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A comprehensive review on phytochemistry and pharmacological activities of *Vernonia amygdalina*

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Abstract
Vernonia amygdalina Delile is a small tree with brittle branches, up to 10 m tall and commonly called as bitter leaf due to its bitter taste. It is native of tropical Africa but widely found on riverside and lakes areas, in woodland and grassland up to 2800 m altitude, in areas where the average rainfall is 750-2000 mm. The plant is considered as a medicinal herb and mostly used in traditional medicine system. The principal phytoconstituents of the plant are oxalate, phytates, tannins, saponins, flavonoids, cyanogenic glycosides, alkaloids, terpenes, anthraquinone, steroid, coumarins, lignans, xanthones, edotides and sesquiterpenes and phenol. The plant attributed with anti-cancer, antidiabetic, anti-malarial, anti-inflammatory, cathartic, hepatoprotective, antimicrobial, antioxidant, chemo protective and cytotoxic, Analgesic, anthelmintic, Anti-pyretic, hypolipidaemic properties and also used as Hemolytic, Antimutagenic, Anti-leishmanial, Spermatogenic, anti-platelet and abortifacient agent. The plant was traditionally used as appetizer and against the problems like constipation and diarrhoea. It is considered as a treatment for alcohol induced hepatotoxicity.

Keywords: bitter leaf, flavonoids, anti-diabetic, *Vernonia amygdalina* Delile

Introduction
Vernonia amygdalina Delile (*Gymnanthemum amygdalinum* (Delile) (VA) is a native to tropical Africa and commonly known as 'African bitter leaf' or bitter leaf plant. The species is widely cultivated in Yemen and Ethiopia, South Uganda, Kenya and Tanzania, Brazil [1]. *VA* (Tribe Vernonieae of family Asteraceae) is a shrub or small tree of 2-5 m. It grows well under full sunlight in humid conditions as well as fairly well in drought conditions. It can be found on all soil types, but performs best in humus-rich soils. In cultivation it is mostly pruned to a shrub or hedge. Plant grows in wide ecological zones in Africa and produces large mass of forage used for medicinal and vegetable use [2, 3]. It is well reported for large number of medicinal uses [4]. The leaves are green with a characteristic odour and a bitter taste. Peak growth period for plant is May-August; propagation by cuttings is most successful in July-August. Flowering is induced by short days in dry season in early February-March. In India this species is presumed to be a recent introduction and found under sporadic cultivation in Bihar, Madhya Pradesh, Odisha and West Bengal for medicinal uses [5, 6]. In areas of Bihar, it is popular for its anti-diabetic properties and grown commonly in the courtyard for domestic use. Besides, this plant has also been widely used as fuel wood, stakes, fodder, construction poles, fencing of agroforestry buffer zone and as ingredient for compost. Due to its bitterness, it also can be used as a bittering agent, a hop substitute and for the control of microbial contamination in beer brewing without affecting the quality of malt. In Ethiopia, it is used to make honey wine called Tei [7-12].

Taxonomical classification of *Vernonia amygdalina*:
Kingdom: Plantae
Division: Angiosperms
Order: Asterales
Family: Asteraceae
Genius: Vernonia
Species: amygdalina
Botanical Name: *Vernonia amygdalina*

Distribution and habitat
VA grows naturally on riverside and lakes areas, in woodland and grassland up to 2800 m altitude, in areas where the average rainfall is 750-2000 mm. humus-rich soils are favorable for the proper growth of plant but is can adapt in all types of soil and it needs full sunlight and

humid environment. It is found at Kona national reserve in Tana River district (Kenya) (420 m), in the eastern side of Mbololo forest in Taita (1400 m), and in Narok (2100 m). D. A [13].

Botanical description

VA is a small tree of height up to 10 m tall. The bark of plant is usually light grey or brown and it have brittle branches. Leaves of plant usually 10-15 x 4-5 cm in size and colour is medium to dark green, with or without sparse hairs above, with fine, soft, pale hairs below and conspicuous red-veining; apex and base tapering, base always almost symmetric, margin entire or very finely toothed; petiole usually very short but may be 1-2 cm long. Flower heads are thistle like, small, creamy white, 10 mm long, grouped in dense heads, axillary and terminal, forming large flat clusters, 15 cm in diameter, sweetly scented [14].

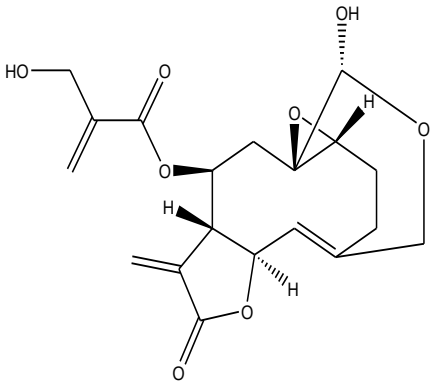
Phytochemistry

The results from the phytochemical studies of VA showed the presence of components such as oxalate, phytates, tannins, saponins, flavonoids, cyanogenic glycosides, alkaloids, terpenes, anthraquinone, steroid, coumarins, lignans, xanthones, edotides and sesquiterpenes and phenol [15, 16]. The various phytochemicals present in VA are concluded in table 1. The saponins like stigmastane present in VA leaves could be responsible for bitter taste. This category of saponins include Vernoniosides A1, A2, A3, A4, B1, B2, B3, C, D, E, are reported to be present in leaves [17, 18, 19, 20]. The leaves of this plant are also found to have abundance of flavonoids such as luteolin, luteolin 7-*O*-β-glucoroniside, luteolin 7-*O*-β-glucoside [22, 25, 27] Other than flavonoids there are some sesquiterpene lactones such as vernolide, vernodalol, vernolepin, vernodalin, vernomygdin, hydroxyvernolide, are present in VA [18, 28, 29, 30]. There are lot of research studies which confirm the presence of these phytochemicals in VA and these studies reveals the presence of some other phytochemicals such as Terpenes, coumarins, phenolic acids, lignans, xanthones, and Anthraquinones [21, 22]. Besides these all phytochemicals there are some peptides present in VA leaves. These phytoconstituents may have synergetic response to give results against life threatening disorders.

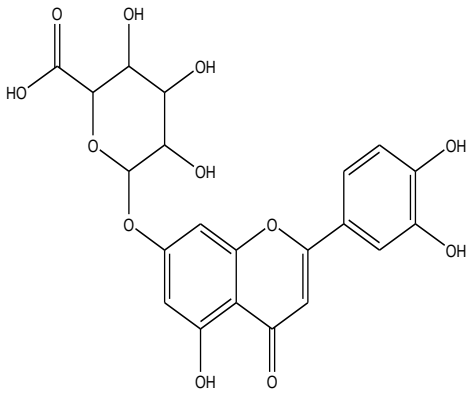
Some studies have specified the concentration of these phytochemical in particular extract. The concentrated was expressed as mg/100 g of extract [16] and the results are shown in table 2. These studies have justified the traditional use of this plant and quantify the extract required to get better treatment results. This plant is traditionally valued as nutritional supplement. The studies have reported the existences of crude protein in VA. The protein concentrate of plant was assessed to estimate the concentration of various amino acids present in concentrate [32] and the results are as given in table 3.

Table 1: The phytochemicals identified from *Vernonia amygdalina*:

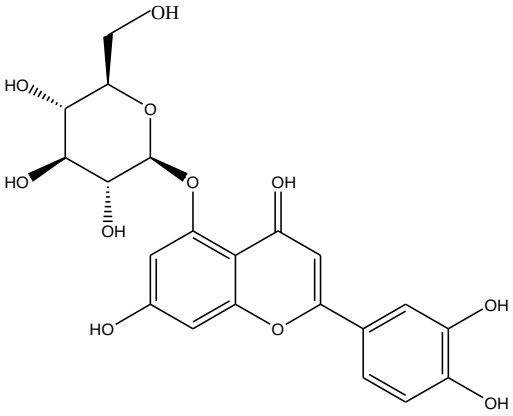
Phytochemicals	Authors
Stigmastane-type saponins Vernoniosides A1, A2, A3, A4, B1, B2, B3, C, D, E	Ohigashi *et al*.[17] and Jisaka *et al*.[18] Kamperdick *et al*.[19] and Ohigashi *et al*. [17]
Terpenes, coumarins, phenolic acids, lignans, xanthones, Anthraquinones	Wall *et al*.[21] and Tona *et al*.[22]
Steroidal saponins	Rwangabo *et al*. (1986), Ohigashi *et al*. (1991), Jisaka *et al*.,[18] Jisaka *et al*.[20], Igile *et al*.[26] and Igile *et al*.[25]
Flavonoids Luteolin, luteolin 7-*O*-β-glucoroniside, luteolin 7-*O*-β-glucoside	Igile *et al*.[26], Udensi *et al*.[27] and Tona *et al*.[22]
Sesquiterpene lactones Vernolide, vernodalol, vernolepin, vernodalin, vernomygdin, Hydroxyvernolide	Kupchan *et al*.[28] and Jisaka *et al*. [20] Koshmizu *et al*.[29] and Erasto *et al*.[30]
Edoties (peptides)	Izevbigie EB [31]



hydroxyvernolide



luteolin 7-O-?-glucoroniside



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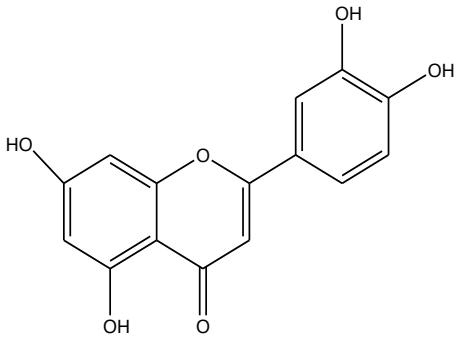
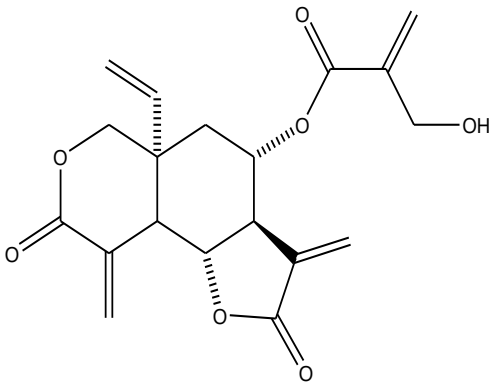
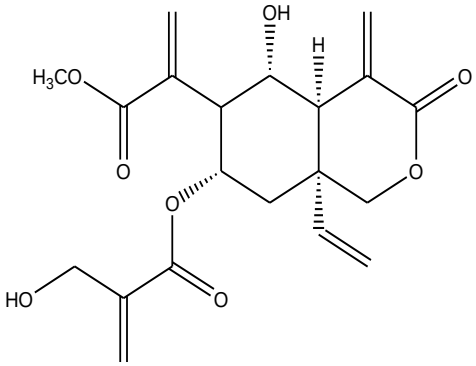


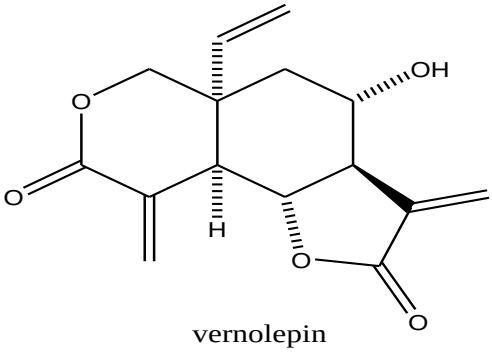
Luteolin



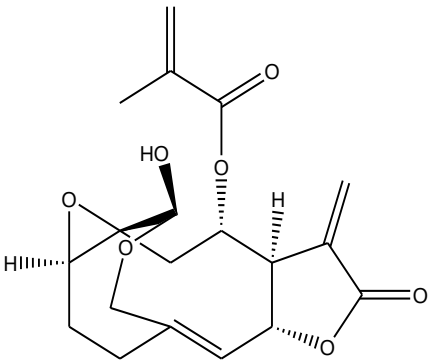
vernodalin



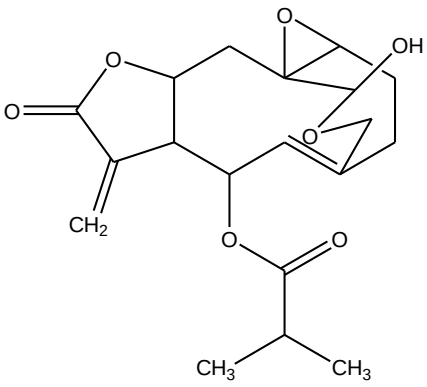
vernodalol



vernolepin



Vernolide



vernomygdin

Fig 1: Chemical structures of some phytochemicals present in *Vernonia amygdalina*.

Table 2: Concentration of various phytoconstituents in mg/100g extract of *Vernonia amygdalina* leaves.

Phytochemicals	Concentration in mg/100g
Oxalate	3.48
Phytates	3.95
Tannins	9.62
Saponins	5.97
Flavonoids	4.89
Cyanogenic glycoside	1.11
Alkaloids	2.16
Anthraquinone	0.14
Steroids	0.38
Phenol	3.24

Table 3: Amino Acid Content of *Vernonia amygdalina* Leaf Protein Concentrates.

Amino acid	Values
Lysine	5.23
Histidine	2.56
Arginine	5.51
Aspartic acid	8.65
Threonine	2.75
Serine	3.67
Glutamic acid	13.97
Proline	3.56
Glycine	1.83
Alanine	2.98
Cyctine	1.76
Valine	5.21
Methionine	1.45
Isoleucine	4.74
Leucine	9.49
Tyrosine	4.56
Phenylalanine	3.38

Traditional uses: VA is used as a medicinal herb from the time when zoo pharmacologists observed that the chimpanzees sucked juice of VA to enhance their body fitness and increase strength. Their appetite was also found to be enhanced with reduction in problems like constipation and diarrhoea [33, 34, 35, 20, 29]. The Bitter taste of plant was ideal identification to choose the appropriate plant and plant part for intake [29]. The native of Africa specially the illiterate and economically weak patient used to use this plant for the medicinal purpose, due to cultural and economic reasons [36]. VA was used traditionally for various diseased conditions such asleaves and roots of plant were used for Stomach disorder, skin wound, diarrhea, scabies, ascariasis, tonsillitis, fever and worms infection [37]. It is also useful in Malaria, fever, constipation, diabetes, anti-helminth [25].

Pharmacological activities: 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; anti-cancer, antidiabetic, anti-malarial,anti-inflammatory, cathartic, hepatoprotective, antimicrobial, antioxidant, chemo protective and cytotoxic, Analgesic activity, anthelmintic, Anti-pyretic activity, hypolipidaemic, Hemolytic properties, Antimutagenicity, Anti-leishmanial activity, Spermatogenic effect, anti-platelet and abortifacient activities.

Anti-cancer activity: VA is reported as effective candidate for breast cancer treatment. VA potently inhibit the proliferation of MCF-7 and MDA-MB-231 cells in a time- and dose-dependent manner. It inhibited the expression of anti-apoptotic Bcl-2 family members such as Bcl-xL and Bcl-2, and activated pro-apoptotic proteins like Bax and Bak. It further activated caspase-8 and caspase-9 which subsequently induced caspase-3 and/or caspase-7 activation, resulting in PARP cleavage.

VA extracts counteract against cancer cells in many ways

- VA inhibited the proliferation of MCF-7 and MDA-MB-231 cells in a time- and dose-dependent manner. The ER-negative MDA-MB-231 cells were shown to be slightly more sensitive to VA-induced growth inhibition than MCF-7 cells. The cytotoxic action induced by VA may be independent of the estrogen receptor.
- In MCF-7 cells, VA induced time- and dose-dependent growth arrest in the G1 phase of the cell cycle, concomitant with a significant decrease in the S phase cells. While, in MDA-MB-231 cells, the effect on cell progression after VA treatment was negligible.
- VA induced apoptosis in MCF-7 cells is at least partially caspase dependent. Results from Annexin V-FITC/PI assay revealed that z-VAD-fmk remarkably reduced the VA-induced apoptotic cell numbers in MCF-7 cells.
- VA-induced cell cycle arrest was dependent on up-regulation of p53 and that the inhibition of p53 would protect cells from VA-induced growth arrest. Hence, the role of p53 in VA-induced cell cycle arrest was verified via a pharmacological approach by using a specific wild type p53 inhibitor, pifithrin-a (PFT-a). Results indicate that the process of VA-induced apoptosis in MCF-7 cells did not involve a p53 transcriptional dependent pathway.
- The exposure of MCF-7 cells to VA for 24 h resulted in the down-regulation of ER-a expression as early as 12 h, and the suppression was even greater at 24 h. This result suggests that VA could be an ER ligand with the ability to inhibit its expression. While MDA-MB-231 cell line has been known to be an ER negative breast cancer cell line, a very low expression of ER-a can still be detected and VA treatment inhibited the ER-a expression significantly [38].

Anti-diabetic activity: The previous studies shows the flavonoid rich fraction of VA leaf extracts shows significant anti-diabetic effect. The blood glucose level was significantly low in crude extract and fraction treated group in comparison to diabetic control group. Out of al fractions, 30% methanol flavonoid rich fraction and 100 5 methanol glycoside rich fraction showed higher antidiabetic effect. The histology of pancreas of different treatment groups shows that the extract has reversed the pancreatic beta cell damage. The 30 % methanol fraction treated group showed regeneration of islet mass and 50 and 100 % fractions showed partial regeneration. Thus the possible mechanism for anti-hyperglycemic activity of VA should be the regeneration of damaged pancreatic beta cells [40].

Anti-malarial activity: VA was traditionally used for the treatment of malaria in southern region of Nigeria. There are also some reported studies which show the activity of ethanolic extract of plant against *Plasmodium berghei* in the dose dependent manner, Omoregie [41]. The aqueous extract of VA possesses anti-malarial activity on *Plasmodium falcifarum, Plasmodium vivax, Plasmodium ovale* and *Plasmodium malaria* [42].

Anti-inflammatory activity: The anti-inflammatory effect of extract of VA was assessed in comparison to acetylsalicylic acid. The inflammation was induced by four parts of pyridine, 1 part distilled water, 5 parts of di ethyl ether and 10 parts croton oi in diethyl ether (v/v). Six hours after applying the vehicle on right ear, all animals were lightly anesthetized and ears were cut and weighed on sensitive weighing balance. The inflammatory response was quantified and compared with in the groups. The VA extract significantly reduced the inflammatory response compared to the inflammatory response produced when croton oil alone was applied [43].

Cathartic effect: The methanolic extract of VA was assessed for the Cathartic activity. The treatment with VA extract showed significant improvement in motility of charcoal meal in mice. The also promote the gastric empting in mice. The effect of extract was not as that of carbachol (1 mg/kg) which was used as standard. The VA extract increase the total number of faeces in a dose dependent manner. The extract produced the contractile effect comparable to acetylcholine. And this was blocked by atropine, so it can be assumed that the VA may act as muscarinic receptor agonist [44] the phytochemistry also shows the presence of saponins, tannins and glycosides in VA [45], which are reported to have purgative response. So the VA is the eligible candidate to use for constipation and gastric discomfort.

Hepato protective: The study of hepatoprotective effect ethanolic leaf extract of VA against acetaminophen (Paracetamol) induced hepatotoxicity in rats shows significant decrease in plasma transaminase activities in VA treated animals group. This indicates the effect of VA against paracetamol damage [46]. The VA extract also showed significant effect against carbon tetrachloride induced hepatotoxicity in rats. The treatment with CCl_4 (1.2 g/kg) for 3 week led to hepatic injury in rats and their serum enzymes such as ALT, AST, SALP, and ϒ-GT were increased in CCl4 treated group. The levels of these enzymes were significantly decreased in extract treated groups the effect was highly expressed at 250 and 500 mg/kg [47]. Another study has shown the aqueous extract of VA protects against alcohol induced hepatotoxicity. The enzyme levels were decreased significantly ($p<0.001$) due to extract administration and the decrease of transaminase level can predict the regeneration of the hepatocytes. The VA extract also improve the activity of anti-oxidant enzymes such as SOD, CAT, GSH and GPx and help to overcome the oxidative stress [48].

Antimicrobial activity: The extract of VA was found to have anti-bacterial activity against *S. aureus*, *P. aeruginosa* and *E. coli*. The hot extract of VA was most effective at *P. aeruginosa* with the zone of inhibition of 13.00mm at 200 mg/ml concentration. And at the same concentration the zone of inhibition for *S. aureus* was 6 mm. in the case of cold extract the only organism against which the extract is effective is, *P. aeruginosa* with the zone of inhibition 10.50mm. The hot ethanolic extract has shown bactericidal effect at the dose of 50 and 125 mg/mg [49].The ethanolic extract of VA showed more affect against *S. mutans* in comparison to aqueous extract. At different doses of 25, 50, 100 and 200 mg/ml, the zone of inhibition was 2.00mm, 3.00 mm, 6.00 mm and 10.00 mm respectively while the *S. aureus* was not much sensitive to ethanolic extract of VA [50].

Anti-oxidant activity: The spray dried water extract of VA was tested with DPPH radical scavenging activity and the results of this study showed significant increase in IC_{50} in comparison to vitamin C and E treated groups. In this study, the activity of antioxidant enzymes such as SOD, MDA level and total antioxidant capacity was evaluated for 14 days. The sod activity was significantly increased in in both VA extract and vitamin c treated groups. This effect was associated with decreased MDA level, which is a major metabolite of lipid peroxidation. The anti-oxidant ability of VA extract that can enter in blood plasma is far more in comparison to vitamin C **[51]**. The reason for the antioxidant activity of VA should be the presence of flavonoids such as (Luteolin, luteolin 7-O-β-glucuronoside and luteolin 7-O-β- glucoside) [52] which are reported to have antioxidant properties [53].

Analgesic activity: The study has shown that the aqueous extract of VA (100 and 200 mg/kg) had significantly decreased the number of writhes produced by acetic acid. The extract showed the significant analgesic effect in comparison to standard drug i.e. indomethacin. The aqueous leaf extract of VA (100 and 200 mg/kg) also decreased the number of paw licks induced by formalin in comparison to control animals. The analgesic effect of the extract at both doses was comparable to standard drug indomethacin. Indomethacin (10 mg/kg) also showed the significant ($p<0.05$) decreased in paw licks in comparison to control. The significant reduction in paw licks indicates the analgesic effect of both indomethacin and plant extract [54].

Anti-pyretic activity: The aqueous leaf extract of VA and the saponins fractions possesses the anti-pyretic effect. The procedure used for inducing fever was as described by, Okokon and Onah [55]. Acetylsalicylic acid was used as standard drug. The results showed that the extract and saponins fraction has significantly ($p<0.05$) lowered the anal temperature at 100 and 200 mg/kg. The study revealed the anti-pyretic activity was observed to be higher in case of leaf extract then root extract [56].

Hypolipidaemic activity: The methanolic extract of VA when administered to hypolipidaemic animals caused significant ($p<0.001$) increase in plasma HDL cholesterol levels. The hypercholestrolemic condition was successfully induced by administering cholesterol (30 mg/0.3 ml/animal). The administration of cholesterol leads to significant increase ($p<0.05$) in plasma and total cholesterol level. The methanolic extract of VA at dose of 200 mg/kg had significantly decreased ($p<0.05$) the hyperglycemia induced by dietary cholesterol. The cholesterol induced decrease in reduced glutathione levels were significantly ameliorated by VA administration [57]. Another study showed that the aqueous leaf extract of VA has also lowered plasma TC, TAG, LDL-c and VLDL concentration with significant increase in HDL-C concentration. The study was conducted in albino New Zealand rabbits and hyperlipidemia was induced with non-phosphorylated egg yolk extract. Treatment with aqueous VA extract results in decrease in plasma TC, LDL-C, TAG and VLDL and increase the concentration of plasma HDL-C concentration. These results indicate that the VA is an eligible candidate for to control the altered blood lipid levels and treat cardiac heart diseases [58].

Anxiolytic and sedative activity: The aqueous leaf extract of VA was found to have anxiolytic activity VA (50 mg/kg) showed significant increase in frequency of head dips in hole

board apparatus. On T-maze apparatus, latency to withdrawal from the closed arm was significantly decreased with significant increase in latency to withdrawal form open arm. These results indicate the anxiolytic effect of VA extract. VA extract at 100 mg/kg and 200 mg/kg doses significantly reduced the rearing and locomotion in the open field apparatus, decreased the head-dips frequency on the hole-board apparatus and significant increased both inhibitory avoidance and one-way escape tasks on the elevated T-maze apparatus, increased the amylobarbitone-induced sleep duration in dose dependent manner and decreased sleep latency. These parameters indicated the sedative effect of VA at doses 100 and 200 mg/kg [59].

Antimutagenicity

Petroleum ether extract was the most active, followed by methanol extract and then the ethyl acetate fraction. The petroleum ether, methanol and ethyl acetate extract of VA were evaluated for anti-mutagenic activity on ethyl methane sulfonate induced mutation on salmonella typhimurium TA100. The results indicate that the extracts were able to significantly inhibit His-to His+ mutation more than 60 %. The antimutagenic ability of petroleum extract was then both extract and ethyl acetate extract had least activity [60].

Anti-leishmanial activity: Anti leishmanial activity of chloroform and methanol extract was evaluated out of which the chloroform extract showed higher activity on promastigotes then that of methanolic extract. The chloroform extract cause decrease in motility within 24 hours at 81 mg/ml and the methanolic extract did the same in 48 hours. Besides that the chloroform extract had more toxicity in host cell in comparison to methanolic extract. Another study showed that the hexane and aqueous extract of VA also have ability to suppress the infection rate of leishmaniosis. It causes the significant reduction in the lesion size and less tissue damage in skin spleen and liver after inoculation with promastigotes from leishmanial parasite in Balb/c mice. The flavonoids present in VA are supposed to be responsible for anti-leishmanial activity [62].

Hemolytic activity: The VA was investigated for the in vitro hemolytic activity and the results showed the significant ($p<0.05$) hemolysis of erythrocyte. The most responsible suspect for the hemolysis of erythrocyte due to VA was genotype SS (1024) and genotype AS (512 were moderately susceptible to hemolysis induced by VA. But the genotype—AA (256) was found to be resistant to VA induced hemolysis [63]. However, there was 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 Hemoglobin concentration (Hb), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC) [64]. Also, there was no significant changes in the platelet, neutrophil, Total White Blood Cell count (TWBC), lymphocyte, eosinophil, and monocytes values relative to their respective controls.

Spermatogenic effect: The Spermatogenic studies of aqueous extract of VA showed that the extract at 50 mg/kg and 100 mg/kg cause significant ($p<0.05$) improvement in sperm concentration, sperm motility, percentage number of live sperms and percentage normal morphology of male wistar rats treated for 30 days when compared to control group of rats. These results indicate the improvement of sperm quality due to administration of aqueous extract of leaves of VA [65]. VA is supposed to increase the glucose metabolism, which results in production of pyruvate responsible for activity and survival of sperm cells [66, 67]. The anti-oxidant potential of phytochemicals like flavonoids and vitamins could maintain the morphology survival and function of sperm. The results reveal that there was no significant change in the level of Leutinizing Hormone (LH) and testosterone. But level of Follicle Stimulating Hormone (FSH) was found to be decreased. In contrast, administration of VA for longer period of time at higher dose of 200 mg/kg 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. The alkaloidal content of VA may release its metabolites which binds to cell molecules and cross linked DNA, this could be the possible mechanism for the untoward effect of VA at higher doses, but the exact mechanism of action remains unknown [68, 69].

Conclusion

Vernonia amygdalina is a potent ethno medicinal plant that can be used to treat various life threatening disorders. The review of literature reveals that the *Vernonia amygdalina* is a plant with huge therapeutic potential. The phytochemistry and pharmacological studies on this plant had explored it to the level where it can be called as lifesaving herbal plant and it can be incorporated as health supplement for human benefits. But a lot more is yet to be explored because the full potential of the plant has not been fully exploited.

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