *V. amygdalina* and their bioactivities.

Compounds in <i>V. amygdalina</i>	Class of compound	Part of the plant	Uses	References
Vernolide	Sesquiterpene lactone	Aerial part/root/pith/leaf	Antimicrobial, antioxidant, antitumor, antiplasmodial, antischistosomal, antibacterial	(25,49)

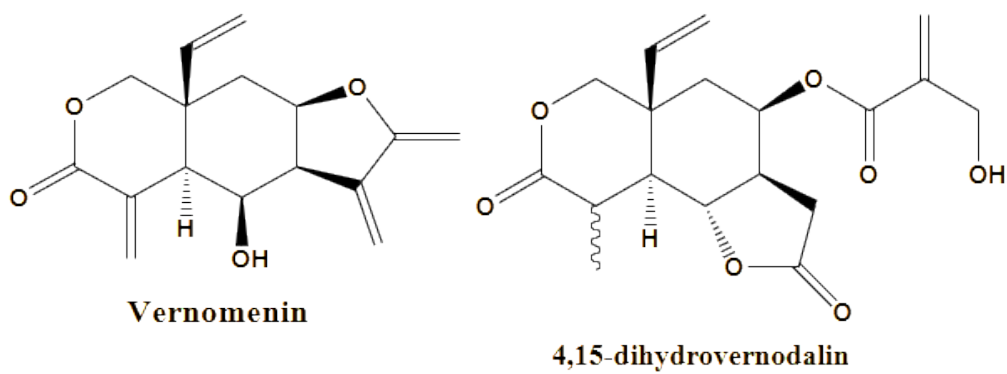
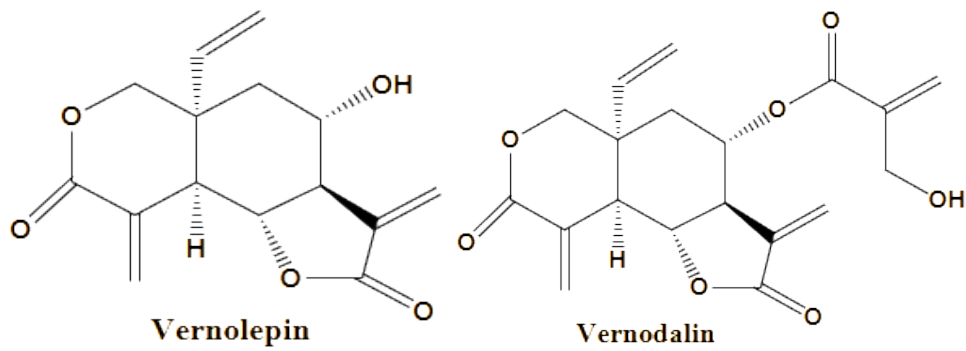
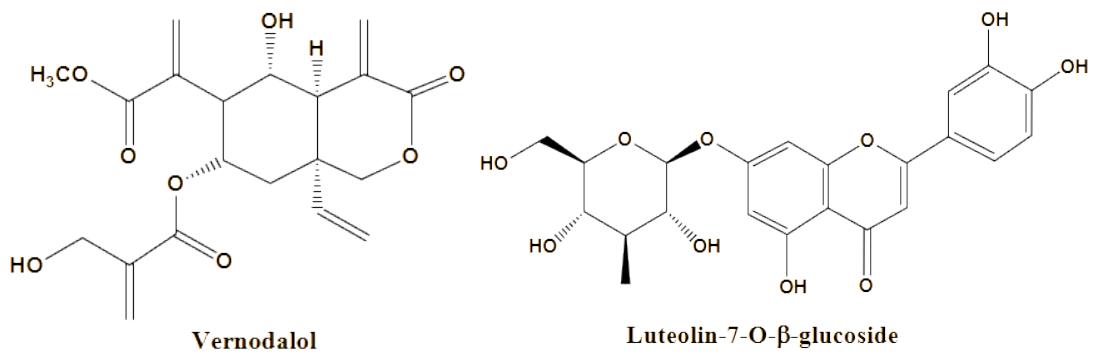
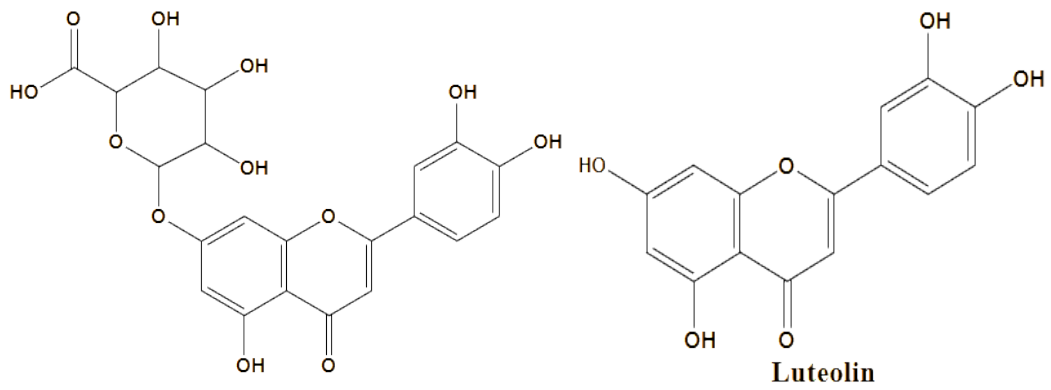
Compounds in <i>V. amygdalina</i>	Class of compound	Part of the plant	Uses	References
Vernodalol	Sesquiterpene lactone	Aerial part/root/pith/leaf	Antimicrobial, antioxidant, antitumoral, antiplasmodial, antischistosomal, antibacterial	(21,50)
Vernodalin	Sesquiterpene lactone	Aerial parts/root/leaf	Antitumor, insecticidal, antileishmanial	(24,51-53)
Vernomygdin	Sesquiterpene lactone	Aerial part	Anticancer	(21, 52, 54)
Vernodalinol	Sesquiterpene lactone	parts/root/pith	Anticancer	(21, 52, 54)
Epivernodalol	Sesquiterpene lactone	Aerial parts	Anticancer	(21, 52, 54)
Vernolepin	Sesquiterpene lactone	Aerial parts/root/pith	Antiplatelet, anticancer, antibacterial	(24, 55, 56)
Vernomenin	Sesquiterpene lactone	Aerial parts/root/pith	Anticancer, antibacterial	(51)
11,13-Dihydrovernodalol	Sesquiterpene lactone	Aerial parts/root/pith	Insecticidal	(53)
Hydroxyvernolide	Sesquiterpene lactone	Aerial parts/root/pith	Antiplasmodial, antitumor, antischistosomal, antileishmanial, antiprotozoal	(57,58)
3'Deoxyvernodalol	Sesquiterpene lactone	Aerial parts/root/pith	Anti-inflammatory and antioxidant	(49)
Luteolin	Flavonoid	Leaf/whole plant	Anticancer, antioxidant	(33, 34, 43, 59)
Luteolin 7-O- β -glucuroniside	Flavonoid	Leaf/whole plant	Antioxidant	(33, 34, 43)
Luteolin 7-O- β -glucoside	Flavonoid	Leaf/whole plant	Antioxidant	(33, 34, 43)
Isorhamnetin	Flavonoid	Flower	Antioxidant and antibacterial	(40)
Vemonioside A1	Steroid Glucoside	Leaf/stem/pith/Root	Antiplasmodial, antischistosomal	(51, 57, 60)
Vemonioside A2	Steroid Glucoside	Leaf/stem/pith/Root	Antiplasmodial, antischistosomal	(51, 57, 60)
Vemonioside A3	Steroid Glucoside	Leaf/stem/pith/Root	Antiplasmodial, antischistosomal	(51, 57, 60)
Vemonioside A4	Steroid Glucoside	Leaf/stem/pith/Root	Antiplasmodial, antischistosomal	(57, 60)
Vemonioside B1	Steroid Glucoside	Leaf/stem/pith/Root	Antiplasmodial, antischistosomal	(51, 57, 60)
Vemonioside B2	Steroid Glucoside	Leaf/stem/pith/Root	Antiplasmodial, antischistosomal, antitumor	(51, 57, 60)
Vemonioside B3	Steroid Glucoside	Leaf/stem/pith/Root	Antiplasmodial, antischistosomal	(57, 60)
Vemonioside D1	Steroid Glucoside	Leaf/stem/pith/Root	Antiplasmodial, antischistosomal	(57, 60)
Vemonioside D2	Steroid	Leaf/stem/pith/	Antiplasmodial,	(57, 60)

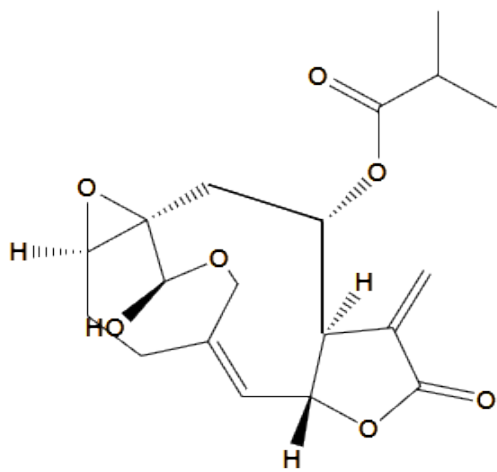
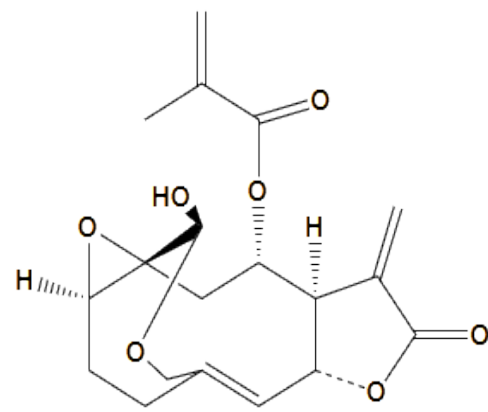
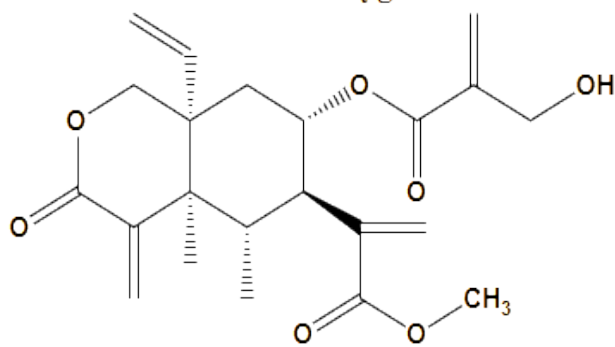
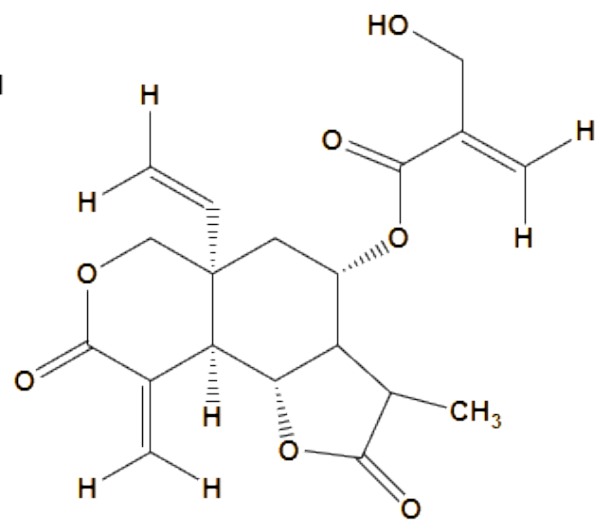
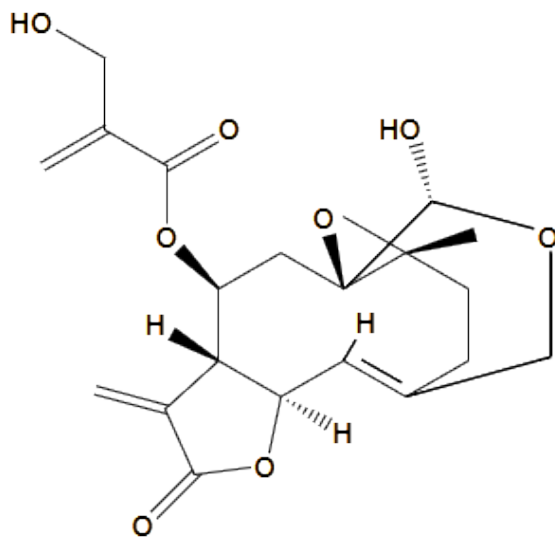
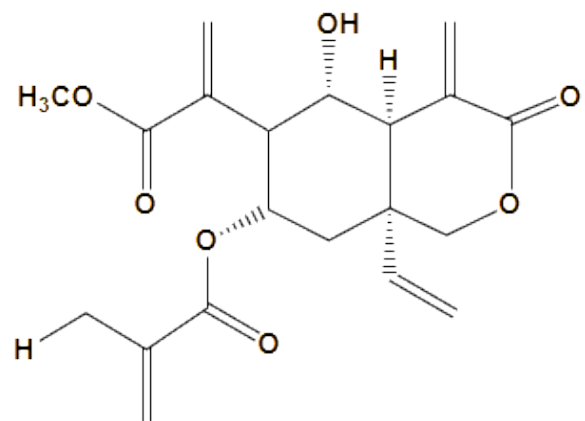
Compounds in <i>V. amygdalina</i>	Class of compound	Part of the plant	Uses	References
	Glucoside	Root	antischistosomal	
Vemonioside E	Steroid Glucoside	Leaf/stem/pith/Root	Antiplasmodial, antischistosomal	(57, 60)
Vernoamyosides A	Steroid saponins	Leaf	Anti-inflammatory	(20)
Vernoamyosides B	Steroid saponins	Leaf	Anti-inflammatory	(20)
Vernoamyosides C	Steroid saponins	Leaf	Anti-inflammatory	(20)
Vernoamyosides D	Steroid saponins	Leaf	Anti-inflammatory, antitumor	(20, 28)
Vernoniamyoside A	Steroid saponins	Leaf	Antitumor	(28)
Vernoniamyoside B	Steroid saponins	Leaf	Antitumor	(28)
Vernoniamyoside C	Steroid saponins	Leaf	Antitumor	(28)
Vernoniamyoside D	Steroid saponins	Leaf	Antitumor	(28)
Edotides	Peptides	Leaf/root		(41,61)
4 α -Hydroxy-n-pentacanoic acid	Hydroxy fatty acid	Stem bark	Anti-plasmodial	(42)
10-geranilanyl-O- β -D-xyloside	Terpene glycoside	Stem bark	Anti-plasmodial	(42)
11 α -Hydroxy-5,12-dien-28-oic acid-3 α ,25-olide	Steroid	Stem bark	Anti-plasmodial	(42)
1-Heneicosenol-O- β -D-glucoopyranoside	Fatty acid glycoside	Stem bark	Anti-plasmodial	(42)
Vernoniaolideglucoside	Fatty-keto glycoside	Stem bark	Anti-plasmodial	(42)
Glucoronolactone	Lactone	Stem bark	Anti-plasmodial	(42)
Others	Terpenes, coumarins, phenolic acid, lignans, xanthenes, and anthraquinones	Leaf		(34)

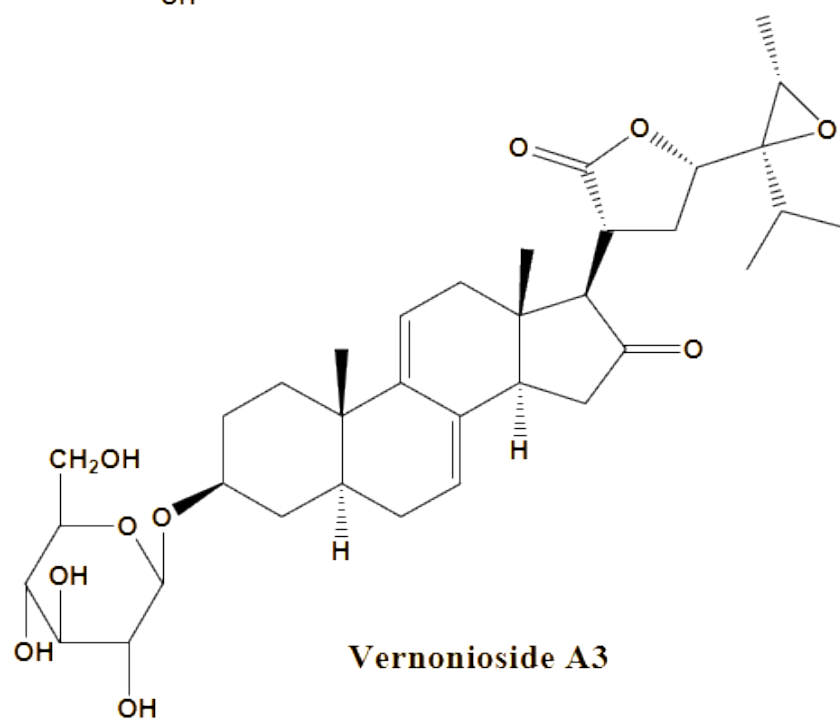
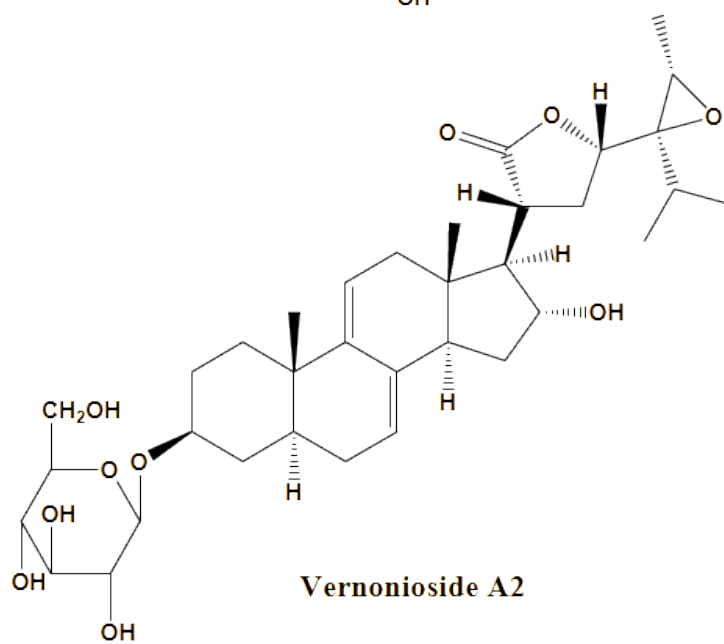
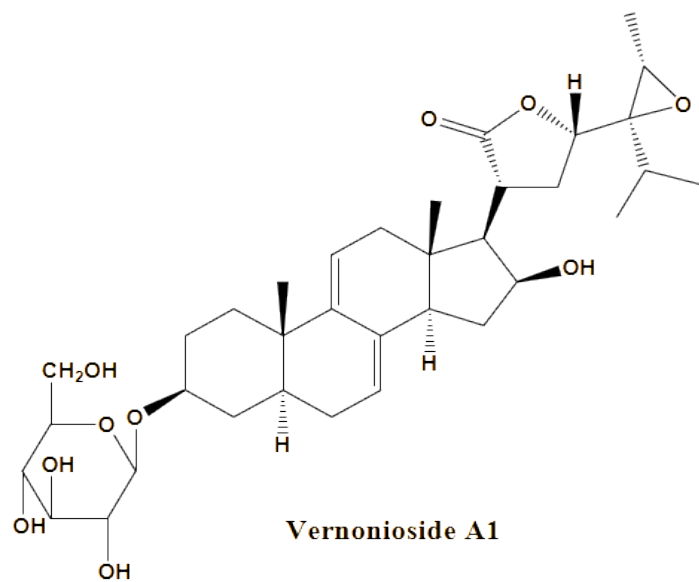
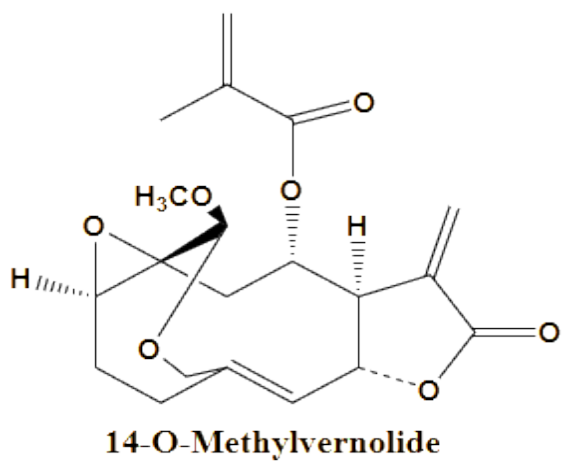
5. ESSENTIAL OIL FROM *V. amygdalina*

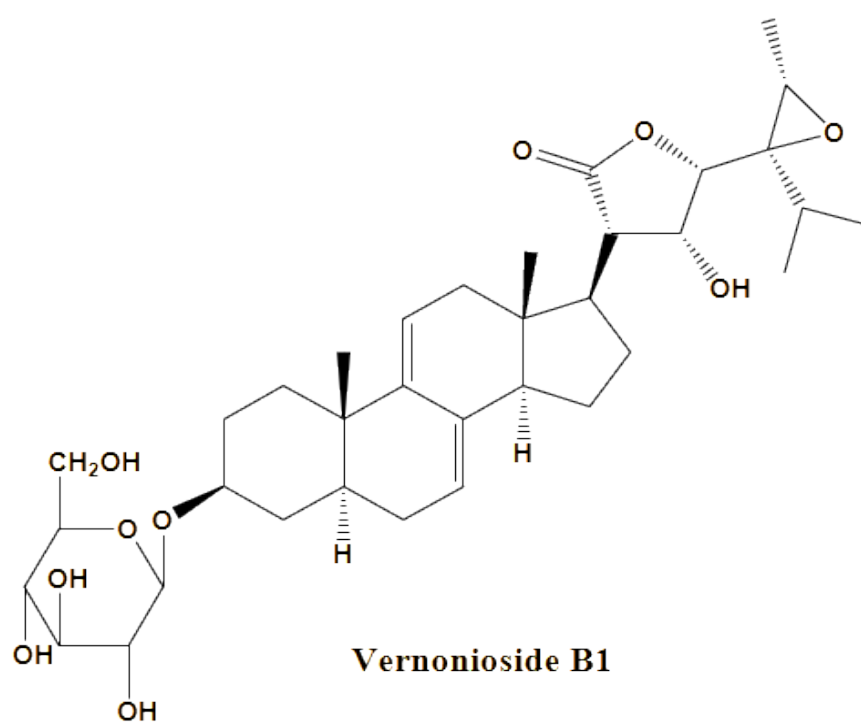
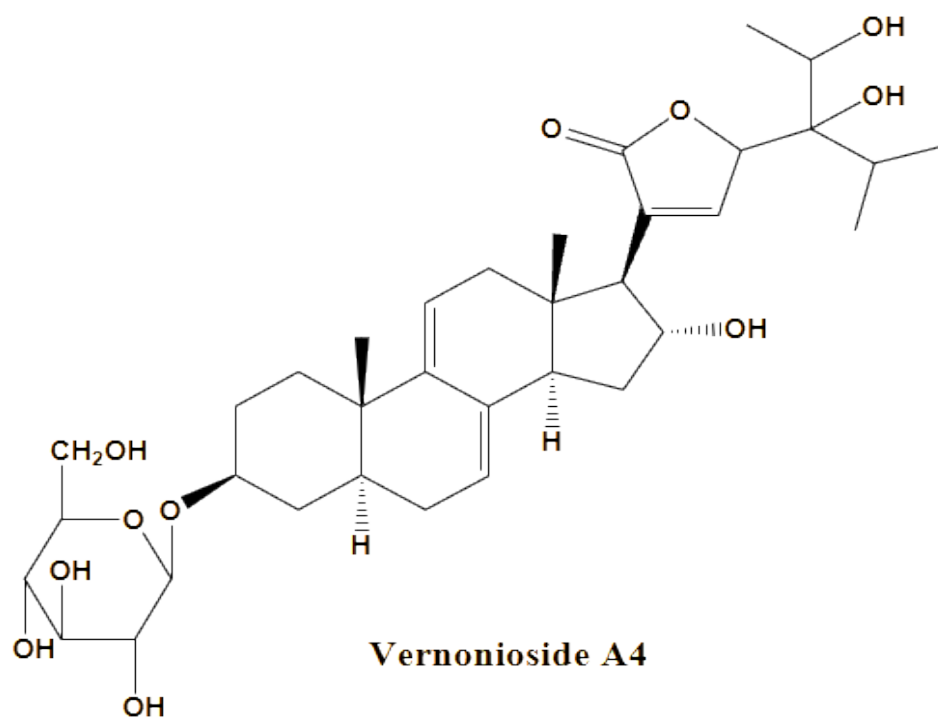
Essential oil obtained from the leaves of *V. amygdalina* via hydrodistillation contained eucalyptol (1,8-cineol, 25%), β -pinene (14.5%), and myrtenal (6.5%) as the major constituents, along with other minor constituents. α -muurolol (45.7%) was the major essential oil extracted from the aerial part (62, 63). Twenty-two percent palmitic acid, 21.5% α -linoleic acid (Omega-3) and 15.8% linoleic acid (Omega-6) were the main fatty

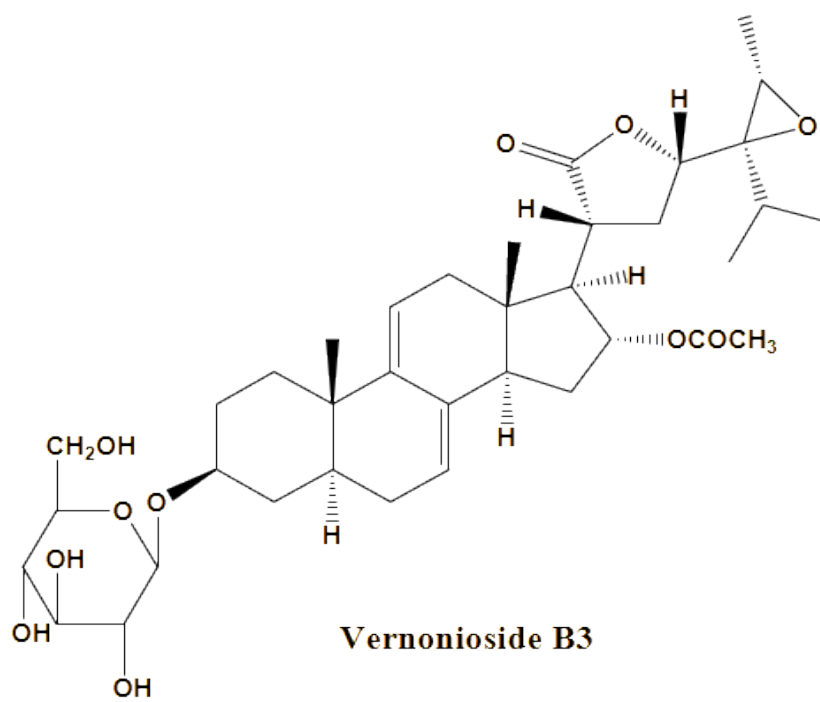
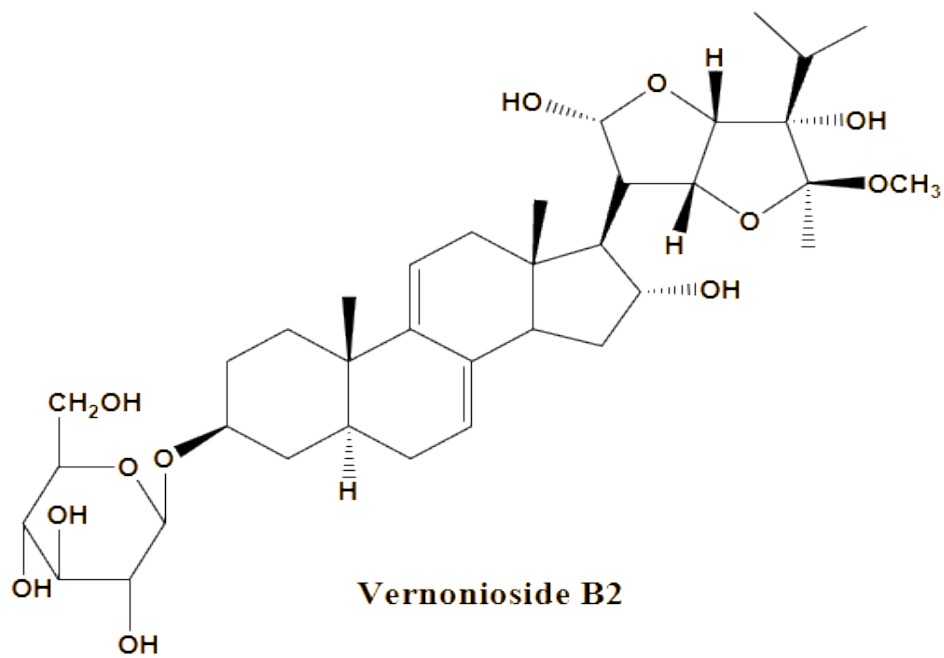
acids obtained from the hexane/isopropanol extract of *V. amygdalina* leaves (64). Furthermore, another report of the composition of the essential oil of the plant indicated that the major constituents include α -muurolol (45.7%), thymol (27.0 %), phytol (15.7 %), *o*-cymene (12.7 %), β -selinene (8.1 %), γ -terpinene (4.4 %), β -caryophyllene (3.9 %), and apiole (3.8 %) (65). While more than 40 compounds have been identified in the essential oil of the plant, the main constituent of oils from the plant obtained in Nigeria was α -muurolol (45.7%) (63).

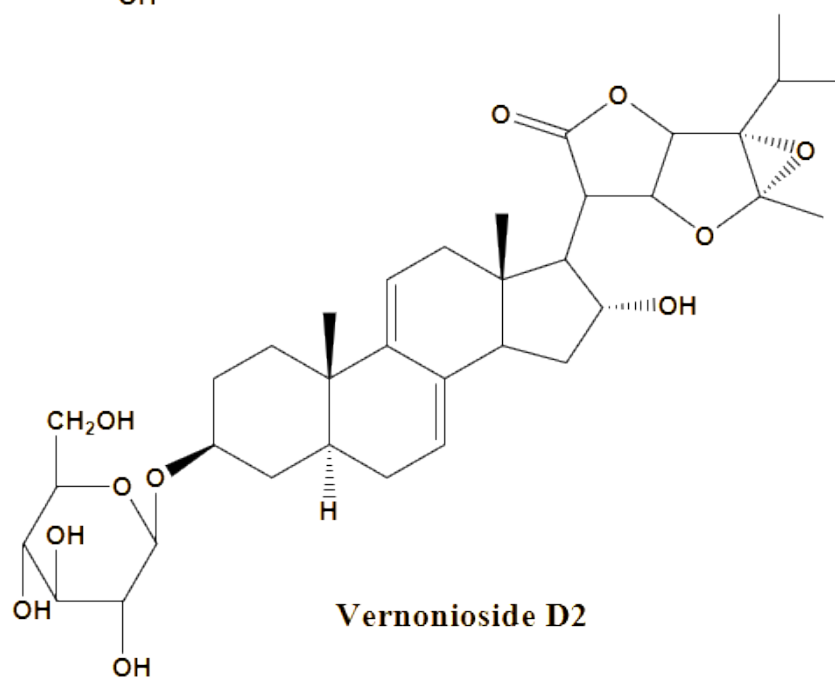
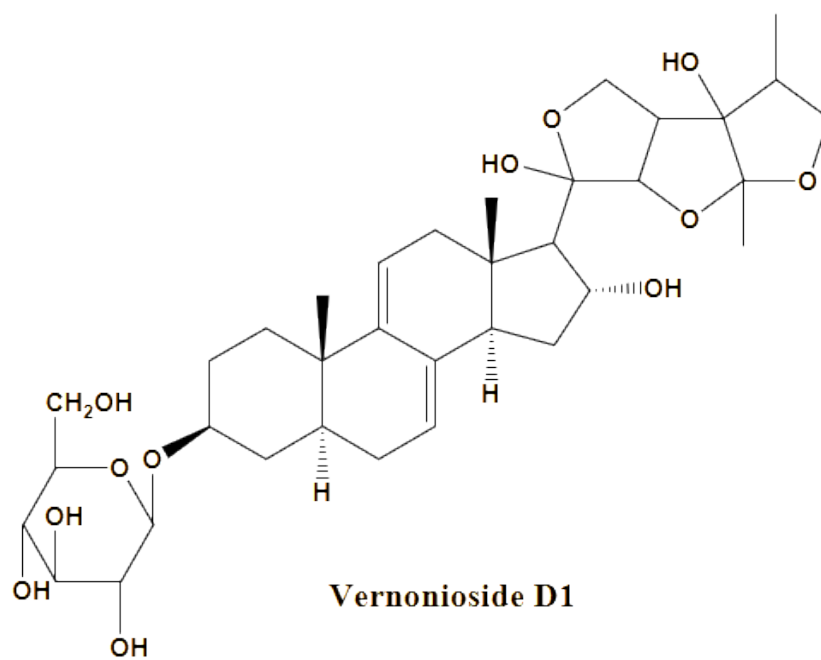


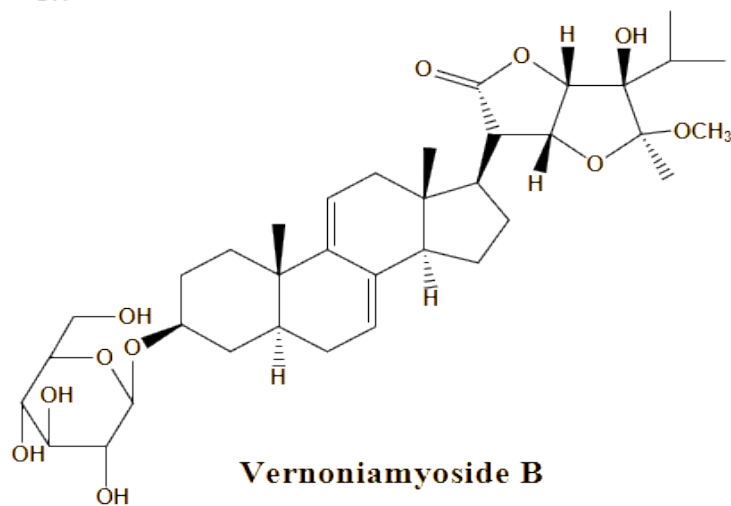
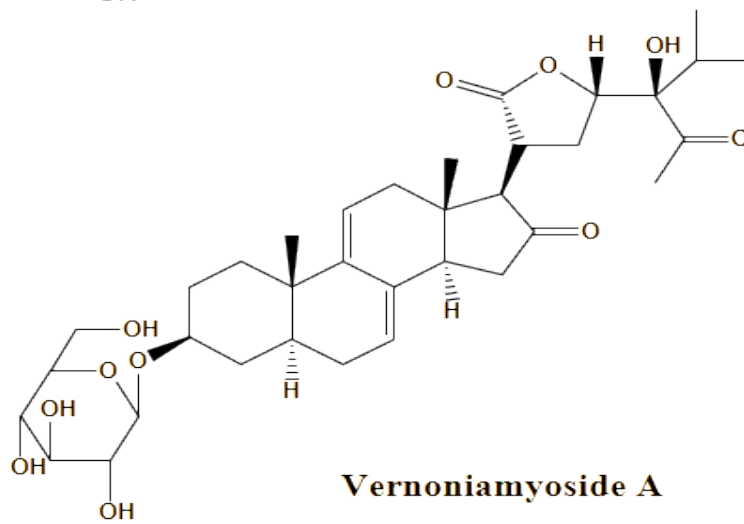
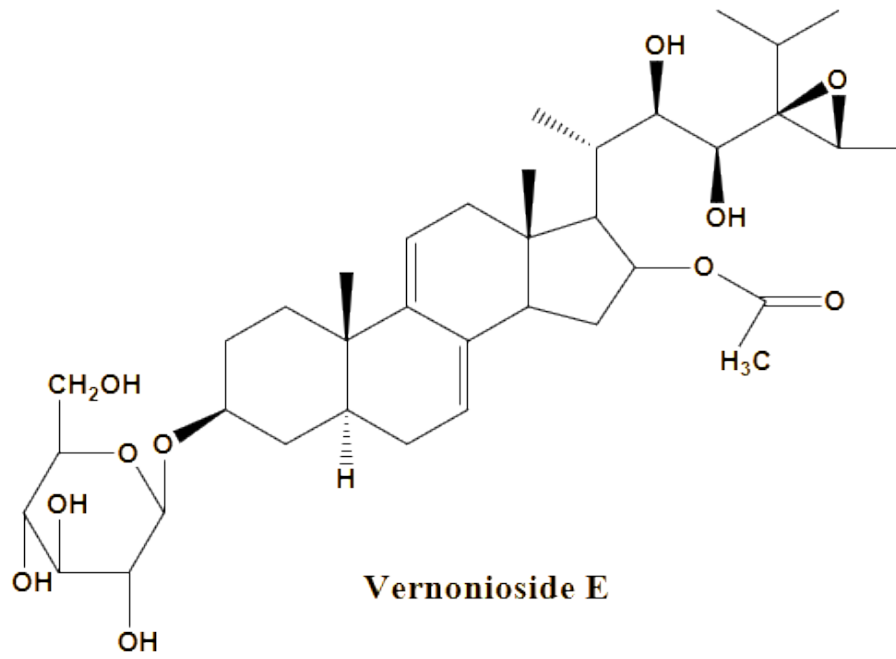
**Vernomygdin****Vernolide****Epivernodalol****11,13-dihydrovernodalol****Hydrovernolide****3'-deoxyvernodalol**

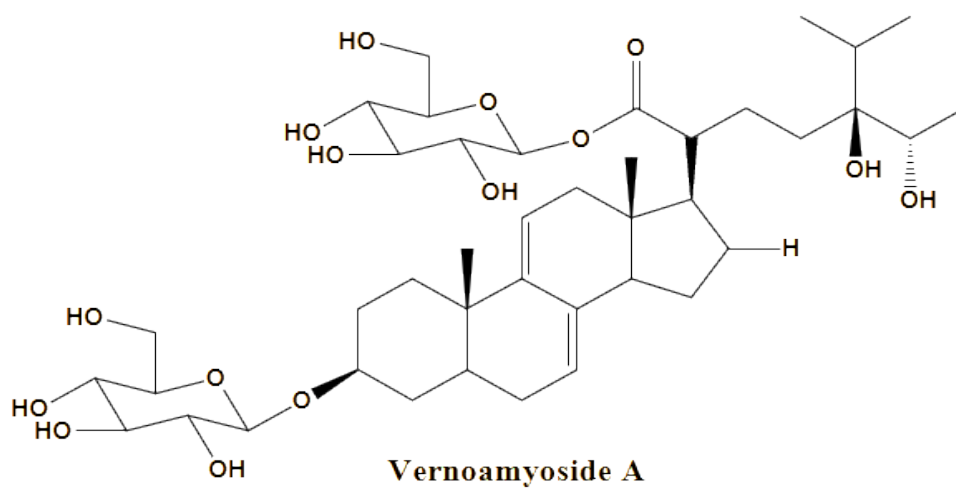
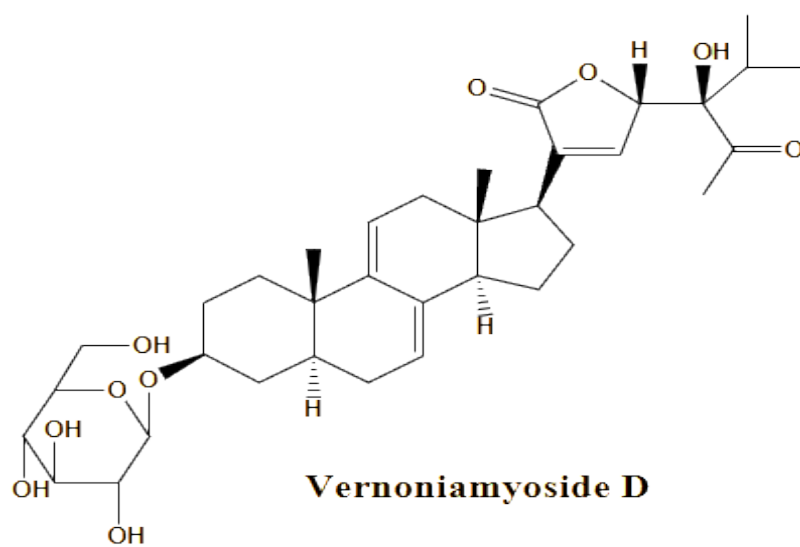
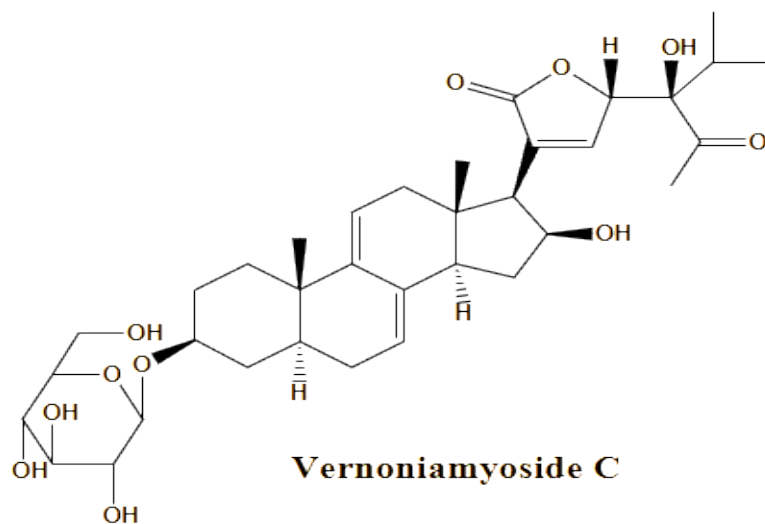


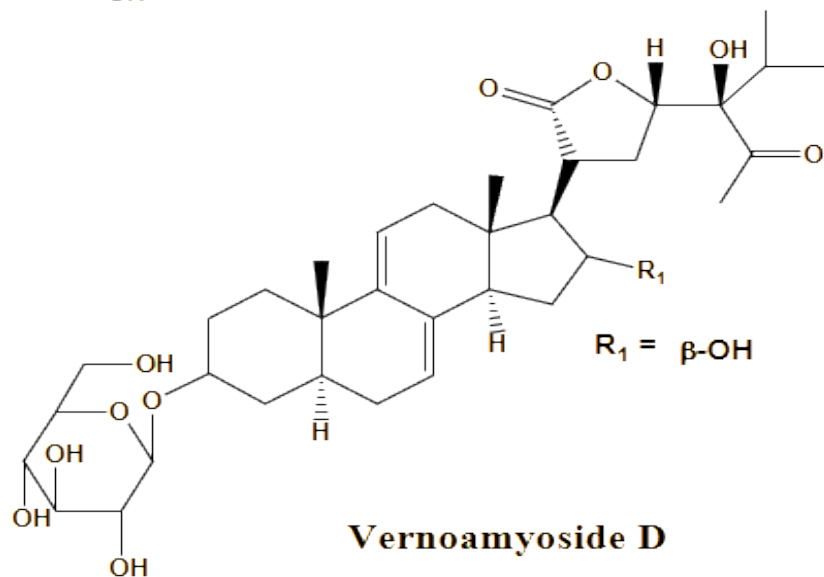
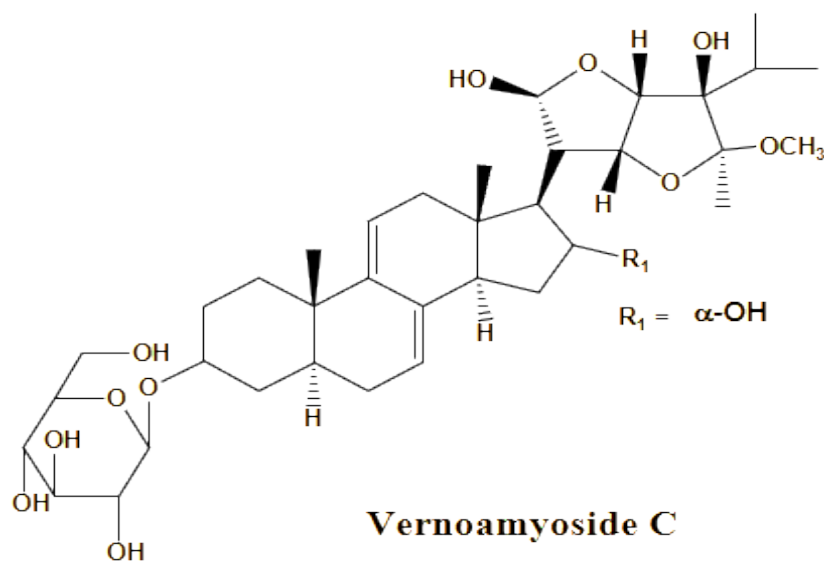
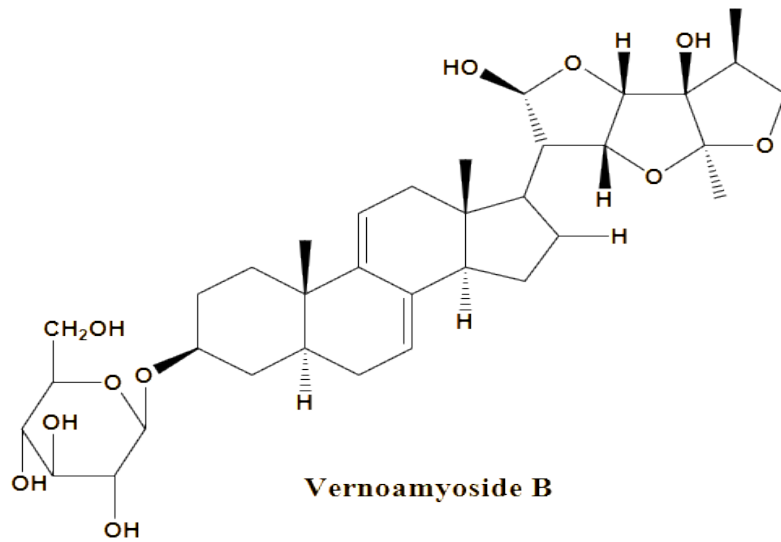


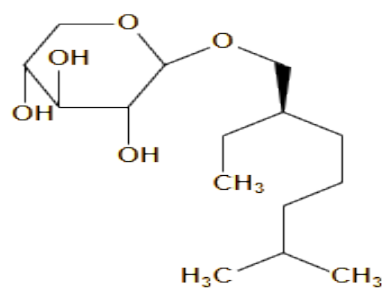
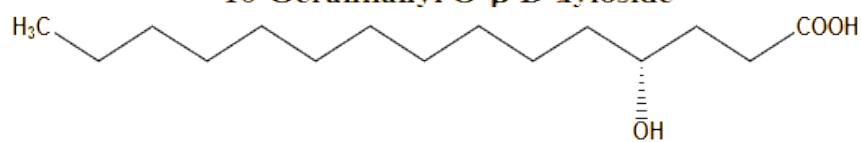
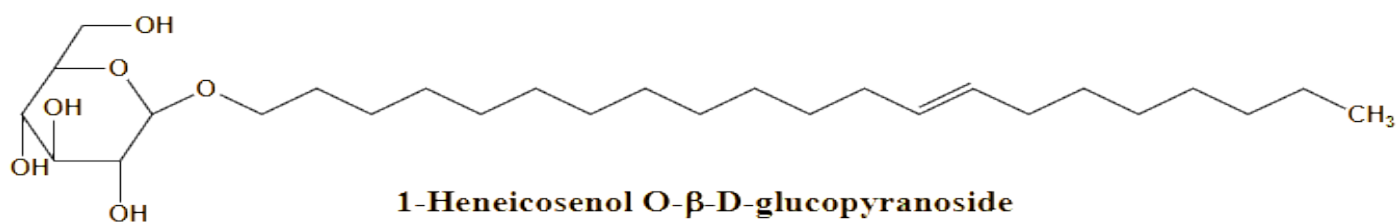
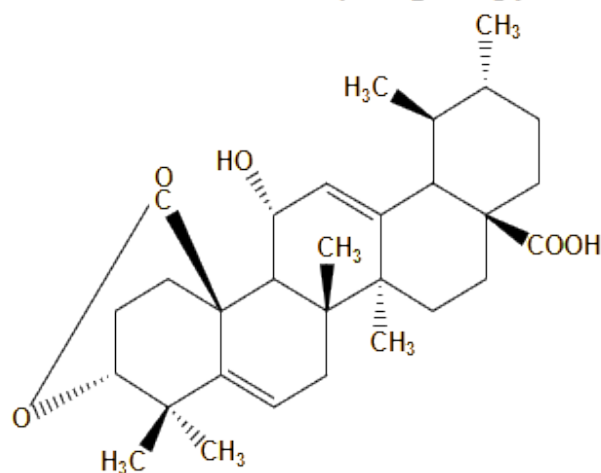
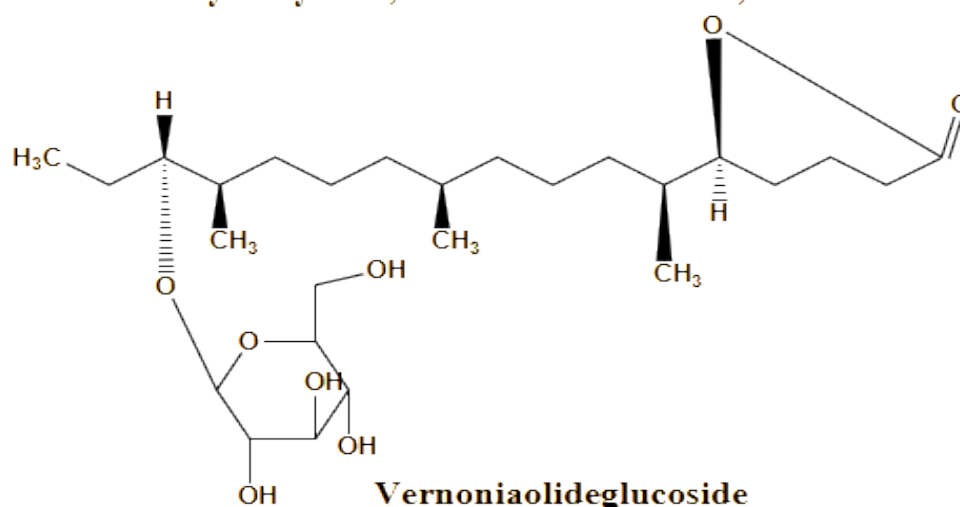










**10-Geranylanyl O- β -D-xyloside****4 α -Hydroxy-n-pentadecanoic acid****1-Heneicosenol O- β -D-glucopyranoside****11 α -Hydroxyurs-5, 12-dien-28-oic acid-3 α , 25-olide****Vernoniaolideglucoside**

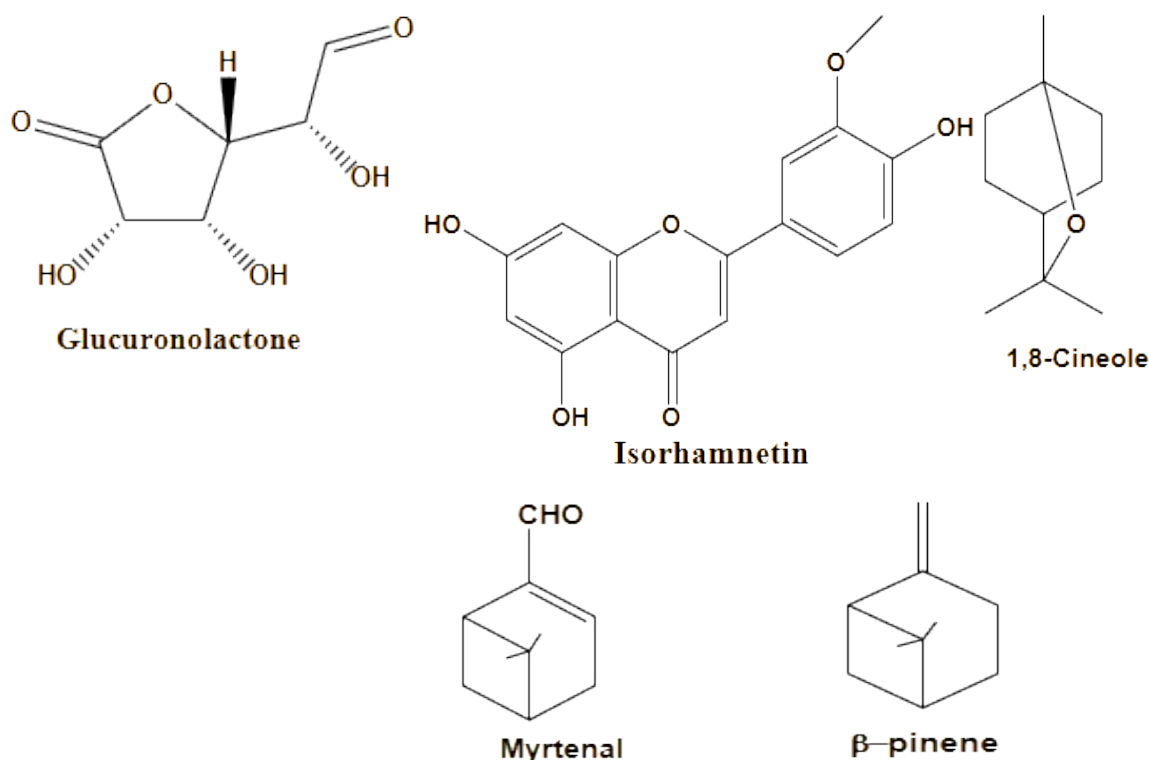


Figure 2: Structures of some phytochemicals isolated from *V. amygdalina*.

6. PHARMACOLOGICAL ACTIVITIES OF *V. amygdalina*

The pharmacological properties of *V. amygdalina* have been investigated to validate the wide traditional uses of the plant as a therapeutic agent. Several studies have indicated that *V. amygdalina* possesses anticancer, antidiabetic, antimalarial, anti-inflammatory, cathartic, hepatoprotective, antimicrobial, antioxidant, chemoprotective and cytotoxic, analgesic, anthelmintic, antipyretic, hypolipidemic, hemolytic, antimutagenic, antileishmanial, spermatogenic, antiplatelet and abortifacient activities (12, 66-68). The bioactive compounds in the plants could act independently or in synergy with each other to exert the pharmacological activities of interest or otherwise (43).

6.1. Antioxidant Activity

Since many synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), are suspected to be tumorigenic (69, 70), plant sources have been viable sources of antioxidants (70-75). The health-promoting activity of *V. amygdalina* is linked to the antioxidant capacity of flavonoids and luteolin. It has been reported that *V. amygdalina* glucosides luteolin 7-O- β -glucuronide and luteolin 7-O- β -glucoside possess significant antioxidant activities. Luteolin is reported as a strong antioxidant compound (32, 76). Studies have revealed that the aqueous and ethanol extracts of the plants' leaves, at about 200 mg/kg dosage, exhibit antioxidant properties, including scavenging free radicals, inhibiting the bleaching of β -carotene, and ameliorating serum malondialdehyde in both in vitro and rat models (38, 77-81).

6.2. Antidiabetic Activity

The aqueous extract of the leaves of *V. amygdalina* at about 80 mg/kg dosage has been reported to have significantly lower blood sugar (82-84). This claim was corroborated by many other reports from similar research on the plant that used various standards and *in vivo* assays (85-91). The sugar-lowering potential of the plant was observed to be independent of the mode of extract administration (92). Furthermore, the administration of the combined extracts of *V. amygdalina* and *Azadirachta indica* A. Juss. was reported to produce synergistic effects that attenuated blood glucose levels, regardless of the diabetic status of test animals (rats) (93).

The fermented extract of the plant exhibited significant antihyperglycemic potency in a rat model (94). The effect was further enhanced with the incorporation of *Ocimum gratissimum* L. leaf extract, suggesting that the potential of *V. amygdalina* leaf extract to inhibit diabetes progression could be synergistically improved with selected plant extracts.

Erukainure *et al.* (2018) examined the potential of *V. amygdalina* leaf infusion to inhibit α -glucosidase and intestinal glucose absorption (95). This inhibition was demonstrated as a potent antidiabetic mechanism, attenuating the breakdown of dietary carbohydrates and slowing the postprandial rise in blood glucose levels. The leaf infusion consequently stimulated muscular glucose absorption and conversion both in the absence and presence of insulin (95). This underscores the importance of glucose uptake and utilization by the muscles, a

major mechanism shared with many conventional antidiabetic drugs, such as metformin.

6.3 Anti-inflammatory Potential

V. amygdalina has also been reported to have anti-inflammatory activities *in vitro* and *in vivo*. A stigmastane-type steroidal saponin, isolated from the leaves and stem barks of the genus, exhibited anti-inflammatory activity (96, 97). The coadministration of *V. amygdalina* and *Azadirachta indica* extracts on normal and diabetic rats yielded a positive anti-inflammatory response (98). The aqueous extract of *V. amygdalina* leaves reportedly inhibited carrageenan-induced rat paw edema and xylene-induced ear edema in treated animals (99). Likewise, 100 µg/mL ethanolic extract of *V. amygdalina* exacerbated polymorphonuclear and mononuclear cell activities without impairing structural cell integrity (100). Young and old leaf extracts of the plant reportedly exhibited anti-inflammatory potential in a carrageenan-induced inflammation model in rats (101). The leaf extract reportedly repressed inflammatory potentials through reductions in leucocyte migration and lipid peroxidation in a mouse model (102). Additionally, *V. amygdalina* leaf extract, which contained compounds including vernonioside D, caffeoyl-quinic acids, luteolin, flavanone-O-rutinoside, and apigenin derivative, suppressed inflammation, collagenases, pain, and cartilage degradation while improving cartilage matrix synthesis when examined in cartilage explant assays and a postmenopausal osteoarthritis (OA) animal model (103). Furthermore, vernonioside V from the leaf extract strongly attenuated the secretion of inflammatory cytokines, including IL-6, IL-8 and TNF α , at a low dose (104).

6.4 Anticancer, Antiproliferative, and Cytotoxic Activities of *V. amygdalina*

The cytotoxicity and anticancer activity of *V. amygdalina* have been widely investigated (105, 106). It was further reported that the aqueous leaf extracts of the plant induced cell death in human hepatoblastoma (HBL), human breast tumors (MCF-7), and urinary bladder carcinoma (UBC) cell lines via a pathway that involved a reduction in the extracellular signal, among others (105). Similarly, peptides from plant leaves have been shown to exert anticancer activity, activating protein kinases (42, 107).

Other studies have shown the potency of plant extracts against cells, including MCF-7, BT-549, and human breast cancer cells (108-111). Vernoniomyosides A and B and vernonioside B₂ isolated from leaves of *V. amygdalina* exhibited strong cytotoxicity towards BT-549 cell lines, while vernoniomyoside C, vernoniomyoside D, and vernoamyoside D exhibited different levels of cytotoxic activities on HeLa, MCF-7, and MDA-MB-231 cells (20). The ethyl acetate extract of the leaves exhibited anticancer activity on 4T1 breast cancer cells via the induction of apoptosis, facilitated cell accumulation in G₂/M phases of the cell cycle, and attenuated the secretion of mTOR and PI3K (112). The cytotoxicity and

antiproliferative potential of the plant continued to be explored (113).

6.5 Neuroprotective Properties of *V. amygdalina*

Neurodegenerative diseases such as Alzheimer's and Parkinson's diseases are of major public health concern globally. These diseases are associated with neuronal death and have tremendous effects on movement, speech, intelligence, sleep, memory, and appetite (114). The neuroprotective role of *V. amygdalina* has been reported in several studies (54, 115). It was also reported that the aqueous leaf extract of *V. amygdalina* improved learning and memory in rats by modulating cholinergic neurotransmission (115). The leaves were demonstrated (116) to exert protective effects against neurodegeneration in the rat brain by modulating the activities of Na⁺/K⁺ ATPase, ecto-5'-nucleotidase, monoamine oxidase (MAO), acetylcholinesterase, butyrylcholinesterase, and oxidative stress. The methanolic extract of the leaves has been reported to protect against gamma radiation in the brain by improving antioxidant status and tissue morphology in the brains of rats exposed to gamma rays (54). It was demonstrated that leaf infusions stimulate glucose uptake and utilization in isolated brains (117). The neuroprotective potential of alkaloid-rich leaf extract has been reported in transgenic fruit fly (*Drosophila melanogaster*) model and scopolamine-induced amnesia rats (118).

6.6 Antibacterial and Anti-fungal Properties of *V. amygdalina*

Extensive investigation, particularly on the stem bark and leaf extracts of *V. amygdalina*, adds credibility to the folkloric claim of the antibacterial and antifungal potencies of the plant (119, 120). In a study, ethanolic and aqueous extracts of *V. amygdalina* leaves exhibited significant MIC inhibitions on *Streptococcus mutans* at 25 and 55 mg/mL, respectively. Ethanolic and aqueous extracts of *V. amygdalina* parts (leaf, bark, root, and honey) exhibited significant *in vitro* antibacterial and antifungal effects against various human pathogens, including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Aspergillus niger*, and *Escherichia coli* (17).

In addition, the stem bark or wood of *V. amygdalina* has been recommended for use as a chewing stick due to the confirmed activities of the extract against a broad spectrum of human pathogenic bacteria (121). The recommendation is in tandem with the documented traditional use of the plant as a chewing stick for maintaining clean and healthy teeth. The antidermatophytic and antimycoplasmal properties of *V. amygdalina* have also been reported (122, 123).

6.7 Antiviral Activities

Antiviral activities are not common properties of the family. However, a few antiviral studies have been reported for *V. amygdalina*. The ethanolic extract of

the plant's leaf is reportedly potent against some viral infections, including poliovirus (124).

6.8 Antimalarial Properties

Traditionally, *V. amygdalina* is used for the prevention and management of malaria (125-127). While *in vitro* anti-plasmodium activities have been reported (128-130,43) and *in vivo* potential has been confirmed (131-133), clinical trials have also been reported in some cases (134,135) and reported that the aqueous extract of the leaves of *V. amygdalina* inhibited 73% of *Plasmodium berghei* in mice when given 200 mg/kg daily for 4 days. Antihelminthic and antimalarial properties, as well as anti tumorigenic properties, have also been reported for extracts from the plant (17).

6.9 Hepato- and Nephroprotective Properties of *V. amygdalina*

Aqueous and ethanolic leaf extracts of *V. amygdalina* reportedly protect against tetrachloromethane-, alcohol- and aflatoxin-induced hepatotoxicity, among others, in mouse and rat models (78, 136-138). The extracts also had the potential to reverse hepatic damage in rats (46). Supplementation of a rat diet with the leaves of *V. amygdalina* was associated with no acute hepatotoxicity (139). However, a report indicated that the extract could trigger hyponatremia in rats (89). It was further confirmed that other selected plant extracts co-administered with the *V. amygdalina* extract reversed hyponatremia but exacerbated hypophosphatemia, which was diabetes-induced (140). In aspirin-triggered gastric ulcers in rats, leaf extract was observed to induce gastroprotection and prevent renal damage (141, 142).

7. ZOO-PHARMACOLOGY

In instances of severe parasitic infestations, including nematodes, some wild animals, such as chimpanzees, are known to deliberately ingest the leaves of *V. amygdalina* for treatment (143, 144). Pigs and other animals are also fed with the plant to expel parasites such as helminths (22, 143, 145). Some experimental research output added credence to the claim of the use of the leaves of the plant for the expulsion of parasites in animals (24).

8. PHYTO-COSMECEUTICAL APPLICATIONS

Phyto-cosmetics are primarily plant-derived products applied to improve the external look, radiance, beauty, and general health of skin, hair, and teeth. Generally, phytocosmeceuticals, as herbal products, contain bioactive phytochemicals that retard skin aging, acne, and skin wrinkling, among others (71, 74, 75, 146, 147). Prominent phytochemicals with bioactive properties, such as antioxidant, anti-inflammatory, and antityrosinase properties, include phenolics, flavonoids, terpenoids, saponins, and lipids (73, 148-150). *V. amygdalina* has been reported for its cosmetic application due to the presence of a great variety of phytochemicals, including phenolics and saponins, that support its usage in the preparation of traditional cosmetics,

astringents, emollients, skin moisturizers, photoprotection, or excipients in cosmetic preparations (151).

The leaf extract of *V. amygdalina* is used in Eastern Cameroon for the preparation of hair cream (151). Additionally, in the southwestern part of Nigeria, the leaves are said to be used as body cream after being dried, crushed into fine powder, and mixed with oil of choice (146). As a result of its antimicrobial potency, the stem and root are chewed in Nigeria for oral hygiene and prevention of dental decay (152). It was reported that the use of the chewing stick made from *V. amygdalina* resulted in fewer incidences of oral lesions compared to those who used toothbrushes (153).

9. OXYTOCIC POTENTIAL OF *V. amygdalina*

Traditionally, *V. amygdalina* aqueous leaf extract is used in child delivery to enhance uterine dilation and motility (154). Hence, some studies have confirmed the oxytocic potential in animal models (155, 156). The administration of the plant extract reportedly enhanced uterine motility and initiated contraction in rabbits, thereby confirming the oxytocic capability. Furthermore, *V. amygdalina* aqueous extract improved milk production while enhancing uterine contraction in an animal model (157). The results obtained add credence to oxytocic potency, as acclaimed by local midwives.

10. TOXICOLOGICAL EVALUATIONS OF *V. amygdalina*

The toxicity of the various parts of *V. amygdalina* has been severely investigated (158). The toxicity of the plant has been reported by various authors. The acetone extract reportedly showed toxicity only at higher dosages with an LD₅₀ of 824.6 mg/kg, while the methanol extract did not show any toxicity up to 2000 mg/kg in albino mice (159). The essential oils of the plant exerted a good measure of toxicity against *Sitophilus zeamais* Motschulsky (160). The polar extract of the plant attenuated Cd-induced hepatic injury in a wistar rat model (161).

10.1. *In silico* Toxicological Evaluation

The safety of plants in folk medicine is often based on the toxicity profile of the phytochemical compounds in the plant. In order to predict toxicity, various *in silico* approaches exist. Here, we performed *in silico* toxicity evaluations of the compounds using the ProTox-II and SuperCYPsPred platforms developed at Charite, University of Medicine. These computational platforms incorporate molecular similarity, fragment propensities, most frequent features, and machine-learning algorithms to predict 46 endpoints (models) in total. Various toxicity endpoints, such as acute toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity, immunotoxicity, adverse outcome (Tox21) pathways, toxicity targets, and cytochrome inhibition prediction of five major CYP isoforms, 3A4, 2C19, 2C9, 2D6, and 1A2, were identified. Based on these predictions (Table 3), the compounds were found to be safe (inactive

for toxicity endpoints) and had no relevant interactions with cytochromes. Table 3 reports the acute toxicity class profiles of these compounds. The ProTox-II acute toxicity prediction classes are defined according to the globally harmonized system of classification and labelling of chemicals (GHS). LD₅₀ values are given in [mg/kg]:

Class I: fatal if swallowed (LD₅₀ ≤ 5); Class II: fatal if swallowed (5 < LD₅₀ ≤ 50); Class III: toxic if swallowed (50 < LD₅₀ ≤ 300); Class IV: harmful if swallowed (300 < LD₅₀ ≤ 2000); Class V: may be harmful if swallowed (2000 < LD₅₀ ≤ 5000); Class VI: nontoxic (LD₅₀ > 5000).

Table 3: Acute toxicity class (predicted LD₅₀ oral in rat) analysis of the compounds using ProTox-II computational platform.

Name	ProTox-Acute Toxicity class	Prediction score (%)	Predicted LD ₅₀ value (mg/kg)
Vernolide	3	75.0	100
Vernodalol	4	69.80	1335
Vernolepin	3	68.07	150
Vernomenin	3	69.36	150
11,13-Dihydrovernodalol	4	69.46	452
Hydroxyvernolide	2	69.26	7
3'Deoxyvernodalol	NA	NA	NA
Luteolin	5	70.95	3919
Luteolin 7-O-β- glucuroniside	NA	NA	NA
Luteolin 7-O-β-glucoside	5	70.97	5000
Isorhamnetin	5	70.67	5000
Vemonioside A1	5	69.26	4000
Vemonioside A2	5	69.26	4000
Vemonioside A3	5	69.26	4000
Vemonioside A4	3	69.71	52
Vemonioside B1	5	69.26	4000
Vemonioside B2	6	70.01	8000
Vemonioside B3	5	69.26	4000
Vemonioside D1	6	69.26	8000
Vemonioside D2	5	78.00	3220
Vemonioside E	6	79.55	8000
Vernoamyosides A	4	73.34	1500
Vernoamyosides B	5	77.75	3220
Vernoamyosides C	3	73.43	53
Vernoamyosides D	3	71.77	52
Vernoniamyoside A	5	77.08	4000

Vernoniamyoside B	6	76.68	8000
Vernoniamyoside C	NA	NA	NA
Vernoniamyoside D	4	75.36	4000
Edotides	NA	NA	NA

NA: Not available.

From the data obtained (**Table 3**), all the compounds except hydroxyvernonolide were classified into classes III to VI, implying that most compounds were saved at $50 < LD_{50} \leq 5000$. These results apparently showed the relative safety of the plant when consumed at moderate doses. Hydroxyvernonolide, a sesquiterpene lactone obtained from the aerial part of *V. amygdalina* and from the leaf of *Vernonia cinerascens Sch.Bip.*, is reported to possess antitumor, antitrypanosomal, antiprotozoan, antileishmanial, and antiplasmodial activities (57, 58,162).

11. OTHER APPLICATIONS

It is apparent that numerous *in vitro* and *in vivo* have been done on the plant. *V. amygdalina* leaf extract was reported to exhibit strong antidiarrheal activity in a diarrheal model that was castor oil-induced. It was found to delay the onset of diarrhea and attenuate the frequency of stools and the weight of feces at 400 mg/kg bw (163). Other authors have also investigated the potential of various extracts of *V. amygdalina* to inhibit diarrhea (164). The plant is also reported for its cathartic (165), antitrypanosomal and antileishmanial (166), antiplaque (167), antinociceptive (102), antiphlogistic (99), antileukemia (106) and antiquorum sensing activities (168).

12. CONCLUSION

V. amygdalina is obviously a multimedicinal plant with high nutritional and pharmacological value. The plant is greatly endowed with important phytoconstituents that could be a source of lead candidates for drug development in the pharmaceutical industry. Plants that are used as vegetables in many climes hold potential for the ready and affordable management and prevention of chronic diseases such as diabetes and other cardiovascular-related diseases. The renowned activities of the plant, which include antioxidant, anti-inflammatory, anticancer, and antidiabetic activities, cannot be overemphasized. The high safety threshold of the plant makes it a candidate for further exploration. The underexplored parts, which include the root and flowers, need more scientific investigation.

13. CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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