https://dx.doi.org/10.4314/bajopas.v4i1.2

Bajopas Volume 4 Number 1 June, 2011

Bayero Journal of Pure and Applied Sciences, 4(1): 10 - 16
Received: February, 2011
Accepted: May, 2011
ISSN 2006 - 6996

PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITIES OF SOME MEDICINAL PLANTS USED IN EBIRALAND

[1*]Alli, A. I., [2]Ehinmidu, J.O. and [2]Ibrahim, Y.K.E.
[1]Applied Microbiology Unit, Dept. of Applied Science, Kaduna Polytechnic, Kaduna.
[2]Dept. of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria.
*Corresponding author: ihimalli@yahoo.com

ABSTRACT
Dried leaves of Abrus precatorius, Desmodium ramosissimum, Scoparia dulcis and aerial parts of Phyllanthus amarus used in folkloric medicine of Ebiraland were extracted with hexane, methanol and water sequentially using soxhlet apparatus. The extracts were screened for antimicrobial activities against Bacillus subtilis, E. coli, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus and Candida albicans using the agar-cup diffusion protocol. All the extracts except the hexane extract of Abrus precatorius were active against at least two of the test microorganisms at the highest concentration of 100 mg/ml used in this study. The aqueous and methanolic extracts of Phyllanthus amarus were active against all the test microorganisms. The methanolic extract of Phyllanthus amarus also showed a broad spectrum of activity with a minimum inhibitory concentration (M.I.C.) of 1.56 mg/ml against all the test microorganisms. The extracts were also screened for secondary metabolites and the result indicated the presence of alkaloids, saponins, tannins and terpenoids. The results of this investigation, appears to justify the ethnomedicinal uses of these plants for the traditional treatment of infectious diseases. However, further investigation is required to obtain more information on their antimicrobial potentials and also to isolate their bioactive compounds.

Keywords: Medicinal plants, antimicrobial activity, minimum inhibitory concentration, phytochemical screening.

INTRODUCTION

Medicinal plants have been used since time immemorial in virtually all societies as source of medicine to combat various ailments including infectious diseases. The World Health Organization (W.H.O.) reported that over 85% of the populations in Sub-Sahara Africa, including Nigeria still depend on herbal traditional medicine for their healthcare needs (W.H.O., 2002). The organization advocates the exploitation of those aspects of it that provides safe and effective remedies for use in primary health care. The organization emphasized, in particular, the importance of scientific investigations into herbal medicines. Hoareau and Da Silva (1999) noted that medicinal plants and herbal remedies are reemerging medical aids whose contribution and significance in the maintenance of good health and well-being is widely accepted. Alves and Rosa (2007) also attest to the important role of the folk or traditional medicinal use of plants in modern drug discovery. They noted that there are about 121 pure chemical substances extracted from about 130 species of higher plants in the modern pharmacopeias throughout the world. Out of these, 89 plant-derived drugs currently used in modern medicine were originally discovered through the study of traditional cures and folk knowledge of indigenous people.

Infectious diseases are currently the world's leading causes of premature deaths, killing almost 50,000 people every day (W.H.O., 2002). The control of these diseases has pose new challenges because of the emergence of multiple drug resistance among several pathogens to some of the antimicrobial drugs commonly used in the treatment of infectious diseases. The problem is further compounded by the indiscriminate use of antibiotics (Davis, 1994). In addition to this problem, some antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression, allergic reactions and even loss of hearing (Ahmed *et al.*, 1998). This situation necessitates the continued search for new antimicrobial substances. Much attention is now focused on plant extracts with biologically active compounds from plant species used in traditional herbal medicine. Many workers have presented data relating to comprehensive investigations of extracts from different plants, which has resulted in successful inhibition of many microorganisms. Fabricant and Farnsworth (2001) noted that plant based antimicrobials represent a vast untapped source of antimicrobials. Antimicrobials of plant origin have enormous potentials. They are noted to be effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Iwu *et al.*, 1999). In this study, extracts(aqueous, hexane and methanolic) of four plants extensively used in herbal medicine either singly or in combinations in Ebiraland, North – Central Nigeria, were screened for their antimicrobial activity

against five species of bacteria and one fungal species(*Bacillus subtilis, E.coli, Pseudomonas aeruginosa, Salmonella typhi Staphylococcus aureus* and *Candida albicans)*. This was done in order to confirm their potency and to determine their antimicrobial potentials.

MATERIALS AND METHODS

Ethno medicinal Survey of Ebira-land

Information on the plants was gathered through structured questionnaire (Sofowora, 2008) and oral interviews with some local medicine men who claimed to have effective prescriptions for common infectious diseases, from Adavi, Ajaokuta, Okehi and Okene Local Government Areas of Kogi State, Nigeria.

Collection, Identification and Authentication of the Plants

The plants were collected by accompanying these local medicine men in the various localities to the field. The plants were then identified at the Department of Biological Sciences, Ahmadu Bello University, Zaria where voucher specimens were deposited.

Extraction of Plant Materials

The leaves of *A. precatorius, D. ramosissimum* and *S. dulcis* were used while the entire aerial part of *P. amarus* was used. The plant materials were dried at ambient temperature for between 5-9 days. The dried plants were pulverized into fine powder using porcelain pestle and mortar. The powdered material (500g each) was packed into soxhlet extractor and extracted exhaustively and successively with n-hexane, methanol and distilled water. The various extracts were respectively concentrated using rotary evaporator at 40^{O}C. The extracts were subsequently transferred into clean containers. The weight of the crude extracts were measured and recorded as percentage yield.

Crude Extract Preparation

100 mg/ml of the various extracts were prepared in distilled water. Where an extract is not soluble in water, 10% solution of Dimethyl sulphoxide (DMSO) in water was used.

Phytochemical Screenings

Screening for carbohydrates, tannins, alkaloids, saponins, flavonoids, steroids/terpenoids, cardiac glycosides and anthraquinone was carried out by standard methods (Trease and Evans, 1989).

Test Microorganism

Standard strains of the following microorganisms sourced from the stock of Dept. of Pharmaceutics and Pharm. Microbiology, A.B.U., Zaria and Dept. of Pharm. Microbiology /Biotechnology, National Institute of Pharmaceutical Research and Development, Abuja. These include *Bacillus subtilis, Candida albican, E. coli, Pseudomonas aeruginosa, Salmonella typhi, and Staphylococcus aureus.*

Preparation of Media/ Inocula

The bacteria strains were tested for sterility on Nutrient agar and then grown in Nutrient broth at 37 ^{oC} for 24hr while the fungal species was tested on Sabouraud Dextrose Agar and cultured in Sabourand Dextrose Liquid Medium at 25^{oC} for 24hr. The overnight cultures were subsequently diluted and standardized spectrophotometrically to give approximately 10^6 cfu/ml.

Antimicrobial Susceptibility Test

The agar cup diffusion method was adopted for both bacteria and fungi. Nutrient Agar and SDA plates were flooded with the appropriately standardized culture of the organisms and the excess drained off. Wells (7mm) were bored into the inoculated plates using sterile cork borer. The wells were filled with the extracts with the aid of Pasteur pipette. Standard antibiotics were used as positive controls while sterile distilled water and DMSO served as negative controls. Diameters of zones of inhibition were determined after incubating plates at 37^{oC} for 24 hr (bacteria) and 25^{oC} for 48hr (fungus). The experiments were replicated and the zones of inhibition (mm) expressed as the mean and standard errors on means recorded.

Determination of Minimum Inhibitory Concentration (M.I.C.)

M.I.C. was determined by a modification of the agar dilution method (NCCLS, 2000).The extracts were sterilized using Corning sterile syringe filter (0.2µm pore size). 10ml of the double strength of the various extract concentrations (100, 50, 25.0, 12.5, 6.25, 3.125, 1.56, and 0.78mg/ml) were incorporated into 10ml of double strength molten agar at 50^{o}C and aseptically poured into Petri-plates. After setting, sterile paper discs (6mm) in duplicates were aseptically applied to the surface of the set agar containing the various extract concentrations. 10µl of each standardized inoculum (10^6cfu/ml) was then spot-inoculated onto each disc and allowed to diffuse for 20 mins before incubating at 37 ^{oC} for 18 hrs (bacteria) and 25 ^{oC} for 24 hrs (fungi). The first lowest concentration that showed no visible growth of the inoculated test organism was recorded as the M.I.C. of the extract for the test organism.

Determination of Minimum Bactericidal / Fungicidal Concentration (M.B.C. / M.F.C)

All inoculated paper discs showing no visible growth from the M.I.C. determination were transferred to 5ml of sterile Nutrient Broth containing 5% Tween 80 to neutralize the effect of the extracts and incubated for another 18 hrs. The discs from the lowest concentration of each plant extract that showed no visible growth (cloudiness) was taken as the M.B.C. of that plant extract against the test organism.

RESULTS

Table 1 provides the Ethnobotanical information gathered about the plants. It includes the botanical names, local names, parts used percentage frequency of mention and the traditional therapeutic uses of the selected plant species in Ebiraland. The characteristics and percentage yield of the various solvent extractions from the test plants are presented in Table 2. The result of the preliminary phytochemical screening is presented in Table 3.

The aqueous and methanolic extracts from the four plant species showed abundance of carbohydrates, flavonoids, and tannins. The hexane extracts were very rich in steroids / terpenoids. Cardiac glycosides were found only in the methanolic extracts of *S. dulcis* and *P. amarus* and in aqueous extract of *A. precatorius*. The *in vitro* antimicrobial activities of the extracts are shown in Table 4.

Aqueous extract of *A. precatorius* inhibited all the test bacteria but has no effect on the fungal strain. The methanolic extract of this plant inhibited only two of the test bacteria while the hexane extract has no activity against the entire test microorganisms.

Hexane extract of *D. ramosissimum* was active against all the test bacteria but has no activity against the fungus. The aqueous and methanolic extracts of *D. ramosissimum* were active against two bacteria each. The aqueous extract of S. dulcis was active against four of the test bacteria only. The methanolic and hexane extract of S. dulcis inhibited three of the test bacteria and the fungal strain. The aqueous and methanolic extracts of *P. amarus* were active against all the test microorganisms. The hexane extract was less active.

The result of the determination of minimum inhibitory concentration is shown in Table 5. The M.B.C. /M.F.C. values are presented in Table 6.

Table 1: Ethnobotanical information on the plant species.

Plant species	Local name (Ebira)	Voucher No	Part used	Percentage Frequency of Mention (%)	Ethnomedicinal uses
Abrus precatorius	Ohinohine – orupa	932	Leaves	80	Tuberculosis, Sore throat, Cough, Aphrodisiac and Diabetes
Desmodium ramosissimum	Ema (oweyi)	879	Leaves	60	Diarrhea, Dysentery, Fever, Pulmonary troubles, Cough, Venereal diseases and Jaundice
Phyllanthus amarus	Avi – ogogirema	3073	Aerial	100	Tuberculosis, Diarrhea, Venereal infection and Poison antidote
Scoparia dulcis	Ohinohine – sesere	555	Leaves	80	Tuberculosis, Sore throat, Cough, Gonorrhea and Diabetes

Table 2: Characteristic and percentage yield of crude extracts.

Plant sp	Extracting solvent	Colour/texture of extract	Percentage yield (%)	Extract code
A. Precatorius	Water	brown solid	2.48	012
,,	Methanol	black solid	3.24	01
,,	hexane	greenish black solid	1.02	02
D. ramosissimum	Water	black solid	0.84	03
,,	Methanol	brown solid	6.42	04
,,	Hexane	black congealed mass	2.08	05
S. dulcis	Water	black solid	2.44	06
,,	Methanol	black solid	5.20	07
,,	Hexane	black congealed mass	1.244	08
P. amarus	Water	brown solid	3.36	011
,,	Methanol	black solid	3.40	09
..	Hexane	black congealed mass	2.00	010

Table 3: Phytochemical Screening of the Various Extracts.

Extract Code	Metabolites								
	Alkaloid	**Saponin**	**Tannin**	**Cardiac glycoside**	**Steroid/ Terpenoid**	**Anthra-quinone**	**Flavanoids**	**Phenol**	**CHO**
01	+ +	+ +	+ +	+	+	+	+ +	-	+
02	-	-	-	-	+ +	-	-	-	-
03	-	-	+	-	-	-	+	+	-
04	-	+	+ +	-	-	-	+ +	+	+ +
05	-	-	-	-	+	-	-	+	-
06	+	-	+	-	-	+	+ +	-	+
07	+	+ + +	+	+ +	+	+	+	+	+ +
08	-	-	-	-	+ +	-	-	+	-
09	+ +	+ +	+	+	+ +	-	+	+	+
010	+ +	-	-	-	+ + +	-	-	-	-
011	+	+ +	+ + +	-	-	-	+	+	+ + +
012	-	+	+ +	+	-	+	+	-	+ +

Key: CHO ⇒ Carbohydrate **+ + +** ⇒ Appreciable amount **+ +** ⇒ Moderate amount
+ ⇒ Trace amount - ⇒ Absent

Table 4: Susceptibility Profile of Test microorganisms in Response to 100mg/ml/cup of The Four Plants Extracts.

EXTRACT CODE	PLANT	EXTRACTING SOLVENT	TEST ORGANISMS					
			Bs	***Ec***	***Pa***	***St***	***Sa***	***Ca***
			Zone of Inhibition (mm)/ SD					
12	*Abrus precatorius*	Aqueous	18 ± 0.0	19.25 ± 1.5	22 ± 0.0	18 ± 1.16	18.5 ± 0.86	-
01	*Abrus precatorius*	Methanol	16 ± 0.0	-	16 ± 0.0	-	-	-
02	"	Hexane	-	-	-	-	-	-
03	*Desmodium ramosissimum*	Aqueous	-	15.75 ± 0.83	14 ± 0.0	-	-	-
04	"	Methanol	18 ± 0.0	-	18 ± 0.0	-	-	-
05	"	Hexane	19 ± 1.0	13.5 ± 0.58	15.5 ± 0.5	13.5 ± 0.58	16 ± 0.0	-
06	*Scoparia dulcis*	Aqueous	20 ± 0.0	16 ±0.0	19 ± 0.86	-	18.5 ± 0.86	-
07	"	Methanol	17 ± 1.0	-	18 ± 0.0		20 ± 0.0	16 ± 1.63
08	"	Hexane	19.5 ± 0.86	-	14.5 ± 0.5	-	17 ± 1.0	17 ± 0.0
09	*Phyllanthus amarus*	Methanol	23 ± 1.0	15.75 ± 0.83	24 ± 0.0	17 ± 0.82	22 ± 0.0	16 ±0.0
10	"	Hexane	12 ± 0.0	-	14 ± 0.0	-	15 ± 1.0	-
11	"	Aqueous	21.5 ± 0.86	17.75 ± 0.83	21 ± 0.86	18 ± 0.82	22.5 ± 0.86	18 ± 0.0
Positive Controls	OXOFLOXACIN DISK(5µg)		23.6 ± 2.08	23 ± 0.0	25.33 ± 2.30	30 ± 0.0	27 ± 3.0	
	GRISEOFULVIN (100mg/ml)		-	-	-	-	-	26 ± 0.0
Negative Controls	DMSO		-	-	-	-	-	-
	Diethyl ether		-	-	-	-	-	-

Bs – *B. subtilis;* Ec – *E. coli;* Pa – *P. aeroginosa;* Sa – *S. aureus;* St – *S. typhi;* Ca-*C. albicans*

Table 5: Minimum Inhibitory Concentration (M.I.C.) of the Crude Extracts against Test Microorganisms.

Plant Species	Extracting Solvent	M.I.C. (mg/ml)					
		Bs	***Ec***	***Pa***	***St***	***Sa***	***Ca***
Abrus precatorius	Water	6.25	12.5	25.0	25.0	1.56	ND
,,	Methanol	1.56	ND	25.0	ND	ND	ND
,,	Hexane	ND	ND	ND	ND	ND	ND
Desmodium ramosissimum	Water	ND	25.0	50.0	ND	ND	ND
,,	Methanol	3.125	ND	50.0	ND	ND	ND
,,	Hexane	12.5	12.5	12.5	12.5	12.5	ND
Scoparia dulcis	Water	1.56	3.125	12.5	100.0	1.56	ND
"	Methanol	1.56	ND	1.56	100.0	1.56	6.25
"	Hexane	25.0	ND	25	ND	50.0	12.5
Phyllanthus amarus	Water	1.56	12.5	12.5	12.5	1.56	25.0
	Methanol	1.56	1.56	1.56	1.56	1.56	1.56
	Hexane	25.0	ND	25.0	ND	25.0	ND

Bs – *B. subtilis;* Ec – *E. coli;* Pa – *P. aeroginosa;* Sa – *S. aureus;* St – *S. typhi;*
Ca – *C. albicans.* ND-No Antimicrobial Activity @ >100 mg/ml

Table 6: Minimum Bactericidal/ Fungicidal Concentration (M.B.C. /M.F.C.) of the Crude Extracts against Test Microorganisms.

Plant Species	Extracting Solvent	M.B.C./M.F.C (mg/ml)					
		Bs	***Ec***	***Pa***	***St***	***Sa***	***Ca***
Abrus precatorius	Water	12.50	25.0	6.25	100	1.56	ND
,,	Methanol	3.125	ND	50.0	ND	ND	ND
,,	Hexane	ND	ND	ND	ND	ND	ND
Desmodium ramosissimum	Water	ND	50.0	100	ND	ND	ND
,,	Methanol	6.25	ND	100	ND	ND	ND
,,	Hexane	25.0	100	25.0	25.0	50.0	ND
Scoparia dulcis	Water	100	25.0	50.0	100	50.0	ND
"	Methanol	12.5	ND	25.0	ND	12.5	12.5
"	Hexane	50.0	ND	100	ND	100	ND
Phyllanthus amarus	Water	50.0	25.0	100	100	3.125	ND
"	Methanol	12.5	50.0	6.25	100	3.125	3.125
"	Hexane	50.0	ND	50.0	ND	50.0	ND

Bs – *B. subtilis;* Ec – *E. coli;* Pa – *P. aeroginosa;* Sa – *S. aureus;* St – *S. typhi,* Ca – *C. albicans*
ND-No Antimicrobial Activity @ >100 mg/ml

DISCUSSION

Infectious diseases are still a major challenge to health issues all over the world. The emergence of resistance to antibiotics has further compounded the problem. The need to source for new antimicrobials has become imperative and urgent to curtail this menace. The use of medicinal plants can play a vital role in improving human health. However, there is the need to use plant extracts with known antimicrobial properties to improve efficiency of treatment regimes in traditional medicine.

Response to this ethno-botanical survey showed that all the test plants are used for treating infectious diseases such as tuberculosis, venereal disease, diarrhea and dysentery. *Phyllanthus amarus* was the most popular with the herbalists sampled. Igoli *et al* (2005) have earlier reported that *A. precatorius, S. dulcis* and *P. amarus* are widely used among the Igede people of North-central Nigeria for treating various ailments such as cough, tuberculosis, eye problems, and venereal diseases. The three plants were also reported to be used extensively in herbal medicine in South-eastern Nigeria as remedy for cough, sore throat and gonorrhea (Edeoga,*et al*,2005). *D. ramosissimum* was also reported to be used for traditional treatment of dysentery, eye diseases and fever in Bauchi state of Nigeria (Adamu *et al,* 2005).

The highest extract yield was obtained from methanol extraction of *D. ramosissimum* (6.42%) while the least extract yield (0.84%) was obtained from water extraction of the same plant. Methanol consistently gave higher extract yields from all the test plants compared to water and hexane extracts.

The *in vitro* antimicrobial activities of the extracts showed that only the hexane extract of *A. precatorius* displayed no antimicrobial activity against any of the test microorganisms. All the other extracts inhibit at least two of the test microorganisms. The aqueous extract of *A. precatorius* inhibited all the test bacteria but has no activity against the fungal strain. The zones of inhibition range between 18.0 to 22.0 mm. The methanolic extract of this plant showed marginal activity (16.0mm) against *B.subtilis* and *P. aeruginosa*. Our result is at variance with those of Parekh and Chanda (2005) who reported that the leaf extracts of *A. precatorius* displayed no antibacterial activity, but it is in accordance with that of Adelowotan *et al* (2008) who also found out that the leaf extract was active against some selected bacteria pathogens.

The hexane extract of *D. ramosissimum* was found to be the most active against all the test bacteria with zones of inhibition ranging from 13.5 to19.0mm. The extract was not active against the test fungus. Aqueous and methanolic extracts of this plant were less active in that they both inhibited two of the test bacteria only. *E. coli* and *P. aeruginosa* were marginally susceptible to the aqueous extract while the methanolic extract has equal activity (16.0mm) against both *B. subtilis* and *P. aeruginosa*. This probably suggested that the bioactive substances were soluble in non-polar organic solvents, and therefore, not present in water and methanol extracts as earlier observed by De-Boer *et al* (2005). Despite few published reports (Trout, 1997 and Adamu *et al*, 2005) on the ethno botanical information of this plant, no previous report of the antimicrobial activity of this plant species could be found in literature. This investigation probably reported the antimicrobial activity of *D. ramosissimum* for the first time.

All the three extracts from *S. dulcis* were active against some of the test microorganisms. The aqueous extract of this plant inhibited four of the test bacteria only while both the hexane and methanol fractions inhibited three of the test bacteria and the fungal strain. Similar antimicrobial activities of this plant have earlier been reported by Latha *et al* (2006) and Yisa (2009). However, our result differs from those of Latha *et al* (2006) who observed that *Salmonella typhi* was susceptible to the methanolic extract of this plant.

All the extracts obtained from *P. amarus* displayed varied levels of antimicrobial activities. The aqueous and methanolic extracts of *P. amarus* exhibited a broad spectrum of activity against all the test microorganisms with zones of inhibition ranging from16.0 to24.0mm. The methanolic extract of this plant compared favourably in terms of the zone of inhibition with the standard antibiotic disc (oxofloxacin) against both *Bacillus subtilis* and *P. aeruginosa* (Table 4). The hexane extract was less active with marginal inhibitory activity against three of the test bacteria only. Several authors (Adegoke *et al*, 2010; Okoli, *et al*, 2009; Olufemi and Debiri, 2008; Sule and Agbabiaka, 2008; Onocha *et al,* 2003 and Odetola & Akojenu, 2000) have all reported on the antimicrobial properties of this plant. However, the solvents used for extraction and the microorganisms tested were not entirely the same with this present study.

The result of the determination of minimum inhibitory in Table 5 showed that the methanolic extract of *P. amarus* demonstrated a more potent inhibitory effect against all the test microorganisms with M.I.C. value of 1.56mg/ml. This suggested that the extract possessed a broad spectrum of activity against both the bacteria and the fungal strain as earlier observed by Yisa, 2009 and Latha *et al*, 2006. The M.I.C. and M.B.C. values (Table 6) were equal for only the aqueous extract of *A. precatorius* against *S. aureus* indicating that the extract could be bactericidal to this organism. The M.B.C. values of the methanolic extract of *P. amarus* were slightly higher than their M.I.C. values for both *S. aureus* and *C. albican* suggesting possible bactericidal and fungicidal activity against these test organisms respectively. All the remaining M.B.C. /M.F.C. values were found to be far above their corresponding M.I.C. values indicating that the extracts were merely bacteriostatic or fungistatic.
In general, the most susceptible test bacterium to the extracts was *P. aeruginosa* which was inhibited by eleven of the extracts. On the contrary, *S. typhi* proved to be the most difficult to inhibit with only slight susceptibility to four of the extracts. The fungal strain (*C. albicans*) was also marginally susceptible to only four of the extracts.

CONCLUSSION

From this study, it can be conclude that all the plants possessed antimicrobial properties. They, however, differ widely in their antimicrobial activities. This investigation provides scientific support to the ethnomedicinal uses of these plants for managing infectious diseases and, therefore, merits further studies to isolate the bioactive compounds responsible for the observed antimicrobial activities.

REFERENCES

Adamu, H.M., Abayeh, O. J., Agho, M.O., Abdullahi, A.L., Uba, A., Dukku, H.U. and Kebede, B.M.(2005). An Ethnobotanical survey of Bauchi State herbal plants and their antimicrobial activity. *Journal of Ethnopharmacology.* Vol. 97(1):421-427.

Adegoke, A.A., Iberi, P.A., Akinpelu D.A., Aiyegoro, O. A., Mboto C.I. (2010). Studies on phytochemical screening and antimicrobial potentials of *Phyllanthus amarus* against multiple antibiotic resistant bacteria. *International Journal of Applied Research in Natural Products.* Vol. 3 (3): 6-12.

Adelowotan, O., Aibinu, I., Adenipekun, E., and Odugbemi, T. (2008). The *in vitro* antimicrobial activity of *Abrus precatorius* (L) fabaceae extract on some clinical pathogens. *Nigeria Postgraduate Medical Journal.* Vol. 15 (1): 32-37.

Ahmed,I.; Mehmood, Z.and Mohammed, F. (1998). Screening of some Indian medicinal plants for their antimicrobial properties. *Journal of Ethnopharmacology.* Vol.62: 183-193.

Alves, R.R.N. and Rosa, I.L. (2007). Biodiversity, traditional medicine and public health : where do they meet? *Journal of Ethnobiology and Ethnomedicine.* Vol. 3: 14 -44.

Davis, J. (1994). Inactivation of antibiotics and the dissemination of resistance genes. *Science.* Vol. 264: 375-382.

De Boer, H.J., Kool, A., Mziray, W.R., Heldberg, I. and Levenfors, J.J. (2005). Antifungal and Antibacteria activity of some herbal remedies from Tanzania. *Journal of Ethnopharmacology.* Vol. 96(10: 461-469.

Edeoga, H.O., Okwu, D.E. and Mbaebie, B.O. (2005). Phytochemical constituent of some Nigerian medicinal plants. *African Journal of Biotechnology.* Vol. 4 (7): 685-688.

Fabricant, D.S. and Farnsworth, N.R. (2001). The value of plants used in Traditional medicine for drug discovery. *Environmental Health Perspective.* Vol. 11: 69-75.

Hoareau, L. and DaSilva, E.J. (1999). Medicinal plants: a re-emerging health aid. *Electronic Journal of Biotechnology,* (online), 15th August 1999, Vol. 2(2). [Cited 7th December 2001]. Available from : http://www.ejbiotechnology.info/content/vol2/issue2/full/2/index.

Igoli, J.O,,Ogaji, O.G., Tor-Anyin, T.A. and Igoli, N.P. (2005).Traditional medicine amongst the Igede People of Nigeria. Part. II. *African Journal of Traditional, Complementary and Alternative Medicine.* Vol.2 (2):134-152.

Iwu, M.W. Duncan, A.R. and Okunji, C.O. (1999). New antimicrobials of plant origin. In: Janick, J. (Ed). Perspective on new crops and new uses. A.S.H.S. Press, Alexandria, V.A. pp 457-462.

Latha, M., Ramkumar, K.M., Pari, L., Damodaran, P.N., Rajeshkannam, V. and Suresh, T. (2006). Phytochemical and Antimicrobial Study of an Antidiabetic Plant: *Scoparia dulcis. Journal of Medicinal Food.* Vol.9(3):391-39.

NCCLS-National Committee for Clinical Laboratory Standards. (2000). Methods for Dilution Antimicribial Susceptibility Tests for Bacteria That grow Aerobically: Approved standard-Fifth Edition. *NCCLS Document M7-A5.* Pennsylvania, U.S.A.

Odetola, A.A. and Akojenu, S.M.(2000). Anti-diarrhoeal and gastro-intestinal potentials of the aqueous extract of *Phyllanthus amarus*. *African Journal of Medicine and Medical Sciences.* Vol. 29(2):119-121.

Okoli, R.I., Turay, A.A., Mensah, J.K.and Aigbe, A.O.(2009). Phytochemical and Antimicrobial Properties of Four Herbs from Edo State, Nigeria. *Report and Opinion.* Vol.1 (5): 67-73.

Oluwafemi,F.and Debiri,.F.(2008)**.** Antimicrobial Effect of *Phyllanthus amarus* and *Perquentina nigrescens* on *Salmonella typhi*. *African Journal of Biomedical Research.* Vol. 11:215 – 219.

Onocha, P.A.; Opegbemi, A.O.; Kadri, A.O.; Ajayi, D.A. and Okorie D.A.(2003). Antimicrobial Evaluation of Nigerian Euphorbiaceae Plants 1: *Phyllanthus amarus and Phyllanthus muellerianus* Leaf Extracts. *Nigeria Journal of Natural Products and Medicine.* Vol. (1): 9-12.

Parekh, J. D. and Chanda, S. (2005). Efficacy of aqueous and methanolic extracts of some medicinal plants for potential antibacterial activity. *Turkish Journal of Biology.* Vol. 29:203-210.

Sule, I.O. and Agbabiaka, T.O. (2008). Antibacterial Effect of some Plant Extracts on Selected Enterobacteriaceae. *Ethnobotanical Leaflets.* Vol.12: 1035-42.

Sofowora, A.(2008). Medicinal Plants and Traditional Medicine in Africa. 3rd Edition. Ibadan. Spectrum Books Limited. Pp 179-180.

Trease, C.E. and Evans, W.G. (1987). Pharmacognosy. 18th Edition. London. Balliere Tindal. Pp 315-679.

Trout, K. (1997). Trout's notes on the Genus *Desmodium* (chemistry, ethnomedicine, pharmacology, synonyms and miscellany. *Trout and Friends.* London. Pp.8

W.H.O. (World Health Organization) (2002). Traditional medicine strategy 2002-2005. [www.who.int/medicines/library/trm/trm_trat_eng.pdf].

Yisa, J. (2009). Phytochemical analysis and Antimicrobial activity of *Scoparia dulcis* and *nympaea lotus*. *Australian Journal of Basic and Applied Sciences.* Vol. 3(4): 3975-3979.