Annals of Microbiology and Infectious Diseases
Volume 2, Issue 4, 2019, PP 01-07
ISSN 2637-5346

Phytochemical Screening and Antibacterial Activity of Bitter Leaf (*Vernonia amygdalina*)

Muhammad Ali[1*], Sani U. Diso[2], Sumayya A. Waiya[3] and Muhammad S. Abdallah[4]

[1]*Department of Microbiology, Federal University Gusau, Nigeria*
[2]*Department of Pharmaceutical Technology, School of Technology, Kano State Polytechnics*
[3]*School of Hygiene Kano, Nigeria*
[4]*Desert Research Monitoring and Control Centre, Yobe State University Damaturu, Nigeria.*

***Corresponding Author:** Muhammad Ali, Department of Microbiology, Federal University Gusau. Email: alimuhd4real@gmail.com*

ABSTRACT

Herbal medicine is the oldest form of healthcare known to mankind. The study was aimed to determine the phytochemical constituents and antibacterial activity of Vernonia amygdalina leaf extracts against some bacteria associated with gastroenteritis. Five (5) different bacterial isolates associated with gastroenteritis namely; Klebsiella pneumoniae, Salmonella typhi, Shigella sp, Escherichia coli, and Staphylococcus aureus were obtained from Pathology Laboratory of Murtala Muhammad Hospital Kano. Phytochemical screening of the leaf extract was conducted to ascertain the presence and amount of bioactive components present in the leaves. The antimicrobial assay of the leaves extracts was performed by disc diffusion method. The qualitative and quantitative phytochemical screening of the extract indicated the presence of Alkaloid, terpenoid, flavonoids, steroid, phenol, saponin and tannin, with flavonoid being the most abundant constituent making about 12.2% followed by steroid, alkaloid and phenol constituting 4.8, 4.6 and 3.6% respectively. The result shows that the extracts were active against the microorganisms. The Methanol extract showed higher activity (12.2 mm) against the bacterial isolates than aqueous extracts (10.2 mm). Statistical analysis of the result shows no significant different on the activity of the extract against the test isolates at $p<0.05$. It is concluded that the application of V. amygdalina leaf in ethno medicine is justified.

Keywords: *Antibacterial activity, bacteria, gastroenteritis, phytochemical, Vernonia amygdalina*

INTRODUCTION

Herbal medicine is the oldest form of healthcare known to mankind and over 50% of all modern clinical drugs are of natural products origin and natural products play important roles in drug development in the pharmaceutical industry [1]. The World Health Organization (WHO) estimates that approximately 80% of the world's inhabitants rely on traditional or herbal medicines for their primary health care and plants have long formed the basis of sophisticated traditional medicine systems and purportedly provide excellent leads for new drug developments [2,3]. The rediscovery of the connection between plants and health is responsible for the launching of a new generation of multi- component botanical drugs, dietary supplements and plant produced recombinant proteins [3]. However, the increasing problems of multi-drug resistant (MDR) bacteria is of great concern to both the clinicians and pharmaceutical industries and this has made it significant to search for newer drugs that are highly effective, affordable, acceptable and available[4].

Vernonia amygdalina commonly called bitter leaf because of its bitter taste is a member of the Asteraceae family. Ethno-medically, it is consumed either as a vegetable (macerated leaves in soup) or aqueous extracts as tonics for the treatment of various diseases [5]. *V. amygdalina* is a medicinal plant use in folk medicine to manage several ailments [6]. The leaves are characteristically bitter although the bitterness can be abated by boiling or by soaking in several changes of clean water [7]. The bitter taste had been associated with the presence of saponins, alkaloids, tannins, and glycosides. These made them act as a bittering agent and a hop substitute used for controlling microbial contamination in beer brewing without reducing the quality of malt [8]. The roots and leaves are used in ethno-medicine to treat fever, hiccups, kidney problems and

stomach discomfort among several other uses [7]. Both aqueous and alcoholic stem, root and leaf extracts are reported to be extensively used as purgative and anti-malarial as well as in the treatment of eczema [9]. The plant has acquired special relevance recently, having been shown in human medicine to possess potent anti-tumorigenic properties [10]. Nutritionally, *V. amygdalina* is used mainly in soup making in the tropics and also as an appetizer and febrifuge [11] and has successful been used as a supplement in weaning foods [12].

Pharmacological studies have shown that the leaf extract of the plant has both hypoglycemic and hypolipidaemic properties in experimental animals and so could be potentially useful in the management of diabetes mellitus [13]. Several studies carried out on this plant had suggested that it contains different bioactive compounds, including, flavonoids, saponins, alkaloids tannins, phenolics, terpenes, steroidal glycosides, triterpenoids, and several types of sesquiterpene lactones [14]. These bioactive compounds made them possess different pharmacological properties like antimicrobial, antimalarial, antithrombotic, antioxidant, anti-diabetic, laxative, hypoglycemic, antihelmintic, anti-inflammatory, cathartic, anticancer, antifertility, anti-fungi, antibacterial, and among others [15].

The aqueous and ethanol extracts of *V. amygdalina* leaves had shown antimicrobial effects against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella* spp., and *Candida albicans* with the MIC values ranged between 12.5 and 50 mg/ml [16]. In another study, ethanolic and aqueous extracts of *V. amygdalina* leaves had shown a higher value of MIC inhibitions on *Strep to coccus*mutants at 25 and 55 mg/ml, respectively [17,18]. The study was aimed to determine the phytochemical constituents and antibacterial activity of *Vernonia amygdalina* leaf extracts against some bacteria associated with gastroenteritis.

Material and Methods

Ethical Approval

An approval (MOH/off/797/T.I/50) for the study was obtained from Research and Ethic Committee Kano State Ministry of Health. The approval was based on the consent of the Hospitals Ethical Committees of Murtala Mohammed Specialists Hospital (MMSH), Kano.

Bacterial Isolates

Five (5) different bacterial isolates associated with gastroenteritis namely;*Klebsiella pneumoniae, Salmonella typhi, Shigella* sp, *Escherichia coli,* and *Staphylococcus aureus* were obtained from Pathology Laboratory of Murtala Muhammad Hospital Kano. The isolates were characterized to specie level at Microbiology of Kano University of Science and Technology Wudil. The isolates were characterized using different procedures including Gram's stain, cultural characterization and Biochemical tests (Indole, Methyl red, Voges Proskauer, Catalase, Citrate utilization and coagulase tests) as described by Holt *et al*.[19]. The isolates were maintained on Nutrient agar slants for further use.

Collection and identification of *V. amygdalina* leaves

The plant's leaves used in this study (*Vernonia amygdalina*) were obtained from Sabon-gari Market in Kano metropolis in Kano, Nigeria. Identification and authentication of the leaves was conducted at Herbarium in the Department of Plant science, Bayero University Kano with the following voucher number BUKHAN 0235 and voucher specimen were deposited in the herbarium for references. The leaves were air dried for 2 weeks, then grounded into fine powder under laboratory condition using sterile pestle and mortar and stored inair tight container for further use.

Extraction of *V. amygdalina* Leaf

The leaf extracts were prepared according to the method adopted by Ali *et al*. [20]. Fifty grams of powdered sample of the plant leaf were extracted exhaustively (cold maceration) using distilled water and methanol for 3 seven days. The extracts were filtered using Whatman No. 2 filter paper and concentrated at to powder form in water bath and rotary evaporator for aqueous and methanol extract respectively. The samples were kept in the refrigerator at 4°C until use.The driedpowdered leaf was measured into McCartney bottles andappropriate volume of Dimethylsulphoxide (DMSO) was added to make a stock solution of 100mg/ml. Sterilization of the extract was done using 0.65 membrane filter by suction pump. The sterilized extract was stored in sterile McCartney bottle and kept inthe refrigerator at 4°C before use.

Phytochemical screening

Phytochemical screening of the leaf extract was conducted to ascertain the presence of bioactive components present in the leaves. Presence of

alkaloid, terpenoid, flavonoids, steroid, phenol, saponin and tannin were determined using procedure described by Sofowora [2] and Trease and Evans [21].

Quantitative Phytochemical Analysis

Different methods were used in evaluating the quantity of phytochemical constituents of the plant materials used. Spectrophotometric method was used to determine terpenoid, tannin and steroids. Folin-Ciocalteu procedure was used to determine phenol content. Flavonoids, alkaloids and saponins were determined by the methods described by Adeniyi *et al*.[22].

Standardization of Bacterial Isolates

Standardization of bacterial inoculum was done by pickinga loop full colonies of each organism into nutrient broth and incubated at 37°C for 24 hours. Turbidity produced by the isolates was adjusted to match 0.5 McFarland standards [20].

Antibacterial Activity of the Extracts

The antimicrobial assay of *V. amygdalina* leavesextracts was performed by disc diffusion method as described by Kirby-Bauer [23]. The Mueller Hinton agar plates were inoculated separately with of each of the test bacterial strain culture (Equivalent to 0.5 Mac Farland) and evenly spread on entire surface of each plate. The sterile discs (6 mm diameter), were dipped aseptically in different extracts (25, 50, 75 and 100 mg/ml) for one minute and placed over the Mueller Hinton agar plates already seeded with bacterial culture. The plates were left at ambient temperature for 30 minutes and then incubated at 37°C for 24 hours and observed for zone of inhibition [24]. Commercially available Ciprofloxacin discs (10 µg, Oxoid, UK) were used as control. The diameter of inhibition zones was measured in millimeters. The experiment was performed in triplicate and the resulting zones of inhibition were recorded as mean ± standard error.

Statistical Analysis

The data of average zone of inhibition produced by the isolates against the extracts used was analyzed using One-Way ANOVAs and the statistical program SPSS 21.0 (Statistical Package for the Social Sciences). The results were presented as the means ± standard deviation. Significance level for the differences was set at $p<0.05$.

RESULTS

Phytochemical screening

The qualitative and quantitative phytochemical screening of *V. amygdalina* leaf extract is presented in Table 1. The result of qualitative phytochemical screening indicated the presence of Alkaloid, terpenoid, flavonoids, steroid, phenol, saponin and tannin. Quantitatively, flavonoid was found to be the abundant constituent in *V. amygdalina* leaf making about 12.2% followed by steroid, alkaloid and phenol constituting 4.8, 4.6 and 3.6% respectively.

Table1. *Qualitative and quantitative phytochemical screening of V. amygdalina leaf extract*

S/N	Phytochemical	Qualitative analysis	Quantitative analysis
1.	Alkaloids	+	4.60±0.23
2.	Flavonoid	+	12.20±1.30
3.	Saponin	+	2.70±0.50
4.	Steroids	+	4.80±0.25
5.	Terpenoid	+	1.70±0.04
6.	Phenol	+	3.60±0.20
7.	Tannin	+	1.20±0.03

Key: + = Presence of phytochemical, - = Absence of phytochemical.

Antibacterial Activity of the Extracts

Aqueous Extract

The average antibacterial activity of aqueous *V. amygdalina* leaf extract is presented in Table 2. The results showed that zones of inhibition recorded by the isolates depend on the type of bacterial isolates and concentration of the extracts. Highest zone of inhibition is demonstrated by *Shigella* sp (14.3mm) at 100mg/ml. The zone of inhibition of the control (Ciprofloxacin 10 µg) ranges from to 19-23 mm

Table2. *Average Antibacterial Activity of aqueous V. amygdalina leafextract*

	Concentration (mg /ml)/zone of inhibition (mm)				
Isolates	**25**	**50**	**75**	**100**	**Control**
Escherichia coli	0.00±0.00	10.2±0.12	11.0±0.18	12.5±0.13	22

Salmonella typhi	8.3±0.12	10.4±0.16	12.4±0.19	13.7±0.20	21
Staphylococcus aureus	0.00±0.00	9.2±0.25	11.2±0.26	11.8±0.31	20
Klebsiella pneumoniae	8.9±0.14	10.5±0.23	12.5±0.19	13.6±0.18	22
Shigella sp	9.3±0.10	11.8±0.18	13.8±0.34	14.3±0.21	23

Methanol extract

The average antibacterial activity of Methanol *V. amygdalina* leaf extract is presented in Table 3. The results showed that zones of inhibition recorded by the isolates depend on the type of bacterial isolates and concentration of the extracts. Highest zone of inhibition is demonstrated by *E. coli* (15.2 mm) at 100mg/ ml. The zone of inhibition of the control (Ciprofloxacin 10 µg) ranges from to 19-23mm

Table3. *Average Antibacterial Activity of MethanolV. amygdalina leaf extract*

	Concentration (mg/ml)/zone of inhibition (mm)				
Isolates	**25**	**50**	**75**	**100**	**Control**
Escherichia coli	8.4±0.00	14.0±0.12	14.5±0.18	15.2±0.13	22
Salmonella typhi	9.2±0.12	11.8±0.16	14.4±0.19	14.9±0.20	21
Staphylococcus aureus	8.2±0.13	9.7±0.25	12.8±0.26	13.5±0.31	20
Klebsiella pneumoniae	9.5±0.14	11.9±0.23	13.9±0.19	14.4±0.18	22
Shigella sp	9.7±0.10	11.5±0.18	13.2±0.34	14.8±0.21	23

DISCUSSION

The phytochemical screening of the leaves extracts of *V. amygdalina* revealed the presence of Alkaloid, terpenoid, flavonoids, steroid, phenol, saponin and tannin. These phytochemicals exhibit various pharmacological and biochemical actions and found to be beneficial to human health as well as possessing antioxidant activity [25]. Several studies had been conducted in isolating and characterizing some bioactive compounds from *V. amygdalina* leaf extracts [8,26,27,28]. The phytochemical studies had resulted in the isolation of flavonoids, saponins, alkaloids, tannins, phenolics, terpenes, steroidal glycosides, triterpenoids, and several types of sesquiterpene lactones [14,29]. The finding of the study was in conformity with the finding of Atangwho et al. [30] reported *V. amygdalina* leaves contain 0.87% flavonoid, 0.37% tannins, 2.15% saponins and 2.13% alkaloids while Ndukwe et al. [31] reported 0,47% flavonoids, 2.78% Alkaloids, 0.64% saponinsand 0.74 tannins.

Alkaloids are known to play some metabolic roles and control development in living system [32]. It also interferes with cell division, hence the presence of alkaloids in *V. amygdalina* could account for their use as antimicrobial agents. Alkaloids are beneficial chemicals to plants serving as repellant to predators and parasites. This probably endows these group agents its antimicrobial activity [33]. Flavonoids have also been implicated as antioxidants both in physiological and diseased states. For instance tea flavonoids have been reported to reduce the oxidation of law-density lipoprotein, lower the blood level of cholesterol and triglycerides [34]. Flavonoids are also expressed in plants in response to microbial infection suggesting their antimicrobial activity [35]. Saponins are believed to react with the cholesterol rich membranes of cancer cells, thereby limiting their growth and viability [36]. Saponins in medicinal plants are responsible for most biological effects related to cell growth and division in humans and have incivility effect on inflammation [37,38]. Present of saponin in *V. amygdalina* leaves supports the usefulness of the plant in managing inflammation. Steroids are importance in pharmacy as they possess compounds like sex hormones and can be used for drug production [39]. Terpenoid have been found to be useful in the prevention and therapy of several diseases, including cancer. Terpenoid are also known to possess antimicrobial, antifungal, anti-parasitic, antiviral, anti-allergenic, antispasmodic, anti-inflammatory and immunomudulatory properties [40]. It have been reported that phenolics are free radical scavengers that prevent oxidative cell damage, and have strong anticancer activities and they might induce mechanism that affect cancer cells and inhibit tumor invasion [41]. They also lower the risk of heart disease and provide anti-inflammatory activities attributable to their ability to neutralize and quench free radicals [42]. Tannin is known to have potentials anti-viral activity [43] as well as potential prophylactic and therapeutic effect against cancer cells [44].

The present study showed that the leaves of *V. amygdalina*possess antimicrobial potential against some bacterial isolates associated with

gastroenteritis. Several studies were conducted on antibacterial activity of *V. amygdalina* leaf extracts [45,46]. Their results show that there was variation in the degree of antibacterial activities of the extracts. Based on the findings of this study, methanol extracts showed more activity (12.2 mm) against the bacterial isolates than aqueous extracts (10.2 mm). This may be due to the higher volatility of the methanol which tends to extracts more active compounds from the samples than water. The result of this study was in conformity with that of Ogundare [47] who found ethanol extract of *V. amygdalina* leaf effective against *S. aureus* and *Shigella*. This result also justifies the finding of Zubairu et al. [48] who found *V. amygdalina* leaf extract effective against *E. coli*, *S. aureus* and *S. typhi*. On the other hand, the result contradicts the finding ofOgundare [47] who found no activity of *V. amygdalina* leaf extract against *E. coli* and *Salmonella.*

The *V. amygdalina* leaf extracts has demonstrated considerable activities against both Gram-positive and Gram negative bacteria. There was however, more activity against the Gram negative (*E. coli*, *Shigella*, *Klebsiella*and *S. typhi*) organism than the Gram positive. Pelczar et al. [49] suggested that the difference in susceptibility of Gram positive and Gram-negative bacteria to various antimicrobial agents probably depends on structural differences in their cell walls. For example, amount of peptidoglycan, presence of receptors and lipids, nature of cross linking, activity of autolytic enzymes that determined the penetration, binding and activity of the antimicrobial agents. The marked difference in the effects of the extracts on the organism therefore, is suggestive of the activity against cell wall components of the organism. The antimicrobial substance appears to exert antimicrobial activity by inhibiting the growth of and by killing the sensitive bacteria.

CONCLUSION

From the findings of this study, it can be concluded that the extracts of *V. amygdalina* exhibits antibacterial activity against both gram positive and gram negative bacteria with methanol extract being the most effective. The activity of the extracts against the isolates is due to present of bioactive compounds in the extracts such as alkaloids, saponin, tannin and flavonoid. As result, the extracts can be used to develop new herbal formulation for preventing bacterial infection.

REFERENCES

[1] Preethi, RM, Devanathan, VV, Loganathan M. Antimicrobial and antioxidant efficacy of some medicinal plants against food borne pathogens. Adv. Biol. Res. 2010;4: 122-125

[2] Sofowora EA. Medicinal plants and Medicine in Africa (2nd eds). John Wiley and Sons, New York. 1993;116pp.

[3] Akinjogunla OJ, Ekoi OH, Odeyemi AT, Akinjogunla OJ, Etok CA, Oshoma CE. Preliminary phytochemistry and in-vitro antibacterial efficacy of Hydro-Ethanolic leaf extracts of Psidium guajava on common urinary tract bacterial pathogens. Biores. Bull. 2011;5: 329-336.

[4] Martino PD, Gagniere H, Berry H, Bret L. Antibiotic resistance and virulence properties of Pseudomonas aeruginosa strains from mechanically ventilated patient with pneumonia in intensive care unit: Comparison with imipenem- resistant extra-respiratory tract isolates from uninfected patients. Microb. Infect. 2002; 4:613-620.

[5] Igile GO, Wieslaw O, Jurzysta M, Stanislaw B and Fasanmade A. Flavonoids from Vernonia amygdalina and their Antioxidant Activities. Journal of Agricultural and Food Chemistry. 1994;42: 2445–2448.

[6] Allen GH. The genetic basis of disease: In general pathology. Walter, J. B. and Israel, M. S. (Eds). Churchill Living Stone Medical Dictionary, 1987;pp 32-37

[7] Burkill HM. The Useful Plant of West Tropical Africa, 1985;Vol 2, Families A-D Kew Royal Botanic Gardens

[8] Farombi EO and Owoeye O. Antioxidative and chemopreventive properties of Vernonia amygdalina and Garcinia biflavonoid. Int. J. Environ. Res. Public Health. 2011;8:2533–2555. doi: 10.3390/ijerph8062533.

[9] Kupcham SM. Drugs from Natural products. Plant source in drugs discovery, science and development. Ame. Chem. Society. 1971;6: 311.

[10] Izevbigie EB, Bryant JL, Walker A. A novel natural inhibitor of extracellular signal-regulated kinases and human breast cancer cell growth. Experimental Biology Medical (Maywood). 2004;229(2):163-169.

[11] Iwu MM, Okunji E, Akah PA, Tempesta MS and Carley DG. Dioscoretine: The hypoglycemic principles of Dioscorea dumentorium. Planta Medicinca. 1996;56: 119-126

[12] Eleyinmi AF, Fasasi OS, Oyarekua MA. Effect of some traditional processing operations on the functional properties of African breadfruit (Treculia africana) seed. LWT-Food Science Technol., 2005;40: 513-519.

[13] Ebong PE, Atangwho IJ, Eyong EU, Egbung GE. The antidiabetic efficacy of combined extracts from two continental plants: Azadirachta indica (A. Juss) (Neem) and Vernonia amygdalina (Del.) (African bitter leaf). Ame. J. Biochem. and Biotechnol., 2008; 4(3), 239-244.

[14] Luo X, Jiang Y, Fronczek FR, Lin C, Izevbigie, EB, Lee S and Lee KS. Isolation and Structure Determination of a Sesquiterpene Lactone (Vernodalinol) from Vernonia amygdalina Extracts. Pharmaceutical Biology. 2017;49(5): 464–470.

[15] Alara OR, Abdurahman NH, Abdul Mudalip SK and Olalere OA. Effect of Drying Methods on Free Radicals Scavenging Activity of Vernonia amygdalina growing in Malaysia. Journal of King Saud University – Science. 2017

[16] Ghamba P, Balla H, Goje L, Halidu A and Dauda M. In Vitro Antimicrobial Activities of Vernonia amygdalina on Selected Clinical Isolates. International Journal of Current Microbiology and Applied Sciences. 2014;3(4): 1103–1113.

[17] Akinpelu DA. Antimicrobial Activity of Vernonia amygdalina leaves. Fitoterapia. 1999;70(4): 432– 434.

[18] AnibijuwonII, Oladejo BO, AdetitunDO and Kolawole OM. Antimicrobial Activities of Vernonia amygdalina against Oral Microbes. Global Journal of Pharmacology. 2012;6(3): 178–185.

[19] Holt JG, Krieg NR, Sneath PA, Stanley JT and Williams ST. Bergey's manual of systematic bacteriology, 9th edition. Williams & Wilkins Co. Baltimore, Maryland, 1994;p786

[20] Ali M,Yahaya A, Zage AU and Yusuf ZM. *In-vitro* Antibacterial Activity and Phytochemical Screening of *Psidium guajava* on Some Enteric Bacterial Isolates of Public Health Importance. *Journal of Advances in Medical and Pharmaceutical Sciences*,2017;12(3): 1-7. DOI: 10.9734/JAMPS/2017/31126

[21] Trease GE, Evans WC. Phytochemicals. In: Pharmacognosy. 15th ed. Saunders Publishers, London, 2002;pp. 42-44, 221- 229, 246- 249, 304-306,331-332, 391-393.

[22] Adeniyi SA, Orjiekwe CL, Ehiagbonare JE. Determination of alkaloids and oxalates in some selected food samples in Nigeria. African Journal of Biotechnology,2009;8, 110-112.

[23] Kirby-Bauer A. Antimicrobial sensitivity testing by agar diffusion method. *J Clin Pathol*, 1996, pp 44:493.

[24] 18. Ahmed I, Beg AZ. Antimicrobial and phytochemical studies on 45 Indian Medicinal plants against multi-drug resistance human pathogens. J Ethnopharmacol 2001;74(2): 113-123.

[25] Omale J and Okafor P. Comparative antioxidant capacity, membrane stabilization, polyphenol composition and cytotoxicity of the leaf and stem of Cissus multistriata. Afr. J. Biotechnol., 2008;7(17); 3129-3133.

[26] Erasto P, GriersonDS and Afolayan AJ. Evaluation of Antioxidant Activity and the Fatty Acid Profile of the Leaves of Vernonia amygdalina Growing in South Africa. Food Chemistry. 2007;104: 636–642.

[27] Kiplimo, J. J., Koorbanally, N. A. and Chenia, H. Triterpenoids from Vernonia auriculifera Hiern Exhibit Antimicrobial Activity. African Journal of Pharmacy and Pharmacolog. 2011; 5(8): 1150–1156.

[28] Toyang NJ and Verpoorte R. A Review of the Medicinal Potentials of Plants of the Genus Vernonia (Asteraceae). Journal of Ethnopharmacology. 2013;146(3): 681–723.

[29] Quasie O, Zhang Y, Zhang H, Luo J and Kong L. Four New Steroid Saponins with Highly Oxidized Side Chains from the Leaves of Vernonia amygdalina. Phytochemistry Letters. 2016; 15: 16– 20.

[30] Atangwho IJ, Ebong PE, Eyong EU, William IO, Eteng MU, Egbung GE. Comparative Chemical Composition of Leaves Some Anti-diabetic Medicinal Plants: Azadirachta indica, Vernonia amygdalina and Gongronema latifolium. Afri. J. Biotechnol. 2009;8(18): 4685-4689.

[31] Ndukwe OK, Awomukwu D and Ukpabi CF. Comparative Evaluation of Phytochemical and Mineral Constituents of the Leaves of some Medicinal Plants in Abia State Nigeria. International Journal of Academic Research in Progressive Education and Development 2013;2(3); 2013: 244-252

[32] Edeoga HO, Omobuna G and Uche LC. Chemical composition of *Hyotissu aveoleus* and *Ocimum gratissium* hybrids from Nigeria. *African Journal of Biotechnology*,2006;5(910), 892-895.

[33] Usunobun U and Okolie PN. Phytochemical analysis and proximate composition of Vernonia amygdalina, International Journal of Scientific World, 2006;4 (1) 11-14

[34] Erdman JW. Flavonoid and Heart Health (2005): Proceedings of the ILSI North America Flavonoid workshop, May 31 – June 1. J. Nutrition, 2007;137(3): 718s-737s.

[35] Kujumgiev A, Tseveikoval TS, Serkedjivay DE, Bankora V, Christo R, Popov S. Antibacterial, antifungal and antiviral activity of propolis geographic origin. J. Ethno pharmacol., 1999;44: 35-40.

[36] Roa RR, Babu RM and Rao MRV. Saponins as anti-carcinogens. *The Journal of Nutrition*, 1995;125, 717-724.

[37] Okwu DE. and Emenike IN. Evaluation of the phytonutrients and vitamin contents of Citrusfruits. International Journal of Molecular Medicine and Advance Science 2006;2, 1–6.

[38] Prohp TP and Onoagbe IO. Determination of phytochemical composition of the stem bark of *triplochitonscleroxylon k. schum*. (sterculia ceae). *International Journalof Applied Biology and Pharmaceutical Technology*, 2012;3(2), 68-76.

[39] Okwu DE. Evaluation of the chemical composition of indigenous spices and flavoring agents. *Global Journal of Pure and Applied Sciences*, 2001; 7(3), 455-459.

[40] Rabi T and Bishayee A. Terpenoids and breast cancer chemoprevention. *Breast Cancer Res Treat*2009; 115, 223-239.

[41] Ugwu OPC, Nwodo OFC, Joshua PE, Bawa A, Ossai EC and Odo CE. Phytochemical and Acute Toxicity Studies of Moringaoleifera Ethanol Leaf Extract. International Journal of Life Sciences Biotechnology and Pharma Research, 2013; 2013; 2(2), 66-71.

[42] Omale J and Okafor P. Comparative antioxidant capacity, membrane stabilization, polyphenol composition and cytotoxicity of the leaf and stem of Cissus multistriata. Afr. J. Biotechnol., 2008;7(17); 3129-3133.

[43] Cheng HY, Lin CC, Lin TC. Anti-herpes simplex virus type 2 activity of casuarinin from the bark of Terminalia arjuna Linn. Antiviral Research, 2002; 55, 447–455.

[44] Narayanan BA, Geoffrey O, Willingham MC, Nixon DW. Expression and its possible role in GI arrest and apoptosis in allergic acid treated cancer cells. Cancer Letters, 1999; 136(2): 215 - 21.

[45] 45. Akujobi CO, Anyanuwa BN, Onyere GOC and Ibekwe VI, Antibacterial activities and preliminary phytochemical screening of four medicinal plants. Journal of Applied Science,2004;7(3): 4328-4338.

[46] Ijeh, II and Adedokun AT. Effects of administration of ethanolic extract of Vernonia amygdalina Del. on kidney function of experimental rabbit model. Res. J. Biotech., 2006; 1: 34-35.

[47] Ogundare AO. Antibacterial properties of the leaf extracts of Vernonia amygdalina, Ocimum gratissimum, Corchorous olitorius and Manihot palmate. Journal of Microbiology and Antimicrobials, 2011 Vol. 3(4), pp. 77-86

[48] Zubairu AY, Mukhtar M, Saidu I, Ibrahim Z, Isah S, Garga MA and Kebbi HS. Antibacterial activity of methanolic extract of bitter leaf (Vernonia amygdalina) from various component fractions using column chromatography. GSC Biological and Pharmaceutical Sciences, 2019; 7(2), 16-21.

[49] Pelczar M.J, Chan ESC and Krieg NR. Airborne diseases. In: Microbiology Concepts and Applications. McGraw Hill, Inc. U.S.A, 1993;pp: 652.

Citation: *Muhammad Ali et.al, "Phytochemical Screening and Antibacterial Activity of Bitter Leaf (Vernonia amygdalina)", Annals of Microbiology and Infectious Diseases 2(4), 2019, pp.01-07.*