

Review

***Vernonia amygdalina*, an ethnoveterinary and ethnomedical used green vegetable with multiple bio-activities**

Swee Keong Yeap³, Wan Yong Ho¹, Boon Kee Beh², Woon San Liang⁴, Huynh Ky¹, Abdul Hadi Noaman Yousr¹ and Noorjahan Banu Alitheen^{1*}

¹Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia.

²Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia.

³Institute of Bioscience, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia.

⁴Spektra Biotek Sdn Bhd, Kampung Melayu Subang, 40150 Shah Alam, Selangor, Malaysia.

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Today, researches are focusing on the discovery of new therapeutic substances of natural origin with possible low or no toxicity to human, animal and environment, based on ethnomedical and ethnoveterinary practices. *Vernonia amygdalina* is a shrub that has been commonly known as bitter leaf due the bitter taste of the leaves. It is widely used as a daily green vegetable or herb to treat malaria and diabetes. The potential of *V. amygdalina* was first noted when scientists observed chimpanzees use the pith of this shrub for self-deparasitization. Since that discovery, subsequent researches had unveiled more and more bioactivities possessed by different extracts of this plant such as antidiabetic, antibacterial, antimalaria, antifungal, antioxidant, liver protection and cytotoxic effects which are beneficial to health. Compounds including steroid glucosides, sesquiterpene lactones and flavonoids which contributed to its bitter taste and bioactivities have also been isolated from this plant. Toxicology studies documented on this plant shows that *V. amygdalina* has low or no toxicity thereby supporting the safe use of this plant for the benefits of health.

Keywords: *Vernonia amygdalina*, bioactivities, antidiabetic, antioxidant, liver protection

INTRODUCTION

Vernonia amygdalina Del is a multipurpose and rapid regenerating soft wooded shrub of 2 to 10 m tall with petiolate leaves of around 6 mm in diameter (Figure 1). This plant has been named differently by different ethnics around the world (Table 1). It is a species under the genus *Vernonia* Schreb (Family: Compositae; Order: Asterales; S/C: Asteridae; Classes: Dicotyledons) which contains about 1000 species. More than 500 of these *Vernonia* plants are distributed in Africa and Asia, approximately 300 in Mexico, Central and South America

and around 16 can be found in the United States.

However, the taxonomic ranking of this genus at all levels; ranging from its species up to the generic term can be a real challenge. Austin (2000) has found that even taxonomy of *V. amygdalina* from different geographical area (Ethiopia and Cameroon) could be different. One of the proposed solutions is through classification of the active chemical compounds, isolated from each species (Mabry et al., 1975). *V. amygdalina* produces a variety of flavonoids and bitter sesquiterpene lactones which contribute to the bioactivities of this plant (Nangendo et al., 2002; Favi et al., 2008). On the other hand, *V. amygdalina* is also commonly mis-identified as *V. colorata*. However, these two plants can be easily distinguished from each other by the hairy leaves of the

*Corresponding author. E-mail: noorjahan@biotech.upm.edu.my.

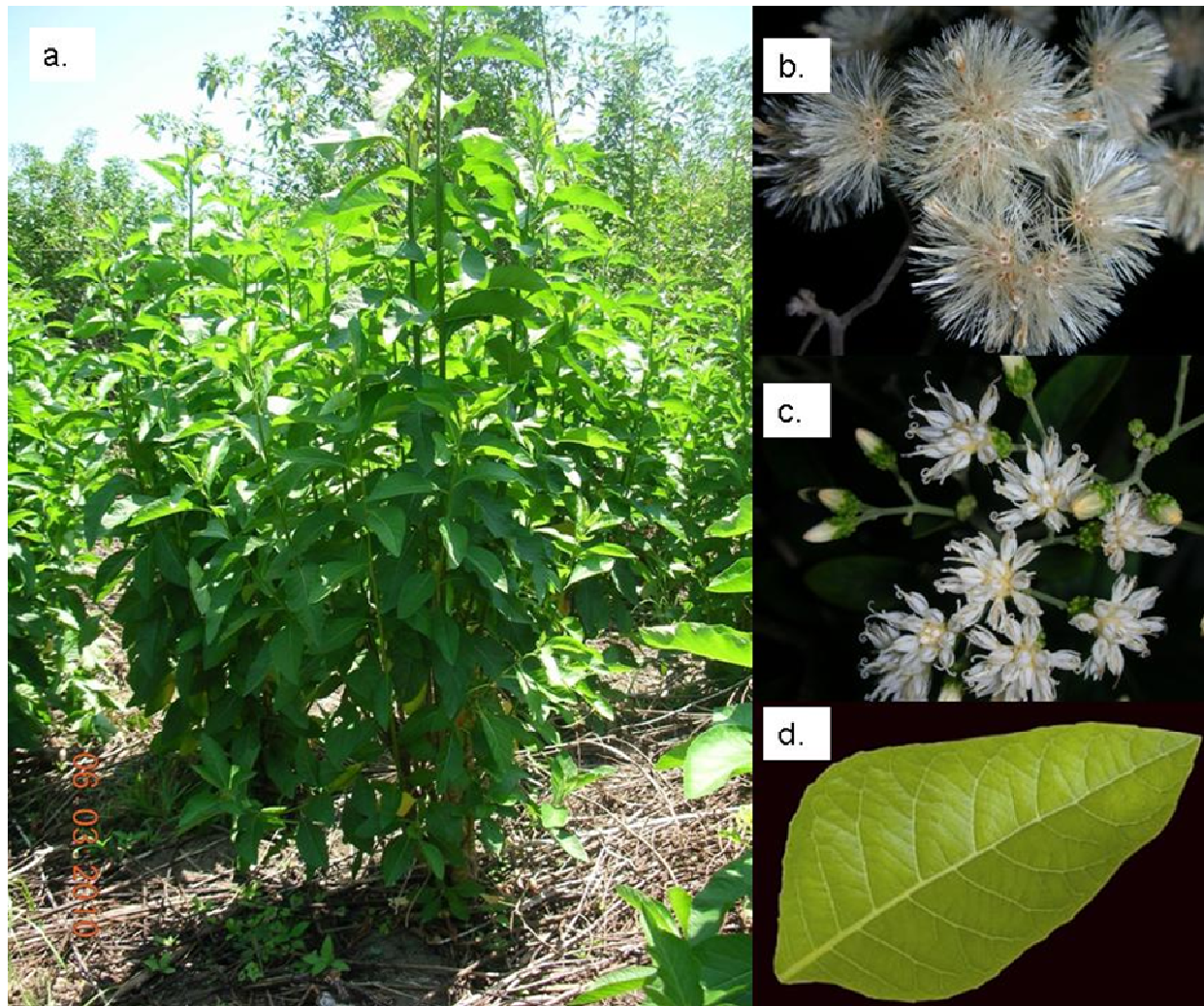


Figure 1. Photos of *V. amygdalina*. 1a. *V. amygdalina* shrub. 1b. 1c. Flower of *V. amygdalina*. 1d. Leaves of *V. amygdalina*. Geographical localisation: *V. amygdalina* nursery, Spektra Biotek Sdn Bhd, Kuala Selangor, Malaysia. 6-3-2010 by Mr. Liang Woon San.

latter (Iwu, 1993). Unlike other species under *Vernonia* family, *V. amygdalina* and *V. ambigua* are the only two species with diploid sets of chromosomes ($2n = 40$ and $n = 20$) (Ayodele, 1999).

V. amygdalina can be commonly found along drainage lines and in natural forests or at home and commercial plantations (Alem and Woldemariam, 2009). *V. amygdalina* is a common homestead farming vegetable and fodder tree in Nigeria (Ndaeyo, 2007) and has been used as an ingredient to prepare Nigerian (Ogbono soup) or Cameroon (Ndole) dish after removal of its bitter taste through soaking in several changes of water or by boiling (Koshimizu et al., 1993; Abosi and Raseroka, 2003; Onabanjo and Oguntona, 2003). In Ethiopian highland, *V. amygdalina* has been classified by the farmer as a multipurpose fodder tree with high biomass yield, easy propagation, high adaptability and high compatibility with

other crops which do not compete with them for soil nutrients or moisture but instead help to improve the soil fertility and growth of perennial crops (Mekoya et al., 2008). *V. amygdalina* does not produce seeds in normal circumstances and is usually propagated by cuttings (Arene, 1972).

However, studies found that some white, fragrant and bee-infested flowers growing on copious corymbose panicles would be formed under drastic growth environment and the seeds from these flowers could then be thrived well in slightly acidic soil with low organic matter and high water holding capacity. Water is the key factor for the growth of its leaves. Thus, high yield can be obtained during rainy season (Kayode, 2004). *V. amygdalina* is a short cycle crop which can be harvested twice per month for up to seven years. Planting *V. amygdalina* can be easy because it is compatible with

Table 1. Different local names of *V. amygdalina* in various countries.

Nation	Local name
English	Bitter leaf
Africa	Akpa gbo, Kossa fina, Mponasere, Ndumburghai, Njenyani, Oriwo
Cameroon	Ated nkol, Suwaaka
China	Ikaruga chrysanthemum tonsils, non-tree south
Democratic Republic of São Tomé and Príncipe	Libo Mucambu, Libo Que
Democratic Republic of Congo	Mpasi nyioso
Ethiopia	Buzut, Giraw, Grawa, Ibicha
Gabon	Ndoki
Ghana	Awonoo, Awonwene, Jankpantire
Kenya	Olulusia, South Africa leaf
Malaysia	South Africa leaf
Nigeria	Etidod, Ewuro, e jije, Onugbu, Olugbu, Shiwaka
Rwanda	Umubilizi
Swaziland	liNyatselo
Tanzania	Mtugutu
Uganda	Ekibirizi, Labwori, Lubilili, Lubirizi, Omubirizi
Zimbabwe	Musikavakadzi

(Abebe, 1984 ; Ajibesin et al., 2008; Akendengue and Louis, 1994; Appiah-Opoku, 1999; Asase and Opong-Mensah, 2009; Boily and Puyvelde, 1986; Bukonya-Ziraba and Kamoga, 2007; Chhabra et al., 1989; Etkin, 2002; Geissler et al., 2002; Giday et al., 2003; Giday et al., 2009; Igile et al., 1994; Iwu, 1993; Kambizi and Afolayan, 2001; Katuura et al., 2007; Langloise-Klassen et al., 2007; Madureira et al., 2002; Nfi et al., 2001; Noumi and Yomi, 2001; Okoli et al., 2003b; Otshudi and Friers, 2000; RGL enterprise, 2010; Stave et al., 2007; Tabuti et al., 2003a;b; Taiwo et al., 1999; Teklehaymanot, 2009).

any type of crop and can be planted in a variety of arrangements (Biggelaar and Gold, 1996).

Furthermore, the presence of bitter principles also protects *V. amygdalina* from most of the animals, insects and microbes where it only suffers from the attack by *Coleoptera curculionidae*, weevil *Lixus camerunus* and *Zonocerus variegates* (which utilized it as a source of protein) (Eluwa, 1979; Idowu and Idowu, 2001). The total area of leaf that is susceptible to insect attack ranges from 0.2 to 12% (Akachuku, 2001). Thus, this plant is suitable to be planted in either small or large scale as a source of income to farmers. The processed *V. amygdalina* was even exported to Europe and North America restaurants for preparation of African dishes (Fontem et al., 2003). *V. amygdalina* with just a little amount of processing can be classified as healthy food because it promotes the healthy development of the body. It contains not only the active drug molecules but also other substances that are necessary for maintaining health and physiological functions of the body without manifestation of toxicity (Iwu, 2002). As a result, *V. amygdalina* serves well as a low cost and readily available source of important nutrients to humans (Ojiako and Nwanjo, 2006).

Besides, this plant has also been widely used as fuelwood, 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 (Babalola and Okoh, 1996; Biggelaar and Gold, 1996; Eleyinmi et al., 2004; Kasolo and Temu, 2008; Okoh et al., 1995; Uraih and Anetekhai, 1991).

The objective of this review paper is to equip researchers and the public who are making use of *V. amygdalina* with knowledge on the bioactivities, efficiency, efficacy and more importantly the safety of this herb.

BACKGROUND AND TRADITIONAL APPLICATION OF *V. amygdalina*

The usage of *V. amygdalina* as medicinal herb started when zoopharmacologists found that sick chimpanzees with empty stomach sucked pith and juice from the unsavoury *Vernonia* plant stalk (which was not their common diet) for self-deparasitization, enhanced body fitness, increased strength or appetite and reduced constipation or diarrhoea especially during rainy season (Clayton and Wolfe, 1993; Huffman and Seifu, 1989; Huffman et al., 1997; Jisaka et al., 1993a; b; Koshimizu et al., 1994). The bitter taste of *V. amygdalina* was suspected as a guide for them to choose for the appropriate plant, plant part and amount of intake (Koshimizu et al., 1994). Other than animals, some of the citizens in Africa especially patients who were less educated with low or middle income also liked to use this plant, due to

cultural and economic reasons (Amira and Okubadejo, 2007). The traditional practices of *V. amygdalina* are summarized in Table 2. Besides its use as a single herbal ingredient, *V. amygdalina* has also been incorporated as an important ingredient in traditional polyherbal formulations to treat various diseases (Table 3).

BIOACTIVITIES OF *V. amygdalina*

Antibacterial

Many experimental studies of *V. amygdalina*, have reported that this plant possesses antibacterial activity. Newbold et al. (1997) showed that this plant has mild antimicrobial effect on rumen bacteria and protozoa while Kambizi and Afolayan (2001) proved that acetone extract of *V. amygdalina* possesses antibacterial activity towards *Bacillus cereus*, *Bacillus pumilus*, *Bacillus subtilis*, *Micrococcus kristinae*, *Staphylococcus aureus*, *Enterobacter cloacae* and *Escherichia coli* growth with minimum inhibition concentration (MIC) of 5 mg/ml. Although, Cos et al. (2002) concluded that *V. amygdalina* was more sensitive towards the gram positive bacteria than gram negative bacteria; some researchers found that the activity of *V. amygdalina* against gram-negative bacteria was comparable to that towards the gram-positive species.

For instance, methanol extract of *V. amygdalina* did not only inhibit growth of the gram positive bacteria such as *B. cereus*, *B. pumilus*, *B. subtilis*, *E. cloacae*, *S. aureus* and *M. kristinae* but was also effective against gram-negative bacteria including *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Shigella dysenteriae* and *E. coli* (Akinpelu, 1999; Kambizi and Afolayan, 2001). Ethanolic extract from the plant also showed antibacterial effect against both gram-negative (*E. coli* and *Salmonella typhi*) and gram-positive (*Clostridium sporogenes*, *Staphylococcus pyogenes* and *S. aureus*) bacteria (Ogbulie et al., 2007; Kola, 2007). However, there were contradicting findings concerning the activity of *V. amygdalina* ethanol extract. Ogbulie et al. (2007) claimed that ethanol and Soxhlet extractions are the best solvent and the best method to give optimum antibacterial effect of this plant. Conversely, despite its inhibitory effect towards *S. aureus*, Pesewu et al. (2008) found that this extract could not inhibit the methicillin-resistant (MRSA UELSHB 102, UELSHB) and methicillin-sensitive (MRSA NCTC 6571) strains of the bacteria while chloroform, water and blender extract of *V. amygdalina* leaves showed low inhibitory effect towards its growth. Among all, only water and blender extract showed effect on *Streptococcus pyogenes*, *E. coli* and *P. vulgaris* with MIC and minimum bactericidal concentration (MBC) higher than 50 mg/ml. On the other hand, *V. amygdalina* root water extract also showed antibacterial activity on *Streptococcus gordonii*, *Porphyromonas gingivalis*, *Porphyromonas nigrescens*, *Prevotella intermedia*,

Fusobacterium nucleatum and *P. aeruginosa* with MIC at 100 mg/ml (Taiwo et al., 1999). Although not showing strong antimicrobial activities, its water extract still remain as the most common practice in traditional medicine and has demonstrated success in treating patients with sexually transmitted diseases (Kambizi and Afolayan, 2001). This may be due to the absence of upper limit in concentration of *V. amygdalina* consumption which contributes to the increased efficacy of this extract in traditional practices.

The antibacterial property of *V. amygdalina* was proved to be beneficial in a few applications. For example, Babalola and Okoh (1996) proposed that this plant may be suitable for use in beer industry, since it was able to inhibit the growth of *Bacillus circulans*, *Aerococcus viridans*, *Clostridium perfringens* and *Micrococcus sp* bacteria but promote alcohol production of brewer's yeast while Kola (2007) claimed that the antibacterial activity of its ethanolic leaf extract against *C. sporogenes* was able to revert the blood, protein and bilirubin urinary to basal level and increase neutrophil and white blood cell count as well as packed cell volume (PCV) in blood, thereby reducing haemolysis caused by infection.

Besides, this plant has also been suggested for use as chewing stick to maintain oral health by dislodging cariogenic micro-organisms (Etkin, 2002), in line with the traditional use of this plant for mouth cleaning. Saliva extracted from *V. amygdalina* chewing stick can maintain oral cleanliness by contributing to gum healing, analgesia, antisickling, haemostasis and antimicrobial activity and plaque inhibiting effect. This was supported by the finding that cold aqueous extract of *V. amygdalina* whole stem, bark and pulp extract showed bactericidal activity against the oral anaerobic bacteria: *Bacteroides gingivalis*, *B. asaccharolyticus*, *B. melaninogenicus* and *B. oralis* (Rotimi and Mosadomi, 1987).

Plants that are frequently used as medicine in traditional practices may not always portray high activity in the corresponding *in vitro* studies. This could be due to contribution from other therapeutic activities of the plant which eventually leads to healing of patients (Madureira et al., 2002). In some cases, the plant can also aid in augmenting the bioactivities possessed by other elements through an indirect way. A good example of this would be the application of *V. amygdalina* as a host to grow *Tapinanthus sessilifolius* which possesses antimicrobial effect and low LD50 on mice (692 mg/kg body weight) (Tarfa et al., 2004).

Antifungal

Most of the researches on antifungal activity of *V. amygdalina* focused on its water extracts. The water extract of *V. amygdalina* leaves can inhibit the growth of *Fusarium moniliforme* on seeds of maize (*Zea mays*) as well as mycelial and conidial growths of *Colletotrichum gloeosporioides* in rubber tree (Ogbebor et al., 2007;

Table 2. Ethnomedicinal usage of *V. amygdalina*.

Country	Preparation	Ailments
Ethiopia	Leaves (not root)	Stomach disorder, skin wound, diarrhea, scabies, hepatitis, ascariasis, tonsillitis, fever, mastitis, tapeworm and worms infection.
	Leaves and root	Stomach ache (worm expulsion)
Democratic Republic of Congo	Leaves and root bark	Diarrhea, dysentery, gastroenteritis, malaria, hepatitis, worms infection
Ghana	Leaves decoction	Malaria, fever, constipation, abortifacient, stomach sores, ulcer, pain, upper respiratory tract infections and dermatitis.
Gabon	Leaves juice	Tinea
Guinea	Leaves decoction	Cough
Nigeria (Hausa tribe of northern)	Leaves, Root and twig	Stomachache, gastrointestinal troubles, oral hygiene, itches, parasitic infection, ringworm, typhoid fever, headache, diabetes, constipation, pile (haemorrhoids) and reduces aflatoxin contamination of storage cobs.
Cameroon	Leaves Maceration of leaves drink many times for 1 day.	Malaria, helminthosis Intestinal diseases
South Africa	Root	Schistosomiasis, infertility, amenorrhoea.
West Africa	Tea	Diuretic, skin infection, constipation, diabetes, metabolic diseases associated with liver
Senegal		Female sterility
Guruve, Zimbabwe	Root	Sexually transmitted disease
India	Leaves	Diabetes
	Stem, Root	HIV (reduce fever, rash, pain, cough, stomachache) Measles, amoebiasis, influenza and mastitis infection.
Uganda	Leaves	Convulsions, cough, painful uterus, inducing uterine contraction, management of retained placenta and post partum bleeding, induced abortion, irregular or painful menstruation, infertility, colic pains bacterial and fungal infections
	Root/Leaves	
Rwanda	Leaves, Fruit	Diarrhea, gastroenteritis, hepatitis, dysentery, malaria, worms infection.
Tanzania	Leaves	Snake bite (chew), fever, stomachache, as appetizer
	Root	Trematode
Kenya	Leaves, Root	“Wuoyo”, “Mbaha”, diarrhoea, “Sihoho” stomachache, bloat, east coast fever, footrot, trematode

(Abebe, 1984; Abo et al., 2008; Adjanohoun, 1993; Agyare et al., 2009; Ajaiyeoba et al., 2003; Ajibesin et al., 2008; Akendengue and Louis, 1994; Akinpelu, 1999; Amole et al., 2006; Appiah-Opoku, 1999; Asase et al., 2005; Aucha et al., 2005; Chhabra et al., 1989; Geissler et al., 2002; Giday et al., 2003; Innocent and Deogracious, 2006; Kamatenesi-Mugisha, 2005; Kambizi and Afolayan, 2001; Katuura et al., 2007; Langloise-Klassen, 2007; Leonard, 2002; Nfi et al., 2001; Noumi and Yomi, 2001; Otshudi and Foirers, 2000; Pesewu et al., 2008; Ssegawa and Kasenene, 2007; Steenkamp, 2003; Tabuti et al., 2003b; Teklehaymanot et al., 2007; Teklehaymanot, 2009; Udo et al., 2000; Vlietinck et al., 1995; Wondimu et al., 2007; Yineger et al., 2007).

Table 3. Polyherbal formulation of *V. amygdalina* in traditional practice.

Medicinal use	Polyherbal	Preparation	Practice	Country
	Roots of <i>V. amygdalina</i> , stem bark of <i>Sapium ellipticum</i> , leaves of <i>Dalbergia nitidula</i> , <i>Desmodium salicifolium</i> and <i>Eriosema psoraleoides</i>	Boiling	Drinking	Tanzania
Malaria	<i>V. amygdalina</i> , <i>Momordica foetida</i> , <i>Cannabis</i> sp., <i>Chenopodium opulifolium</i> , <i>Mangifera indica</i> and <i>Tamarindus indica</i>	Boiling	Adult- 100 to 500 ml, children above 5 years- 100 to 250 ml and children younger than 5 years- 1 to 3 tablespoons) 1 to 3 times daily for 1 to 3 days	Uganda
	<i>V. amygdalina</i> and <i>Citrus limon</i>	Boiling or squeezing in cold water	Drinking or hot bath	Uganda
	leaves of <i>Markhamia lutea</i> , <i>tetradenia riparia</i> and <i>Vernonia amygdalina</i>	Water macerate	Drinking	Rawanda
Antrax infection	Leaves of <i>V. amygdalina</i> , bark of <i>K. senegalensis</i> A. Juss, and <i>S. incanum</i> Linn	Grind with water	Drinking (35 ml) 3 times daily	Nigeria
Worm infection in cattle and chicken	<i>V. amygdalina</i> and <i>Anona senegalensis</i>	Water extract (1.17g/100ml water)	Drinking	Nigeria
	Leaves of <i>V. amygdalina</i> , <i>Elytraria marginata</i> , whole plant of <i>peperomia pellucida</i> and seed of <i>Piper guineensis</i>	Soup	Three tablespoonfuls twice daily	
	<i>V. amygdalina</i> and <i>Ocimum gratissimum</i> fresh leaves and <i>Butyrosperum paradoxum</i>		Topical application on skin twice daily after bath	
Measles	Leaves of <i>Newbouldia laevis</i> , <i>Momordica charantia</i> , <i>Vernonia amygdalina</i> and <i>Ocimum gratissimum</i>	Boiling	Drinking three times daily	Nigeria
	Leaves of <i>V. amygdalina</i> and <i>Ocimum gratissimum</i>	Soak into palm wine (24h)	One teacupful twice daily	
	Leaves of <i>V. amygdalina</i>	Soaked in alcohol	One teaspoonful once daily	
	leaves of <i>Chenopodium opulifolium</i> , <i>Cannabis sativa</i> and <i>V. amygdalina</i>	Infusion with salt	Soup	Uganda
	Leaves of <i>V. amygdalina</i>	Infusion with salt	Drinking	Uganda

Table 3. contd

	Leaves of <i>V. amygdalina</i>	Squeezed to produce juice and mixed with salt	Drinking	
	Leaves of <i>V. amygdalina</i>	Squeezed with water	Drink twice daily	
	Leaves of <i>Peperomia pellucida</i> , <i>Ocimum gratissimum</i> and <i>V. amygdalina</i>	Squeezed with water	Drinking with glass cup thrice daily	
	Fruits of <i>Bidens pilosa</i> fruits and leaves of <i>Vernonia amygdalina</i>	Soaked in water	One glass cup three times daily	
	<i>Vernonia amygdalina</i> mixed with potash salt and honey	Juice	One cup every morning and night	
	Leaves and roots of <i>Vernonia amygdalina</i> , <i>Momordica charantia</i> , <i>Carica papaya</i> , <i>Bidens pilosa</i> and <i>Ocimum gratissimum</i> .	Soaked in alcohol	One glass cup of essence thrice daily.	
	Leaves of <i>Macaranga barteri</i> , <i>Ageratum conyzoides</i> and <i>Vernonia amygdalina</i>	Squeezed with water	Drinking	
	Leaves of <i>Vernonia amygdalina</i> and bulb of <i>Allium cepa</i>	Soaked in water for 5 days	One glass cup daily	
Diabetes mellitus	Seeds of <i>Bidens pilosa</i> seeds, leaves of <i>Vernonia amygdalina</i> , fruits of <i>Momordica charantia</i> , leaves of <i>Ocimum gratissimum</i> and bark of <i>Alstonia congensis</i>	Soaked in local alcohol	Drinking	Nigeria
	Leaves of <i>Vernonia amygdalina</i> , seeds of <i>Bidens pilosa</i> and roots of <i>Uvaria afzelii</i>	Soaked in water	One cup of macerate three times daily	
	Stem and bark of <i>Bridelia micrantha</i> , leaves of <i>Vernonia amygdalina</i> and <i>Ocimum gratissimum</i>	Squeezed with water	Drinking	
	Leaves of <i>Vernonia amygdalina</i> and <i>Ocimum gratissimum</i>	Squeezed with water	Drinking	
	Leaves of <i>Vernonia amygdalina</i> , fruits of <i>Croton lobatus</i> and leaves of <i>Macaranga barteri</i>	Boiled with water	One glass cup daily	
	Leaves of <i>Vernonia amygdalina</i> and <i>Glyphaea brevis</i>	Boiled with water for 20 min (decoction)	Drinking	
	Leaves of <i>Allium sativum</i> , <i>Vernonia amygdalina</i> , <i>Ocimum gratissimum</i> , and potash	Boiled with water	One glass cup daily	

Table 3. contd

Aprophylaxis	Leaves of <i>V. amygdalina</i> and <i>Talinum cuneifolia</i> (Vahl) Willd	Cold water extract	Drinking	
Diarrhea on animal	Roots of <i>Kalimi kambwa</i> , <i>Harrisonia abyssinica</i> and <i>V. amygdalina</i>	Infusion added with salt	Drinking	
Cough	Leaves of <i>Chenopodium opulifolium</i> , <i>Senna occidentalis</i> and <i>V. amygdalina</i>	Infusion	Drinking	Uganda
Bronchitis	Leaves of <i>V. amygdalina</i> , <i>Cannabis sativa</i> L., fruit of <i>Capsicum frutescens</i> L., and fruit peelings of <i>Citrus limon</i> (L) Burm. F	Decoction	Drinking	Uganda
Bacillary white diarrhoea, Fowl pox, Coccidiosis and Newcastle diseases	Leaves of <i>V. amygdalina</i> , <i>Aloe</i> sp and <i>Moringa oleifera</i> Lam.	Cold water extract	Drinking	
	Leaves of <i>V. amygdalina</i> , <i>Aloe</i> sp, <i>Jatropha carcus</i> L. and bark of <i>Allium</i> sp	Decoction	Drinking	
	<i>V. amygdalina</i> , <i>Moringa oleifera</i> Lam., root of <i>Kalanchoe densiflora</i> Rolfe, root and bark of <i>Mangifera indica</i> L.	Decoction	Drinking	
Wound healing	Leaves of <i>V. amygdalina</i> , <i>Aspillia plurisetata</i>	Pounded	Drinking	Kenya
Schistosomiasis	<i>V. amygdalina</i> , root of <i>Vigna sinensis</i>	Cold infusion	Drinking	Kenya

(Alawa et al., 2002; Bukenya-Ziraba and Kamoga, 2007; Gbolade, 2009; Hakizamungu et al., 1992; Moshi et al., 2009; Sonibare et al., 2009; Tabuti et al., 2003a).

Owolade et al., 2000; Suleiman et al., 2008). Cold water extract of stem bark and root bark (but not leaves) was able to inhibit *Colletotrichum capsici* (Synd) isolated from pepper (Nduagu et al., 2008). On the other hand, the juice of *V. amygdalina* showed stronger effect than its cold water extract where its juice could more effectively inhibited seed borne fungi (*F. moniliforme*, *Botryodiplodia theobromae*, *Aspergillus niger* and *Aspergillus flavus* *in vitro* and *in vivo* (Nwachukwu and Umechuruba, 2001).

In crop industry, hot water extract of *V. amygdalina* was able to help to control the infection of *Sclerotium rolfsii* and increased the plant height, shelf life, relative water content, chlorophyll content, leaf area index, number of branches, total dry matter, number of pod per plant, weight and also grain yield on cowpea. However, it induced phytotoxic effect where it reduced the recoverable of photosynthates and transpiration rate of the treated plant. This feature can be used to maintain high water content in cowpea seedlings through

antitranspirants during dry seasons for its survival (Alabi et al., 2005b).

Aside of water extract, only methanol extract of *V. amygdalina* was reported to show strong antifungal activity on *Pseudoperonospora cubensis* and mild activity on *Rhizoctonia solani* (Ohigashi et al., 1991b). Interestingly, the ash from *V. amygdalina* also possesses antifungal property where it was shown to inhibit the growth of *Sclerotium rolfsii* Sacc. mycelial growth on wheat and protected seedlings against post-emergence infection through inhibition of fungal growth within the root and crown zones. This effect was contributed by the high nitrogen level in the ash of *V. amygdalina* which could directly inhibit sclerotial germination and retarding mycelial growth of *S. rolfsii* (Enikuomehin et al., 1998).

In contrary to antifungal property, Okigbo and Emoghene (2003) have found that leaves extract of *V. amygdalina* can be metabolized by *Mycosphaella fijiensis* for growth and induction of sporulation.

Antiparasite activity

From literature, it can be noted that *V. amygdalina* is a powerful plant which shows apparent inhibitory activity against a wide range of parasites. The methanolic extract of this plant possessed antitrichomonas activity with 100% of inhibition against *Trichomonas vaginalis* (Hakizamungu et al., 1992). The methanolic and aqueous leaves extract also possessed antiamebic activity at 100 to 500 µg/ml with 20 to 80% inhibition against *Entamoeba histolytica* after 3 to 4 days incubation (Otshudi and Foriers, 2000; Moundipa et al., 2005). On the other hand, its chloroform and methanol extracts showed antileishmania effect towards amastigotes and promastigotes (*Leishmania aethiopica*) (Tadesse et al., 1993; Carvalho and Ferreira, 2001). Five consecutive days i.p. injection of 1mg/kg petroleum ether and ethanolic leaf extracts *V. amygdalina* possessed antischistosomiasis effect (72.3 and 83.61% reduction of parasite load) on mice infected with *Schistosoma mansoni* type cercariae. In addition, single prophylactic (1 mg/kg) dose of petroleum ether or ethanolic leaf extracts of *V. amygdalina* also reduced 34.06% and 44.57% of parasite load respectively (Ogbole et al., 2000).

Leaf extract of *V. amygdalina* was also able to reduce nematode populations and thus enhanced the growth of cacao seedling in nursery (Orisajo and Dongo, 2005). Powder and water extracts of *V. amygdalina* were able to inhibit root knot nematode (*Meloidogyne incognita*) on sesame *Ceratotherca sesamoides* Endl (Oyedunmade and Olabiyi, 2006; Oyedunmade and Chukwu, 2007). Decomposition in the root and soil of the *V. amygdalina* plants were found to significantly reduce population density of root-knot nematodes. Thus, this plant is suitable to be co-planted with other crops in order to manage nematode population in plantations (Afouda et al., 2008).

The ethanol extract of *V. amygdalina* was mild toxic towards the intestinal threadworm *Strongyloides papillosus* with LC50 0.49 and 0.2% (weight/volume) at 24 and 48 h respectively (Musongong et al., 2004). Its aqueous extract on the other hand, was able to reduce faecal egg count of ascarids (*Toxocara canis*) and hookworm (*Ancylostoma caninum*) in treated puppies (Adedapo et al., 2007). This effect was contributed by the major active constituents' vernodaline (bitter tasting constituent) and vernonioside B1 (non-bitter principle) (Koshimizu et al., 1994). Furthermore, its water extract (1 mg/ml) was able to depress faecal egg output and reduce adult worm population of *Ascaridia galli* in the host (Siamba et al., 2007). On top of these, *V. amygdalina* also showed a few other major antiparasitic activities which will be discussed in more detail in the following sections.

Anthelmintic activity

The anthelmintic property of *V. amygdalina* was

observed when the juice from its pith was found to reduce the number of *Oesophagostomum stephanostomum* eggs in the dung of chimpanzee (Huffman et al., 1997). Anthelmintic efficacy of *V. amygdalina* in ethnoveterinary study was found to be 52.4% (Nfi et al., 1999). Alawa et al. (2000; 2003) had previously reported that the hot water extract of *V. amygdalina* did not possess antihelminthics effect against *Haemonchus contortus* and *Trichostrongylos colubriformis*. They hypothesised that the traditional antihelminthic claim of this plant extract maybe contributed by the cleansing of gastric and intestines through increase of smooth muscle motility.

However, the high extraction temperature and pressure may degrade all the active compounds which further lead to inactivity against the larvae. In another research carried out by Molgaard et al. (2001), the leaves including stem, root and root bark of *V. amygdalina* water extract effectively killed cestodes of *Hymenolepis diminuta* after 24 h of treatment. Abdul et al. (2000) suggested that dissolving *V. amygdalina* water extract in potash (potassium carbonate) is needed in the case of worm treatment. Besides, methanolic extract of *V. amygdalina* possessed median effective dose (ED50) at 3.5 mg/ml against *Ascaris suum*. This extract killed 50% of *Ascaris* after 12 h at 6 mg/ml (Innocent and Deogracious, 2006).

Antimalaria/ antiplasmodial activity

The ethanol, petroleum ether, dichloromethane, ethyl acetate, acetone-water and isoamyl alcohol extracts of *V. amygdalina*, showed antimalarial activity against *Plasmodium falciparum* (Dd2) *in vitro* (Madureira et al., 2002; Masaba, 2000; Tona et al., 2004). The root extract of *V. amygdalina* displayed mild activity against chloroquine-sensitive *P. falciparum* with IC50 of 19 µg/ml but no activity against the chloroquine-resistant strain (Froelich et al., 2006). *P. falciparum* is a parasite that can lead to the most pathogenic form of human malaria which is one of the most dangerous infectious diseases endangering human lives (Anderson et al., 2000).

This antimalarial effect of *V. amygdalina* is contributed by its active compounds, or more specifically sesquiterpene lactones such as vernolepin, vernolin, vernolide, vernodaline and hydroxyvernodaline which exhibited antiplasmodial activity of IC50 value lower than 4 µg/ml (Tona et al., 2004). Besides, Masaba (2000) discovered that acetone-water extract from *V. amygdalina* leaves showed lower IC50 value (25.5 µg/ml) against *P. falciparum* than water extract (76.7 µg/ml) after 48 h. The relatively lower bioactivity of water extract as compared to solvent extraction explains why larger quantity (100 to 200 ml) of *V. amygdalina* decoction is used in traditional practices for defeating the disease. Besides *P. falciparum*, *V. amygdalina* is also effective against another species of *Plasmodium* protozoa which serves as the causative agent of malaria. Ethanolic

leaves and root extract of *V. amygdalina* exerted *in vivo* suppressing effect against *P. berghei* at early stage of infection with ED50 125 mg/kg body weight for leaves and 250 mg/kg for root extract after 3 days treatment. However, the extracts failed to remove the infection completely (Abosi and Raseroka, 2003). Besides, aqueous extract from its leaves also inhibited 73% of *P. berghei* on mice when administrated at 200 mg/kg i.p. daily for 4 days (Njan et al., 2008).

Furthermore, Iwalokun (2008) showed that this aqueous leaves extract (62.5, 125 mg/kg) was able to work synergistically with chloroquine (5 and 30 mg/kg) against both chloroquine-sensitive and resistant *P. berghei* to shorten the parasite clearance time, prolong the recrudescence times and improve curing rate. The study has also suggested that administration of *V. amygdalina* ethanol extract 1 h prior to chloroquine intake can avoid the reduction in chloroquine bioavailability (Igboasoia et al., 2008).

Antiviral activity

Ethanol extract of the fruit (which is rarely found on most of the *V. amygdalina* shrub) possessed antiviral effect on polio virus (Vlietinck et al., 1995).

Pesticidal or Insecticidal activity

Methanol extract of *V. amygdalina* leaf induced 50% of mortality on *Spodoptera litura* through deterrent of feeding activity at 2 mg/ml and induced 50% of mortality on *Culex pipiens pallens* at 10 µg/ml (Ohigashi et al., 1991b). *V. amygdalina* powder possessed pesticidal activity against *Sitophilus zeamais* (LC50 = 0.3 g) and *Callosobruchus maculatus* (LC50 = 0.39 g).

The principle of this effect may be due to the presence of hydrocyanic acid and oxalic acid. However, concentrated extract was recommended to give better response (Kabeh and Jalingo, 2007). One to five grams of *V. amygdalina* dry leaf powder was able to control the growth of *S. zeamais* and *Sitophilus oryzae* on stored maize grains and rice (Enobakhare and Law-Ogbomo, 2002; Law-Ogbomo and Enobakhare, 2007).

Anticancer and cytotoxic effect

Cold water extract of *V. amygdalina* leaf extract exhibited cytostatic action on MCF-7 cell growth and DNA synthesis (Izevbigie, 2003; Izevbigie et al., 2003; Izevbigie et al., 2004) through down regulation of extracellular signal-regulated protein kinase (ERK) signaling (Izevbigie et al., 2003; Izevbigie et al., 2004), induction of cytochrome P450 3A4 (CYP3A4) and microsomal epoxide hydrolase expression (Howard et al., 2003) and alteration of cell membrane permeability and

efflux (Opata and Izevbigie, 2006). The aqueous extract when combined with tamoxifen was able to reduce 10-fold less concentration of tamoxifen to inhibit 50% of cell growth (Izevbigie et al., 2005). In addition, exposure of aqueous extract of *V. amygdalina* at 100 to 300 µg/ml was also able to reverse the ethanol-induced stimulatory response in paclitaxel-sensitive and resistant human breast cancer cell MCF-7 (Howard et al., 2006). These results suggest that *V. amygdalina* may prevent or delay the onset of breast cancer.

However, other researches also reported that cold water extract of the plant possessed moderate cytotoxicity effect with IC50 at 218 µg/ml (Opata and Izevbigie, 2006) or even without IC50 at 1 ~ 2 mg/ml (Yedjou et al., 2008) against MCF-7 cell. This was believed to be attributed by batch variation of the extracts (Opata and Izevbigie, 2006). Liquid chromatography separation indicated that the active constituents of the various solvent fractionated extract showed similar retention times of approximately 2 min. This suggested that HPLC peaks of approximately 2 min may serve as a predictive tool for monitoring the activities of *V. amygdalina* extract (Oyugi et al., 2009).

Aqueous extract of *V. amygdalina* also showed increasing effect against the growth of estrogen receptor negative ductal carcinoma (BT-549) cell line in a concentration dependent fashion with IC50 at 1000 µg/ml through inhibition of DNA synthesis. Although the inhibition concentration was high, *V. amygdalina* has been consumed in large quantities without reported cases of toxicity and thus may be suitable for incorporation into the diet of cancer patients to improve their prognosis or quality of life (Gresham et al., 2008; Robinson et al., 2009). Cold water, hot water and ethanol extract were found to induce apoptosis against acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) from the patients with IC50 ranging between 5 to 10 µg/ml. Ethanol extract was found to be most effective against both ALL and AML when compared to cold and hot water extract (Khalafalla et al., 2009). Petroleum ether/ethyl acetate leaf extract also possessed cytotoxic effect towards human hepatoblastoma (HepG2) and urinary bladder carcinoma (ECV-304) cell lines (Froelich et al., 2006).

Moreover, four-week exposure of *V. amygdalina* water extract (10 to 100 µg/ml/day of mice) was chemoprotective against exposure to alcohol through reduction of CYP1A2 expression (Howard and Samuel, 2005). Chloroform and methanol extract were also active against human leukaemia monocyte THP-1 cell line with IC50 19.1 and 243.4 µg/ml, respectively (Carvalho and Ferreira, 2001; Tadesse et al., 1993).

Antimutagenic activity

Organic solvent extracts of *V. amygdalina* had inhibitory effect (higher than 60%) towards His⁻ to His⁺ reverse-

mutations on *Salmonella typhimurium* TA100 induced by ethyl methanesulfonate. The most effective extract was petroleum ether followed by methanol and ethyl acetate extracts. However, all the extracts induced toxicity against *S. typhimurium* when high concentrations were applied (Obaseiki-Ebor et al., 1993).

Antifertility activity

Ethanol extract of its leaves possessed uterotonic (2%) and anti-implantation (1.54 g/kg) effect (Desta, 1994).

Anticoagulant and antithrombotic activities

V. amygdalina caused reduction of blood pressure and Vernolepin isolated from this plant was identified to cause antiplatelet activity. Methanolic extract of *V. amygdalina* at 100 and 200 mg/kg induced 40 and 50% inhibition against thrombosis in mice. This may be due to the inhibition of platelet aggregation but the mode of action such as the effect on thromboxane A₂ formation and on the level of cyclic AMP in platelets is still unknown (Awe et al., 1998; Igboechi et al., 1984)

Analgesic (reduce pain) and antipyretic (lowering body temperature) effect

Aqueous leaves extract and ethanol root extract of *V. amygdalina* exhibited antipyretic effect, central and peripheral analgesic effect against acetic acid induced writhing, as well as protective effect on formalin and pentylenetetrazole-induced lethality (Iroanya et al., 2010; Njan et al., 2008; Onah and Okokon, 2004; Tekobo et al., 2002).

Anti-inflammatory activity

Leaf aqueous extract of *V. amygdalina* was able to inhibit carrageenan induced rat paw oedema and xylene induced ear oedema in treated rats (Iroanya et al., 2010). Ethanol extract (25 to 100 µg/ml) of *V. amygdalina* was also able to inhibit polymorphonuclear cell and mononuclear cell activity without affecting the cell viability (Koko et al., 2008).

Antioxidant

V. amygdalina ethanol extract was shown to possess antioxidant activity from DPPH radical scavenging test (Ayoola et al., 2008). Total flavonoid and phenolic contents was found to be correlated positively with total antioxidant activity of the plant. At 250 µg/ml, ethanol extract of *V. amygdalina* showed highest reducing

capacity and DPPH radical scavenging activity effect as compared to the same concentrations of its isolated compounds vernodalol and vernolide. This result suggested that the comparatively higher antioxidant activity of *V. amygdalina* ethanol extract may be the result of synergistic effect brought about by both the compounds (Erasto et al., 2007a).

Comparing DPPH radical scavenging activity of different extracts from the root of *V. amygdalina*, ethanol was found to be the best extraction which can inhibit 77% of scavenging activity followed by hot water (63%) and cold water (49%) at 1 µg/ml. Also, all the extracts were able to inhibit bleaching of B-carotene, oxidation of linoleic acid and lipid peroxidation induced by Fe²⁺/ascorbate in a rat liver microsomal preparation (Owolabi et al., 2008; Khalafalla et al., 2009). On the other hand, another study showed that methanol exhibited highest antioxidant activity followed by acetone and water extract (Erasto et al., 2007b). The antioxidant capacity of the methanolic extract was able to protect membrane stability in hemagglutination test (Iwalewa et al., 2005).

This plant contains natural antioxidants against aqueous radicals and reactive species ions (Erasto et al., 2007b). Boiling, sun-drying and blanching processes were found to reduce ascorbic acid content of the plant (Oboh and Akindahunsi, 2004; Oboh, 2005; Odukoya et al., 2007). Blanching of the plant also reduced its reducing capacity and free radical scavenging property but enhanced the taste and reduced toxicity of *V. amygdalina* (Oboh, 2005). In spite of the reduction in ascorbic acid content, the total phenolic content, reducing power and free radical scavenging ability of the plant was significantly increased. Thus, it can be concluded that vitamin C does not play the role as an important antioxidant agent in this plant.

Liver protective

Oral administration of the aqueous extract from *V. amygdalina* leaves could accelerate the reversion of liver damage through reduction of liver marker enzymes, including aspartate aminotransferase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP), glutamate-oxaloacetate transaminase, glutamate-pyruvate transaminase, lactate dehydrogenase and bilirubin indices in liver biochemical tests (Arhoghro et al., 2009; Iwalokun et al., 2006).

Histopathological examination showed that as low as 15% of extract could even completely revert liver change to normal in the treated animals (Arhoghro et al., 2009). Adesanoye and Farombi (2009) reported that oral administration of methanolic extract (at 250 to 750 mg/kg body weight) on mice with or without carbon tetrachloride (CCL₄) increased the level of liver marker enzyme ALT, AST, SALPO but reduced the γ-GT, cholesterol and TG content of the CCL₄ treated rat. In another research, higher concentration of the extract was administered (800

mg/kg body weight) for nine weeks (four weeks pre-radiation and five weeks post-radiation). This study however showed decrease of AST, ALT, ALP, conjugated or non-conjugated bilirubin, serum or liver lipid peroxidation level in the treated mice. The methanol extract was claimed to protect the liver against damage of reactive oxygen species (ROS) with increase in the protein and glutathione content of animals radiated by 400 rads of γ radiation (Adaramoye et al., 2008b).

All of the above studies agreed that both methanolic and aqueous extract of *V. amygdalina* was able to enhance the liver antioxidant level (Adaramoye et al., 2008b; Adesanoye and Farombi, 2009; Iwalokun et al., 2006). Sesquiterpene lactones extracted from *V. amygdalina* were found to be the major contributor to the liver-protective effect of this plant, against CCL4 induced toxicity (Babalola et al., 2001).

Antidiabetic effect

Diabetes mellitus is a chronic disease which affects millions of people worldwide and the prevalence of this disease was projected to reach 300 million before year 2025 (Erasto et al., 2009). *V. amygdalina* was the most popular antidiabetic traditional herbal remedy in Nigeria (Gbolade, 2009). Water and n-hexane/isopropanol extract of the plant had been reported to enhance the glucose utilization of muscle and liver cell cultures but not on adipose cells (Erasto et al., 2009). Scientific studies have confirmed the antidiabetic potential of *V. amygdalina* where oral intake of hot water *V. amygdalina* leaves extract (500 mg/kg) reduced blood glucose concentration of both normoglycaemic and hyperglycaemic rats induced by alloxan (Osinubi, 2007). Consumption of *V. amygdalina* through means of squeeze-wash-drink and raw chewing by normal human subjects were found to control postprandial blood glucose without inducing severe hypoglycemic effect (Okolie et al., 2008). Besides, oral administration of *V. amygdalina* aqueous extract also did not induce severe hypoglycemic effect on non-diabetic rats (Taiwo et al., 2009).

However, its cold water leaf extract was also able to trigger hypoglycaemic, hypolipidaemic and antioxidant effect by reducing fasting blood glucose and triglyceride level in diabetic rats induced by glucose, streptozotocin and alloxan monohydrate (Akah et al., 2004; Nwanjo, 2005; Taiwo et al., 2009). Besides, this extract could also lower plasma oxidation stress (plasma malondialdehyde level) and LDL-cholesterol but not total cholesterol and HDL-cholesterol level of streptozotocin induced diabetic rats (Nwanjo, 2005). Furthermore, one week oral administration of *V. amygdalina* root water extract can ameliorate plasma glucose, triacylglycerol, cholesterol and β -hydroxybutyrate level of diabetic rat induced by alloxan monohydrate while longer feeding period (7 weeks) of its ethanolic root extract brought about

hypoglycaemic, hypoproteinaemic, hypocholesterolaemic and hypolipidaemic properties, against the alloxan induced diabetic rats (Nimenibo-Uadia, 2003; Igbakin and Oloyede, 2009).

On the other hand, ethanol leaf extract of *V. amygdalina* also showed kidney protection and liver protection activities, on top of its hypoglycemic property. This extract was proved to reduce glucose, urea, creatinine, potassium, sodium, chloride and aminotransferase activity in serum and regenerated glomerular tuft, endothelial cells, glomerular capsule and juxtaglomerular of kidney when administered to alloxan induced diabetic rats. The antioxidant activity of *V. amygdalina* was proposed to augment the protective effect on kidney, pancreatic β cell and liver regeneration. Pancreatic β cell regeneration may further help to reduce serum glucose level thereby overcoming hyperglycemia (Atangwho et al., 2007a; b).

Oral administration of 100 mg/kg of *V. amygdalina* methanol extract reduced fasting blood glucose after 2 h but it did not cause hypoglycemic effect on the mice (Ogundipe et al., 2003). However, methanol extract (200 and 400 mg/kg b.w.) of its fermented black tea was shown to possess better antihyperglycemic effect than the unfermented green tea on rats and this effect was further enhanced when flavored with *Ocimum basilicum* and *Ocimum gratissimum* (Okafor et al., 2009).

V. amygdalina reduced the plasma glucose of normal and streptozotocin induced diabetic rats. This may be due to high content of non-starch polysaccharides, that can reduce postprandial blood glucose adsorption or interaction between its secondary metabolites which may contribute to both the enhancement in pancreatic insulin production and in mechanism similar to that of insulin on glucose metabolism (Okolie et al., 2008; Taiwo et al., 2009).

Osinubi (2007) proposed that sesquiterpene lactones and the bitter principle of the plant may be responsible for insulin production, stimulation and release of pancreatic islets from the beta-cells. Moreover, extracts from this plant may also work well with some other type of herbs or even antidiabetic drugs, to bring about improved efficacy. Ebong et al. (2008) had reported that *V. amygdalina* worked synergistically with *Azadirachta indica* to reduce blood sugar and protect the liver more effectively. Besides, the aqueous extract (250 mg/kg b.w.) also enhanced the hypoglycemic effect by tolbutamide (25 mg/kg b.w.) on both diabetic and non-diabetic rats (Taiwo et al., 2009).

On the other hand, tannin, flavonoids glycosides and phytosterols of the plant may also act as alpha glucosidase inhibitor which contributed to the hypoglycemic effect of the plant. However, the significant reduction of serum sodium and potassium in diabetic rats, fed with its ethanol extract indicated a possible induction of dilutional hyponatremia as a side effect (Atangwho et al., 2007a; b).

Hypolipidemia effect

Methanolic extract of *V. amygdalina* leaves also exerted hypolipidemic effect through interference with the lipid metabolic pathway which successfully reduced the plasma and post-mitochondrial fraction cholesterol, low-density lipoprotein (LDL), triglyceride and lipid peroxidation (LPO) level and increased the plasma HDL level (Adaramoye et al., 2007; 2008a; 2009). Ethanol extract of the plant was also able to reduce body weight by decreasing triglyceride level (Ekpo et al., 2007).

Other activities

Despite the wide range of bioactivities depicted by *V. amygdalina*, the plant also showed no activity in certain studies. In a clinical survey carried out in Nigeria, *V. amygdalina* was found not helpful to the hypertensive patients (Amira and Okubadejo, 2007). Juices from its leaves also did not show anti-tick effect against *Boophilus decoloratus* (Regassa, 2000). Besides, *V. amygdalina* was found not affecting the rumen micro-organism activity but was then suitable to be used as animal feed (Osuji and Odenyo, 1997). For example, powder of *V. amygdalina* leaf was able to increase feed conversion efficiency of cockerels without affecting their haematological profile (Olobatoko and Oloniruha, 2009).

Nevertheless, the usefulness of *V. amygdalina* was not only beneficial for medical and healthcare purposes but it also serves as a low cost, environmental friendly, readily available and non-toxic natural inhibitor for the corrosion of mild steel in HCL and H₂SO₄, as well as aluminium alloy in HCL and HNO₃ (Abdel-Gaber, 2006; Avwiri and Igbo, 2003; Loto, 1998; Odiogonyi et al., 2009).

CHEMICAL COMPOSITION OF *V. AMYGDALINA*

V. amygdalina was found to contain 21 to 23% of dry matter (Fafunso and Bassir, 1976; Ifon and Bassir, 1980). Out of the dry matter, it contained 6.5 to 29.2% of crude fibre content (Alabi et al., 2005a; Antia et al., 2006; Ifon and Bassir, 1979; 1980; Oboh, 2006; Okoli et al., 2003a) and 0.137% of chlorophyll content (with 75% of chlorophyll-a) (Faboya, 1985). Higher hemicellulose was found in the dry than the fresh leaves of *V. amygdalina* (Bonsi et al., 1995a; b; Okoli et al., 2003a). *V. amygdalina* contains crude protein (17 to 33 g/100g DW) (Ifon and Bassir, 1980; Mekoya et al., 2008; Oboh, 2006; Okoli et al., 2003a) and fat (2 to 15 g/100g DW with 24.54% saturated and 65.45% polyunsaturated. oleic acid was the major monounsaturated fatty acids) (Alabi et al., 2005a; Eleyinmi et al., 2008; Ifon and Bassir, 1980; Oboh, 2006). Due to its high content of crude protein, it was found to be a good source of protein. High amount of protein is essential for animal growth and increased milk production (Oke, 1965; Tangka, 2003). It was found that

V. amygdalina leaves, when added to soybean meal was the best infant weaning food which helps to gain weight (Agbede et al., 2007).

High ash content (10 to 13 g/100g DW) (Alabi et al., 2005a; Faboya, 1983; Ifon and Bassir, 1979; Ifon and Bassir, 1980; Mekoya et al., 2008; Oboh, 2006; Okoli et al., 2003a) reflected the useful mineral contents (calcium, chlorine, chromium, copper, ferum, potassium, iron, magnesium, manganese, nickel, phosphorus, potassium, sodium, sulphur and zinc) that are present in this plant (Alabi et al., 2005a; Faboya, 1983; Gbaruko and Friday, 2007; Ifon and Bassir, 1979; 1980; Oboh, 2006). Ash of *V. amygdalina* contained high content of nitrogen, phosphorus and other types of exchangeable bases (Calcium, Magnesium, Sodium and potassium) (Enikuomehin et al., 1998). High concentration of sulphur is important for detoxification of cyanide while low sodium content is suitable for obese patients (Ifon and Bassir, 1979). The nutritive values of young and mature leaves did not differ significantly (Akachuku, 2001). Abrasion of the leaf caused a significant decrease, in both the nutrient and antinutrient content of the leaf except in carbohydrate (Oboh, 2006).

On the other hand, high sugar (raffinose, lactose, sucrose, glucose, galactose, fructose, maltose and arabinose) (Alabi et al., 2005a; Ifon and Bassir, 1980), vitamin (thiamine, nicotinamide, thiamine, riboflavin, pyridoxine and ascorbic acid), casein hydrolysate, amino acids (non-essential amino acid: cysteine, glycine and essential amino acid: leucine, valine and phenylalanine), less acid value (10 mg/100g DW) and high iodine (35 mg/100g) value have promoted it as a popular vegetable in Africa and propose its potential in treating goiter. The moisture content of *V. amygdalina* ranged between 79.1 to 82.1%. Fresh leaves contained 280 to 354 mg/100g of vitamin C (Fafunso and Bassir, 1976). However, vitamin C concentration was affected by the soil properties (regular use of fertilizers). Loss of 60% of vitamin C was observed after 8 h and this loss was more drastic when the plant was exposed under sunlight. Keeping in the refrigerator can effectively prevent the loss. Thus, photo-oxidation was identified as the major contributor for the loss of vitamin C (Faboya, 1990). Washing and cooking was found to further reduce 40 to 77% of vitamin C content (Ejoh et al., 2003; Fafunso and Bassir, 1976).

These suggest that *V. amygdalina* should be consumed immediately after harvesting or must be kept in a refrigerator before processing. Most of the active ingredients from this plant were thermostable (Alabi et al., 2005a; Ifon and Bassir, 1979). Other than the common metabolites and minerals, *V. amygdalina* also contain several active secondary metabolites which contribute to its bioactivity.

Stigmastane-type steroid glucoside compounds

One of the major steroid glucoside compounds that has

been identified from *V. amygdalina* is the vernoniosides. These glucoside compounds can be isolated from the leaf, stem, pith and root parts of the plant (Huffman, 2001).

Among all, Vernonioside B1 was found in higher concentrations in the leaves than in the stem and much more abundant in the pith of the plant (Huffman et al., 1993; Koshimizu et al., 1994; Ohigashi et al., 1994). Besides, this compound was also identified to be responsible for the removal of parasites in primates who sucked the young pith of *V. amygdalina*, for the control of gastrointestinal illnesses (Huffman et al., 1993; Igile, 1995b; Koshimizu et al., 1994).

V. amygdalina is well known for its bitter taste. Vernoniosides A1, A2, A3 and A4, were found to be part of the constituents in contributing to this characteristic while vernoniosides B1, B2 and B3, did not show any bitter taste (Jisaka et al., 1992; Jisaka et al., 1993a; b; Koshimizu et al., 1994; Ohigashi et al., 1991a; Osinubi, 2007). The vernoniosides B were found to lack a free hydroxyl end at their C-16, as was present in vernoniosides A. Hydroxylation at C-16 of these steroid glucosides (Figure 2) was therefore hypothesized to play an important role in causing bitterness to this plant (Jisaka et al., 1992; 1993a; 1993b; Ohigashi et al., 1991a).

Sesquiterpene lactones

Another major group of bioactive compounds that has been isolated from *V. amygdalina* are the sesquiterpene lactones, consisting of vernodalol, vernolide, vernolepin, vernomenin, vernomygdin, vernolic, vernodalol, hydroxylvernolide, 11,13-dihydrovernodalol, 11,13-dihydrovernoderline, 4,15-dihydrovernodalol, 7,24(28)-stigmastadien-3 β -ol and 1,2,3,15,11,13,2',3'-octahydrovernodalol (Figure 3).

Vernodalol

Sesquiterpene lactones can be isolated from the leaf stem, pith and root of *V. amygdalina* with the exception of vernodalol which could not be isolated from the pith of the plant. Vernodalol was also found to be more concentrated in young leaves than young stems (Huffman et al., 1993; Ohigashi et al., 1994).

Previous studies showed that vernodalol possess antitumor activity against human nasopharynx carcinoma KB and mouse leukemia P-388 and L-1210 cancer cell lines (Jisaka et al., 1993 b; Kupchan et al., 1969). This compound also showed *in vitro* insecticidal activity against African armyworm (Ganjian et al., 1983), antibacterial effect towards *B. subtilis* (Jisaka et al., 1993 b) and *Micrococcus luteus* as well as antileishmanial activity on *Leishmania infantum* (Koshimizu et al., 1994).

Vernolide

Vernolide is mono-hydroxylated as compared to vernodalol which has 2 hydroxyl groups. Vernolide is therefore less polar and more lipophilic than vernodalol. Lipophilicity was found to be the major influence affecting fungicidal activities of these two compounds. This was confirmed by Erasto et al. (2006) whereby vernolide possessed stronger inhibition against *A. flavus*, *Mucor hiemalis*, *Fusarium oxysporum*, *Penicillium notatum* and *A. niger* than vernodalol at concentrations ranging from 0.05 to 0.5 mg/ml. Vernolide also possess antibacterial activity against the gram positive bacteria *B. cereus*, *B. subtilis*, *Staphylococcus epidermidus*, *S. aureus*, *M. kristinae*, *M. Luteas* and *Streptococcus pyrogens* and the gram negative bacterium *Salmonella pooni* (Erasto et al., 2006; Jisaka et al., 1993b).

Besides, Vernolide has been shown to exert cytotoxic effect towards human nasopharynx carcinoma KB and mouse leukemia (P-388 and L-1210) cancer cell lines and slight activity against Ehrlich's ascites carcinoma cells *in vitro* (Jisaka et al., 1993b; Kupchan et al., 1969; Sayed et al., 1982). This compound also possesses antileishmanial or antiprotozoa effect against *L. infantum* (Koshimizu et al., 1994).

Vernodalol

Vernodalol had been reported to possess antileishmanial activity, *in vitro* insecticidal activity against African armyworm and antimicrobial effect against both bacteria and fungus. This compound was active against the gram positive bacteria (*B. cereus*, *S. epidermidus*, *S. aureus*, *M. kristinae* and *S. pyrogens*) but was inactive against gram negative bacteria with the exception of *S. pooni* which was inhibited at 0.5 mg/ml (Erasto et al., 2006; Ganjian et al., 1983; Koshimizu et al., 1994).

Vernolepin

Vernolepin showed antiplatelet effect in rabbits through the inhibition of arachidonic acid, ADP and collagen-induced platelet aggregation, as well as by interference with ATP-release without the inhibition of cyclooxygenase or lipoxygenase. In addition, vernolepin also demonstrated a time dependent biphasic enhancement/inhibition feature of coaxial stimulation and antagonism against histamine in guinea pig ileum (Laekeman et al., 1983; 1985). On the other hand, vernolepin also showed cytotoxic activity against P-388 and L-1210 mouse leukemic cancer cell lines and antibacterial effect against *B. subtilis* and *M. lutea* (Jisaka et al., 1993b).

Vernomenin

Similar to some other compounds in *V. amygdalina*,

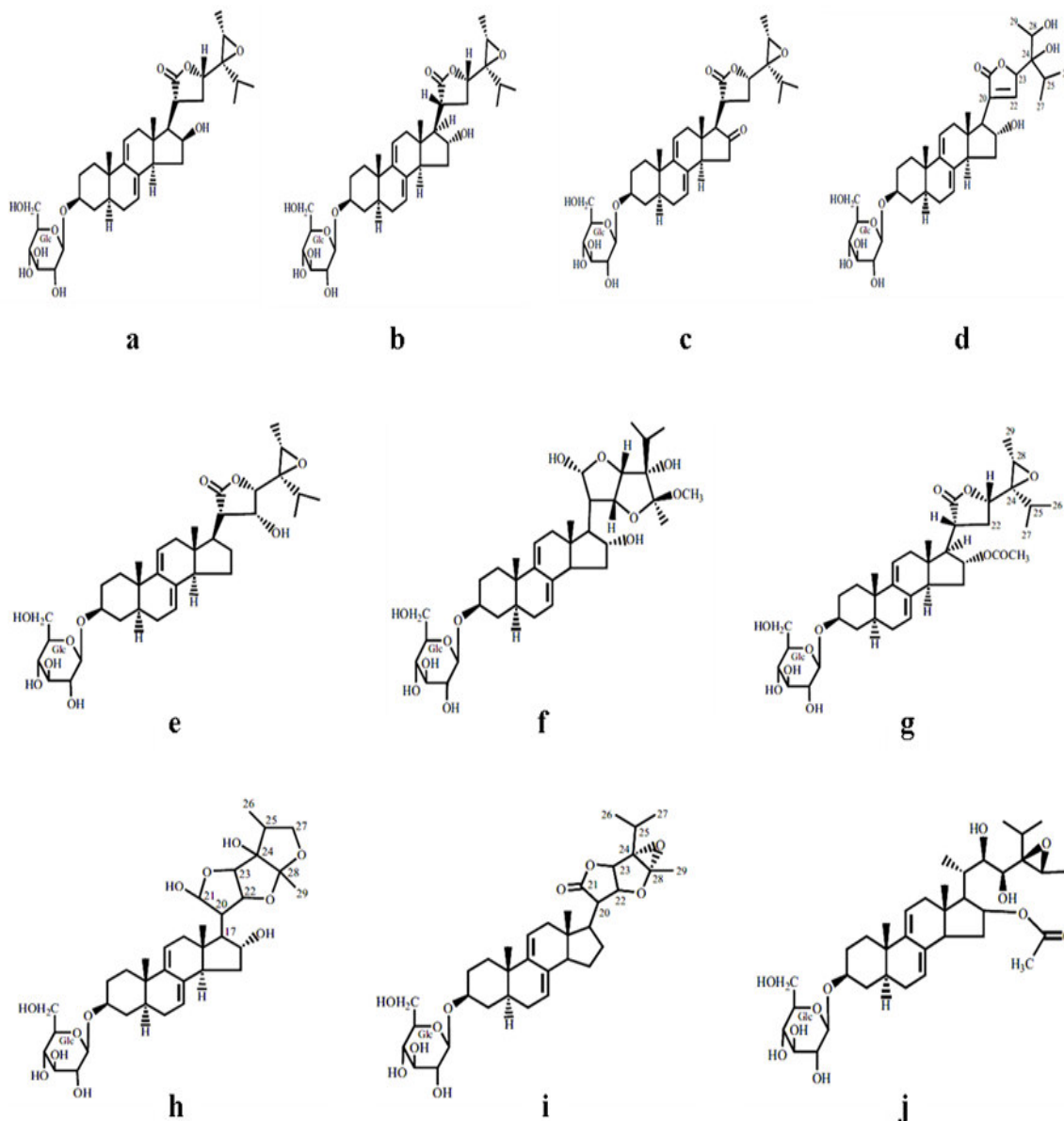


Figure 2. Steroid glucosides isolated from *V. amygdalina*. 2a. Vernonioside A1 ($C_{35}H_{52}O_{10}$) MW: 632. 2b. Vernonioside A2 ($C_{35}H_{52}O_{10}$) MW: 632. 2c. Vernonioside A3 ($C_{35}H_{50}O_{10}$) MW: 630. 2d. Vernonioside A4 ($C_{35}H_{52}O_{11}$) MW: 648. 2e. Vernonioside B1 ($C_{35}H_{52}O_{10}$) MW: 632. 2f. Vernonioside B2 ($C_{36}H_{52}O_{12}$) MW: 680. 2g. Vernonioside B3 ($C_{37}H_{54}O_{11}$) MW: 674. 2h. Vernonioside D ($C_{35}H_{52}O_{12}$) MW: 664. 2i. Vernonioside D2 ($C_{35}H_{50}O_{10}$) MW: 630. 2j. Vernonioside E ($C_{37}H_{58}O_{11}$) MW: 678 (Igile et al., 1995b; Jisaka et al., 1992; 1993a; Ohigashi et al., 1991a; Schmittmann et al., 1994).

vernomenin also showed inhibitory activity against P-388 and L-1210 mouse leukemic cancer cell lines and the bacteria *B. subtilis* and *M. lutea* (Jisaka et al., 1993b).

1,2,3,15,11,13,2',3'-octahydrovernodalol

1,2,3,15,11,13,2',3'-octahydrovernodalol is the most bitter compound of *V. amygdalina*. The bitter taste of the

compounds in the plant was related to either α -methyl- or α -methylene- γ -lactone. On the other hand, α -methylene- γ -lactone was also found to be correlated to the strong antitumoral effect on the P-388 and L-1210 mouse leukemic cancer cell lines.

However, the correlation of α -methylene- γ -lactone to these characteristics did not conclude for the association of the bitterness of sesquiterpene lactones, to their antimicrobial and antitumoral activities. This was shown

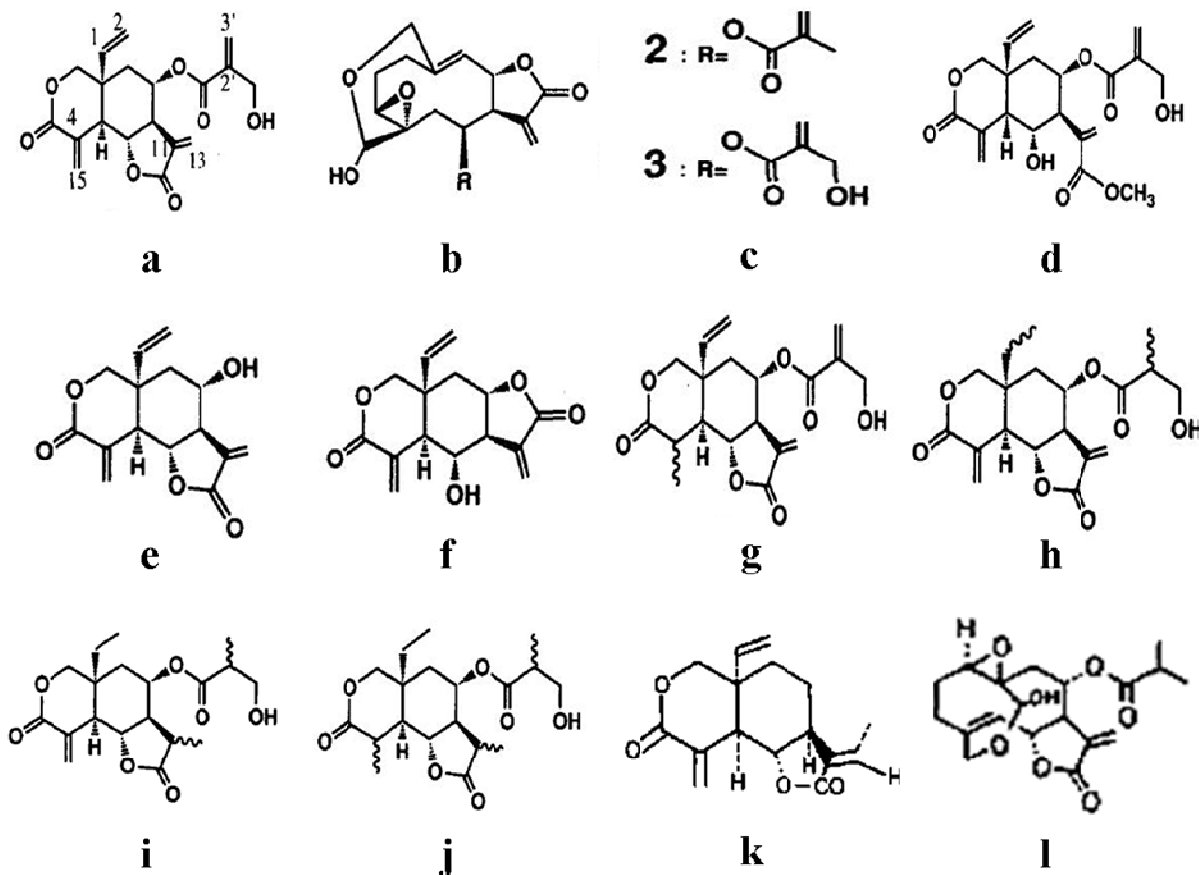


Figure 3. Sesquiterpene lactones isolated from *V. amygdalina*. 3a. vernodalin (C₁₉H₂₂O₇). 3b. Vernolide. 3c. hydroxyvernolide. 3d. vernodalol (C₂₀H₂₄O₈). 3e. vernolepin (C₁₅H₁₆O₅) MW:276.30. 3f. vernomenin. 3g. 4,15-dihydrovernodalol. 3h. 1,2,2',3'-tetrahydrovernodalol. 3i. 1,2,11,13,2',3'-hexahydrovernodalol. 3j. 1,2,3,15,11,13,2',3'-octahydrovernodalol. 3k. 11, 13-dihydrovernodalol. 3l. vernomygdin (Erasto et al., 2006; Ganjian et al., 1983; Jisaka et al., 1993b; Koshimizu et al., 1994 ; Laekeman et al., 1985; Mabry et al., 1975; Osinubi, 2007)

when octahydrovernodalol showed least activity in both antitumoral and antibacterial activities whilst compounds like vernodalin, vernolide, vernolepin and vernomenin which are less bitter in taste acted much more effectively against both the cancer cell lines and on *B. subtilis* and *M. lutea* (Jisaka et al., 1993b).

11,13-dihydrovernodalol

This compound has been shown to possess antifeedant activity which can prevent insects from feeding but does not lead to lethality (Van Beek and Groot, 1986).

4,15-dihydrovernodalol

4,15-dihydrovernodalol exerted the highest activity against P-388 and L-1210 mouse leukemia cancer cell lines and the microbes *B. subtilis* and *Micrococcus lutea* among vernodalin, vernolide, vernolepin and vernomenin

(Koshimizu et al., 1994).

Vernomygdin

Vernomygdin showed inhibitory effect against the human nasopharynx carcinoma (KB) cell line (Kupchan et al., 1969).

11,13-dihydrovernodalol

11,13-dihydrovernodalol which is lack of exocyclic conjugated double bond on the γ -lactone moiety also exhibited *in vitro* insecticidal activity against African armyworm comparable to vernodalin and vernodalol (Ganjian et al., 1983).

Hydroxyvernolide

Hydroxyvernolide also showed antileishmanial

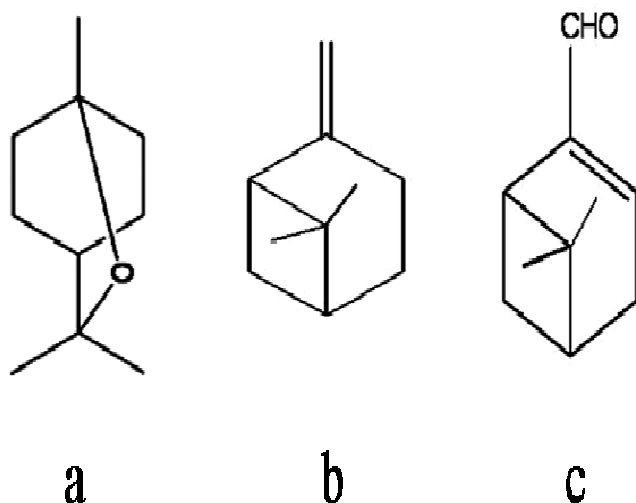


Figure 4. Major compounds present in essential oil of *V. amygdalina*. 4a. 1,8 cineole. 4b. beta pinene. 4c. myrtenal.

(antiprotozoa) on *L. infantum* (Koshimizu et al., 1994).

Vernolic and 7, 24 (28)-Stigmastadien-3 β -ol

Seed fat of *V. amygdalina* was found to contain as much as 58% of Vernolic (12, 13-Epoxyoctadec-9-enoic) (Badami and Patil, 1981) and 7,24(28)-Stigmastadien-3 β -ol, was successfully isolated from light petroleum extract, from the stem of *V. amygdalina* was able to isolate (Arene, 1972).

Other secondary metabolites

The variety of secondary metabolites extracted from *V. amygdalina*, explains well the diversity of the biological activities of this plant extract. Leaf extract of *V. amygdalina* was found to contain reducing sugar, polyphenolics, terpenoids, saponins, alkaloids, cardiac glycosides, steroids or triterpenes, anthraquinone and coumarins without cyanogenic glycoside (Ayoola et al., 2008; Nwanjo, 2005; Otshudi and Foirers, 2000; Ogundipe et al., 2003; Tona et al., 2004).

However, only tannins, glycosides and saponins without flavanoids could be obtained from its root and stem bark extracts (Nduagu et al., 2008). All of these phytochemicals contributed to anticorrosion (Odiogenyi et al., 2009) and antifungal effect (Nduagu et al., 2008) of *V. amygdalina* while its bitter taste was reported to be due to the presence of antinutritional factors such as alkaloids, saponins, tannins and glycosides (Arhoghro et al., 2009).

Phenolic compounds identified in *V. amygdalina* can be grouped into flavonoids, tannins and caffeoyl quinic acid

(Salawu and Akindahunsi, 2007). Flavonoids protect the cell as antioxidant against free radicals and reactive oxygen species. Antioxidant activity of *V. amygdalina* was contributed by the flavonoids which can be extracted from the leaves by using methanol extraction. Luteolin possessed the strongest antioxidant as comparable to the synthetic butylated hydroxytoluene (BHT) at 15 mg/l. Besides, luteolin 7-O- β -glucuronoside (the most abundant compound) and luteolin 7-O- β -glucoside also possess similar antioxidant activity but significantly lower as compared to luteolin. This effect may be due 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 (Igile et al., 1994). Ethanol was found to be more effective in extracting luteolin than hot water (Ola et al., 2009). This may contribute to the higher antioxidant activity of ethanol extract compared to water extract. Caffeoyl quinic acid was the dominant cinnamoyl derivative followed by dicaffeoyl quinic acid and dicaffeoyl quinic acid (Ola et al., 2009).

Essential oil

Essential oil extracted through hydro-distillation of the leaves of *V. amygdalina* contained eucalyptol (1, 8 cineole, 25%), beta pinene (14.5%), myrtenal (6.5%) (Figure 4) and other minority constituents while essential oil from its aerial part contained mainly alpha-muurolol (45.7%) (Asawalam and Hassanali, 2006; Ogunbinu et al., 2009). The essential oil of *V. amygdalina* (0.3%) was able to protect maize from the maize weevil *S. zeamais* by reducing the number of weevil progeny production and by evoking a high repellent action against weevil without damaging the grain (Asawalam and Hassanali, 2006).

Besides, Hexane/isopropanol extract of *V. amygdalina* leaves also yielded 0.31% (w/w) of oil. Palmitic acid (22%) appeared to be the most abundant lipid in the extract and two other essential fatty acids, namely α -linolenic (omega-3, 21.5%) and linoleic acid (omega-6, 15.8%) were also found in high amount in the extract (Erasto et al., 2007b).

TOXICITY AND SAFETY OF *V. amygdalina*

Traditionally, Temma people of Sierra Leone called bitter leaf as "goat killer". It warned the animal to stay away through its extreme bitterness (Engel, 2003). This phenomenon confers a clear sign for the need of careful scientific assessments in order to identify the safe dosage and the possible adverse effect of using this plant.

Toxicology studies had been carried out via both *in vitro* and *in vivo* systems on various subjects to confirm the toxicity of *V. amygdalina* (Table 4). As indicated in Table 4, *V. amygdalina* only induced mild toxic side effect when administrated at very high concentration. More

Table 4. *In vitro* and *in vivo* toxicity of *V. amygdalina*.

Subject	Extract	Mode	LD ₅₀ (mg/kgb.w.)	Remark and References
Mice	Aqueous (500-2000 mg/kg/day for 14 consecutive days)	Oral	-	No signs of toxicity or adverse toxicological effects at all doses except for decrease of red blood cell count and dose dependent increase of serum bilirubin (Njan et al., 2008).
	25% of <i>V. amygdalina</i> dry powder (or equivalent amount of ethanol extract or crude/purified saponins) for 2 weeks	Oral	-	Reduction of body and liver weights and increase of urinary and fecal output associated with stomach and small intestines enlargement (Igile et al., 1995a).
	Aqueous (62.5 and 125 mg/kg)	Oral	-	Serum glutamate oxaloacetate transaminase (sGOT), serum glutamate pyruvate tansaminase (sGPT) and lactate dehydrogenase (LDH) level rose around 6-33%. Increase of serum enzyme markers level was more severe when it was consumed with antimalarial drug chloroquine (Iwalokun, 2008).
	Aqueous (87.53 to 92.57g/kg)	Oral	-	No change in organ damage, blood count and liver enzyme profile (AST and ALT) (Amoela et al., 2006).
	Aqueous (50 and 100 mg/kg)	i.p.	-	No change of liver function diagnostic enzymes level (total bilirubin, conjugated bilirubin, unconjugated bilirubin, alanine aminotransferase, aspartate aminotransferase and alkaline phoosphatase) (Ojiako and Nwanjo, 2006)
	Ethanol (up to 1000 mg/kg for 1 month)	Oral	-	No change in liver (ALT, AST, ALP, total and conjugated bilirubin) and kidney (creatinine) enzymes marker level (Ekpo et al., 2007)
	Cold water (for 24 h)	i.p.	500 to 1265.22	(Nwanjo, 2005; Ojiako and Nwanjo, 2006)
Rat	Aqueous (250 and 500 mg/kg/day for 5 consecutive days)	Oral	-	Reduction of spermatozoa mobility and viability in dosage dependent mode. Hypoplasia of the seminiferous tubules was also observed in the treated rats. (Oyeyemi et al., 2008)
	Aqueous extract of <i>V. amygdalina</i> , <i>Ocimum gratissimum</i> and <i>Gongronema latifolia</i> (ratio 1:1:1 at 16 g/kg b.w. (p.o) and 2.5 g/kg b.w. (i.p.))	Oral i.p	-	No significant change in general behaviour (Iroanya et al., 2010).
	Methanol (50, 100 and 200 mg/kg)	Oral	-	Diarrhea and abortion (Awe et al., 1999) due to cathartic effect through weak contractile effect on smooth muscle.
	Powder in standard food (25-75%)	Oral	-	Skin of the rat turned lighter without alteration of tissues architecture and cellular morphology. (Ibrahim et al., 2001).

Table 4. contd.

Rabbit	Aqueous	i.p.	1112	(Akah and Okafor, 2006)
	Aqueous (0.3 mg/ml)	injection		Increase of uterine, intestine and jejunum contraction which sustained for 30 minutes with elevated concentrations used (Caiment-Leblond, 1957; Kamatenesi-Mugisha et al., 2005; Kerharo and Bouquet, 1950)
Guinea pig	Aqueous (10 and 100 mg/kg)	Oral		Increase of uterine and mammary gland contraction amplitudes and thus increase milk production and help in infant's delivery (Ijeh et al., 2008). This supports the traditional use of <i>V. amygdalina</i> as an oxytocic plant in assistance of child birth traditionally.
Murine macrophages J774	Lipophilic extract	<i>In vitro</i>	IC ₅₀ 6.48 µg/ml	(Ganfou et al., 2008)
<i>Allium cepa</i> root tip	Cold water extract	<i>In vitro</i>	1% after 8 h of incubation	Induced mitodepressive effect. Higher concentration of the extract or extensive incubation caused sticky effect on chromosomes during cell division and protein denaturation lead to nuclear disintegration and cell death. Presence of sesquiterpene lactones were suggested as the major contributors to this effect (Ene-Obong and Amadi, 1987).

i.p.: intra-peritoneal injection; b.w.: body weight

importantly, safe consumption dosage needs to be identified for women at different stages or vitality of pregnancy, to avoid abortion since it may induce uterine contraction.

Plenty of antinutrient elements were found in different extracts of *V. amygdalina* (Table 5). Saponins, glycosides and tannins were found to contribute to the purgative effect through direct stimulation, astringence and surface emulsification of these compounds (Awe et al., 1999). Thus, water extract of *V. amygdalina* induced a cumulative dose-dependent (1 to 66 mg/ml) contraction of ileum and this result has supported the traditional use of the plant in stomach upset and constipation (Owu et al., 2008) but it has also been reported to cause vomiting, diarrhea and even abortion, if being used during first month of pregnancy (Giday et al., 2003; Kamatenesi-Mugisha and Oryem-Origa, 2007).

Generally, *V. amygdalina* is safe to consume and is good for health unless it is consumed in very large quantities and the potential danger of taking this plant is much lower than that of other common vegetables (Ojiako and Nwanjo, 2006). *V. amygdalina* may cause adverse effect over the male reproductive system without controlled regimen.

CULTIVATION AND PROCESS OF *V. amygdalina*

Although *V. amygdalina* is normally propagated by cutting, hairy root of *V. amygdalina* can also be generated *in vitro*

using explant cultures in the presence of auxin. IBA (with concentration ranging between 0.25 to 2 mg/l) was found to be the best growth regulator for its adventitious root induction (Khalafalla et al., 2009).

The herb can be subjected to further processing when harvested but some procedures may alter its nutrient content. The best temperature for drying was 45°C as reported by Ejoh et al., (2005). Blanching and extraction at 100°C can cause at least 56% of total chlorophyll loss but this can be reduced by adjusting the pH to 6 (Faboya, 1983).

Studies showed that squeeze-washing, rinsing and freezing were able to preserve carotenoid, iron, crude protein, total reducing sugar and dietary fibers in the plant (Ejoh et al., 2003; 2005). Increase of washing time from 0 to 20 min too did not significantly change the nitrogen, calcium and magnesium level of the herb but this could increase its potassium, sodium and fibre content which then resulted in the reduction of its fat, phosphorus and ash level (Tangka and Penda, 2007).

COMMERCIALIZATION APPLICATION AND MARKET DEMAND OF *V. amygdalina*

V. amygdalina is an important vegetable in Cameroon, where out of 93,600 tons of leafy vegetables harvested in 1998, 23% (21,549 tons) was bitter leaf (Smith and Eyzaguirre, 2007). Currently, AfriProducts is selling a kilogram of fresh/frozen *V. amygdalina* leaves at USD

Table 5. Anti-nutrient elements potentially identified in *V. amygdalina*.

Anti-nutrient element	Concentration	Remark
Oxalate	95.5 mg/100 g	may contribute to potent human poisons. Fortunately, proper cooking and process can significantly reduce the total oxalate content of the plant.
Piperidine	0.19 µg N/kg	may induce esophagus tumor
n-propylamine	0.26 µg N/kg	
Proline	0.21 µg N/kg	
N-Nitrosamine	0.4 µg/ml (low level)	carcinogenic, immunotoxic and embryotoxic. The presence of N-nitrosamine may be due to use of nitrogen-containing fertilizers and pesticides
Phytate	1466.7 mg/kg	Soaking with salt was found to be able to reduce phytate concentration
Cyanide	1.1 mg/kg	Blanching was found to be able to reduce cyanide concentration
Nitrosodimethylamine	0.4 ppb (low level)	Present only in minute amount
Heavy metal (Hg & Pb)	0.01-0.48 µg/ml	Environmental pollution has been identified as one of the major reasons that contribute to heavy metal accumulation in vegetables. Thus, plantation of <i>V. amygdalina</i> should be away from heavy industrial activity such as oil refinery and petrochemical industry to avoid heavy metal (lead, cadmium, copper, nickel etc.) contamination. Gradual accumulation of these toxins may lead to adverse health effects.
Aflatoxin (B1, B2, G1) and ochratoxin A		Produced by <i>Aspergillus flavus</i> after prolonged storage of <i>V. amygdalina</i> herbal leaf or fresh crude water extract. Can be removed by initial surface decontamination (using alcohol or sodium hypochlorite followed by distilled water) or by converting the crude herbal extract into a more stable powder (using freeze dry or spray dry processes).
Tannins	380-600 mg/100 g	
Hydrocyanic acid (HCN)	6.40-46 mg/g	Plants that produce more than 20mg HCN/100g fresh weight are considered deleterious. <i>V. amygdalina</i> only contains moderate amount of hydrocyanic acid .

(Alabi et al., 2005a; Antia et al., 2006; Atawodi et al., 1993; Efuntoye, 1999; Eriyamremu et al., 2005; Gbaruko and Friday, 2007; Oboh et al., 2005; Oboh, 2006; Okoli et al., 2003a; Uhegbu, 1997; Yusuf et al., 2003)

4.55 and its powder at USD 7.5 (AfriProduct, 2010) while RGL enterprise sells a kilogram of powder at USD 10 (RGL enterprises, 2010).

The processing of leaves prior to sale for consumer's convenience, especially for those living in the cities, depicts great potential in marketing of plants. Plenty health products formulated with *V. amygdalina* have been commercialized. For instance, EdoBotanics under the Jackson State University is selling processed *V. amygdalina* under the name of EdoTide Plus (which contains 600 mg of leaf extract with the suggestion of 600 to 1200 mg oral intake per day) (60 capsules at USD 42) (EdoBotanics, 2010). In Nigeria, "Diabetes 5" (contains 50 to 50% mixture of *V. amygdalina* leaves and *Anisopus manii* leaves) has been sold as an unofficial herb to treat diabetes mellitus. Studies have found that methanol (100

mg/kg) and chloroform (200 mg/kg) extract of "Diabetes 5" possessed similar hypoglycemic activity as Glibenclamide (3 mg/kg) after 24 h of oral administration. However, both extracts and Glibenclamide exerted adverse effects on spleen and kidney of tested animals (Agunu et al., 2008). Thus, more suitable processing and concentrations should be inspected and optimized in the formulation of better health products from *V. amygdalina*, to minimize or eliminate adverse effect to the consumers. *V. amygdalina* was used to prepare feed for rumen traditionally. However, the palatability of this herb was comparatively lower than other plants due to its bitterness which is contributed by alkaloids and saponins. Although the suggestion of adding molasses to *V. amygdalina* (ratio 5:12) may improve the acceptance of the rumen, this would further increase the dependency on

molasses and the cost of commercial supplement. Thus, *V. amygdalina* may not be viable to be used as a single dietary supplement in modern medicine (Bonsi et al., 1995a,b; Hindrichsen et al., 2004).

CONCLUSION

In conclusion, *V. amygdalina* possesses various bioactivities with low or absence of side effects. With its great health promoting effect, it may be more advantageous to incorporate *V. amygdalina* into health supplement for human benefits rather than using it solely as feed for the poultry industry.

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