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Significance of Bitter Leaf (Vernonia Amagdalina) In Tropical Diseases and Beyond: A Review

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Abstract

Vernonia amygdalina Delile (VA), family Asteraceae or Compositae is plants that is consumed locally as food and serve important ethnomedicinal uses. It grows throughout tropical Africa to a height of about 1 – 5 metres and it is indigenous to many West African Countries. Many parts of the plants are useful, they are used locally for the treatment of fever, Stomach disorder, jaundice, worm infestation, constipation, malaria, hiccups, kidney problems, amoebic dysentery, schistosomiasis, cough, wounds, diabetes, laxative, veneral diseases and other bacterial and protozoal infection. This review examines, discusses and summarizes the current evidence of ethnomedicinal uses, phytochemistry and biological activities as well as toxicity of this species with a view to identifying its therapeutic relations and possible contradictions, inconsistencies and gaps that may have arisen in the research literature. This review is based on literature study on journals and books of scientific origin from library, both manual and electronic sources such as PubMed, Science Direct, Elsevier, ACS, google Scholar etc using various combinations of search words. *V. amagdalina* is a tropical plant with a lot of interesting biological and medicinal uses. The plants are relatively not toxic, safe for consumption and possess a great potential as pharmaceutical leads for the treatment of diseases and beyond. This review will stimulate further research in the pharmacology and phytochemistry of *V. amagdalina*.

Keywords: *Vernonia amygdalina*; Plant extract; Pharmacology; Medicinal uses; Phytochemistry; Pharmacotherapy

Introduction

The Asteraceae (Compositae) are herbs, shrubs, or less commonly trees and is arguably the plants largest family of flowering and has approximately 1,620 genera and more than 23,600 species [1]. Vernonia is a genus of about 1,000 species of forbs and shrubs of which *V. amygdalina* is the most prominent specie and one of the pan tropical tribes of the family Asteraceae [2]. It grows predominantly in tropical Africa especially in Nigeria, Zimbabwe and South Africa and it is domesticated in parts of West Africa [3-5]. It is popularly called bitter leaves because of its bitter taste and is used as vegetables or as flavour decoction soups. The bitter taste of VA is as a result of its anti-nutritional components such as alkaloids, saponins, glycosides and tannins [6-8]. In Nigeria, it is known by several local names such as ''Ewuro'' in Yoruba language, ''Onugbu'' in Igbo language, "Oriwo" in Bini language, "Ityuna" in Tiv language, "Chusar doki or fatefate" in Hausa language and "Etidot" in Ibiobio [9]. The roots and leaves decoction of VA are commonly used in ethno medicine to treat fevers, hiccups, kidney problems and stomach discomfort among other several uses [10,11]. It is also used in the treatment of diarrhea, dysentery hepatitis and cough and as a laxative and fertility inducer [11]. The leaves of VA are also commonly used as a treatment against nematodes in humans and chimpanzees as well as other intestinal worms [12,13]. In addition, extracts of the plants have been reported to be used in Nigerian herbal homes as tonic, in the control of tick and treatment of hypertension [14-16]. The reported activity of VA is attributable to the complex active secondary plant compounds that are pharmacologically active [17]. Besides, this review also discusses the toxicity of this species and it gave a reasonable information that no abnormality or toxicity was caused by the administration of some of the extracts as well as single compounds on the organs of the animal samples. Nevertheless, there is need to carry out further study on chemical constituents and their mechanisms of action in other to fully comprehend its full phytochemical profile and complex pharmacological effects. Furthermore, clinical studies on the toxicity of all the plant parts extracts and the compounds isolated from this plant are required to ensure that they are eligible as sources of drugs.

Medicinal Properties

The aqueous and alcoholic crude extracts of the leaves, bark, stem and roots are reported to be widely used as antimalarial, for the treatment of eczema and as a purgative [18-20]. The roots and the leaves of VA are used in traditional medicine to treat fever, stomach discomfort, hiccups and kidney problems [21]. It is known as quinine

substitute because it is widely used for the treatment of fevers [21]. The wood, particularly those from the root is a tooth cleaner, an appetizer, fertility inducer and also for gastrointestinal upset [22]. The root infusion is taken in Nigeria for the treatment of intestinal worms as well as for enteritis and rheumatism [22]. Wild Chimpanzees have been observed to eat both the leaves and stems of the plant as a medication for self-deparasitization [23]. Other documented medicinal uses include the treatment of schistosomiasis, amoebic dysentery, treatment of malaria, wound healing, veneral diseases, hepatitis and diabetes [24,25]. Fresh leaves of VA have been reported to have abortifacient and purgative activities [26]. It is used in some part of Africa to prepare cough remedy [27]. The chopped roots of VA are used for the treatment of sexually transmitted diseases in parts of Zimbabwe [28]. The root of VA is used for its antifertility effect and for the treatment of amenorrhoea [28] (Table 1).

Part	Medicinal uses	References
Whole Plant	Snakebite and insect sting treatment, dyspepsia, influenza, dysentery, malaria and respiratory infections.	Kirtikhar and Basu, Chopra et al.
Leaf	Fever, colic pain, loss of appetite irregular stools and diarrhea, common cold, cough, fever, hepatitis, tuberculosis, mouth ulcers, bronchitis gastro-intestinal disorder and sores .	Selena et al. Panossian et al. Bensky and Gamble, Dymock et al.
Aerial part	Common cold, hypertension, diabetes, cancer, malaria and snakebite, urinary tract infection.	Panossian et al. Bensky and Gamble, Dymock et al. Perry,
Root	Febrifuge, tonic, stomachic and anthelmintic	Chopra et al.

Table 1: Medicinal uses of *VA*.

Phytochemistry

The phytochemical studies of *Vernonia amygdalina* reveals the presence of saponins, flavonoids, alkaloids, terpenes, steroids, coumarins, phenolic acids, lignans, xanthones, anthraquinones, edotides and sesquiterpenes [29] (Table 2).

Compound	Type	Plant part	Reference
Andrographolide	Diterpenoid lactone	Leaves/aerial	Reddy et al. Klaeipool, Puri et al. Matsuda et al. Cheung et al. Du et al. Kumar et al.
Neoandrographolide	Diterpenoid lactone	Leaves/aerial	Chan et al. Rao et al. Jain et al. Yang et al.
14-deoxyandrographolide	Diterpenoid lactone	Aerial parts	Matsuda et al. Kumar et al. Rao et al. Wu et al.
Andrographoside	Diterpene	Leaves/aerial parts	Matsuda et al. Rao et al.
14-deoxy-11, 12-didehydroandrographolide	Diterpenoid lactone	Aerial parts	Balman and Connolly, Wu et al. Jain et al.

19-O-β-D-glucopyranosyl-ent-labda-8(17), 13-dien-15, 16, 19-triol	Ent-labdane diterpenoid lactone	Aerial parts	Zou et al.
8α-methoxy-14-deoxy-17β-hydroxyandrographolide	Ent-labdane diterpenoid lactone	Aerial parts	Ma et al.
Andrographolactone	Diterpenoid lactone	Aerial parts	Xou et al.
3, 13, 14, 19-tetrahydroxy- ent-labda-8(17), 11-dien-16, 15 olide and 3, 19 isopropylidene- 14-deoxy- ent-labda-8(17), 13-diene-16, 15-olide	Diterpenoid lactone	Aerial parts	Xou et al.
14-deoxy-15-isopropylidene-11,12-didehydroandrographolide	Unusual Terpenoid	Aerial parts/roots	Reddy et al.
3,7,19-trihydroxyl-8,11, 13- ent-labdatriene-15, 16-olide and 8α,17β-epoxy-3, 19-dihydroxy-11,13-ent-labdatrien-15, 16-olide	Diterpene lactone	Aerial parts	Ma et al.
Andrograpanin	Diterpene	Leaves	Liu et al.

Table 2: Compounds of *VA*.

Sesquiterpenes

Different types of sesquiterpenes lactones have been isolated from VA, they include vernolide, vernodalol, vernolepin, vernodalin, vernomygdin, hydroxyvernolide, vernodalinol, vernomenin, vernolic, 11, 13-dihydrovernodalin, 11, 13-dihydrovernorodeline, 4, 15-dihydrovernodalin, 1, 2, 3, 15, 11, 13, 2', 3'-octahydrovernodalin and epivemodalol [17,29,30-33].

Stigmastane-type steroid glucosides:

These includes vernoniosides A1 ($C_{35}H_{52}O_{10}$, MW 632), vernoniosides A_2 ($C_{35}H_{52}O_{10}$, MW 632), vernoniosides A3 ($C_{35}H_{50}O_{10}$, MW 630), vernoniosides A4 ($C_{35}H_{52}O_{11}$, MW 648), vernoniosides B1 ($C_{35}H_{52}O_{10}$, MW 632), vernoniosides B_2 ($C_{36}H_{52}O_{12}$, MW 680), vernoniosides B_3 ($C_{37}H_{54}O_{11}$, MW 674), vernoniosides D ($C_{35}H_{52}O_{12}$, MW 664), venonioside D_2 ($C_{35}H_{50}O_{10}$) and vernoniosides E ($C_{37}H_{58}O_{11}$) [34-37].

Steroidal alcohol:

7, 24(28)-stigmastadien-3-β-ol [38].

Flavonoids:

These are; luteolin, luteolin 7-O-β glucoside and luteolin 7-O-glucuronoside, Myricetin [39,40] (Table 3).

Compound	Type	Plant part	Reference
5, 7, 2/, 3/-tetramethoxyflavonone	Flavonone	Whole plant	Rao et al.
5-hydroxy-7, 2/, 3/-trimethoxy flavones	Flavone	Whole plant	Rao et al.
5-hydroxy-7,2/,6/trimethoxyflavone	Flavone	Root	Rao et al.
7-O-methyldihydrowogonin	Flavone	Root/aerial part	Redi et al. Rao et al.
7-O-methylwogonin	Flavone	Root/aerial part/whole plant	Redi et al. Rao et al. Gupta et al. Kuroyanagi et al.
Flavone-1, 2/methylether	Flavone	Root/aerial part/whole plant	Redi et al. Rao et al. Jalal et al.
7-O-methylwogonin-5-glucoside	Flavones	Root/aerial parts	Redi et al. Rao et al. Kuroyanagi et al.
Dihydroskullcapflavone	Flavone	Whole plant	Hari et al.

5-hydroxy-7, 8, 2, 3/ tetramethoxyflavone	Flavone	Whole plant	Kuroyanagi et al. 1987 Rao et al.

Table 3: Flavonoids of *VA*.

Peptides:

The peptides are known as edotides and were first isolated [41] (Table 4).

Arabinogalactan	Protein	Herbs	Singh et al.
1, 8-dihydroxy-3, 7-dimethoxy-xanthone	Xanthone	Root	Dua et al.
4,8-dihydroxy-2,7-dimethoxy-xanthone	Xanthone	Root	Dua et al.
1,2-dihydroxy-6, 8-dimethoxy-xanthone	Xanthone	Root	Dua et al.
3,7,8-trimethoxy-1-hydroxy-xanthone	Xanthone	Root	Dua et al.
Andrographidoid A	Noriridoid	Root	Xu et al.
Andrographidoid B	Noriridoid	Root	Xu et al.
Andrographidoid C	Noriridoid	Root	Xu et al.
Andrographidoid D	Noriridoid	Root	Xu et al.
Andrographidoid E	Noriridoid	Root	Xu et al.

Table 4: Peptides of *VA*.

Essential oil:

Essential oil obtained from hydrodistillation of the aerial parts yields 1, 8 – cineol (eucalyptol) (25.11%), β – pinene (14.54%), myrtanal (6.52%), trans-pinocarveol (6.24%), linalool (4.28%) and α-pinene (4.93%) as the major components as well as other minor components [42]. Palmitic acid (22%), α-linoleic acid (Omega-3, 21.5%) and linoleic acid (Omega-6, 15.8%) were the major fatty acids obtained from hexane/ Isopropanol extract of VA leaves which had a yield of 0.31% w/w [43].

Pharmacology

The Pharmacological properties of VA have been investigated with a view to validate the wide traditional uses of the plant as a therapeutic agent. Several research has shown that VA possesses the following activities; antidiabetic, antiplasmodial, cathartic, hepatoprotective, antimicrobial, antioxidant, chemoprotective and cytotoxic, antihelmintic, hypolipidaemic, anti-platelet and abortifacient activities.

Anti-diabetic:

A number of studies have demonstrated the anti-diabetic properties of VA. Erasto et al. [44] demonstrated the *in vitro* anti-diabetic properties. The *in vivo* anti-diabetic have also be proven. [45-49]. While the clinical studies have also showed that VA improved glucose tolerance, fasting blood sugar and postprandial blood glucose levels in normoglycemic subjects [50]. Ethanolic extract of the leaves of VA improves glucose tolerance in streptozotocin-induced diabetic and normal Wistar rats at a dose of 400 mg/kg, 500 mg/kg metformin and ethanol vehicle served as positive and negative controls respectively. In the experiment, pre-treatment of the Wistar rats test group with 400 mg/kg of VA before inducing diabetics brought about 18.4% increase in the blood glucose of the rats (relative to blood glucose at time zero) compared to 36.6% increase in Diabetic control rat group after 1 hour of glucose loading, though metformin performed better at improving the glucose tolerance by reducing the blood glucose level to 5%, [51]. The possible antidiabetic mechanism of action of VA could be due to the fact that the aqueous extract of VA has been known to enhanced glucose utilization and uptake of muscles and liver cell cultures [44].

Antihelmintic activities:

Hot water extract of VA has shown no significant in vitro anthelmintic activity against *Haemondius Contortus* eggs at concentrations up to 11.2 mg/ml which gave up to 97.5% hatch rate as against 0.0% at concentration of 25μg/ml for albendazole standard [52]. This also corroborates an earlier in vivo trial [53]. Though it has been suggested that wild chimpanzees and gorillas eat VA for self-deparasitization [54] the above *in vitro* and *in vivo* studies does not seem to support the claim. However, since the primates eat the whole plant. It may therefore be argued that it is the purgation after ingesting the plant material that potentially causes the mechanical agitation and expulsion of helminthes.

Antioxidant Properties:

The antioxidant properties of VA are associated with its chemical constituents. Several researchers [55,56] have previously established that the antioxidant activities of fruits and vegetables like VA are related to their phenolic compounds. Natural polyphenols scavenges free radicals, chelates metal catalysts, activates antioxidant enzymes, reduces α-tocopherol radicals and inhibits oxidases, all of which have chain-breaking antioxidant activities and contributing to the prevention of degenerative diseases, cancer and atherosclerosis [57] has established that the DPPH radical scavenging activities of the leaves of VA fractions were significantly higher ($P<0.05$) than that of Glutathions. They also established that the polar polyphenolic compounds from VA were significantly better ($P>0.05$) at DPPH radical scavenging than the non-polar compounds (which mostly contain chlorophyll) present in non-polar acetone eluate. Fasakin et al. [58] demonstrated that leaf extract fractions of VA displayed weaker superoxide scavenging activities against superoxide radicals produced from pyragallol autoxidation when compared to Glutathione. It was also established that the 80% acetone extract fraction (Chlorophyl-enriched) of VA exhibited stronger superoxide radical scavenging ability than 70% ethanol and 70% methanol. This study is important because, though superoxide ions cannot directly initiate lipid oxidation, they are potential precursors of highly reactive species such as hydroxyl radical. Also 80% acetone fraction of VA leaves displayed significantly higher hydroxyl scavenging abilities than Glutathione, unlike ethanol fractions which displayed lower hydroxyl scavenging

activities than Glutathione. Polyphenolic fractions VA also showed a higher metal chelating ability than Glutathione [59-61]. The polyphenolic compounds in the acetone extract leaves of VA extract are good hydrogen and electron donors, this was demonstrated by its reduction of Fe^{3+}-fernicyanide complex to the ferrous state and measured at 700 nm and was found to be directly proportional to the reducing at power of VA [61]. The result showed that the ferric reducing ability of the VA extract fraction is similar to that of Glutathione extract fraction.

Hypolipidemic:

Hypercholesterolemia is a risk factor for cardiovascular diseases such as myocardial infarction and atherosclerosis, which is a common cause of mortality and morbidity [62,63]. Even though several factors such as age, lifestyle, diet rich in cholesterol and hypertension, have been reported to cause heart failure [64], increased levels of cholesterol, especially low density lipoprotein cholesterol, are mainly responsible for hypercholesterolemia. Several researches have shown that hypercholesterolemia is associated with enhanced oxidative stress related to increased lipid peroxidation. High generation of oxidized LDL is a major factor in the vascular damage associated with high cholesterol levels. Thus, the inhibition of oxidative stress under hypercholesterolemia is considered a vital therapeutic approach [65-68]. The screening of VA as hypolipidemic drug was borne out of the desire to discover new drugs from nature that can serve as a credible alternative to the present synthetic drugs such as fibrates, bile acids sequestrants and statins which are currently used for such purpose, but however, has severe adverse effect such as rhabdomyolysis which can be caused by taking a statin with another lipid-lowering drug, particularly fibrates [69]. The lipid-lowering effect of methanol extract of *Vernonia amygdalina* (MEVA) leaves in rats fed on high cholesterol diet, and compared with a standard hypolipidemic drug, Questran have been investigated. The effects of MEVA on the lipid profile were assessed by measuring the levels of total cholesterol, LDL cholesterol, HDL cholesterol, triglyceride, lipid peroxidation (LPO), phospholipid, and glutathione (GSH) in the plasma and liver of the rats. Cholesterol administered at a dose of 30 mg/ 0.3 ml, five times in a week for nine consecutive weeks resulted in a significant increase ($p<0.05$) in plasma and post mitochondrial fraction (PMF) cholesterol levels by 33% and 55%, respectively. But, treatment with MEVA at doses of 100 mg/kg and 200 mg/kg caused a dose dependent reduction in plasma and PMF cholesterol by 20%, 23% and 23%, 29%, respectively. These reductions in cholesterol were similar to the ones obtained in Questran-treated rats. VA enhanced the cholesterol-induced decrease in PMF glutathione levels of the rats [70]. In general, it can be deduced that these results suggest the lipid-lowering effects of VA and as a result could serve as potential natural product for the treatment of hyperlipidaemia.

Anticancer activity:

The different extracts of VA have been investigated against different types of cancer cell lines. Various fractions from different extracts of VA were investigated against cells derived from human carcinoma of the nasopharynx in tissue culture. The compounds from VA were cytotoxic sesquiterpene lactones; vernodalin, vernomygdin and vernolide with activity of ED50 of 1.8, 1.5 and 2.0 µg/ml respectively against KB cell culture [15]. Ethanol extract of VA have also been reported to possess considerable *in vitro* cytotoxic activity (IC50=60.33) against non-cancerous vero cell line (C-1008 kidney fibroblast from African green monkey) by neural red uptake method [71]. Also, the *in vitro* growth inhibitory and cytotoxic evaluation of the epivernodalol, a known sesquiterpene lactone compound from VA leaves against skin melanoma cell line (HT-144) by the sulforhodamine B (SRB) assay have been investigated [72]. It was demonstrated that various concentrations (3 – 100 mg/ml) of water soluble extract of VA potently inhibited extracellular cellular Signal-Regulated Kinase (ERK) activities, DNA synthesis and cell growth in a dose-dependent manner. The results showed that VA in least concentrations of up to 10 mg/ml exhibited cytostatic action to retard the growth of human breast cell cancer cells, both in the presence or absence of serum [41].

Further studies conducted by Adesanoye et al. investigated the *in vitro* mechanisms of VA leaf extracts against breast cancer. The study demonstrated that, in a concentration and time dependent manner, VA-induced cytotoxicity and apoptosis in MCF-cells (breast cancer cell lines) involved phosphatidyl serine externalization followed by secondary necrotic cell death [73]. Kupchan et al. reported that vernomygdin, vernodalin showed inhibitory effects against human nasopharynx carcinoma cell line. Vernomenin, vernodalin and vernolepin also showed inhibitory activity against P-388 and L-1210 mouse leukemic cancer cell lines, while only vernodalin possessed antitumor activity against nasopharynx carcinoma KB cancer cell lines [30]. VA extract inhibited cell proliferation and DNA synthesis of BT-549 Breast cancer cells in *in vitro* antiproliferative study [74,75].

Cathartic effect:

The methanol extract of VA produced significant ($p<0.05$) promotion of gastrointestinal motility of charcoal meal in mice, gastric emptying of gastrointestinal contents was also promoted. Though these effects were not as pronounced as that of carbachol (1mg/kg), VA extract treatment produced a dose-dependent increase of the total number of faeces in rats. The reference drug used, senna, produced defecation to a greater degree than the extract. The increased gastrointestinal motility was attributed to the presence of saponin which may act by direct gastric mucosa irritation [76] or reasonable content of calcium ions [77] which is essential for various physiological responses including glandular secretion and muscle contraction; these findings support the traditional use of VA for constipation and stomach upset [78].

Abortifacient:

The methanol extract of VA when administered to pregnant mice caused abortion within 24 hours [26].

Antifertility:

Recent report indicated that 95% ethanol crude extract of the leaves of VA possesses an *in vitro* anti-implantation effect at doses of 0.385, 0.5 and 1.0 g/kg body weight of mice in isolated mouse uterus compared to control agonist Acetylcholine (1 g/kg). It caused a significant reduction in mean number of implantation sites compared to the control, there was also a significant ($p<0.05$) reduction in the number of live fetuses and survival percentages between the controls and test groups at a dose of 3 mg/kg [78].

Antimicrobial:

A methanol leaf extract (60%) of VA has been found to be active at 25 mg/ml against a panel of microorganisms (8 bacterial isolates and some fungi, but with absence of marked activity against *Candida*

albicans [27]. The secondary plant metabolites such as saponins, flavonoids and alkaloids found in the plant are thought to be responsible for this observed antimicrobial inhibitory activity effective in wound healing. Jisaka et al. showed that vernolepin and vernomenin possessed antibacterial effect against *B. subtilis* and *M. lutea*. 4, 15-dihydrovernodalin demonstrated the highest antibacterial activity against *B. subtilis* and M. lutea when compared to vernolepin, vernolide, vernodalin and vernomenin [17,32]. Vernodalol is also active against *B. cereus*, *S. epidemidus*, *S. aureus, M. kristinae* and *S. pyrogens* (gram positive bacteria), except for *S. pooni* (inhibited at 0.5 mg/ml), which was found to be inactive against gram negative bacteria [79].

Antiplatelet and anticoagulant:

An antiplatelet drug is a member of class of pharmaceuticals that decrease platelet aggregation [80]. Vernolepin is a sesquiterpene lactone compound isolated from VA alcoholic leaves extract and has demonstrated *in vitro* platelet anti-aggregating and disaggregating activity at an optimum concentration of 1 X 10-5 g/ml. it showed a 'stabilizing effect' towards rabbit platelets during freeze-thawing, it inhibited arachidonic acid, ADP and collagen-induced platelet aggregation as well as interferes with ATP-release. Electromicroscopy reveals platelet protection by vernolepin against adhesion and a desaggregating effect. These activities gave a steep dose response relationship and were time dependent [81]. VA extract caused a reduction in blood pressure, vernolepin isolated from this plant was identified to be responsible for its antiplatelet activity. Methanol extract of VA at doses of 100 mg/kg and 200 mg/kg induced a 40% and 50% inhibition against thrombosis in mice [39].

Anti-malarial:

The anti-plasmodial activities of VA have been investigated by several researchers. Isoamyl alcohol fractions of VA at concentrations less than 3µg/ml showed in vitro antiplasmodial activity. The *in vitro* antiplasmodial activity was evaluated by dissolving 5 mg of VA in 5 ml ethanol to obtain a polar extract which was diluted in distilled water to give a series of test concentrations ranging from 0.5 to 500 µg/ml which were tested in triplicate against chloroquine sensitive *Plasmodium falciparium* infected human blood, Quinine 2HCl was used as an antimalarial reference product, with IC_{50} value of 0.25 µg/ml. Krafi et al. demonstrated that ethanol extract of VA showed a high antiplasmodial activity (9.82 µg/ml) against 3D7 chloroquine sensitive clone of NF-54 isolate of *Plasmodium falciparium* using a 48 hours microassay technique [82]. The aqueous and hydroethanol extract however demonstrated significant antiplasmodial activity at IC_{50} of 41.690 µg/ml and 44.03 µg/ml. It was reported that the significant *in vitro* antiplasmodial activities of vernodalin and vernodalol from *Vernonia coloata*; a related species to VA, at IC_{50} values of 1.1-4.8 µg/ml were also determined. Sesquiterpene lactones such as vernolepin, vernolin, vernolide, vernodalin and hydroxyvernodalin isolated from VA leaves have been reported to exhibit antiplasmodial activities (IC_{50}<4 µg/ml) against *P. falciparium* strains [82,83].

Hepatoprotective:

Oral administration of methanol extract of leaves of VA brought about a modulatory effect on the hepatotoxicity of carbon tetrachloride (CCl4) [84]. It brought about a liver protective effect against CCl_4 – induced hepato-toxicity at the tested concentration. Hepatotoxicity was induced in albino Wistar rats by the oral administration of CCl4 at a dose of 1.2 g/kg body weight 3 times a week for 3 weeks, this treatment induced a significant hepatic injury as shown by increased activity of the serum enzymes AST, ALT, SALP and Y-GT. Methanol extract of of VA administered 5 times a week for 2 weeks prior to CCl_4 treatment at 250 and 500 mg/kg doses of the extract reduced the increase in the activities of these enzymes. In the same way, the methanol extract of VA reduced the CCl4-induced increase in the concentrations of cholesterol, phospholipid and triglyceride by 37.8%, 8.5% and 30.6% respectively, as well as reduction in the cholesterol/ phospholipid ratio. Pretreatment with methanol VA extract at a dose of 500 mg/kg reduced CCl4-induced lipid peroxidation by 57.2%. In a similar way, it increased the activities of superoxide dismutase, glutathione S-transferase, but reduced glutathione concentration significantly at 500 mg/kg ($p<0.05$) and catalase activity at 500-1000 mg/kg doses, suggesting that VA leaves possesses protective effect against CCl_4 – induced hepatotoxicity by antioxidant mechanism of action, it was hypothesized that the increase in the activity of aspartate aminoferase alone may be of extra hepatic origin [85].

Analgesic activity:

Ethanol extract of VA has been found to induce a significant ($p<0.05$) dose-dependent analgesic activity in acetic acid induced writhing test in mice, the highest percentage inhibition (71.9%) was found at 100 mg extract/kg against the acetic acid-induced writhing in mice. The activity was higher than that of the standard analgesic; ketonal (67.5%) used at 10 mg/kg [86,87]. The study results tend to justified the ethnomedicinal uses of VA in the management of toothache, gingivitis, rheumatism since it was found to have analgesic activity. Agbaje et al. examines the antinociceptive potential of aqueous leaf extract (50-200 mg/kg), using the acetic acid – induce writhing, formalin test and tail flick test models [88]. It was observed that the extract significantly inhibited acetic acid-induced writhing and formalin test in mice but did not give any significant effect in the tail – flick test, suggestive of central and peripheral analgesic properties of the extract [89].

Anti-inflammatory:

The roots and leaves extracts, and the saponin fractions of VA possessed significant anti-inflammatory activity against xylene-induced acute inflammation in the ear of Wistar rats. The anti-inflammatory response was dose-dependent and the percentage inhibition was higher with the leaf than the root extract. The VA-saponin extract gave a relatively lower, but also significant inhibitory activity compared to the extracts. The anti-inflammatory activity of the extracts were comparable in magnitude to activity of dexamethasone and may partly be explained to be due to the presence of flavonoids, tannins, glycosides and trace elements; copper, manganese and zinc which were shown to be present in previous study [84]. Other studies have reported the anti-inflammatory activity of these substances [90-93].

Anti-pyretic activity:

Investigations proved that the anti-pyretic activities of the leaf, root and saponin fraction from VA. Pyrexia was induced using the procedure demonstrated Oboh et al. 20 hours after the administration of *Saccharomyces cerevitae* (Brewer's yeast) induces pyrexia, the anal temperatures of the animals (Wistar's rat), the anal temperature

reading of each animal was taken before dose administration [94]. The anal temperature reading of each animal was repeated 4 hours after dose administration; this procedure was used for the evaluation of the leaf and root aqueous extract, and the VA saponin fractions. Normal saline (5 ml/kg) was used as placebo and Acetyl Salicylic Acid 250 mg/kg as standard analgesic. The result showed that, just as observed with ASA, all doses of aqueous extracts of leaves and roots and VA-saponin fraction gave a significant anal temperature decrease (but to a lesser extent than ASA), except at 50 mg/kg of VA-saponin B fraction. Anti-pyretic activity was observed to be higher in the leaf than the root extract, but the saponin B fraction showed much lower activity than the root extract at similar dose of 200 mg/kg (108).

Haemolytic properties:

Recent reports investigated the *in vitro* haemolytic properties of VA, results showed that infusion of VA induced a significant ($p<0.05$) haemolysis of human erythrocyte. Human genotype SS (1024) were highly susceptible to haemolysis induced by VA infusion, genotype-AS (512) were moderately susceptible, but genotype-AA (256) were highly resistant to haemolysis induced by the same infusion [95]. However, Alawa et al. reported a non-significant effect of methanol extract of VA in a 30-day treatment of rats on red blood cells (RBC) counts and other indices such as Heamoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) which are related to it, this is in relation to the control [96]. Also, there was no significant changes in the Total White Blood Cell count (TWBC), platelet, neutrophil, lymphocyte, eosinophil, and monocytes values relative to their respective controls.

Antimutagenicity:

Obaseiki –Ebor et al. demonstrated that the Petroleum ether, methanol and ethylacetate extracts of VA were able to significantly (more than 60%) inhibit His-to His+ mutation induced by ethylmethanesulfonate on *Salmonella typhimurium* TA100 [97]. Petroleum ether extract was the most active, followed by methanol extract and then the ethylacetate fraction.

Anti-leishmanial activity:

Methanol, hexane and aqueous extract of VA leaves have demonstrated both *in vitro* and *in vivo* experiments to suppress the infection rate of leishmaniasis [32], it delayed onset of the disease with significant reduction in lesion size and attenuation of the histopathological outcome characterized by intact epidermis and less tissue destruction in skin, spleen and liver after inoculation with metacyclic promatigotes from *Leishmania* major parasite in Balb/c mice. The methanol extract containing the highest concentration of flavonoids produced the highest activity, thus suggesting that, the flavonoids are responsible for the anti-leishmanial activity of VA (120) [32]. Hydroxylvernolide, a compound isolated from the leaves of VA showed a significant antileishmanial effect on *Leishmania infantum* [32].

Spermatogenic effect:

Recent report showed that the aqueous extract of the leaves of VA significantly ($p<0.05$) improved sperm concentration, motility, percentage normal morphology and percentage number of live sperm at doses of 50 mg/kg and 100 mg/kg in dose dependent manner in groups of male Wistar rats treated over a duration of 30 days when compared to the control group of rats. This observation suggests that the administration of aqueous extracts of the leaves of VA successfully improved the sperm qualities [98] since there were significant improvements observed in all the sperm parameters of rats treated with the extract. The possible mechanism of VA induced improvement in sperm parameters could be as a result of the ability of VA to increase glucose metabolism, leading to the production of pyruvate which is known to be the preferred substrate essential for the activity and survival of the sperm cells [99,100]. It could also be as a result of or in addition to its antioxidant potential, the flavonoids and vitamins in VA leaf extract could maintain sperm morphology, sperm survival and sperm function, thus, could be regarded as a source of supply of additional nutrients to the treated group. However, there was a decrease in the level of Follicle Stimulating Hormone (FSH), but no significant increase in the level of Leutinizing Hormone (LH) and testosterone. In contrast, administration of VA at higher dose of 200 mg/kg for longer period of time provoked varying degrees of testicular degeneration, ranging from significant reduction in sperm motility, concentration, percentage normal morphology, percentage number of live sperm, to a significant increase in number of percentage of abnormal sperm. Though it was suggested that the possible mechanism by which higher doses of VA exert untoward effect against spermatogram and its relevant hormone may be as a result of its alkaloidal content which releases its metabolites that binds to cell molecules and cross-linked DNA thereby causing cytotoxicity, but the exact mechanism of action remains unknown [98,101].

Effect on CD4+ cell count (HIV/AIDS):

Aqueous extract of leaves of VA showed a dose-dependent increase in CD4+ cells in a statistically significant manner ($p<0.005$) when compared to the control group. The exact mechanism by which this is done is currently not known, though, it could be related to its antioxidant contents of tannins, saponins and flavonoids, each of which has been individually shown to possess antioxidant property. Another possible mechanism of action could be as a result of enhanced early maturation and releases of leucocytes, since transient interaction between leucocytes have long been known to be critical for the normal function of the immune system [102]. It was therefore concluded that since doses of 200-800 mg/kg body weight of aqueous extract of leaves of VA for as long as 21days will have a positive effect on the CD4+ cells of the Wistar rats used in the study when compared to standard group of Wistar rats, it could be advised that aqueous extract of the leaves of VA can be used as immune booster in immune compromised health conditions [103].

Toxicity:

Lower doses of ethanol extract of leaves of VA (100 mg/kg per oral) showed no significant toxicity in the testis of male albino rats, higher doses (300 mg/kg and 600 mg/kg per oral), however, showed significant ($p<0.05$) testicular toxicity as demonstrated by reduction in tubular diameter, cross-sectional area, number of tubular profiles per unit area and the mean numerical density of seminiferous tubules [89]. The experimental model used showed that the toxicity limit was far higher than the limit for substances considered highly toxic (toxicity at less than 1mg/kg. Adiukwu et al. reported that VA caused no clinical signs of toxicity or adverse toxicological effects of VA at doses of 500-2000 mg/kg/day for 14 consecutive days [89].

Conclusion

The review summarizes the pharmacotherapeutic effects of VA. The phytochemistry and pharmacology was also discussed in detail. VA is a potent ethnomedicinal plant that may be used in the management of cancer and other tropical diseases. The full potential of this plant has not been fully exploited. Hence, this review will stimulate further scientific research into the biological activities, with the view to discovering novel or lead pharmaceutical agents.

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