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PHYTOCHEMICAL AND PHARMACOLOGICAL PROPERTIES OF *Vernonia amygdalina*: A REVIEW

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ABSTRACT

Vernonia amygdalina has been a known food and medicinal plants used in Asia and Africa (West Africa) due to its pharmacological effects (antioxidant, anti-diabetes, anti-inflammatory, anticancer, anti-malaria, and among others). The phytochemical studies revealed that this plant is enriched with proteins, fats, fibres, amino acids, minerals, vitamins, and carbohydrates. Many bioactive compounds had been isolated from its extracts. Therefore, this review summarized the overview of the bioactive compounds, phytochemical and pharmacological properties of *V. amygdalina* as well as its mechanism of action and toxicology.

Keywords: Bitter leaf; anti-diabetic; anti-microbial; antioxidant; toxicology.

1.0 INTRODUCTION

Bitter leaf (Figure 1) scientifically known as *V. amygdalina* is one of the most famous plants found in Africa and Asia. It is the most cultivated species of the genus Vernonia that is about 1,000 species of shrub (Njan *et al*., 2008; Agbogidi and Akpomorine, 2013; Toyang and Verpoorte, 2013; Egharevba *et al*., 2014). *V. amygdalina* has been the most prominent species in the family of Asteraceae that had been studied in Africa (Ankit *et al*., 2010; Nwaoguikpe, 2010; Farombi and Owoeye, 2011; Ijeh and Ejike, 2011). Normally, *V. amygdalina* does not produce seeds but its cultivation is usually done by stem planting and mostly grow in tropical areas. This plant is found majorly along the drainage, commercial plantation or forest (Yeap *et al*., 2010).

V. amygdalina is a wooded shrub of about 2 to 10 m height that regenerates rapidly after planted. The leaves are petiolated in shape with a bitter taste of which its name "Bitter leaf" spring up. *V. amygdalina* are being called different local names which vary from country to country (Table 1). The bitter taste had been associated with the presence of saponins, alkaloids, tannins, and glycosides. These made them act as a bittering agent and a hop substitute used for controlling microbial contamination in beer brewing without reducing the quality of malt (Ayoola *et al*., 2008; Ankit *et al*., 2010; Adama *et al*., 2011; Farombi and Owoeye, 2011).This plant can be harvested twice per month for the period of seven years. They are popularly used for food and traditional medicine, their characteristic odour and bitter taste can be reduced either by washing in several changes of water or by boiling before consumption (Nwaoguikpe, 2010; Agbogidi and

Akpomorine, 2013; Toyang and Verpoorte, 2013; Alara *et al*., 2017a). The Medical Traditional Healer Association in Rukararwe, Uganda produced the greenish powder packed in sachet and consume as tea by patients suffering from malaria (Njan *et al*., 2008), it has also been reported to be used in soup (Ogbono and Orugbo soups) in Nigeria and Cameron (Ndole dish) (Yeap *et al*., 2010; Oduah, 2012; Agbogidi and Akpomorine, 2013; Oguwike *et al*., 2013). The honey wine called Tei are being produced from the bitter leaf in Ethiopia (Yeap *et al*., 2010). The leaves and roots decoctions have been used in ethnomedicine to treat hiccups, fevers, kidney problems and stomach disorder (Igile *et al*., 1994; Yeap *et al*., 2010; Sha, 2011; Oduah, 2012).

Several studies carried out on this plant had suggested that it contains different bioactive compounds, including, flavonoids, saponins, alkaloids, tannins, phenolics, terpenes, steroidal glycosides, triterpenoids, and several types of sesquiterpene lactones (Erasto *et al*., 2006; Farombi and Owoeye, 2011; Kiplimo *et al*., 2011; Toyang and Verpoorte, 2013; Adedapo *et al*., 2014; Quasie *et al*., 2016; Luo *et al*., 2017). These bioactive compounds made them possess different pharmacological properties like antimicrobial, antimalarial, antithrombotic, antioxidant, anti-diabetic, laxative, hypoglycemic, antihelmintic, anti-inflammatory, cathartic, anticancer, antifertility, anti-fungi, antibacterial, and among others (Igile *et al*., 1994; Akinpelu, 1999; Iwalokun *et al*., 2006; Erasto *et al*., 2007; Gresham *et al*., 2008; Khalafalla *et al*., 2009; Ilondu, 2010; Farombi and Owoeye, 2011; Anibijuwon *et al*., 2012; Ngatu *et al*., 2012; Adetunji *et al*., 2013; Atangwho *et al*., 2013; Akinyele *et al*., 2014; Ezeadila *et al*., 2015; Udochukwu *et al*., 2015; Alara *et al*., 2017c).

The objective of this review paper is to outline and discuss the studies that had been done on the bioactive compounds, phytochemical and pharmacological properties of *V. amygdalina*. Besides, this review also expatiates the mechanism of action and toxicological effects of *V. amygdalina* extracts.

Figure 1: *Vernonia amygdalina*
Source: (Yeap *et al*., 2010)

Table 1: Different local names of *Vernonia amygdalina*

Country	**Local name(s)**
English	Bitter leaf
Tanzania	Omjunso
Nigeria	Onugbo, Ewuro, Etidot, Ityuna, Oriw, Chusa-doki Shiwaka
Malaysia	South Africa leaf
Rwanda	Umubilizi
Cameron	Suwaaka
Uganda	Labwori, Omubirizi, Ekibirizi
Ghana	Awonoo, Awonwene, Jankpantire
Congo	Mpasi nyioso
Zimbabwe	Musikavakadzi
Gabon	Ndoki
China	Ikaruga
Kenya	Olulusia
Ethiopia	Grawa, Graw

Source: (Igile *et al*., 1994; Alabi *et al*., 2005; Iwalokun *et al*., 2006; Njan *et al*., 2008; Nwaoguikpe, 2010; Akpaso *et al*., 2011; Farombi and Owoeye, 2011; Owen, 2011; Oduah, 2012; Toyang and Verpoorte, 2013; Egharevba *et al*., 2014)

2.0 PHYTOCHEMICAL PROPERTIES OF *V. amygdalina*

Nutritional Value

The analysis had shown that *V. amygdalina* leaves, stems, and root is enriched with proteins, fats, fibres, amino acids, minerals vitamins, and carbohydrates (Igile *et al*., 1994; Alabi and Amusa, 2005; Owu *et al*., 2008). The nutritional compositions of *V. amygdalina* leaf, root and stems varied from one study to another, probably due to different geographical location, genetic, environmental, harvest conditions, and ecology of the plant. Early studies reported that *V. amygdalina* leaves contain carbohydrates (4.31 mg/100 g), proteins (20.2 mg/100 g), lipids (15.0 mg/100 g), acids (10.26 mg/100 g), iodine (35.82 mg/100 g), hydrocyanic acid (0.46 mg/100 g), total oxalate (0.62 mg/100 g), amino acid, viz: thiamine (170 mg/100 g), pyridoxine (2.6 mg/100 g), ascorbic acid (20.49 mg/100 g), glycine (4.63 mg/100 g), cysteine (1.84 mg/100 g), casein hydrolysate (96.99 mg/100 g), nicotinamide (1.65 mg/100 g). In another study, there was a variation in the nutritional values of *V. amygdalina* leaf, stem and root as summarized in Table 2.

Table 2: *V. amygdalina* leaves chemical and nutritional compositions

Leaf		**Stem**		**Root**	
Composition (mg/100 g dry weight)	Value	Composition per 100 g dry weight	Value	Composition per 100 g dry weight	Value
Sugar contents		Vitamins and minerals		Vitamins and minerals	
Glucose	7.20	Vitamin A (mg/100 g)	21.5	Vitamin A (mg/100 g)	30.90
Sucrose	13.20	Vitamin C (mg/100 g)	49.0	Vitamin C (mg/100 g)	10.30
Fructose	6.00	Vitamin E (mg/100 g)	106.20	Vitamin E (mg/100 g)	35.83
Lactose	2.61	Thiamin (mg/100 g)	0.50	Thiamin (mg/100 g)	0.37
Galactose	6.56	Riboflavin (mg/100 g)	0.13	Riboflavin (mg/100 g)	0.15
Arabinose	9.25	Niacin (mg/100 g)	0.03	Niacin (mg/100 g)	0.05
Raffinose	5.10	Saponins (%)	13.21	Saponins (%)	28.52
Maltose	7.24	Alkaloid (%)	7.02	Alkaloid (%)	6.11
Mineral ash and fibre content		Flavonoid (%)	1.02	Flavonoid (%)	0.51
Na	8.48	Fe (mg/100 g)	0.12	Fe (mg/100 g)	0.09
Ca	67.39	Cu (mg/100 g)	0.021	Cu (mg/100 g)	0.022
K	60.90	Zn (mg/100 g)	0.14	Se (mg/100 g)	0.016
P	60.90	Proximate values		Zn (mg/100 g)	0.26
Mg	88.10	Crude fat (%)	34.03		
				Proximate values	
Mn	5.56	Moisture (%)	18.50	Crude fat (%)	30.15
Fe	14.20	Crude protein (%)	6.71	Moisture (%)	12.00
Cu	6.01	Ash (%)	17.99	Crude protein (%)	7.30
Zn	8.05	-	-	Ash (%)	11.01
Ash	10.22	-	-	-	-
Fibre	9.75	-	-	-	-
		-	-	-	-
Vitamins and amino acids					
Polyphenols (mg/100 g)	9.75	-	-	-	-
Vitamin C (mg/100 g)	228.40	-	-	-	-
Vitamin A (IU/100g)	345.50	-	-	-	-
Vitamin E (IU/100g)	37.30	-	-	-	-

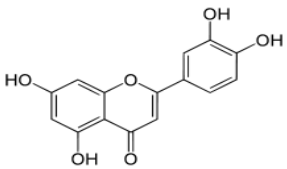
Vitamin B_1 (%)	1.00	-	-	-	-
Vitamin B_2 (mg/100g)	3.10	-	-	-	-
Niacin	0.41	-	-	-	-
Oxalic acid (mg/100 g)	5.36	-	-	-	-
Carotenoids (mg/100 g)	30.00	-	-	-	-
Saponins (mg/100 g)	1.425	-	-	-	-
Ihiamine (mg/100 g)	170.00	-	-	-	-
Pyrdoxine (mg/100 g)	2.06	-	-	-	-
Glycine (mg/100 g)	4.63	-	-	-	-
Cysteine (mg/100 g)	1.84	-	-	-	-
Casein hydrolysate (mg/100 g)	96.99	-	-	-	-
Nicotinamide (mg/100 g)	1.65	-	-	-	-
Average nutritive value	1.10	-	-	-	-
Proximate values		-	-	-	-
Digestible protein (g)	16.58	-	-	-	-
Moisture (%)	79.92	-	-	-	-
Crude protein (g)	19.23	-		-	-
Total carbohydrate (g)	68.35	-	-	-	-
Total lipids	4.70	-	-	-	-
Reducing sugar (g)	14.31	-	-	-	-
Dry matter (%)	20.08	-	-	-	-
Ash (g)	7.72	-	-	-	-
Dietary fibre (g)	25.47	-	-	-	-
Energy (Kcal)	392.67	-	-	-	-
Total oxalate (mg/100g)	0.62	-	-	-	-
Iodine	35.82	-	-	-	-

(mg/100g)

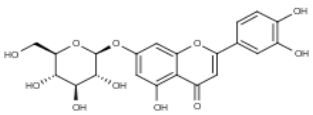
Source: (Alabi and Amusa, 2005; Alabi *et al*., 2005; Nwaoguikpe, 2010; Eyong *et al*., 2011; Toyang and Verpoorte, 2013)

Bioactive Compounds in *Vernonia amygdalina*

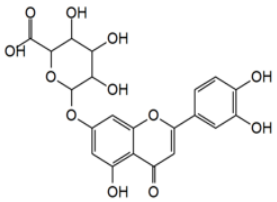
Several studies had been done in isolating and characterizing some bioactive compounds from *V. amygdalina*. The phytochemical studies had resulted in the isolation of flavonoids, saponins, alkaloids, tannins, phenolics, terpenes, steroidal glycosides, triterpenoids, and several types of sesquiterpene lactones (Erasto *et al*., 2006; Farombi and Owoeye, 2011; Kiplimo *et al*., 2011; Toyang and Verpoorte, 2013; Adedapo *et al*., 2014; Quasie *et al*., 2016; Luo *et al*., 2017). Figure 2 shows some of the isolated bioactive compounds reported in the literature. Sesquiterpene lactones (vernodalinol, vernolepin, vernomygdin, hydroxyvernolide, vernolide and vernodalol) had been reported to inhibit breast cancer cell growth, possessed antitumoral and antimicrobial properties, and exhibited a significant bactericidal activity against gram bacteria (Jisaka *et al*., 1993; Erasto *et al*., 2006; Amodu *et al*., 2013; Luo *et al*., 2017). Isolated vernoniosides from *V. amygdalina* leaves exhibited anti-inflammatory property and used in the treatment of gastrointestinal disorder when tested with murine macrophage cell line and wild chimpanzees, respectively (Huffman *et al*., 1993; Quasie *et al*., 2016). Flavonoids, tannins, saponins, and triterpenoids had been studied to possess antioxidant and hypolipidaemic effects (Igile *et al*., 1994; Erasto *et al*., 2007; Ayoola *et al*., 2008; Farombi and Owoeye, 2011; Atangwho *et al*., 2013; Alara *et al*., 2017b).



Luteolin

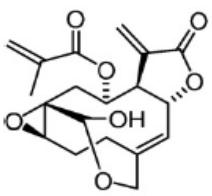


Luteolin 7-O-β-glucoside

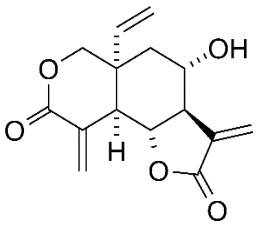


Luteolin7-O-glucuronide

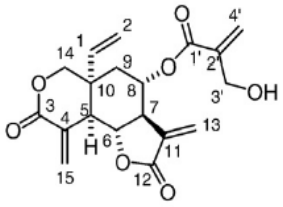
(a)



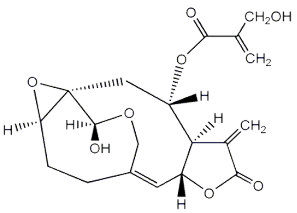
Vernolide



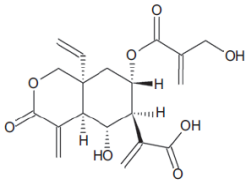
Vernolepin



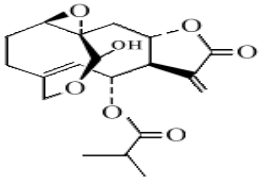
Vernodalin



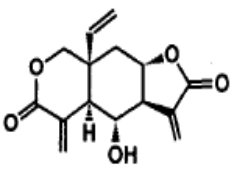
Hydroxyvernolide



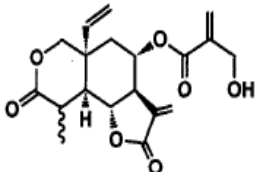
Vernodalol



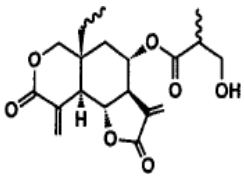
Vernomygdin



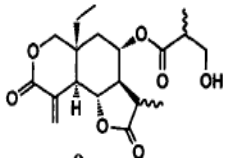
Vernomenin



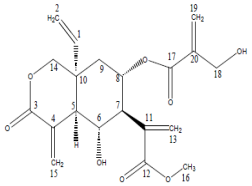
4,15-dihydrovernodalin



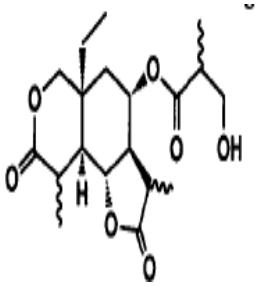
1,2,2′,3′-tetrahydrovernodalin



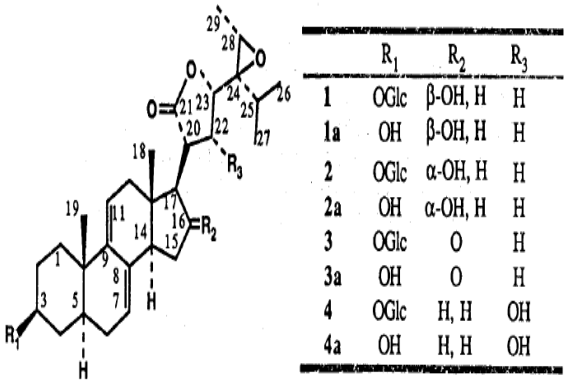
1,2,11,12′,3′-hexahydrovernodalin



Epiverodalol



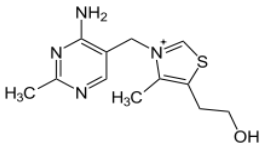
1,2,4,15,11,13,2′,3′-octahydrovernodalin



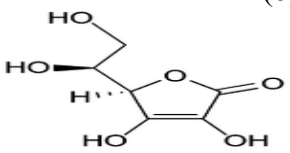
	R_1	R_2	R_3
1	OGlc	β-OH, H	H
1a	OH	β-OH, H	H
2	OGlc	α-OH, H	H
2a	OH	α-OH, H	H
3	OGlc	O	H
3a	OH	O	H
4	OGlc	H, H	OH
4a	OH	H, H	OH

Vernonioside A1, A2, A3, A4, B1, B2, B3, and B4

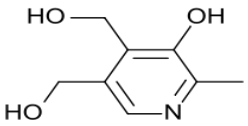
(b)



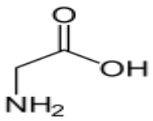
Thiamin



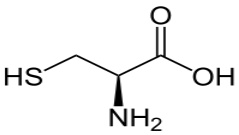
Ascorbic acid



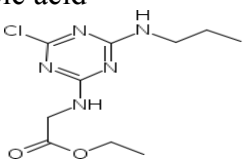
Pyridoxine



Glycine



Cysteine



Casein hydrolysate

(c)

Figure 2: Isolated flavonoids (a), sesquiterpenes lactones (b) and triterpenes (c) from *V. amygdalina* (Erasto *et al*., 2006; Farombi and Owoeye, 2011; Kiplimo *et al*., 2011; Toyang and Verpoorte, 2013; Adedapo *et al*., 2014; Quasie *et al*., 2016; Luo *et al*., 2017)

Flavonoids

Flavonoids are a group of natural compounds found in plants with variable phenolic structures. They are present virtually in all parts of the plant. Flavonoids are responsible for the taste, colour, protection of vitamins and enzymes, and prevention of fat oxidation (Yao *et al*., 2004). The pharmacology importance of flavonoids, including, antioxidant, hepatoprotective, antibacterial, anti-inflammatory, anticancer, and antiviral activities are indispensable in the human body (Yao *et al*., 2004; Shashank and Abhay, 2013). *V. amygdalina* possesses flavonoids which can be extracted from the leaves using organic solvents. Different flavonoids had been isolated from the crude extracts of *V. amygdalina* leaf. In the early study, three flavones (luteolin, luteolin 7-O-β-glucuronoside and lutelin7-O-β-glucoside) were identified (Figure 2a). The identified flavones showed strong antioxidant activities (Igile *et al*., 1994). Luteolin exhibited stronger antioxidant activity in comparison to the synthetic butylated hydroxytoluene

(BHT) at 15 mg/l. Besides this, luteolin 7-O-glucuronoside (the most abundant compound) and luteolin 7-O-glucoside also possess similar antioxidant activity but significantly lower as compared to luteolin. This may be attributed to the blockage of glycosides with glucose or uronic acid linked at the 7-O position compared with the unsubstituted 5,7- OH of luteolin. Higher antioxidant inhibition was observed in the ethanolic extraction of luteolin than aqueous extract (Yeap *et al*., 2010). This may contribute to the higher antioxidant activity of ethanol extract compared to water extract. The chemical structures of the isolated flavones are shown in Figure 2a.

Sesquiterpene lactones

This is another group of phytochemicals isolated from *V. amygdalina*. Sesquiterpenes lactones are secondary metabolites widely found in family Asteraceae. They possess pharmacology properties like antibacterial, anaesthetic, antifungal, anti-inflammatory, antiprotozoal, and antimicrobial. Most of the isolated sesquiterpene lactones from *V. amygdalina* were from leaf, stem, and root. The isolated compounds were vernolide, vernolepin, vernodalin, hydroxyvernolide, vernodalol, vernomygdin, vernomenin, 4,15-dihydrovernodalin, 1,2,11,12′,3′ hexahydrovernodalin, 1,2,4,15,11,13,2′,3′ octahydrover nodalin, epivernodalol, and vernonioside A1, A2, A3, A4, B1, B2, B3, and B4 (Huffman *et al*., 1993; Jisaka *et al*., 1993; Khalafalla *et al*., 2009; Owoeye *et al*., 2010; Quasie *et al*., 2016; Luo *et al*., 2017). The chemical structures of these sesquiterpenes lactones are showed in Figure 2b.

Vernodalin and vernolide had been reported to exhibit potent activity (IC_{50} for P-388 and L-121O cells: 0.11 and 0.17 µg/ml for vernodalin and 0.13 and 0.11 µg/ml for vernolide, respectively), while the activity of hydroxyvernolide and vernodalol was weak. The lower activity of hydroxyvernolide, vernolepin and vernodalol could be explained by the loss of hydrophobicity in the acyl moiety (Jisaka *et al*., 1993). In another study, vernolide showed a significant inhibition against gram-positive bacteria *B. cereus*, *Staphylococcus epidermidus*, *B. subtilis*, *S. aureus*, *M. luteus, M. kristinae*, and *Streptococcus pyrogens* and the gram-negative bacterium *Salmonella pooni*. These showed its antibacterial activity (Erasto *et al*., 2006; Luo *et al*., 2017). More so, vernodalin and vernomygdin had been reported to possess antitumor activity against human nasopharynx carcinoma KB, L-1210 cancer cell lines and P-388 leukaemia mouse.

The antifeedant activity was noticed from the isolated 11,13-dihydrovernorodeline, this can resist insects from feeding on the plant but does not result in lethality (Yeap *et al*., 2010). Vernolepin had also been reported to show antiplatelet property against ADP, arachidonic acid and collagen-induced platelet aggregation in rabbits (Erasto *et al*., 2006; Yeap *et al*., 2010). Similar to other isolated sesquiterpenes lactones, vernomenin inhibited L-1210 cancer cell lines and P-388 leukaemia mouse (Jisaka *et al*., 1993; Yeap *et al*., 2010).

Triterpenoids

Triterpenoids are widely distributed in medicinal and edible plants. This phytochemical group is being evaluated for use in new functional foods, cosmetics, foods, and healthcare products. They have been isolated from the leaf, root and stem of *V. amygdalina*. They possess diverse pharmacological properties including anticancer, anti-inflammatory, hepatoprotective, antioxidant, antibacterial, antileukaemia, analgesic

and anti-nociceptive (Igile *et al*., 1994; Alabi *et al*., 2005; Nwaoguikpe, 2010; Yeap *et al*., 2010; Wong *et al*., 2013). Some of the isolated triterpenes are thiamine, ascorbic acid, pyridoxine, glycine, cysteine, casein hydrolysate, eucalyptol, beta piene, myrtenal, and alpha-muurolol (Figure 2c).

3.0 PHARMACOLOGICAL EFFECTS OF *V. amygdalina*

V. amygdalina had been reported to possess several pharmacological effects like antimicrobial, antimalarial, antithrombotic, antioxidant, anti-diabetic, laxative, hypoglycemic, antihelmintic, anti-inflammatory, cathartic, anticancer, antifertility, anti-fungi, antibacterial, and among others (Igile *et al*., 1994; Akinpelu, 1999; Iwalokun *et al*., 2006; Erasto *et al*., 2007; Gresham *et al*., 2008; Khalafalla *et al*., 2009; Ilondu, 2010; Farombi and Owoeye, 2011; Anibijuwon *et al*., 2012; Ngatu *et al*., 2012; Adetunji *et al*., 2013; Atangwho *et al*., 2013; Akinyele *et al*., 2014; Ezeadila *et al*., 2015; Udochukwu *et al*., 2015; Alara *et al*., 2017c).

Antioxidant Effect

The crude extracts from *V. amygdalina* had been studied to possess an antioxidant property by scavenging the free radicals cells. The aqueous extracts from the leaf showed a significant reduction in the malondialdehyde levels of oxidative stressed streptozotocin-induced diabetic rats (Nwanjo, 2005). The leaves extracts had been examined to scavenge 75-99.3% DPPH radicals and 96.2-100% of the ABTS radicals (Erasto *et al*., 2007). The presence of flavonoids in the *V. amygdalina* extracts had been attributed to their antioxidant property (Igile *et al*., 1994; Ayoola *et al*., 2008; Farombi and Owoeye, 2011). In vivo biochemical analysis of *V. amygdalina* leaf extracts on the rats showed an appreciable increase in the level of the antioxidants, superoxide dismutase, catalase, glutathione, and malondialdehyde. In addition, daily administration of the extracts to rats resulted in the reduction of their lipid profile when compared to the control (Imaga and Bamigbetan, 2013). The chemopreventive effects of *V. amygdalina* extracts had been attributed to their ability to scavenge free radical cells, interfere with DNA binding of some transcription factors, and induced detoxification (Amodu *et al*., 2013). Moreover, the extracts from this plant had been found to inhibit bleaching B-carotene, lipid peroxidation induced by iron ion ascorbate in a rat liver microsomal preparation, and linoleic acid (Khalafalla *et al*., 2009; Yeap *et al*., 2010).

Anti-diabetic Effect

Diabetes mellitus has been associated with a fasting venous plasma glucose concentration higher than 7.8 mmol/l (140 mg/dl) 2 h after an oral ingestion of 75 g glucose equivalent or carbohydrate meal (Nwanjo, 2005; Letchuman *et al*., 2010; Jan Mohamed *et al*., 2015). Studies had shown that aqueous extracts from *V. amygdalina* leaves reduced the blood glucose, increased the serum triglyceride levels and serum MDA, increased the LDL-cholesterol, and normalized cholesterol concentrations in streptozocin-induced diabetic rats (Nwanjo, 2005). In another study on the effect of *V. amygdalina* leaf extracts on blood glucose of diabetic rats, the results showed that decrease in blood glucose after administration of the extracts may be associated with the presence phytochemicals, vitamins and other nutrients in the extracts (Osinubi, 2008; Nwaoguikpe, 2010; Ejike *et al*., 2013). The aqueous extracts had been administered to alloxan-diabetic rats, the blood glucose and serum triglyceride levels were significantly reduced (Akah *et al*., 2004). Justin *et al*. 2012 had reported that decoction of *V, amygdalina* and *Azadirachta indica* leaves promptly lowered blood glucose and

maintained a relatively steady level over the study period. Likewise, the study on the activities of *V. amygdalina* leaves aqueous extracts on the haemostatic, haematological and biochemical profile of induced male diabetic albino rats showed a significant reduction in the glucose level (Oguwike *et al*., 2013).

Anti-allergic Effect

The extracts from *V. amygdalina* leaves had been reported to inhibit and prevent atopic or eczema dermatitis syndrome in mice (Ngatu *et al*., 2012).

Anti-inflammatory Effect

V. amygdalina leaves extracts had been reported to possess anti-inflammatory activity when applied to the ear of rat suffering from inflammation. It produced a significant reduction when compared with the application of acetylsalicylic acid (Georgewill and Georgewill, 2010). The percentage of inhibition of leaves extracts was higher than roots extracts (Egharevba *et al*., 2014).

Anticancer Effect

Breast cancer has been the second leading cause of deaths of women in the world (American Cancer Society, 2016). *V. amygdalina* leaves extracts had been reported to inhibit the proliferation of MCF-7 and MDA-MB-231 which involved the stimulation of cell-type specific G1/S phase cell cycle arrest in only MCF-7 cells but not in MDA-MB-231 cells given an approximate of 70% of diagnosed breast cancer express ER-α (Opata and Izevbigie, 2006; Gresham *et al*., 2008; Wong *et al*., 2013). Owoeye *et al*. 2010 had also reported the presence of epivernodalol in the methanolic extract of *V. amygdalina* leaf which was active against HT-144 (skin melanoma) cell line. On the other hand, the aqueous extracts were administered to mice for 4 weeks at dose 10 to 100 µg/ml per day, there was a significant reduction in CYP_1A_2 expression. Methanol and chloroform extracts were as well inhibited human leukaemia monocyte THP-1 cell line with IC_{50} values of 19.1 and 243.4 µg/ml, respectively (Yeap *et al*., 2010).

Antimicrobial Effect

The aqueous and ethanol extracts of *V. amygdalina* leaves had shown antimicrobial effects against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella spp*., and *Candida albicans* with the MIC values ranged between 12.5 and 50 mg/ml (Ghamba *et al*., 2014). In another study, ethanolic and aqueous extracts of *V. amygdalina* leaves had shown a higher value of MIC inhibitions on *Streptococcus mutans* at 25 and 55 mg/ml, respectively (Akinpelu, 1999; Anibijuwon *et al*., 2012).

Anti-malaria Effect

Leaves and roots of *V. amygdalina* extract possessed antimalarial effect against drug sensitive *Plasmodium berghei* in mice which resulted in 67% and 53.5% suppression of parasitaemia after the four days of administration, respectively (Audu *et al*., 2012). The leaves extracts had exhibited a significant antiplasmodial effect in mice against *Plasmodium berghei* with 73% inhibition (Njan *et al*., 2008). In the same vein, isolated sesquiterpene lactones from *V. amygdalina* had been reported to show antiplasmodial property with $IC_{50} < 4$ µg/ml against *Plasmodium falciparum* (Egharevba *et al*., 2014). *V. amygdalina* leaf extracts dose had dependently restored the efficacy of chloroquine against *Plasmodium berghei* in mice which had developed resistance (Iwalokun, 2008).

Significant results were obtained antiplasmodial effects of ethanolic and aqueous extracts at IC_{50} values 44.03 and 41.690 µg/ml, respectively (Egharevba *et al*., 2014).

Antifertility Effect

The antifertility effect of 95% ethanolic extracts of *V. amygdalina* leaves on the isolated mouse uterus had been reported at doses of 0.385, 0.5, and 1.0 g/kg body weight of mice when compared with the control agonist acetylcholine (1 g/kg). Significant reduction in a mean number of implantation sites, the number of live foetuses and survival percentage were recorded (Egharevba *et al*., 2014).

Antifungal Effect

Sesquiterpene lactones had been known to be highly antifungal (Barrero *et al*., 2000; Wedge *et al*., 2000). The presence of vernodalol and vernolide which belongs to sesquiterpene lactones in the *V. amygdalina* leaves extracts made them exhibit higher antifungal effect against *Penicillium notatum* and *Aspergillus flavus* with LC_{50} values of 0.4 mg/ml each (Erasto *et al*., 2006).

Antibacterial Effect

Sesquiterpene lactones from *V. amygdalina* leaves had exhibited antibacterial against five gram-positive bacteria with the MIC value of 0.25 mg/ml, but lack efficacy against gram-negative bacteria (Erasto *et al*., 2006). Likewise, the ethanolic stem extracts inhibited antibacterial effect against *Staphylococcus aureus* with 50 mg/ml concentration (Akinyele *et al*., 2014). The aqueous, ethanolic and methanolic extracts of the leaves had been reported to exhibit strong potency against clinical bacteria: *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* (Alo *et al*., 2012; Adetunji *et al*., 2013).

Anti-leukaemia Effect

The root culture of cold water, hot water, and ethanolic *V. amygdalina* extracts had been tested on the patients suffering from acute leukaemia and myeloid leukaemia. The extracts showed a higher level of anti-leukemia activity on the patients (Khalafalla *et al*., 2009).

4.0 MECHANISM OF ACTIONS OF *V. amygdalina* EXTRACTS

V. amygdalina leaves extracts had been reported to possess protective effect against carbon tetrachloride-induced hepatotoxicity by the antioxidant mechanism of action (Adesanoye and Farombi, 2010). Whereas, the administration of *V. amygdalina* aqueous extracts to wistar rats showed a dose-dependent improvement on CD4+ cells in comparison to control group. It was suggested that the mechanism could be related to the presence of antioxidant bioactive compounds (saponins, tannins, and flavonoids). Another possible mechanism of action could be due to early maturation and release of leucocytes (Momoh *et al*., 2010). Therefore, the used of *V. amygdalina* leaf aqueous extracts were suggested as an immune booster in related health conditions (Egharevba *et al*., 2014).

5.0 TOXICOLOGY OF *V. amygdalina*

The toxicology of *V. amygdalina* leaves extracts had been studied on mice and the results showed no clinical signs of toxicity or toxicological effects in the treated groups, except for a significant decrease in red blood cell count and a dose-dependent increase

in serum bilirubin (Njan *et al*., 2008). Owen *et al*. 2011 had also reported that there was no indication of toxicity in the *V. amygdalina* leaves extracts upon biomolecules evaluations when administered to broiler finisher chickens. Likewise, there was no appreciable difference in the glucose level, haematological profile, liver, and kidney function of the tested rats when the extracts were administered to rats (Imaga and Bamigbetan, 2013). Egharevba *et al*. (2014) reported that the toxicity limit was insignificant when compared with the highly toxic substances (toxicity at less than 1 mg/kg). Studies on the acute toxicity of the leaf extracts resulted in LD_{50} of 5.1523 g/kg when administered orally and this showed that the extracts were non-toxic (Adiukwu *et al*., 2012). In contrary, Temma people of Sierra Leone called bitter leaf as 'goat killer', this makes the animals to stay away from it due to its bitterness (Yeap *et al*., 2010). Table 3 shows some of the in vivo and in vitro toxicity of *V. amygdalina*.

Table 3: In vivo and in vitro toxicology of *Vernonia amygdalina*

Animal	Type of extract	LD_{50} (mg/kg b.w.)	Mode of administration	Consequences	Reference(s)
Rabbit	Aqueous	1122	Intra-peritoneal injection	-	Akah and Okafor (2006)
Rat	Methanol leaf extract (28 days at doses of 80, 160 and 320 mg/kg)	-	Intravenous injection	The histopathological studies showed no significant abnormalities in all the vital organs of the rats.	Akah *et al*. (2009)
			Intra-peritoneal injection	No effect on glycolysis	Atangwho *et al*. (2014)
	Chloroform fraction (7-14 days at 55 mg/kg b.w.)	-	Intra-peritoneal injection	-	Asante *et al*. (2016)
	Ethanol (dosage at 40 mg/kg)	-	Oral	Any apparent toxic symptom or mortality was not observed after the 24 h	Mansurah *et al*. (2013)
	Aqueous of combined *V.*				

	amygdalina and *O. gratissimum* (24 h at doses from 10-5000 mg/kg)				
Broiler finishers'	Leaf meal (28 days using 0%, 5%, 10% and 15% VALM)	-	Mixed with broilers' meals	The leaf meals have no adverse effect on the kidney, since the creatinine levels were not significantly altered.	Owen (2011)
Murine macrophages J774	Lipophilic extract	IC_{50} value of 6.48 µg/ml	-	-	Ganfon *et al*. (2008)
Guinea pig	Aqueous (0.3 mg/ml)	Injection	-	Increase of uterine, intestine and jejunum contraction which sustained for 30 min with elevated concentrations used	Yeap *et al*. (2010)
Albino mice	Aqueous (7 days at doses of 125 mg/kg)	-	Intra-peritoneal injection	Increase of serum enzyme markers level was more severe when it was consumed with antimalarial drug chloroquine	Iwalokun (2008)

6.0 CONCLUSION

This review reveals that *V. amygdalina* is endowed with different bioactive compounds that possess several pharmacological properties. Its medicinal potential has been explored, proven very effective with no toxicity to health. *V. amygdalina* is an ethnomedicinal plant that may be used in managing tropical diseases. However, other

pharmacological properties of this plant abound which can require genetic evaluation. In addition, several works had been carried out on the phytochemical, pharmacological properties, toxicology and mechanism of action of *V. amygdalina* leaf but few studies had been done on the stems and roots. Therefore, further studies are required to isolate and validate the potential of the *V. amygdalina* stems and roots.

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