IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN: 2278-3008, p-ISSN:2319-7676. Volume 10, Issue 1 Ver. II (Jan -Feb. 2015), PP 115-120
www.iosrjournals.org

In Vitro Antibacterial Activities of *Cochlospermum planchonii* Roots Crude Extracts

Okechukwu Edith Chinenye[1] Umeh Ebere U[2] Aguora Sunday Okechukwu[3]
Department of Biological Sciences, College of Science, Federal University of Agriculture Makurdi, Benue State, Nigeria.
Department of Biological Sciences, College of Science, Federal University of Agriculture Makurdi, Benue State, Nigeria.
Department of Pharmacology and Therapeutics, College of Health Sciences, Nnamdi Azikiwe University Nnewi Campus, Nigeria.

Abstract: *The antibacterial activities of the methanolic, hot water, chloroform and petroleum ether of Cochlospermum planchonii root extracts on some clinical bacterial isolates and reference organisms were investigated using conventional microbiological and microdilution indicator technique. Phytochemical screenings were also carried on the extracts. The root extracts of the plant exhibited antibacterial activities against reference strains and clinical isolates of Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Shigella flexneri, and Salmonella typhii. However, the susceptibility pattern of the bacteria did not differ significantly from each other (p>0.05). The methanolic root extracts exhibited the highest antibacterial activity, its minimum inhibitory concentration (MIC) ranging between 1.25 mg/ml and 5.00mg/ml; and its zones of inhibition diameter on the various test microorganisms ranging between 8mm and 12mm. The petroleum ether extracts had the weakest antibacterial activity, with minimum inhibitory concentration of 5.00mg/ml and its zones of inhibition diameter ranging between 4mm and 7mm. The bioactive constituents in the plant were alkaloids, tannins, saponins, cardiac glycosides, and sterols. The methanolic extracts of root appeared to be more biologically active than other extracts and may be more useful in treating human infections caused by these pathogens.*

Keywords: *Cochlospermum planchonii root, antibacterial activities, zone of inhibition, minimum inhibitory concentration, phytochemical constituents.*

I. Introduction

Antimicrobials can be referred to as agents or substances sourced from plants, animals and even micro-organisms that have the ability to inhibit or prevent the growth of pathogens. Efforts have been made to discover new antimicrobial compounds from various sources. One of such resources is the folk or traditional medicine. The use of plant compounds to treat infections is an age-old practice in a large part of the world, especially in developing countries, where there is dependence on traditional medicine for a variety of diseases (Serrentino, 1991; Shiba *et al.*, 2005; and Gangoue-Pieboji *et al.*, 2006).

There has been a great interest in exploration and use of natural antimicrobial compounds of plant origin to treat diseases because of the increasing prevalence of multidrug resistant strains of pathogens that reduces the effectiveness of the antibiotics, fakeness, expensive treatment regimen of synthetic drugs already in practice and their gross side effects due to indiscriminate use (Ody, 1997; Sharif, 2001; Tomoko *et al.*, 2002; Shiota *et al*., 2004; Abu-Shanab *et al.*, 2004; Umeh *et al.*,2005). Every culture on earth, through written or oral tradition, has relied on the vast variety of natural chemistry found in healing plants for their therapeutic properties (Serrentino, 1991).

Cochlospermum planchonii exhibit strong fungitoxicity against *Colletotrichum capci*si and have potential for being formulated into products for the control of anthracnose of sweet pepper (Nduagu *et al.*, 2008). Rhizomes of *Cochlospermum tinctorium* are used against fever, hepatitis, abdominal pain, helminthes and bilharzias infestations (Ekanem, 1994).

Aqueous extracts of *Cochlospermum planchonii* Hook family (Cochlospermaceae) rhizomes are used by native medical practitioners in northern Nigeria to treat jaundice (Aliyu *et al.*, 1995).

The aim of this research is to evaluate the antibacterial activities of *Cochlospermum planchonii* root extracts against clinical isolates and reference organisms and also validate its claims in the treatment of some common infections among locals.

II. Materials And Methods

2.1 Test plant: The roots of *Cochlospermum planchonii* were collected in August 2008 from the farmland behind Block B, South-Core of Federal University of Agriculture Makurdi. The plant was identified by Mr. P.O. Ekwuno of the Department of Forestry, University of Agriculture Makurdi, Benue State, Nigeria.

2.2 Test Organisms: The microorganisms used were clinical strains of *Staphylococcus aureus, Escherichia coli, Salmonella typhii, Pseudomonas aeruginosa and Shigella Flexner, and* reference strains of *Staphylococcus aureus* (ATCC 28923), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) used as controls.

2.3 Extraction procedure

The root of the plant *Cochlospermum planchonii* were collected, sun dried and pulverized by pounding. One hundred grams (100g) of the powdered roots of *Cochlospermum planchonii* were placed in a corked bottle, and 500 ml of solvent (methanol, petroleum ether, chloroform or hot water) were added in the cold (cold extraction). The resulting suspension was allowed to stand in a tightly covered bottle for 48 hours at room temperature after which it was filtered using Whatman's No.1 filter paper into a round bottom flask. The flask containing the filtrate was placed in the water bath and allowed to evaporate off the extraction solvent to obtain the crude extract. The crude extract was placed in sterile sample bottles and labeled appropriately. The yield was weighed and recorded in grams.

2.4 Determination of minimum inhibitory concentration (MIC):

The microdilution assay and disc diffusion were carried out using the Ciprofloxacin against the test organisms both the reference strains and clinical isolates to confirm the sensitivity of Ciprofloxacin against the test organisms.

2.5 Microdilution Assay Technique

The antibacterial activities of the various extracts were assayed using modified microdilution techniques of Drummond and Waigh (2000), as described by Satyajit *et al*. (2007). Micro titre plates were prepared under aseptic conditions. A sterile 96-well microtitre plate was labeled. One hundred microlitres (100 µL) of test material in 10%(v/v) dimethylsulpoxide (a stock concentration of 10mg/ml) was pipetted into the first row of wells in triplicates. Sterile nutrient broth (50 µL) was added to all other wells. The extract was serially diluted two-folds using a multichannel pipette. Tips were discarded after use so that each well had 50 µL of the test material in serially descending concentrations. To each well 10 µL of resazurin indicator dye solution in sterile water was added. Bacterial suspension (10 µL) in nutrient broth ($5x10^6$ cfu/ml) was added to each well to achieve a concentration of $5x10^5$ cfu/ml.

Two columns were used as controls: positive control (comprising broad-spectrum antibiotic Ciprofloxacin) that prevented bacterial growth; and negative control containing bacterial suspension, resazurin indicator dye and 10%v/v dimethylsulphoxide without the test extract. Finally each plate was sealed with paraffin film to ensure that the bacteria did not become dehydrated. Plates were incubated at 33°C for 18-22hrs. The plates were then observed macroscopically for colour change and turbidity under the reading mirror. The minimum inhibitory concentration, which is the lowest concentration of the extract that caused bacterial growth inhibition, was read off.

2.6 Minimum bactericidal concentration

The minimum bactericidal concentration was determined by sub culturing the minimum inhibitory concentrations onto a sterile surface of drug free Mueller-Hinton agar plates. The plates were incubated at 33^0C for 18-22hours. The plates were observed for colonies and non colonies. Plates without growth colonies indicate minimum bactericidal concentration.

2.7 Disc Diffusion Assay Technique

Antimicrobial screening using the disk diffusion method as described by the NCCLS (2004) was carried out. Twenty milliliters (20 ml) of molten sterile Mueller-Hinton agar was poured into sterile Petri dishes and was allowed to solidify. A loopful of inoculums containing $5x10^5$ cfu/ml bacterial suspension was uniformly streaked on the surface of the agar. Pre-sterilized filter paper discs of 3 mm diameter were impregnated with the different concentrations of extracts, and were placed on the seeded agar. Ciprofloxacin discs (10µg) used as a positive control and discs saturated with sterile water (negative control) were placed at the center of the seeded plates and incubated for 18-22hrs at 33 to 37^oC. At the end of incubation period, diameter of inhibition zones in all three replicates were measured in millimeters using measuring slide and the mean of the three was determined (Barry *et al*., 2001).

2.8 Phytochemical Test

Phytochemical test was carried out on the extracts for the following bioactive constituents: tannins, saponins, cardiac glycosides, alkaloids, sterols and flavonoids. Chemical tests were carried out on the extracts

of the root using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (2002), and Harborne (1998).

2.8.1 Test for Tannins: Small portion of extracts of root each was stirred with 1ml absolute methanol and $FeCl_3$ (aq) added in a test tube. A blue black precipitate is indicative of the presence of tannins.

2.8.2 Test for Saponins:

i. Frothing test; Small portion of extracts of root each was shaken with water in a test tube. Frothing which persisted on warming is indicative of the presence of saponins.

ii. Emulsion test ; Five drops of olive oil was added to 3ml of extracts of root in a test tube and the mixture was vigorously shaken to form stable emulsion. Formation of stable emulsion indicates the presences of saponins.

2.8.3 Test for Flavonoids: 2ml of solution of extracts was added to Magnesium chip and few drops of H_2SO_4 in a test tube. Light yellow precipitate in brown solution is indicative of flavonoids.

2.8.4 Test for Alkaloids:

i). Small portion of the extracts of root was dissolved in 2ml 0.1 HCl (aq) in a test tube. To this was added 2 drops of Mayer's reagent, a light yellowish white precipitate indicates the presence of alkaloids.

ii). Dragendroff's reagent, 0.85g basic bismuth nitrate in 10ml glacial acetic acid into 40ml water was added to small portion of extracts of root in a test tube and the mixture was heated for 2minutes. Faint yellowish orange precipitate indicates the presence of alkaloids.

2.8.5 Test for Cardiac glycosides: Keller-Killani test; To a small portion of solution of extracts in glacial acetic acid containing 2 drops of $FeCl_3$ (aq) was added 1.5ml conc. H_2SO_4. Lower conc. H_2SO_4 layer that is colourless, with upper acetic acid layer is indicative of the presence of glycosides.

2.8.6 Test for Sterols: The extracts were dissolved in 2ml of acetic anhydride and cooled ice conc. H_2SO_4 was carefully added. A colour change from violet to blue then to green indicates the presence of sterols.

2.9 Statistical Analysis: Inferential and descriptive data analyses were carried out using SPSS version 15.0. The t-test was used to determine mean differences (zones of inhibition diameter) of the extracts, clinical isolates and reference strains of the test bacteria, and Gram-positive and Gram-negative test bacteria. F-statistic (ANOVA) was used to determine the mean differences between extraction solvents, namely, hot water, methanol, chloroform, and petroleum ether.

III. Results

Table 1 showed the minimum inhibitory concentrations of root extracts of *C. planchonii* using modified indicator based micro-dilution technique. The MIC of methanolic root extracts was between 1.25 and 5.00mg/ml. *E.coli* ATCC 25922 was 1.25mg/ml and *S. aureus* ATCC 28923 and *Pseudomonas aeruginosa* ATCC 27853 were 1.25mg/ml respectively. Petroleum ether extract had the weakest antibacterial activity of 5.0mg/ml. The MIC of the hot water root extract for *Staphylococcus aureus* ATCC 28923 and *Escherichia coli* ATCC 25922 could not be determined because there was no change in colour in the wells.

The results of the antibacterial susceptibility test of the plant root extracts using the disc diffusion method are shown in Table 2. The extracts of different solvent screened showed varying inhibitory effects of methanolic extracts (8-12mm), chloroform extracts (4-10mm), petroleum ether extracts (4-7mm) and hot water (4-10mm) against the test organisms. Ciprofloxacin used as the positive control had (11-15mm) gave the largest zone of inhibition.

The results of the qualitative phytochemical screening of *Cochlospermum planchonii* root investigated as presented in Table 3, revealed some of the bioactive constituents that were present in the root extract such as alkaloids, saponins, tannins, cardiac glycosides and sterols. Flavonoids were absent in the root extracts.

The results showed that the control containing only the resazurin dye and dimethylsulphoxide did not exhibit any antimicrobial activity indicating no zone of inhibition diameter (Tables 1 and 2).

The result of the statistical significance test at 0.05 level showed that there is no significant difference ($P > 0.05$) between the roots extract and the organisms as shown on Table 4. The *Choclospermum planchonii* roots are shown in Fig.1. The minimum inhibitory concentrations and zones of inhibition diameter of *Choclospermum planchonii* root extracts against reference organisms and clinical isolates are shown in Fig 2 and 3 respectively. The results showed that the minimum inhibitory concentration of 1.25mg/ml of the methanol extract was observed for *Staphylococcus aureus* ATCC 28923, *Escherichia coli* ATCC 25922, *Pseudomonas*

aeruginosa ATCC 27853 and *Salmonella typhii*, and 2.5mg/ml was observed for *Escherichia coli* and *Pseudomonas aeruginosa* (fig 2). 12mm was observed for *Escherichia coli* ATCC 25922. 11mm was for *Escherichia coli.* 10mm was observed for *Staphylococcus aureus* ATCC 28923, *Pseudomonas aeruginosa* ATCC 27853 and *Salmonella typhii*. 9mm was observed for *Pseudomonas aeruginosa* and *Shigella flexneri*. 8mm was observed for *Staphylococcus aureus* (Fig. 3).

Table 1: Minimum Inhibitory Concentrations (mg/ml) of Root Extracts obtained by the Microdilution Technique.

Organisms	Methanol	Hot Water	Chloroform	Petroleum Ether	Ciprofloxacin	Viability Control
Reference Strains						
Staphylococcus aureus ATCC 28923	1.25	Nd	2.50	5.00	+	-
Escherichia coli ATCC 25922	1.25	Nd	2.50	5.00	+	-
Pseudomonas aeruginosa ATCC 27853	1.25	5.00	2.50	5.00	+	-
Clinical Isolates						
Staphylococcus aureus	5.00	5.00	5.00	5.00	+	-
Escherichia coli	2.50	5.00	5.00	5.00	+	-
Pseudomonas aeruginosa	2.50	5.00	2.50	5.00	+	-
Salmonella typhi	1.25	2.50	2.50	5.00	+	-
Shigella flexneri	5.00	2.50	5.00	5.00	+	-

Key: nd = Not determined; +: growth inhibited; - = growth uninhibited

Table 2 : Zone of Inhibition Diameter (mm) of Bacterial Growth by Root Extracts

Organisms	Methanol	Hot Water	Chloroform	Pet. Ether	Ciprofloxacin	Viability Control
Reference Strains						
Staphylococcus aureus ATCC 28923	10	4	5	4	13	-
Escherichia coli ATCC 25922	12	5	6	7	15	-
Pseudomonas aeruginosa ATCC 27853	10	5	4	4	12	-
Clinical Isolates						
Staphylococcus aureus	8	5	5	5	11	-
Escherichia coli	11	6	10	7	14	-
Pseudomonas aeruginosa	9	6	6	4	12	-
Salmonella typhi	10	10	7	6	14	-
Shigella flexneri	9	6	7	6	13	-

NOTE: each value represent mean of three different observations; - means uninhibited growth

Table 3: Phytochemical Constituents of Root Extracts

Test	Methanolic Extract	Chloroform Extract	Pet. Ether Extract	Hot Water Extract
Alkaloid	+	-	-	-
Saponin	+	-	+	+
Tannins	+	+	-	-
Flavonoids	-	-	-	-
Cardiac glycosides	+	-	+	-
Steroids	+	+	-	-

+: Presence of constituent; -: Absence of constituent.

Table 4 : Test of Significant Difference Between Organism Strains (Root Extracts)

Anova: Single Factor						
SUMMARY						
Groups	Count	Sum	Average	Variance		
Reference Strains	3	9.27084	3.09028	0.090421		
Hospital Strains	5	20.3125	4.0625	0.683594		
ANOVA						
Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	1.772272	1	1.772272	3.647629	0.104726	5.9873742
Within Groups	2.915218	6	0.48587			
Total	4.68749	7				
Ho: F≤ Fcrit					**α = 0.05**	
Ha: F> Fcrit						
		No significant Difference				

Fig. 1 *Cochlospermum planchonii* roots.

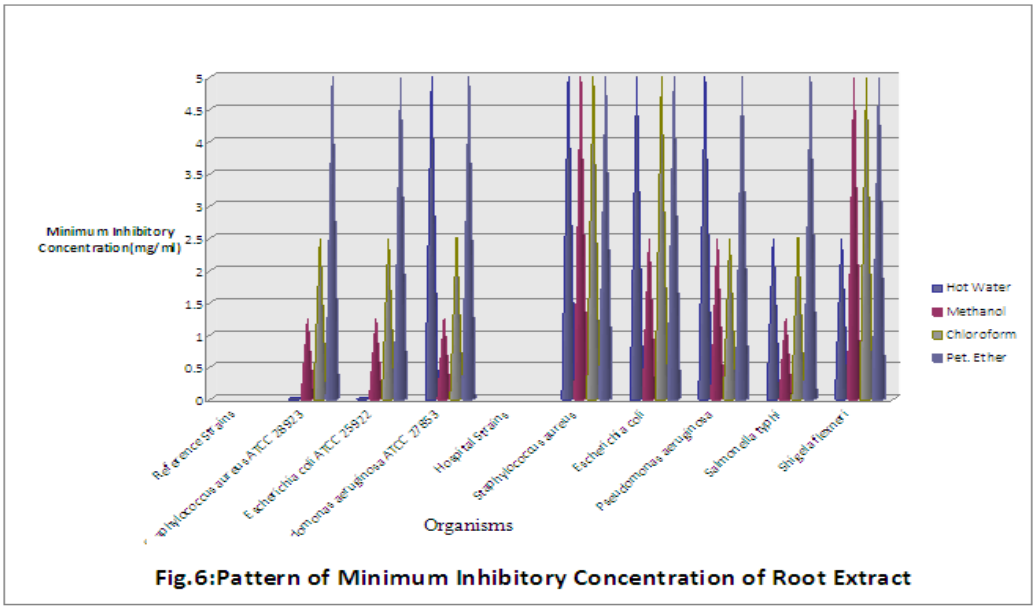

Fig. 2 Pattern of Minimum Inhibitory Concentration of root extracts.

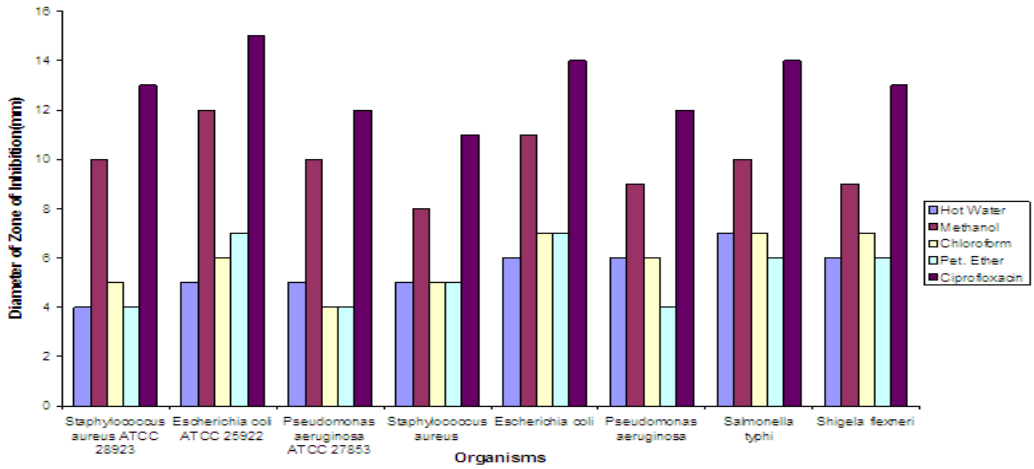

Fig 3. Pattern of zone of inhibition diameter of root extracts

IV. Discussion

Although, there is a growing interest in the use of medicinal plants only about 2,400 plant species among more than 250,000 higher plants have been screened for phytochemicals (Oluwalana and Adekunle, 1998). Few studies concerning the phytochemical constituents of *C. planchonii* roots have been found (Benoit-Vical *et al.*, 1999). The preliminary qualitative test of *C. planchonii* roots revealed the presence of some phytochemicals such as saponins, tannins, alkaloid and glycosides. This finding agrees with the previous studies related to *C. planchonii* and reported by Ouattara *et al.* (2007). *C. planchonii* root extracts exhibited

antibacterial activity against some clinical isolates of *E. coli* and *P. aeruginosa* and their reference strains which is in conformity with previous reports on *C. planchonii* (Ouattara *et al.*, 2007)**.**

Antibacterial activity and susceptibility assay of *C. planchonii* varied according to extraction solvent. This indicates that the minimum inhibitory concentration and zone of inhibition diameter of the root extracts were affected by the solvent used for extraction. For example the methanolic extract, the variation may probably be due to the type of bioactive compounds present in the different extracts as suggested by Abiodun *et al.* (2007).

These results confirm the evidence in previous studies (Ahmad *et al.*, 1998; and Cowan, 1999) that alcoholic solvents like methanol and ethanol are more suitable than other solvents such as water in extracting components of medicinal plants. .

V. Conclusion

The significant antibacterial activity of *Cochlospermum planchonii* roots could be attributed to the presence of bioactive compounds in the extracts. Therefore it is suggestive that the plant could serve as a potential broad spectrum antibacterial agent for treatment of bacterial infections.

Acknowledgement

The authors are grateful to the staff of the Department of Microbiology, National Veterinary Research Institute (NVRI) Vom Jos, Plateau State, Nigeria and staff of the Department of Microbiology and Biotechnology of National Institute of Pharmaceutical Research and Development (NPRID) Idu Industrial Layout Abuja, Nigeria for their immense support to see to it that this research was successful.

References

[1]. Abu-Shanab B., Adwan G., Abu-Safiya D. (2004). Antibacterial activities of some plant extracts utilized in Palestine in popular medicine. *Turk J Biol* 28: 99-102.
[2]. Aliyu R, Okoye ZS, and Shier WT. (1995). The hepatoprotective cytochrome P-450 enzyme inhibitor isolated from the Nigerian medicinal plant Cochlospermum planchonii is a zinc salt. *J Ethnopharmacol*, 48(2): 89.
[3]. Barry, A.L. and Thomsberry, C. (1985). Susceptibility test: diffusion test procedures. In Lennette.E.H. et al (Eds).Manual of Clinical Microbiology, 4th ed, pp 978-987.
[4]. Ekanem, J. O. (1994). Treatment regimens for malaria as a function of level of chemo-resistance in Africa Malaria/Paludisme et maladies infectieuses, **1:** 96-97.
[5]. Gangoue-Pieboji J., Pegnyemb D.E., and Niyitegeka D. (2006). The in-vitro antimicrobial activities of some medicinal plants from Cameroon. *Ann Trop Med Parasitol* 100: 237-243.
[6]. Harborne, J.B. (1998). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. (3rd Edition). Chapman and Hall Co., New York. 1998, 88-190p.
[7]. Nduagu C, Ekefan E.J and Nwankiti A.O. (2008). Effect of some crude plant extracts on growth of Colletotrichum capsici (Synd) & Bisby, causal agent of pepper anthracnose. *Journal of Applied Biosciences* 6(2): 184-190.
[8]. NCCLS (2004). Performance standards for antimicrobial susceptibility testing, fourteenth informational supplement. M100 – S14. NCCLS, Wayne, P. A. USA.
[9]. Ody P. (1997). The Complete Medicinal Herbs. Dorling Kindersley Limited London,: 8.
[10]. Satyajit, D.Sarker, S. Nahar, L. and Kumarasamy, Y. (2007). Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. Methods 42 (4): 321-324.
[11]. Serrentino J. (1991). How Natural Remedies Work. Point Robert, W.A.: Harley and Marks Publishers, 20 -22.
[12]. Sharrif, Z.U. (2001). Modern herbal therapy for common ailments. Nature Pharmacy Series (vol 1),Spectrum Books Limited, Ibadan, Nigeria in Association with Safari Books (Export) Limited,United Kingdom pp 9-84.
[13]. Shiba H, Kondo K, and Katsuyama, R. (2005). Alkyl Gallates, Intensifiers of b-Lactam Susceptibility in Methicillin-Resistant Staphylococcus aureus. *Antimicrob Agents Chemother* 49: 549-555.
[14]. Shiota S, Shimizu M, and Sugiyama J. (2004). Mechanisms of action of corilagin and tellimagrandin I that remarkably potentiate the activity of beta-lactams against methicillin-resistant Staphylococcus aureus. *Microbiol Immunol* 48: 67-73,
[15]. Sofowara A. (1993). Medicinal plants and Traditional medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria. p. 289.
[16]. Trease, A., and Evans, W.C. (2002). A textbook of Pharmacognosy (14th edition) Bailliere Tindall London, 76-84p.
[17]. Tomoko, N. and Takashi, A. (2002). Antibaterial activity of extracts prepared from tropical and subtropical plants on methicillin-resistant Staphylococcus aureus. *J. Health Sci.* 48: 273-276.
[18]. Umeh, E.U., Oluma, H.O.A., and Igoli, J.O. (2005). Antibacterial screening of four local plants using an indicator based microdilution technique, *Afr. J. Traditional, Complementary and Alternative Medicines* 2(3):238-243.