Phytochemical and Antimicrobial Properties of *Vitellariaparadoxa* Seed Oil Extracts on *Staphylococcus Aureus* and *Candida Albicans*

John Walter Chinaka, Nanlir Janfa, Oladejo Afolabi, Ya'u Hussaini, Sunday Oladipo

Federal college of forestry Jos, PMB 2019, Jos, Plateau state, Nigeria

Abstract- This research was carried out to evaluate the phytochemical and antibacterial effect of ethanol extract of *Vitellaria paradoxa* seed on *Staphylococcus aureus* and *Candida albicans*. The research was conducted at Microbiology laboratory of Federal college of Forestry, Jos.*V. paradoxa* seeds were collected from Bida, Niger State, Nigeria. Clinical isolates of *S.aureus*and Candida albicanswere obtained from the stock culture of Microbiology Diagnostic Laboratory, N.V.R.I., Vom, Plateau State. Susceptibility testing was carried out using agar well method.100, 50 and 25gm/mlconcentrations of the extract was used to determine zone of inhibition and water served as control. Minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) was conducted using tube method at double fold dilution. MIC and MBC of the extract were tested at concentrations of 25, 12.5, 6.25, 3.125, 1.5625 and 0.7812mg/ml. Ciprofloxacin 20mg/mlserved as positive control and replicated three time.The statistical analysis (One- way ANOVA) was employed at p>0.05, todetermine the significant differences between the different concentrations used. The result of the phytochemical of the plant seed revealed the presence of Glycosides, Tannins, Saponins, Alkaloids, Phenols, Steroids,and flavonoid. At the highest extract concentration (100mg/ml), the seed oil extract exhibited the highest antimicrobial activity of 20.7mm, while lower activity of 10.3mmwas noted at the concentration of 25mg/ml against the tested isolates. The result obtained from MIC, MBC and MFCshowed MIC at 3.125mg/ml, MBC at 6.25mg/ml and MFC at 12.5mg/ml respectively. The result showed significant difference between the concentrations used.The finding of this work suggest that seed oil extract of *V. Paradoxa* can be used against *S.aureus and C. albicans*

Index Terms- *Staphylococcus aureus, Candida albicans, Vitellariaparadoxa,* Antibacterial, Antifungal, Phytochemical.

I. INTRODUCTION

World Health Organization (WHO) investigation showed that more than 80% of the world's population use traditional medicine for treating illness, Ammara,*et al*., 2009 [1]. Use of herbal medicines in Nigeria reveals a long evidence of human interactions with the nature. The medicinal important of herbal plantslies in some chemical compounds that produce a specific chemical action on the human body. These bioactive compounds of plants of medical importance include alkaloids, flavonoids, tannins and phenolic compounds, Edeoga,*et al*., 2005[2]. Developing countries like Nigeriarelies on plant materials specially for herbal medicines, food, forage, construction of houses, making household equipments, sleeping mats, and for fire and shade. The use of botanicals as drugs is well known in rural areas of many developing countries, Sandhu and Heinrich, 2005[3] and Gupta et al., 2005 [4]. Herbal practitioners believes that their medicine is cheaper, more effective and causes less side effects as compared to synthetized drugs. In developing countries like Nigeria, poor people such as farmers, rural dwellers and native communities use traditional medicine for the treatment of common illness, Rojas,*et al*., 2006 [5].

The increasing microbial resistance to drugs has become a worrisome issue worldwide,Gardam, 2000 [6]. Medical practitioners consider drug-resistant microorganisms a major problem in the treatment of sick individuals, Lepape,*et al*., 2009 [7]. Expanding microbial resistance is necessitating a desire in research of the antimicrobial role of botanicala against resistant strains, Alviano and Alviano, 2009 [8]; Hemaiswarya,*et al*., 2008 [9]. High number of medicinal plants have been seen as important resources of organic antimicrobial substances, Mahady, 2005 [10]. These botanicals are said to contain plenty medical valuessuch as antimicrobial, antimutagenic, anticarcinogenic, antithrombotic and vasodilatory activities, Bidlack*et al*., 2000 [11]. It is therefore the aim of this study is to verify the antimicrobial activity of *Vitellariaparadoxa*plant so as to find an alternative for the common antibiotics presently in use.

II. MATERIALS AND METHODS

A. Study area

Plateau state is located between Latitudes 8.50°-100.46°North and Longitudes 8.20°-10.36°East in the North-Central zone of Nigeria (Dashe*et al*., 2013). The area falls under Natural Region II of Nigeria's agro-ecological zones, the climate of the area is humid with an average annual rainfall and temperature between 140-1480 mm and 10^0-32^0C respectively.

B. Preparation of the Seeds for Extraction

Shea butter (*V. paradoxa*) seeds (nuts) were obtained from Bida, Niger State, Nigeria. The seeds were pulverized and dried under room temperature for a 24hrs and followed byoven drying for three hours at 50 °C to ensure adequate removal of moisture content.

C. Extraction of Oil from Seeds

The collected seeds were dried in Oven at 70 ^{O}C to obtain a constant weight, pulverized. Soxhlet extractor with ethanol as solvent was used. The mixture was allowed to cool after

extraction, followed by filtration to remove solid particles. The resideu was concentrated under vacuum in a rotary evaporator, Akpan,*et al*., 2005 [12]. The obtained oil undergophysicochemical analysis to detect physical and chemical properties of the extracted seed oil. Standard reagents were used all through.

D. Gum removal and Purification

This was done according to the method of Akpan, *et al*. 2005 [12]. The extracted oil was subjected to 60^0C heating, followed by addition of activated carbon(purchased from the market) into the heated oil, this causes oil to change colour. Thedecolorized oil was mixed with sterilizeddistilled water thoroughly and subjected to heating again at 60^OC, stirred thoroughlyfor 15 minutes, sieved, cooled and the residue on the filter paper was discarded. The oil finally obtained was transferred into a 50ml conical flask and kept in a refrigerator until needed.

E. Preparation of Ethanolic Extracts

V. paradoxa seed oil extract (100g) was introduced into an air-tight sterile jars containing 100 ml of the solvent (95% ethanol) and kept on a shaker (150 rpm) with uniform shaking for 24 hours at 25^oC. The ethanol containing the extracts was removed using muslin cloth and followed with Whatman no. 1 filter paper respectively. Further extraction of the powdered samples was done with same volume of 95% ethanol, separated and filtered two more times. The filtrates from each round of extraction were combined and were evaporated to dryness in small, open - mouth jars and then packed in separate clean dry bottles and kept at 25^oC until need, Ajijolakewu and Awarun, 2015 [13].

Figure 1: Ethanolic extract

F. Quantitative analysis

For the separation of various fraction, 350g of *Vitelallariaparadoxa* seed oil extract was dissolved in 300ml of 70% ethanol and allowed to stand 24 hours in a separating funnel clipped to a stand. The tap was then opened to allow the dissolved extract to drain into an evaporating dish. The filtrate was concentrated to dryness at 60^0C using a water bath until the liquid portion was removed. About 25.3g of dried solid portion obtained was re-dissolved in 250ml of distilled water and shaken vigorously. The suspension was filtered and the filtrate used for separation into various phytochemical fractions. Glycoside fraction was extracted with 5% KOH in n-butanol, saponin was extracted with 10% HCL in n-butanol while alkaloid was extracted in chloroform. Flavonoid were obtained from the aqueous fraction of glycoside, saponin and alkaloid.

Figure 2: Quantitative analysis test.

G. Sterility of Extracts

Each of the extracts was examined for sterility. This was carried out by making serial dilution of 1 g of each extract $10^{-1}$to 10^{-6}. Ten microliters (10 µl) of the samplewere inoculated aseptically on the sterilized Nutrient Agar plates and incubated at 37 °C for 24 hours. The incubated media plates were observed for microbial growth. Absence of microbial growth on the media plate indicated sterility. Sterile extracts were used to test for antimicrobial study.

H. Sources of Bacterial and Fungal Isolate

Clinical isolates of *Staphylococcus aureus* and *Candida albicans* were obtained from the stock culture of Microbiology Section of Central Diagnostic Laboratory, N.V.R.I., Vom, Plateau State.

I. Standardization of the Test Organism

Five colonies of bacterial and fungal isolates were inoculatedinto a prepared sterilized nutrient broth and incubated at 37^o C for 24 and 48hrs respectively. Turbidity produced was adjusted to 0.5 McFarland Standard using a Nephelometer (TREK Diagnostics) for the bacteria while the fungal isolate was set at 10^6 CFU/ml, Agada,*etal* 2012 [14].

J. Determination of Antimicrobial Activity

Antimicrobial activity of the ethanolic extract of the plant sample was evaluated by the dics diffusion method. Four ditches were bored using a cork borer equidistant from each other. Using a micropipette, 100ul of the extract at predetermined concentrations (100mg/ml, 50mg/ml and 25mg/ml) was introduced into the ditches. The standardized bacterial inoculum was flooded on nutrient agar, while the fungal isolate was flooded on potato dextrose agar. There were done in triplicates, alongside the controls. Plates were incubated at 37^o C and 30^o C for bacteria and fungal isolates respectively for 24 h. Antibacterial and antifungal activity of the oil was examined by measuring zone of inhibition, Agada*etal.*, 2012 [14]. The antimicrobial effect of the ethanolic oil extract was expressed as the mean of the zones of inhibition (in millimeter).

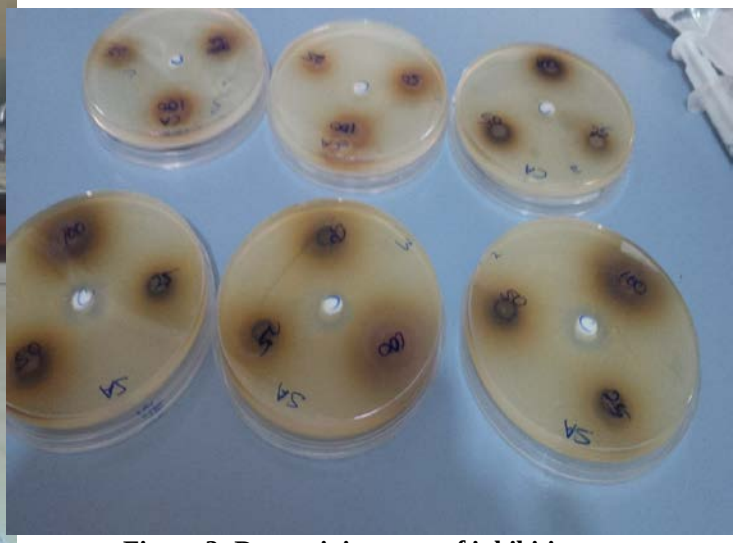

Figure 3: Determining zone of inhibition.

K. Determination of Minimum Inhibitory Concentration (MIC)

MIC of the oil was examined for each of the testmicroorganisms in triplicates. 0.5ml of different concentrations of the extracts (50, 25, 12.5, 6.25, 3.125 mg/ml), was added to 4.5ml of nutrient broth and then 100ul of the test organism were inoculated into the tubes. The bacteriological peptone was poured into test tube in appropriate was poured into test tube in appropriate volumes. Tubes one will contain only 1.0ml of the stock concentration. 10.0ml of stock was added in test tubes numbered 2 to 4. 1m1 of each extract was added into each of test tubes (numbered 2 to 4). Using pasture pipette, to achieve even dilution and distribution of extract within the broth, the contents were mixed thoroughly. 1.0ml of the mixture was withdrawn from tube 2 and transferred into tube 3 number 3 evenly mixed and incubated at 37^{0}C for 24 hours, after which microbial growth was observed. Those which were clear (no turbidity), do not have microbial growth. This was considered the minimum inhibitory concentration of the extract (the lowest concentrated that prevented visible growth).The experiment for positive control was conducted on the test organisms using purchased antibiotics (ciprofloxacin and ketoconazole, 20mg/ml for bacterial and fungal isolates respectively). Nutrient broth without extract inoculated with the test organisms as described above to serve as control. Tubes containing bacterial cultures were then incubated at 37^0 C for 24 h while tubes containing fungal isolates were incubated for 72h at 30^0 C. After 24 and 72hr, the incubated samples were then examined for visible turbidity indicating microbial growth,Agada,*etal.*, 2012 [14].

Figure 4: Determining MIC

M. Determination of Minimum Bactericidal and Fungicidal Concentration (MBC and MFC)

Using a sterile wire loop, aloopful of broth from incubated tubes showingno growth were streaked on sterile nutrient agar and potato dextrose agar for bacterial and fungi respectively. Nutrient agar and potato dextrose agar were streaked with the test organisms to serve as control. Plates inoculated with bacteria were then incubated at 37^{O} C for 24 h while fungi inoculated plates were incubated at 30^{O} C for 72 h. After incubation, the concentration at which no growth was observed was recorded as the MBC, Agada,*etal.*, 2012 [14].

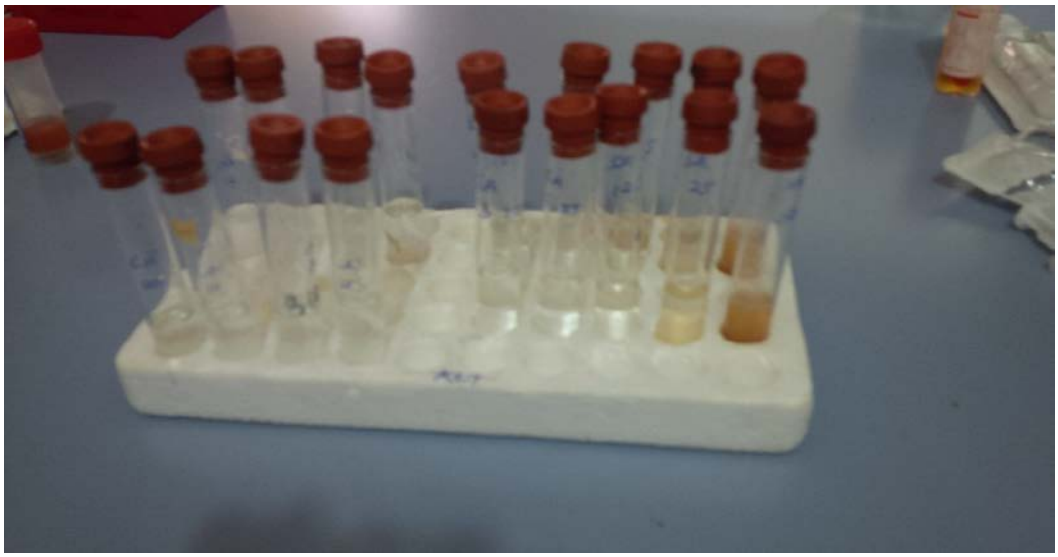

Figure 5: Determining MBC and MFC.

N. Statistical Analysis.

Data collected were analyzed using analysis of variance, ANOVA to determine the significant difference between the concentrations of the *Vitellariaparadoxa* plant extract and mean separation were made using Duncan multiple ratio test, DMRT.

III. RESULTS AND DISCUSSION

A. Results

Table 1: Phytochemical and quantitative composition of *Vitellaria*paradoxa seed oil extract

s/no	Sample	constituent	Test Reaction	Colour observed	Result	Quantity (%)
1	**V.paradoxa**	Saponins	Frothing	-	+	0.41
2	**seed oil**	Tannins	Precipitate	Dark green	+	0.27
	extract (25g)	Alkaloid	Precipitate	Reddish brown	+	0.25
3		Flavonoid	-	-	-	-
4		Phenols Steroid	Precipitate	Light brown	+	0.28
		Carbohydrate	-	-	-	-
5		Resins	Forming	Brown	+	0.27
6			Forming	Bluish	+	0.24
7						

8

Key :(+) = present (-) = absent.

Table 2: Effects of difference of concentrationsextract (mg/ml) on *Candida albicans and Staphylococcus aureus*

Concentration (mg/ml)	***Candida albicans*** **(mm)**	***Staphylococcus aureus*** **(mm)**
100	16.7[a]	20.7[a]
50	13.0[b]	17.6[b]
25	10.3[c]	13.6[c]
Control	0.00[d]	0.00[d]

Means followed by the same superscript are not significantly different (p>0.05) LSD at (0.05)

Table 3: Effects of Ethanolic Extract on Minimum Inhibitory Concentration against *Candida. Albicans*

	25	12.5	6.25	3.125	1.5625	0.7812	**MIC (mg/ml)**
C. albicans	-	-	-	+	+	+	**6.25**
+ control	20	10	5	2.5	1.25	0.625	**MIC (mg/ml)**
	-	-	-	-	-	+	**1.25**

Key: - no visible turbidity, + visible turbidity

Table 4: Effects of Ethanolic Extract on Minimum Fungicidal Concentration against *C. albicans*

	25	12.5	6.25	3.125	1.5625	0.7812	**MFC (mg/ml)**
C. albicans	-	-	+	+	+	+	**12.5**
+C.	20	10	5	2.5	1.25	0.625	
	-	-	+	+	+	+	**10**

Key: - no growth; + growth

Table 5: Effects of Ethanolic Extract on Minimum Inhibitory Concentration against *Staphylococcus aureus*

	25	12.5	6.25	3.125	1.5625	0.7812	**MIC (mg/ml)**
S. aureus	-	-	-	-	+	+	**3.125**
+ control	20	10	5	2.5	1.25	0.625	**MIC (mg/ml)**
	-	-	-	-	-	-	**0.625**

Key: - no visible turbidity, + visible turbidit

Table 6: Effects of Ethanolic Extract on Minimum Bactericidal Concentration against *Staphylococcus aureus*

	25	12.5	6.25	3.125	1.5625	0.7812	**MBC (mg/ml)**
S. aureus	-	-	-	+	+	+	**6.25**
+ C.	20	10	5	2.5	1.25	0.625	
	-	-	-	-	-	+	**1.25**

Key: - no growth; + growth

POSITIVE CONTROL: *C. albicans*Ketoconazole 20 mg/ml, *S. aureus* Ciprofloxacin 20mg

B.Discussion

Analysis of the plant extracts revealed the presence of phytochemicals such as tannins, flavonoids, saponins, glycosides, steroids, resins and alkaloids.The presence of tannin, alkaloid, Saponin, has been reported to possess antimicrobial activities on different organism,Akujobi,*et al*., 2004 [15]. Bioactive components of plant extracts affect the cell membrane integrity microorganisms, Ibekwe*et al.*, 2000 [16].

The zone of inhibition with highest diameter as indicated in table 4 was obtained using 100mg of the extract which gave 20.7mm and the lowest ZOI is 10.3mm at the lowest concentration (25mg). Decrease indifferent extract concentration led to a direct reduction of the inhibition zone around each test microorganism. Similar observationby Adamu,*et al.*2013 [17] and Arekemase,*et al*. 2013 [18]had suggested that higher concentrations of antimicrobial compounds gave appreciable antimicrobial efficacy.

The pattern of antimicrobial activities of the plant extracts and the zone of inhibition (mm) of the bacteria and fungi are in agreement with Adegboye,*etal*.2008 [19], who revealed varying degrees of against gram negative bacteria. The plant had profound activities against both fungi and bacteria. There was however, more activity against the bacteria organism than the fungi.Pelczar*et al*. 1993 [20] suggested that the difference in susceptibility of microoganismsto various antimicrobial agents probably depends onstructural differences in their cell walls. For example,amount of peptidoglycan, presence of receptors andlipids, nature of cross linking, activity of autolyticenzymes that determined the penetration, binding andactivity of the antimicrobial agents.The marked difference in the effects of the extracts onthe organism therefore, is suggestive of the activityagainst cell wall components of the organism. Theantimicrobial substance appears to exert antimicrobialactivity by inhibiting the growth of and by killing the sensitive microbe. This particular finding was alsoencountered by Emeruwa, 1982 [21] in his study on theantimicrobial substance from *Carica papaya* fruit extract.

Staphylococcus aureus and *Candida albicans* were susceptible to ethanolic seed oil extract of *V. paradoxa* at 50mg/ml and 25mg/ml, concentrations. This corroborated the claims of Esimone*et al*.2008 [22], that wider range of susceptibility are usually recorded by gram negative bacteria. The lowest inhibitory concentration of 6.25mg/ml and 12.5mg/ml recorded for *Candida albicans*and *Staphylococcus aureus* were similar to 12.5mg/ml of Combretumsp reported by Esimone*et al.* 2008 [22], inhibition against *Salmonella typhi*.

The lowest minimum bactericidal and fungicidal concentrations 6.25 and 3.125mg/ml respectively of the seed oil extracts against test organisms confirmed the findings of Anibijuwon and Udeze, 2009 [23] who posited that methanolic extracts of *Combretumadenogonium* showed activities against *S.aureus* This result also correlates with the work of Mann *etal*. 2011 [24] who reported that the methanolic and hexane extracts of *Bombaxbuonopozense* had antimicrobial activity against *Aspergillusniger*, *Staphylococcus aureus* and *Escherichia coli.*

IV. CONCLUSION

The result obtained in this research work indicatesthat the leaves of *Vitellariaparadoxa*containantimicrobial substances which is both bacteriostaticand bacteriocidaland fungistatic and fungicidal on *Staphylococcus aureus* and *Candida albicans.*However the varied widely in their degree of susceptibility to the plant extracts. Through such research as this, natural product of plant origin have been obtained for human use. For instance, *Aloe vera*product from Ghana are a source of export earning for that country. Several herbal products are known to be produced in india today including herbal toothpaste which are in Nigeria markets.Further studies involving other concentrations not considered in the current effort should be carried out.Other vegetative parts of the plant should be screened for their phytochemicals and their antimicrobial potentials.

ACKNOWLEDGMENTS

The authors thank the management of Federal College of Forestry, Jos for technical support through-out the study and NVRI Jos for supply of standard strain of bacteria and fungi used in the study.

REFERENCES

[1] H. Ammara, R. Salma, D. Farah, M. Shahid. 2009. Antimicrobial activity of some plant extracts having hepatoprotective effects. Journal of Medicinal Plants Research,2009, Vol. 3(1), pp. 020-023.

[2] H.O. Edoga, D.E. Okwu, B.O. Mbaebie. Phytochemicals constituents of some Nigerian medicinal plants. Afr. J. Biotechnol., 2005, 4(7): 685-688.

[3] D.S. Sandhu,M. Heinrich. The use of health foods, spices and other botanicals in the Sikh community in London. *Phytotherapy Res.*2005, 19:633–42.

[4] M.P. Gupta, P.N. Solis, A.I. Calderon, F. Guionneau-Sinclair,M. Correa,C. Galdames,C. Guerra,A. Espinosa,G.I. Alvenda,G. Robles,R. Ocampo. “Medical ethnobotany of the Teribes of Bocas del Toro, Panama”. J.Ethnopharmacol. 2005, 96:389–401.

[5] J.J Rojas,V.J. Ochoa,S.A Ocampo,J.F. Muñoz. Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: A possible alternative in the treatment of nonnosocomial infections. BMC Compelmentary and Alternative Medicine. 2006, 6:2.

[6] M.A Gardam. Is methicillin-resistant *Staphylococcus aureus*an emerging community pathogen? A review of the literature. Can. J. Infect. Dis. 2001, 11:202-211.

[7] A.D. Lepape. Monnet on behalf of participating members of the European Society of Intensive Care Medicine (ESICM). Experience of European 68 intensive care physicians with infections due to antibiotic-resistant bacteria. Euro Surveill. 2009, 14(45): pii=19393. Accessed November 10, 2011, at http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19393.

[8] D.S. Alviano, C.S. Alviano. Plant extracts: search for new alternatives to treat microbial diseases. Curr. Pharm. Biotechnol. 2009, 10:106-121.

[9] S. Hemaiswarya, A.K. Kruthiventi, and M. Doble. Synergism between natural products and antibiotics against infectious diseases. Phytomedicine2008, 15:639-652.

[10] G.B. Mahady. Medicinal plants for the prevention and treatment of bacterial infections. Curr. Pharm. Des. 2005 11:2405-2427.

[11] W.R. Bidlack, S.T. Omaye, M.S. Meskin, and D.K.W. Topham.*Phytochemicals asBioactive Agents*. CRC press, Boca Raton, FL. 2000.

[12] U.G. Akpan, A. Jimoh,A.D. Mohammed. Extraction, characterization and modification of castor seed oil. Leonardo Electronic Journal of Practices and Technologies, 2005.

[13] K. A.Ajijolakewu, F. J.Awarun. Comparative Antibacterial Efficacy of Vitellariaparadoxa(Shea Butter Tree) Extracts against Some Clinical Bacterial Isolates. *Not SciBiol*, 2015, 7(3):264-268.

[14] G.O.A. Agada, S.C. Chollom, J.G. Gotep, N.N. Gambo, A.D. Tyem, I.O. Okeke, O.O. Nwankiti,A.E.J. Okwori. Evaluation of Antimicrobial Potential of Ethanolic Leaf and Stem Bark Extracts of *Tamarindusindica. International Journal of Applied Microbiology Science*, 2012, 1, 26-34.

[15] C.O. Akujobi, B.N. Anyanuwa, G.O.C. Onyere and V.I. Ibekwe. Antibacterial activities and preliminary phytochemical screening of four medicinal plants. Journal of Applied Science, 2004, 7(3): 4328-4338.

[16] V.I. Ibekwe, K.C. Ubochi and B.N. Anyanwu. Prevalence of Penicillia Resistance in organisms that cause sexually transmitted diseases in Port Harcourt, Nigeria. Int. J. Environmental Health Res., 2000, 10: 251-255.

[17] H.M. Adamu, O.A.Ushie, E.Nansel. Antimicrobial activity of oil from *Butyrospermumparkii*seed (Shea butter). International Journal of Modern Biology and Medicine, 2013,3(2):50-59.

[18] M.O. Arekemase, G.P.Oyeyiola, K.I.Balogun. Assessment of bitter leaf (*Vernoniamygdalina*) on some selected pathogenic organisms from University of Ilorinteaching hospital. Journal of Microbiology, Biotechnology and Food Sciences2013, 2(5):2360-2365.

[19] M.F. Adegboye, D.A. Akinpelu,.A.Okoh. The bioactive and phytochemical properties of Garcinia kola (Heckel) seed extract on some pathogens. Afr. J. Biotechnol., 2008, 7(21): 3934-3938.

[20] M.J. Pelczar, E.S.C. Chan and N.R. Krieg. Airborne diseases. In: Microbiology Concepts and Applications. McGraw Hill, Inc. U.S.A, 1993, pp: 652.

[21] A.I. Emeruwa. Antibacterial substance from *Carica papaya* fruit extracts. J. Nat Prod, i982, 45(2): 123-127.

[22] C.O. Esimone, M.U. Adikwu, C.S. Nworu, F.B. Okoye, D.C. Odimegwu. Adaptogenic potential of Camellia sinensis leaves, Garcinia kola and Kola Nitida seeds. Sci. Res. Essays, 2008, 2(7):232-237.

[23] I.I. Anibijuwon, A.O. Udeze. Antimicrobial effects. Activity of *Carica papaya* (Pawpaw Leaf) on some pathogenic organisms of Clinical Origin from South-Western Nigeria. Ethanobotanical Leaflets, 2009, 13: 850-864.

[24] A. Mann, F.B. Salawn, I. Abdulrauf,. Antibacterial activity of Bombaxbuonopozense P. Beauv. (Bombacaceae) edible floral extracts. European Journal of Scientific Research, 2011, 48(4):627-630.

AUTHORS

First Author – JOHN WALTER C., MSc MICROBIOLOGY, FEDERAL COLLEGE OF FORESTRY, JOS. NIGERIA
Second Author – NANLIR JANFA, B. TECH. BOTANY,FEDERAL COLLEGE OF FORESTRY, JOS. NIGERIA
Third Author - OLADEJO AFOLABI, MS.c ENTOMOLOGY AND PARASITOLOGY,FEDERAL COLLEGE OF FORESTRY, JOS. NIGERIA
Fouth Author – YA'U HUSSAINI, BSc CHEMISTRY**,** FEDERAL COLLEGE OF FORESTRY, JOS. NIGERIA
Fifth Author – SUNDAY OLADIPO, MSc PHYSICS, FEDERAL COLLEGE OF FORESTRY, JOS. NIGERIA
Correspondence Author – JOHN WALTER CHINAKA, wchinaka@yahoo.com, 08036137846