

PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF BITTER LEAF (*VERNONIA AMYGDALINA*) COLLECTED FROM LAPAI, NIGER STATE, NIGERIA ON SOME SELECTED PATHOGENIC MICROORGANISMS

Audu Yunusa^{1,2}, Shuaibu Babaji Sanusi^{2*}, Alona Cuevas Linatoc², Murtala Muhammad Mainassara², and Jiya Janet Awawu¹

¹Department of Biology, Faculty of Applied Sciences and Technology, Ibrahim Badamasi Babangida University Lapai, Niger State.

²Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia (UTHM), Pagoh Educational Hub, 84600 Pagoh, Johor, Malaysia.

*Corresponding Author Email Address: sanusishuaibu@gmail.com

ABSTRACT

Vernonia amygdalina called 'Shuwaka' in Hausa is commonly used to treat stomach-ache and gastrointestinal troubles in Northern Nigeria ethno- medicine. This study was aimed to verify the claimed antimicrobial activity of *V. amygdalina* collected from Lapai, Niger State, Nigeria and analyse its phytochemicals. Phytochemical investigation and studies on antimicrobial activity of stem barks and roots extract of *V. amygdalina* were carried out on *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Aspergillus niger* using agar-well diffusion assay. Preliminary phytochemical screening revealed the presence of alkaloids, steroids, glycosides, flavonoids, tannins, saponins, phlobatannins, and phenolics in the stem bark and roots extract. The largest diameter of inhibition zone was observed on the acetone extract of stem bark against *S. aureus* (16 mm) and *P. aeruginosa* (16 mm). All the tested pathogens were resistant to the water extract of stem bark and root, except for *S. aureus* which showed inhibitory activity with 7 mm diameter of inhibition zone on stem bark extract. The outcomes of this work suggest the presence of pharmacologically active compounds in the extracts with antimicrobial activity against the pathogenic strains which justifies its ethno- medicinal use.

Keywords: *Vernonia amygdalina*, phytochemical, antimicrobial activity, pathogens

INTRODUCTION

With the recent trend of high percentage resistance of multiple-drug resistant microbial strains, efforts have been intensified by researchers to search for possible alternatives (Adetunji *et al.*, 2013). Medicinal plants and traditional preparation with antimicrobial activities have been used extensively in the West African regions. These plants of medicinal important have been proven to be very effective even where treatments with antibiotics failed (Oshim *et al.*, 2016). *Vernonia amygdalina* commonly called bitter leaf (English), Shuwaka (Hausa), Ewuro (Yoruba), Oriwo (Edo), and Olubu (Igbo) is a perennial shrub belonging to the family Asteraceae (Ghamba *et al.*, 2014; Gashe & Zeleke, 2017). *V. amygdalina* is a shrub that can grows to 10 m tall with petiole leaf of about 6 mm in diameter and elliptic in shape and grows throughout tropical Africa and has been domesticated in various parts of West Africa including Nigeria, where it is locally

used as vegetable in soups (Etim *et al.*, 2012; Habtamu & Melaku, 2018). In some African countries including Nigeria, this plant species is traditionally used to treat many ailments including diabetes (Akah & Okafor, 1992), malaria, helminth infections, fever, (Magadula & Erasto, 2009), promote wound healing (Adetutu *et al.*, 2011) and to treat microbial infections (Noumedem *et al.*, 2013). Also, the Hausa tribe of the northern part of Nigeria used the root and twig of *V. amygdalina* to treat stomach-ache and gastrointestinal troubles (Akinpelu, 1999). It is also prescribed to nursing mother as it improves lactation (Anibijuwon *et al.*, 2012).

The pharmacological study of *V. amygdalina* have been reported to demonstrated Antihelminthic and Antimalarial properties (Abosi & Raseroka, 2003), antitumorigenic properties (Izevbigie *et al.*, 2004), analgesic and antipyretic activities (Tijjani *et al.*, 2017), hypoglycemic and hypolipidaemic effect in experimental animals (Nwanjo, 2005). The reported biologically active phytoconstituents from *V. amygdalina* are alkaloids, flavonoids, terpenes, saponins, coumarins, xanthenes, phenolic acids, lignans, steroids, anthraquinones (Tona *et al.*, 2004), edotides (Izevbigie, 2003), and sesquiterpene lactone (Kupchan *et al.*, 1969). Despite the vast traditional used of *V. amygdalina*, it is still considered among the under exploited crops of economic significance. Thus, this study is aimed at determining the antimicrobial activity and the phytochemical constituents of *V. amygdalina* collected from Lapai, Niger State, Nigeria.

MATERIALS AND METHODS

Collection and preparation of plant materials

Stem barks and roots of *V. amygdalina* plant were collected in a sterile polythene bags from the Federal Low-cost, Lapai, Niger State, Nigeria. The samples were taken to the Department of Biology, Faculty of Natural Science, Ibrahim Badamasi Babangida University, Lapai, Niger state, Nigeria for proper identification and authentication. The samples were washed with distilled water chopped into pieces and air-dried for two weeks after which they were grounded with mortar and pestle.

Sample extraction

Aqueous Extract

Ten grams each of the grounded stem barks and roots was added

to 100 mL of sterile distilled hot water in order to obtain aqueous extracts (100 mg/mL) and mixed thoroughly after which the plant residue was filtered through a muslin cloth and the obtained filtrate was further sterilised by filtration through membrane filter HC type. The sterile extract obtained was then transferred into sterile capped McCartney bottles and refrigerated at 4°C until further use.

Ethanolic extract

Ten grams each of the ground stem barks and roots was separately added to 100 mL of 70 % ethanol or acetone in order to obtain ethanol and acetone extracts (100 mg/mL).

The extraction was done for 72 h (Newton *et al.*, 2002). The muslin cloth was used to filter the plant residue and the obtained filtrate was further sterilised by filtration through membrane filter HC type. The sterile extract obtained was then transferred into sterile capped McCartney bottles and refrigerated at 4°C until further use.

Phytochemical Screening of the Stem Barks and Roots of *V. amygdalina*

Phytochemical screening of the *V. amygdalina* stem bark and root extracts for major constituents including tannins, alkaloids, saponins, steroids, phenols, phlobatannins, flavonoids, and glycosides was carried out using standard qualitative method as previously described by Trease and Evans (1989).

Sterility test of the plant extracts

Each of the extract (aqueous, ethanol, and acetone extract) was tested for sterility after sterilization by inoculating 1 mL of each extract on sterile nutrient agar and incubated at 37°C for 24 h. The plates were observed for growth. No growth in the extracts after incubation indicates that the extracts were sterile.

Test organisms

The test organisms employed in this study were medical isolates of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus niger*. They were obtained from Department of Microbiology, Ibrahim Badamasi Babangida University, Lapai.

Standardization of the bacterial cell suspension

Few colonies of each tested organism were picked into sterile test-tube containing sterile nutrient broth and incubated at 37°C for 24 h. The turbidity of the suspensions was adjusted to 0.5 turbidity McFarland standard.

Determination of Antimicrobial Activity of Various Crude Extract

The antimicrobial activity of the crude extracts was done using agar-well diffusion method described by (Gashe & Zeleke, 2017) with few modifications. Briefly, 0.2 mL of the adjusted suspensions were separately inoculated using a sterile wire loop on the solidified media plates (Nutrient Agar (NA) for bacteria and Potato Dextrose Agar plate (PDA) for fungi) and spread uniformly using a sterile glass rod. The agar medium was punched out using a sterile hole cork-borer of 8 mm diameter and cut agar discs were aseptically and carefully removed with sterile forceps. A sterile Pasteur pipette was used to introduce 0.5 mL of the crude plant extract samples into the 8 mm holes bored on the

surface of the agar media containing the cultures. The plates were allowed to stand for one hour at room temperature to allow for diffusion of the substrates to proceed before the growth of the organisms commenced. The plates were finally incubated at 37°C for 24 h for bacteria and at room temperature for fungi. The presence of zone of inhibition around the holes containing the extracts indicates the antimicrobial activity against the test organisms. Antimicrobial activity was expressed in terms of diameter of zones of inhibition (mm).

RESULTS

The root and stem bark of the plant were found to be rich in alkaloids, steroids, glycosides, flavonoids, saponins and Phlobatannins, though tannins and phenols were only present in the stem bark as shown in table 1 below.

Table 1: Phytochemical Constituents of Root and Stem bark of *V. amygdalina*

Phytochemical Compounds	Root	Stem bark
Alkaloids	++	++
Steroids	+++	+
Glycosides	+++	+++
Flavonoids	++	+
Tannins	-	+++
Saponins	++	+++
Phlobatannins	++	++
Phenolics	-	+++

Key: +: present, -: Absent, ++: moderately present, +++: Highly present

Table 2 below shows the zone of inhibition of ethanol extract of *Vernonia amygdalina* stem bark and root against different pathogenic organisms. When tested with the extract of stem bark, the sensitivity of the tested organisms showed that *S. aureus*, *C. albicans*, and *A. niger* produced the same diameter of inhibition zone of 8 mm (38.10%). *P. aeruginosa* produced the lowest diameter of inhibition zone of 5 mm (17.24%). For the ethanol extract of root, *A. niger* produced the largest diameter of inhibition zone with 8 mm (47.06%), followed by *S. aureus* with 6 mm (35.29%) and *C. albican* 2 mm (11.77%) diameter of inhibition zone.

Table 2: Sensitivity Patterns of Some Pathogenic Organism to Ethanol Extract as Measured by Zone of Inhibition.

Organisms	Stem bark	Percentage (%)	Root	Percentage (%)
<i>S. aureus</i>	8 mm	38.10	6 mm	35.29
<i>P. aeruginosa</i>	5 mm	17.24	1 mm	5.88
<i>C. albicans</i>	8 mm	38.10	2 mm	11.77
<i>A. niger</i>	8 mm	38.10	8 mm	47.06

The sensitive pattern of the tested pathogenic organism towards

acetone extract of *Vernonia amygdalina* stem bark and root and their percentage zone of inhibition are presented in table 3 below. It was shown that *S. aureus* and *P. aeruginosa* produced the largest diameter of inhibition zones of 16 mm (37.21%) when tested with acetone stem bark extract of *Vernonia amygdalina*, followed by *C. albicans* with diameter of inhibition zones of 7 mm (16.28%). *A. niger* produced the lowest diameter of inhibition zones with 4 mm (9.30%). Interestingly, *A. niger* produced the largest diameter of inhibition zones of 8 mm (30.77%) when exposed to acetone root extract of *Vernonia amygdalina*, while *S. aureus* and *C. albicans* produced the same size of diameter of inhibition zones of 6 mm (23.08%).

Table 3: Sensitivity Patterns of Some Pathogenic Organism to Acetone Extract as Measured by Zone of Inhibition

Organisms	Stem bark	Percentage (%)	Root	Percentage (%)
<i>S. aureus</i>	16 mm	37.21	6 mm	23.08
<i>P. aeruginosa</i>	16 mm	37.21	2 mm	7.69
<i>C. albicans</i>	7 mm	16.28	6 mm	23.08
<i>A. niger</i>	4 mm	9.30	8 mm	30.77

The result of the hot aqueous extract showed that except for *S. aureus* which exhibited activity with the diameter of inhibition zone of 7 mm, all other organism were resistant to the extract (Table 4).

Table 4: Sensitivity patterns of some pathogenic organism to hot aqueous extract as measured by zone of inhibition.

Organisms	Stem bark	Percentage (%)	Root	Percentage (%)
<i>S. aureus</i>	7 mm	100	R	-
<i>P. aeruginosa</i>	R	-	R	-
<i>C. albicans</i>	R	-	R	-
<i>A. niger</i>	R	-	R	-

DISCUSSION

It has been revealed that all the tested plant extracts possesses antimicrobial properties. *S. aureus* (gram positive) was more susceptible to the various extracts; ethanol, acetone, and aqueous extracts and thus the higher size of diameter of Inhibition zones. It was previous reported that plant extracts displayed stronger antimicrobial effect on the gram positive strains than on their gram- negative counterparts (Desta, 1993; Okigbo & Omodamiro, 2008; Chekole *et al.*, 2016). *P. aeruginosa*, *C. albicans* and *A. niger* were inhibited by ethanolic and acetone extracts but not inhibited by the aqueous hot extracts as shown in (Table 2, 3 and 4). This is supported by the previous studies by Ibekwe *et al.* (2001); Adetunji *et al.* (2013); Adetutu *et al.* (2011); Koduru *et al.* (2006), and Moreno *et al.* (2006) which showed that the ethanolic and acetone extracts possesses more antimicrobial activity than aqueous extract. In this study, the hot water decoction was used to mimic the traditional way of preparation. The extracts obtained from water decoction in this study displayed weak or no antimicrobial activity, which is contrary to the anticipation. Water is used as a solvent in traditional medicinal probable because it is more convenient to prepare in a household environment using either decoction or infusion. Earlier, Eloff (1998) reported that due to the inability of water to extract nonpolar compounds, the water preparation is usually not suitable

for antimicrobial discovery. Another reason for organic extract to be more active than water extract is due to the better solubility of the active components in organic solvents (Boer *et al.*, 2005; Doughari *et al.*, 2007). The presence of alkaloids, steroids, Glycosides, flavonoids, tannins, saponins, phlobatannins, and phenolics in the extracts of *V. amygdalina* may explain the reason for its antimicrobial actions since the antimicrobial properties of most of these phytoconstituents have previously been documented (Taleb-contini *et al.*, 2003; Mandalari *et al.*, 2007; Nenaah, 2013; Jasim *et al.*, 2015; Al-Harbi *et al.*, 2017; Jin *et al.*, 2017).

Conclusion

This study highlighted the antimicrobial effects of *V. amygdalina* extracts on some human pathogens thereby verifying the traditional healer's claim. Sooner than expect, broad-spectrum drug which might be able to cure various human ailments could be developed from *V. amygdalina*. Further work should be carried out in order to isolate the active compounds for further antimicrobial, pharmacological and clinical testing.

REFERENCES

- Abosi, A. O. & Raseroka, B. H. (2003). In vivo antimalarial activity of *Vernonia amygdalina*. *British Journal of Biomedical Science* 60: 89–91.
- Adetunji, C. O., Olaniyi, O. O. & Ogunkunle, A. T. J. (2013). Bacterial activity of crude extracts of *Vernonia amygdalina* on clinical isolates. *Journal of Microbiology and Antimicrobials* 5: 60–64.
- Adetutu, A., Morgan, W. A. & Corcoran, O. (2011). Ethnopharmacological survey and *in vitro* evaluation of wound-healing plants used in South-western Nigeria. *Journal of Ethnopharmacology* 137: 50–56.
- Akah, P. A. & Okafor, C. L. (1992). Blood Sugar Lowering Effect of *Vernonia amygdalina* Del, in an-Experimental Rabbit Model. *Phytotherapy Research* 6: 171–173.
- Akinpelu, D. A. (1999). Antimicrobial activity of *Vernonia amygdalina* leaves. *Fitoterapia*, 70: 432–434.
- Al-Harbi, R., Al-Wegaisi, R., Moharram, F., Shaaban, M. & El-Rahman, O. A. (2017). Antibacterial and anti-hemolytic tivity of tannins from *Pimenta dioica* against methicillin resistant *Staphylococcus aureus*. *Bangladesh Journal of Pharmacology* 12: 63–68.
- Anibijuwon, I. I., Oladejo, B. O., Adetunju, D. O. & Kolawole, O. M. (2012). Antimicrobial activities of *Vernonia amygdalina* against oral microbes. *Global Journal of Pharmacology* 6: 178–185.
- Chekole, G., Wedajo, B. & Mulat, M. (2016). *In vitro* antibacterial activities of some traditional medicinal plants against food-borne bacterial pathogens in Woldia District, Ethiopia. *Biotechnology International* 9: 209–222.
- de Boer, H. J., Kool, A., Broberg, A., Mziray, W. R., Hedberg, I. & Levenfors, J. J. (2005). Anti-fungal and anti-bacterial activity of some herbal remedies from Tanzania. *Journal of Ethnopharmacology* 96: 461–469.
- Desta, B. (1993). Ethiopian traditional herbal drugs. Part II: Antimicrobial activity of 63 medicinal plants. *Journal of Ethnopharmacology* 39: 129–139.
- Doughari, J. H., Elmahmood, A. M. & Manzara, S. (2007). Studies on the antibacterial activity of root extracts of *Carica papaya*

- L. *African Journal of Microbiology Research* 1: 37–41.
- Eloff, J. N. (1998). Which extractant should be used for the screening and isolation of antimicrobial components from plants? *Journal of Ethnopharmacology* 60: 1–8.
- Etim, K. H., Ofor, S. E., Mandor, U. A. & Ogar, M. (2012). Evaluation of the antibacterial potential of *Vernonia amygdalina* on foodborne pathogens isolated from kunu sold in Calabar, Nigeria. *Journal of Microbiology and Biotechnology Research* 2: 778–782.
- Gashe, F. & Zeleke, G. (2017). Antimicrobial activities of *Vernonia amygdalina* Del and *Prunus africana* extracts against multidrug resistant clinical strains. *Research Journal of Medicinal Plants* 11: 142–147.
- Ghamba, P. E., Balla, H., Goje, L. J., Halidu, A. & Dauda, M. D. (2014). *In vitro* antimicrobial activities of *Vernonia amygdalina* on selected clinical isolates. *International Journal of Current Microbiology and Applied Sciences* 3: 1103–1113.
- Habtamu, A. & Melaku, Y. (2018). Antibacterial and antioxidant compounds from the flower extracts of *Vernonia amygdalina*. *Advances in Pharmacological Sciences*, vol. 2018, Article ID 4083736, 6 pages.
- Ibekwe, V. I., Nnanyere, N. F. & Akujobi, C. O. (2001). Studies on antibacterial activity and phytochemical qualities of extracts of orange peels. *Int. J. Environ. Health Human Dev* 2: 41–46.
- Izevbigie, E. B. (2003). Discovery of water-soluble anticancer agents (edotides) from a vegetable found in Benin City, Nigeria. *Experimental Biology and Medicine* 228: 293–298.
- Izevbigie, E. B., Bryant, J. L. & Walker, A. (2004). A novel natural inhibitors of extracellular signal-regulated kinases and human breast cancer cell growth. *Experimental Biology and Medicine* 229: 163–169.
- Jasim, H., Hussein, A. O. & Hameed, I. H. (2015). Characterization of alkaloid constitution and evaluation of antimicrobial activity of *Solanum nigrum* using gas chromatography mass spectrometry (GC-MS). *Journal of Pharmacognosy and Phytotherapy* 7: 56–72.
- Jin, Z., Gao, L., Zhang, L., Liu, T., Yu, F., Zhang, Z. & Wang, B. (2017). Antimicrobial activity of saponins produced by two novel endophytic fungi from *Panax notoginseng*. *Natural Product Research* 31: 2700–2703.
- Koduru, S., Grierson, D. S. & Afolayan, A. J. (2006). Antimicrobial Activity of *Solanum aculeastrum*. *Pharmaceutical Biology* 44: 283–286.
- Kupchan, S. M., Hemingway, R. J., Karim, A. & Werner, D. (1969). Tumor inhibitors. XLVII. Vernodalin and vernomygdin, two new cytotoxic sesquiterpene lactones from *Vernonia amygdalina* Del. *The Journal of Organic Chemistry* 34: 3908–3911.
- Magadula, J. J. & Erasto, P. (2009). Bioactive natural products derived from the East African flora. *Natural Product Reports* 26: 1535–1554.
- Mandalari, G., Bennett, R. N., Bisignano, G., Trombetta, D., Saija, A., Faulds, C. B. & Gasson, M. J. (2007). Antimicrobial activity of flavonoids extracted from bergamot (*Citrus bergamia* Risso) peel, a byproduct of the essential oil industry. *Journal of Applied Microbiology* 103: 2056–2064.
- Moreno, S., Scheyer, T. & Romano, C. S. (2006). Antioxidant and antimicrobial activities of rosemary extracts linked to their polyphenol composition. *Free Radical Research* 40: 223–231.
- Nenaah, G. (2013). Antimicrobial activity of *Calotropis procera* Ait. (Asclepiadaceae) and isolation of four flavonoid glycosides as the active constituents. *World J Microbiol Biotechnol*, 29: 1255–1262.
- Newton, S. M., Lau, C., Gurcha, S. S., Besra, G. S. & Wright, C. W. (2002). The evaluation of forty-three plant species for *in vitro* antimycobacterial activities; isolation of active constituents from *Psoralea corylifolia* and *Sanguinaria canadensis*. *Journal of Ethnopharmacology* 79: 57–67.
- Noumedem, J. A. K., Mihasan, M., Kuate, J. R., Stefan, M., Cojocar, D., Dzoyem, J. P. & Kuete, V. (2013). *In Vitro* antibacterial and antibiotic-potential activities of four edible plants against multidrug-resistant gram-negative species. *BMC Complementary and Alternative Medicine* 13: 190.
- Nwanjo, H. U. (2005). Efficacy of aqueous leaf extract of *Vernonia amygdalina* on plasma lipoprotein and oxidative status in diabetic rat models. *Nigerian Journal of Physiological Sciences*, 20: 39–42.
- Okigbo, R. N. & Omodamiro, O. D. (2008). Antimicrobial Effect of Leaf Extracts of Pigeon Pea (*Cajanus cajan* (L.) Millsp.) on Some Human Pathogens. *Journal of Herbs, Spices & Medicinal Plants* 12: 37–41.
- Oshim, I. O., Desmond, C. O., Anyi, R., Nwobu, U., Ezugwu, U. M. & Urama, E. U. (2016). Kinetics of minimum inhibitory concentration, minimum bactericidal concentration and minimum fungicidal concentration of *Vernonia amygdalina* (Bitter leaf) on microorganisms isolated from wound infections. *International Journal of Surgical Research* 5: 8–14.
- Taleb-contini, S. H., Salvador, M. J., Watanabe, E. & Ito, I. Y. (2003). Antimicrobial activity of flavonoids and steroids isolated from two *Chromolaena* species. *Brazilian Journal of Pharmaceutical Sciences* 39: 403–408.
- Tijjani, M. A., Mohammed, G. T., Alkali, Y. T., Adamu, T. B. & Abdurahman, F. I. (2017). Phytochemical analysis, analgesic and antipyretic properties of ethanolic leaf extract of *Vernonia*. *Journal of Herbmed Pharmacology* 6: 95–99.
- Tona, L., Cimanga, R. K., Mesia, K., Musuamba, C. T. & Bruyne, T. (2004). *In vitro* antiplasmodial activity of extracts and fractions from seven medicinal plants used in the Democratic Republic of Congo. *Journal of Ethnopharmacology* 93: 27–32.
- Trease, G. E. & Evans, W. C. (1989). *Pharmacognosy*. Eleventh Edition. Brailiar Trindel Can. Macmillan Publishers, London.