ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

PHYTOCHEMICAL CONSTITUENTS AND ANTIMICROBIAL ACTIVITIES OF THE ROOT BARK EXTRACTS OF *Massularia acuminata spp.*

U. S. Ukekpe[1,2,*], H. M. Adamu[2], E.O. Ekanem[2] and A. A. Saleh[3].

1. Department of Science Laboratory Technology, Federal Polytechnic, Damaturu, Nigeria.
2. Chemistry programme, Abubakar Tafawa Balewa University, Bauchi, Nigeria.
3. Biological Sciences Programme, Tafawa Balewa University, Bauchi, Nigeria.

Manuscript Info

Manuscript History:

Received: 18 June 2015
Final Accepted: 29 July 2015
Published Online: August 2015

Key words:

Phytochemical, root bark, extracts, antibacterial, antifungal, medicinal values.

Corresponding Author

U. S. Ukekpe

Abstract

The root bark extracts of *Massularia acuminata* from Akwa Ibom State, Nigeria, (locally known as "okok edi") have been investigated for its phytochemical constituents and antimicrobial activities. Air dried pulverized root bark of the plant part was sequentially solvent extracted with hexane, chloroform, ethyl acetate, acetone, ethanol and methanol. The phytochemical screening of the extracts show the presence of alkaloids, anthraquinones, saponins, phlobatannins, cardiac glycosides, tannins, terpenoids, steroids, flavonoids and resins, but not across all the extracts. Ethyl acetate, acetone, ethanol and methanol extracts were assayed for their antimicrobial activities and all were found to be active against the clinical isolates, *Salmonella spp. Staphylococcus aureus, Eschurichia coli*, *Aspergillus nigger* and *Candida albicans* but at varied level. The presence of some phytochemicals with known pharmacological effects in the plant part extracts and their antimicrobial activities justify the traditional use of the plant part for medicinal purpose.

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Many of these isolations were based on the uses of the agents in traditional medicine (Doughari *et al*., 2008). Plants are potent biochemical factories and have been components of phytomedicine from the onset and their medicinal actions are unique to particular plant species and groups (Ara and Nur, 2009).

It is observed that certain diseases do not have drugs that can be used for routine management and also Hostettman (1997), noted that the efficacy of existing antifungal drugs is rather limited,

there is therefore the need for continuous search for new drugs, and medicinal plants are one of the useful areas of research in this regard (Ching *et al*., 2008).

The use of medicinal plants and their potency or otherwise is due to the presence of some chemical compounds in the plants that have physiological effects. Edeoga *et al*. (2005) stated that the medicinal value of plants lies in some chemical substances that produce a definite physiological action in human body. In many cases these chemical compounds serve as the molecules of plant defence against predation by micro-organisms, insects and herbivores (Mallikharjuna *et al*., 2007), and several of these compounds possess medicinal properties (Cowan, 1999).

The success story of chemotherapy can only lie in the continuous search for new drugs to counter the challenge posed by resistant strains of microorganisms (Doughari *et al*., 2008). If not, even some of the ailments that have cure may later be classified as having no cure due to drug resistant problem. Hence, the search for pharmacologically active compounds is imperative and many have been isolated from plants and introduced into clinical medicine.

The focus on plants for new drugs in recent years is mostly due to the diverse pharmacological properties of medicinal plants (Karthikumar *et al*., 2007). *Massularia acuminata* (D.Don) Bullock ex Hoyle, is a medicinal plant used for its antimicrobial property. It is a medium size shrub or small tree, growing up to 5m high (Iwu, 2014 and Yakubu and Omoniwa, 2012). It is a tropical plant found usually in the undergrowth of closed moist forest (Yakubu *et al*., 2008). It is a plant native to the tropical region of West Africa (Wong, 2014). In this work, the root bark of *Massularia acuminata spp* is investigated for its phytochemical constituents and antimicrobial activities. The result can show why the plant part is traditionally used for medicinal purpose.

METHODS.

Plant identification

The plant *Massularia acuminata (spp),* locally known as okok edi in Annang dialect of Akwa Ibom State was indentified through analysis of its macro-morphological features and other botanical profiles in the Botanical laboratory of University of Uyo, Uyo, Akwa Ibom State, Nigeria.

Sample collection and treatment

Fresh roots of *Massularia acuminata* were collected within Udi and Nto Etuk Udo villages in Ika Local Government Area of Akwa Ibom State. They were washed in water and re-washed in distilled water. The barks were then peeled-off from the roots, air-dried and ground to fine powder.

Extraction.

Soxhlet extractions were carried out using the solvents hexane, chloroform, ethyl acetate, acetone, ethanol and methanol sequentially in that order. Each extract was then concentrated using rotary evaporator, dried in moist free environment and kept for further use.

Detection of Phytochemicals.

Detection of Alkaloids: 5.0cm^3 of 2M HCl was added to 0.5g of each extract and warmed in a boiling water bath. The solution was then filtered and the presence of alkaloids in the extract was detected using Dragendroff's, Mayer's and Wagner's tests as described by Tiwari *et al*. (2011) and Goyal *et al*. (2013).

Detection of phenols: Ferric chloride test: A solution of 0.5g of each extract in 5cm^3distilled water was boiled and a few drops of 1% iron (III) chloride solution added. A blue black colouration indicated the presence of a phenolic hydroxyl group (Tiwari *et al*., 2011).

Detection of flavonoids: (a) 0.5g of extract was dissolved in 5cm^3 of distilled water and $NaOH_{(aq)}$ was added drop wise to 2cm^3 of the solution until a yellow colouration was obtained. A change in colour of the solution from yellow to colourless on addition of $HCl_{(aq)}$ indicated the presence of flavonoid (Ajayi *et al*., 2011).

(b) Lead acetate test**:** 0.5g of each extract was dissolved in 5cm^3 of distilled water and 1cm^3 of 10% lead acetate solution added. Observed colour change to yellow indicated the presence of flavonoids (Tiwari *et al*., 2011).

Detection of phlobatannins: Each plant extract was boiled in 1% HCl. The formation of red precipitate indicated the presence of phlobatannins (Edeoga, *et al*., 2005).

Detection of Terpenoids: The presence of terpenoids in the plant extracts was detected using Salkowski's test (Edeoga *et al*., 2005) and Harbone's test (Sofowora, 2008).

Detection of Anthraquinones: 0.5g of each plant extract was taken in a dry test-tube and 2cm^3 of chloroform was added and shaken for 5 minutes. The solution was filtered and equal volume of 10% ammonia solution was added to the filtrate and shaken. Observed red colour in the ammoniacal layer indicated the presence of anthraquinones.

Detection of Cardiac glycosides: Keller-Killiani test (Sofowora, 2008) was employed for the detection of cardiac glycosides.

0.5g of the plant extract was dissolved in 2cm^3 glacial acetic acid containing 1 drop of ferric chloride solution. 1cm^3 of concentrated H_2SO_4 was added gently through the side of the test-tube to the solution. Formation of a brown ring at the inter-phase indicated the presence of cardiac glycoside.

Detection of Saponins: The foam test (Sofowora, 2008) was used for the detection of saponins.

5cm^3 of distilled water was added to 0.5g of each plant extract in a test-tube and shaken vigorously. A stable persistent froth indicated the presence of saponins.

2 drops of olive oil was then added to the frothing solution. Formation of emulsion confirmed the presence of saponins.

Detection of Steroids: 0.5g of each extract was dissolved in $2cm^3$ of chloroform and $1cm^3$ acetic anhydride solution added followed by 2 drops of concentration H_2SO_4 added. An observed pink colour which changed to bluish green on standing indicated the presence of steroids/terpenes.

Detection of Tannins: Ferric chloride test (Sofowora, 2008) was used for the detection of tannins. 0.5g of each plant extract was dissolved in $10cm^3$ of distilled water and boiled. It was then filtered and a few drops of 0.1% ferric chloride added to $2cm^3$ of the filtrate. A colour change to brownish-green or blue black indicated the presence of tannins.

Biological activities of the extracts.

The antimicrobial activities of the plant extracts were measured against the following clinical isolates, *Salmonella spp*, *Staphylococcus aureus* and *Eschurichia coli* for antibacterial test, *Aspergillus niger* and *Candida albicans* for anti-fungal test.

The modified plate-well diffusion assay described by Uduak and Kola (2010) and Babu *et al.* (2007) was used for the tests. Wells were made in nutrient agar and sabourand dextrose agar (SDA) plates which had been previously incubated with bacteria and fungi respectively. The extracts ($0.2cm^3$) were introduced into the wells and left to pre-diffuse for 30 minutes. Distilled water was used as negative control and standard drugs, streptomycin for bacteria and fulcin for fungi were used as positive controls.

The bacterial plates were incubated at 37^oc for 24h, while the fungi plates were incubated at 25^oc for 72 h.

The degree of inhibition was determined by the size of the zone of inhibition measured in mm and was taken as evidence of antimicrobial activity of each of the extracts.

Minimum Inhibitory Concentration (MIC) and sensitivity test.

The method of Igbinosa, Igbinosa and Aligoro (2007) and Akinpelu and Kolawole (2004) was used in the estimation of minimum inhibitory concentration (MIC). $2cm^3$ aliquot of different concentrations of the extracts were added to $18cm^3$ of pre-sterilized molten nutrient agar and sabourand dextrose agar (SDA) for bacteria and fungi respectively at 40^oC. The medium was then poured into sterile petri-dishes and allowed to set. The bacterial and fungal cultures were then streaked on the surfaces of the media. The plates were then incubated at 37^oC for 24h for bacteria and 25^oC for 72 h for fungi. The plates were afterward examined for the presence or absence of growth. The lowest concentration that inhibited the growth of the test organisms was taken as the MIC and indication of lowest sensitive concentration.

RESULTS.

Table 1: Phytochemical constituents of extracts of root bark of *Massularia acuminata*.

Phytochemicals	Extracts					
	Hexane	Chloroform	Ethyl acetate	Acetone	Ethanol	Methanol
Alkaloids	-	-	-	-	-	+
Anthraquinones	-	-	-	-	+	+
Saponins	-	-	++	++	++	++
Phlobatanins	-	-	-	+	+	+
Tannins	-	-	-	+	+	+
Terpenoids	+	+	+	+	+	+
Steroids	-	-	-	+	+	+
Flavonoids	-	-	-	+	+	+
Cardiac Glycosides	+	+	+	+	+	+

(+) – Presence, (++) – Strong presence, (-) – Absence.

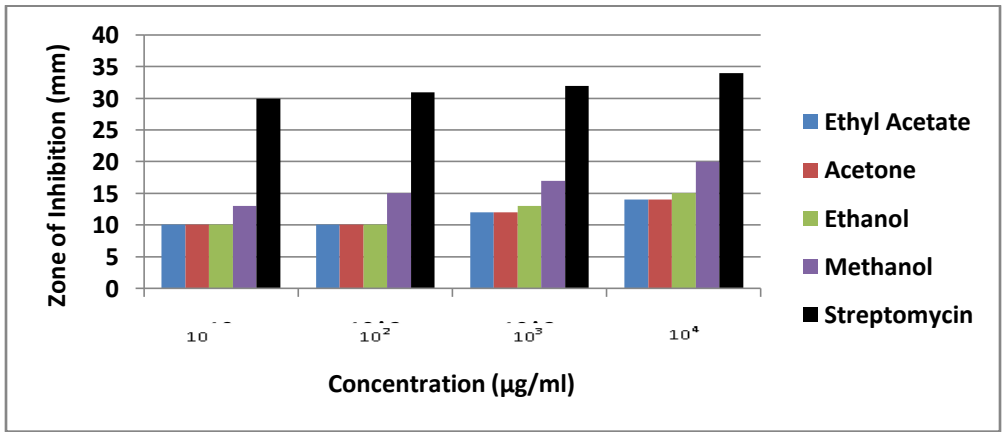

Figure 1: Activities (zone of inhibition) of the extracts against *Salmonella spp.*

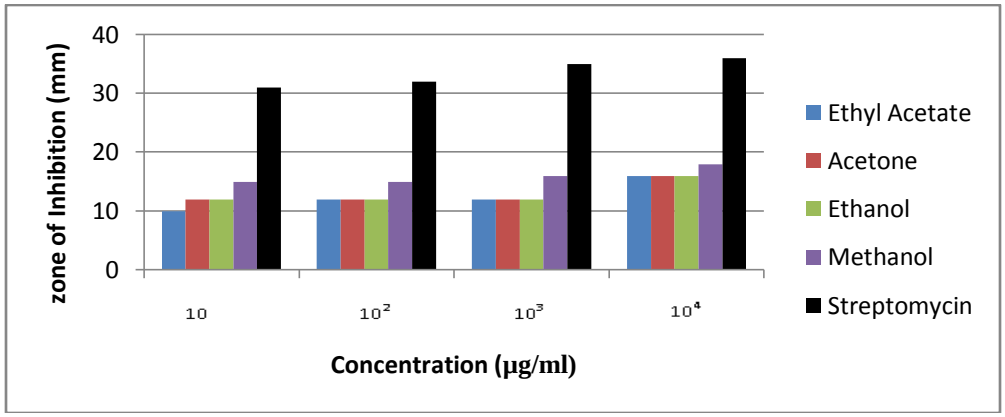

Figure 2: Activities (zone of inhibition) of the extracts against *Staphylococcus aureus.*

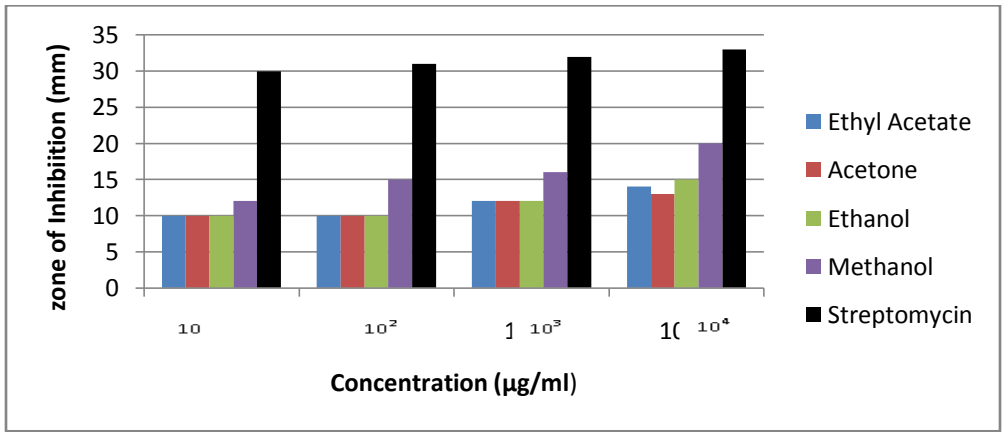

Figure 3: Activities (zone of inhibition) of the extracts against *Escherichia coli.*

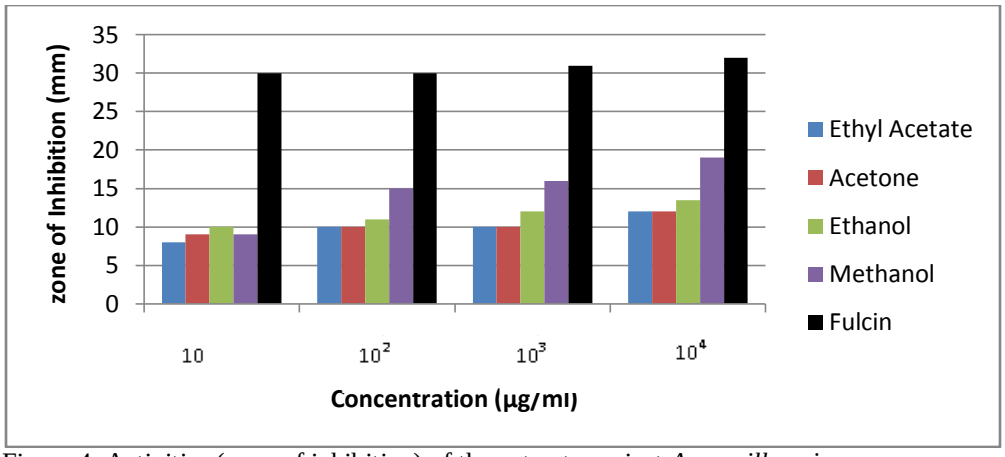

Figure 4: Activities (zone of inhibition) of the extracts against *Aspergillus nigger*.

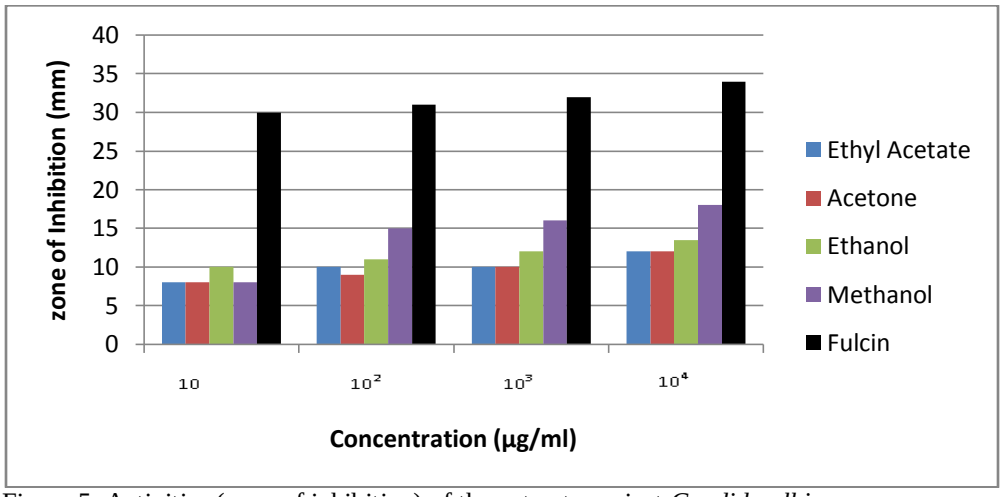

Figure 5: Activities (zone of inhibition) of the extracts against *Candida albicans*.

Table 2: Minimum inhibitory concentration (µg/ml) of Root bark Extracts of *Massularia acuminata* against some clinical isolates.

Test Organism	Extracts			
	Ethyl acetate	Acetone	Ethanol	Methanol
Salmonella Spp.	10^3	10^3	10^3	10
Staphylococcus aureus	10^2	10	10	10
Escherichia coli	10^3	10^3	10^3	10
Aspergillus nigger	10^4	10^4	10^2	10^2
Candida albicans	10^4	10^4	10^2	10^2

Table 3: Sensitivity test of Root bark extracts of *Massularia acuminata* at different concentrations (µg/ml) against some Clinical isolates.

Test Organism	Extract Conc. (µg/ml)	Extracts			
		Ethyl acetate	Acetone	Ethanol	Methanol
Salmonella Spp.	10^4	S	S	S	S
	10^3	S	S	S	S
	10^2	R	R	R	S
	10	R	R	R	S
Staphylococcus aureus	10^4	S	S	S	S
	10^3	S	S	S	S
	10^2	S	S	S	S
	10	R	S	S	S
Escherichia coli	10^4	S	S	S	S
	10^3	S	S	S	S
	10^2	R	R	R	S
	10	R	R	R	S
Aspergillus nigger	10^4	S	S	S	S
	10^3	R	R	S	S
	10^2	R	R	S	S
	10	R	R	R	R
Candida albicans	10^4	S	S	S	S
	10^3	R	R	S	S
	10^2	R	R	S	S
	10	R	R	R	R

KEY:
S = SENSITIVE
R = RESISTANT

DISCUSSION.

The results of phytochemical screening (Table 1) show Terpenoids and cardiac glycosides to be present in all the extracts. The analysis revealed the presence saponins in ethyl acetate, acetone, ethanol, and methanol extracts. Phlobatannins, tannins, steroids, and flavonoids were detected in acetone, ethanol and methanol extracts, while anthraquinones were present only in ethanol and methanol extracts, and alkaloids in only methanol extract. The variation in the phytochemical compostion of the extracts is as a result of the fact that secondary metabolites have different degrees of polarity (Yalavarthi and Thiruvengadarajan, 2013) and hence solubility in different solvents of varying polarity. These variations in the phytochemical composition with solvents have also been reported by others working on different parts of the plant. Akande and Ajao (2011) identified the phytochemicals anthraquinones, anthocyanins, flavonoids, saponins, steroids and tannins as the phytochemicals of *Mussularia acuminata* in their work. They have reported the absence of alkaloids and phlobatannins in both aqueous and ethanol extracts, and also the absence of steroids and anthraquinones in aqueous extract of *Massularia acuminata* stem. Bankole *et al.*, (2012) have reported the presence of tannins, anthocyanin and phlobatannins in aqueous stem extract, and along with saponins, glycosides, anthraquinones and flavonoids in ethanol extract but reported the absence of alkaloids in both aqueous and ethanol extracts, the absence of cardiac glycosides and anthraquinones in aqueous extract and the absence of phlobatannins in ethanol extract.

The presence of the detected phytochemicals in the extract can give medicinal property to the plant part as their presence and nature make plants to possess therapeutic activity and medicinal value (Ahmad, 2007).

The strong presence of saponins in the plant part could be part of the reasons why the plant is used as chewing stick. Kolapo *et al.,* (2009) have noted that plants used as chewing sticks are carefully selected for such properties as foaminess, hardness or bitterness, and saponins are the phytochemicals associated with foaminess. This strong presence is also expected to contribute fairly enough to the medicinal property of the plant part as pharmacological and medical properties are among the diverse range of properties of saponins (Vincken *et al*., 2007).

The results of antibacterial and antifungal assays performed for ethyl acetate, acetone, ethanol and methanol extracts against the clinical isolates *Salmonella spp*, *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus niger* and *Candida albicans* (Figures 1-5) show that all the five extracts were active at appropriate concentrations against the microorganisms. This is as expected based on the phytochemical composition of the extracts, Arif, *et al*., (2007), Arabski, *et al*., (2012) and Abad, *et al*., (2009) have listed saponins, terpenoids, flavonoids, phenolic compounds and steroids among the phytochemicals with antimicrobial effects and these phytochemicals are present in the extracts (Table 1).

The results show increase in antibacterial activity with increase in polarity of the solvent used in the extraction. This could mean that the concentrations of the phytochemicals were higher in more polar solvent extracts than the less polar solvent extracts. It is also noted that while some photochemical were not present in ethyl acetate extract, such were present in more polar solvent extracts.

The extracts generally show more activity against the bacteria than the fungi and each extract varied in activity towards each of the microorganisms compared with another extract. It is known that compounds of plant origin do not have the same antimicrobial effect on individual microorganisms, while some could be highly antibacterial, some could be highly anti-fungal.

Methanol extract show more activity against the microorganisms than the other extracts. It showed MIC of 10µg/ml against the bacteria, *Salmonella spp*, *Staphylococcus aureus* and *Escherichia coli*, and 100µg/ml for the fungi *Aspergillus niger* and *Candida albicans* being sensitive to the organisms at these concentrations. Ethanol extract show different MIC and sensitivity in respect of *Salmonella spp* and *Escherichia coli* from that of methanol extract, with acetone extract having similar MIC and sensitivity concentrations (Tables 2 and 3) with ethyl acetate extract except against *Staphylococcus aureus*.

CONCLUSION.

The findings of the study have shown the presence of some phytochemicals with attributes of medicinal properties in the root bark extracts of *Massularia acuminata*. The presence of the phytochemicals and the activities of the extracts against the microorganisms, *Salmonella spp.*, *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus nigger* and *Candida albicans* though at varied degrees show the plant part to possess medicinal value as it has been used traditionally.

REFERENCES.

Abad, M. J., Ansuategui, M. and Bermejo, P. (2007). Active Antifungal Substances from Natural sources. *ARKIVOC*. **VII** :116-145.

Ahmad, S. (2007). Pharmacognosy: Introduction of Plant Constituents and Their Tests. Retrieved March 25, 2012 from http://nsdl.niscair.res.in/jspui /bitstream/123456789/708/1/ corrected%20Introduction%20to%20 different%20 group%20of%20plant%20constitutes.pdf.

Ajayi, I. A., Ajibade, O. and Oderinde, R. A. (2011). Preliminary Phytochemical Analysis of Some Plant Seeds. *Research Journal of Chemical Sciences.* **1**(3): 58-62

Akande, T.A. and Ajao, A.T. (2011). Chemotherapeutic Values of Four Nigerian Chewing Sticks on Bacteria Isolates of Dental Infection. *Global Journal of Science and Frontier Research.* **11**(8): 91 – 95.

Akinpelu, D.A., Kolawole, D.O. (2004). Phytochemical and Antimicrobial Activity of Leaf Extract of *Poliostigma thonningii* (Schum). *Sc. Focus J*. **7 :**64 – 70.

Ara, N. and Nur, H. (2009) In-vitro Antioxidant Activity of Methanolic Leaves and Flowers Extract of *Lippia Alba*. *Research Journal of Medicine and Medical Sciences*. **4 (1)**: 107 – 110.

Arabski, M., Wegierek-Liuk, A., Czerwonka, G., Lankoff, A. and Kaca, (2012). Effect of Saponins Against Clinical *E.coli* strains and Eukaryotic cell live. *Journal of Biomedicine and Biotechnology*. **2012** : 1-6.

Arif, T. et al. (2009). Natural Products- Antifungal Agents from plants. Journal of Asian Natural Products Research **2**(7): 621-638.

Babu, S., Satish, S., Mohana, D.C., Raghavendra, M.P. and Raveesha, K.A. (2007) Anti-bacterial Evaluation and Phytochemical Analysis ofSome Iranian Medicinal Plants Against Plant Pathogenic *Xanthomanas pathovars*. *Journal of Agricultural Technology*. 307 – 316.
Bankole, P.O., Adekunle, A. A., Oyede, R.T., Faparais, F. and Adewole, A. (2012)
Antimicrobial Activities and Phytochemical Screening of Two Tropical Nigerian Chewing Sticks. *International Journal of Applied Sciences and Technology*. **2** (6): 131 – 138.

Ching, F., Omogbai, E. K. I, Ozolua, R. I. and Okpo, S. O. (2008). Antidiarrhoeal Activities of Aqueous Extract of *Sterespermum kunthianum* (Cham, Sandine Petit) Stem Bark in Rodents. *African Journal of Biotechnology*. **7 (9)**: 1220 – 1225.
Cowan, M. M. (1999). Plant Products as Antimicrobial Agents. *Clinical Microbiology Reviews*. **12**(4): 564-582.
Doughari, J. H., El-Mahmood, A. M. and Tyoyina, I. (2008). Antimicrobial Activity of Leaf Extracts of *Senna obtusifolia* (L). *African Journal of Pharmacy and Pharmacology.* **2** (1): 007-013.
Edeoga, H.O., Okwu, D.E. and Mbaebie, B.O. (2005). Phytochemical Constituents
of Some Nigerian Medicinal Plants. *African Journal of Biotechnology*. **4** (7): 685 – 688.

Goyal, A.K., Middha,S.K. and Sen,A.(2010). Evaluation of the DPPH Radical Scavenging
Activity, Total Phenols and Antioxidant Activities in Indian Wild *Bambusa vulgaris* "vittata"
Methanolic Leaf Extract. *Journal of Natural Pharmaceuticals*. **1**(1): 40-45.

Hostettmann, K. (1997) Strategy for the Biological and Chemical Evaluation of Plant Extracts. A lecture presented at the International Conference on Biodiversity and Bioresources: Conservation and Utilization on 23-27 November 1997. At Phaket, Thailand. Retrieved June 20, 2013 from htt://www.iupac.org/sympsion/proceeding/phaket97/hostettmaun.html.

Igbinosa, O. O., Igbinosa, E. O. and Aiyegoro, O. A. (2009). Antimicrobial Activity and Phytochemical Screening of Stem Bark Extracts from *Jatropha curcas* (Linn). *African Journal of Pharmacy and Pharmacology*. **3** (2): 58- 62.
Iwu, M. M. (2014) Handbook of African Medicinal Plants. Boca Raton. CRC Press, Taylor & Francis group. Pp253-254.
Karthikumar, S., Vigneswari, K., and Jegatheesan, K. (2007). Screening of Antibacterial and Antioxidant Activities of Leaves *Eclipta Prostrate* (L). *Science Res. Essay.* **2**(4): 101 – 104.

Kolapo, A. L., Okunade, M. B., Adejumobi, J. A. and Ogundiya, M. O. (2009). Phytochemical
Composition and Antimicrobial Activity of *Prosopis Africana* **5** (1): 90 – 93.

Mallikharjuna, P. B., Rajanna, L. N., Seetharam, Y. N. and Sharanabasappa, G. K. (2007). Phytochemical studies of *Strychnos potatorum* L.F. of Medicinal Plant. E-Journal of Chemistry. **4**(4): 510-518.
Sofowora, A. (2008). *Medicinal Plants and Traditional Medicine in Africa.* Ibadan. Spectrum Books Limited. Pp 182, 199-204.
Tiwari, P., Kumar, B., Kaur, M., Kaur, G, Kaur, H. (2011) Phytochemical Screening and
Extraction: A review. *International Phamaceutica Scienca.* **1**(1): 98-106.

Uduak, A. E. and Kola, K. A. (2010) Antimicrobial Activities of Some Enphobiaceae Plants Used in the Traditional Medicine of Akwa Ibom State of Nigeria. *Ethnobotanical Leaflets*. **14**: 654 – 664.

Vincken, J., Heng, L., de Groot, A. and Gruppen, H. (2007). Saponins, Classification and Occurrence in the Plant Kingdom. *Phytochemistry*. **68**: 275-297.
Wong, C. (2014). The Benefits of *Massularia Acuminata*. Retrieved December 12, 2014 from http.// altmedicine. About.com/od/iterbal Remedies /H/ The-Benefits-of-Massularia-Acuminata, htm.
Yakubu, M. T., Akanji, M, A., Oladiji, A. T. Adesokan, A. A. (2008). Androgenic Potentials of Aqueous Extracts of *Massularia acuminata* (G. Don) Bullock ex. Hoyle. Stem in Male Wistar Rats. *Journals of Ethnopharmacology*. **118**: 508-513.

Yakubu, M.T. and Omoniwa, B.P. (2012) Effects of Aqueous Stem Extract of Massularia Acuminata on Some Liver Function Indices of Male Rats. *Iranian Journal of Toxicology*. **6**: 716-722.

Yalavarthi, C. and Thiruvengadajan, V. S. (2013). A Review of Identification Strategy of Phyto Constituents Present in Herbal Plant. *International Journal of Research in Pharmaceutical sciences*. **4** (2): 123-140.