

Phytochemical and *in vitro* Antibacterial Evaluation of the Ethanolic Extract of the Stem bark of *Entada africana* Guill. & Perr. and *Sarcocephalus latifolus*

ABSTRACT

Aims: The phytochemical composition and the *in vitro* antibacterial activity of the ethanolic extract of the stem bark of *Entada africana* Guill.& Perr. and *Sarcocephalus latifolus* on *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli* and *Bacillus subtilis* were investigated.

Study design: The plants stem bark samples were collected from Idu, Industrial Area of the Federal Capital Territory, Abuja, and were characterized by Mrs. Jamilat at the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. The bacteria isolates were gotten from the Microbiology Laboratory, Anambra State University, Uli, Nigeria.

Place and Duration of Study: Analysis on the plant samples was done at the Department of Biochemistry and Department of Microbiology, Anambra State University, between January 2014 and February 2014.

Methodology: Phytochemical screening was done following standard methods. Agar well diffusion technique was used to screen the extract for antibacterial activity. MIC and MBC for the various extracts were determined by the tube dilution technique and was done to establish the antimicrobial activity of these extracts. Ciprofloxacin, a standard antibiotic was used as control.

Results: The result of the phytochemical analysis revealed the presence of tannins, alkaloids, terpenes, resins, sterols, anthraquinone, phenols and saponins in *E. africana* while *S. latifolus* contained alkaloids, saponins, flavonoids, glycosides, anthraquinone, terpenes, phenols, resins and saponins. The *in vitro* antibacterial analysis revealed that the ethanolic extract of *E. africana* inhibited the growth of *S. typhi* and *B. subtilis* significantly while that of *S. latifolus* inhibited the growth of *E. coli* as well *B. subtilis* significantly with the zones of inhibition ranging from (12.00±0.02) to (0.80±0.01). The MIC and MBC for the organisms were at 10mg/ml.

Conclusion: The ethanolic extract of the stem bark of the plant samples exhibited antibacterial activity and thus could serve as a source for useful drugs.

Keywords: Phytochemicals, *Entada africana* Guill. & Perr., *Sarcocephalus latifolus*, Bacteria.

1. INTRODUCTION

All over the world, medicinal plants serves as a potent weapon in the diagnosis of diseases, making of traditional and modern medicines and in the treatment of various diseases which are caused by microbial agents such as bacteria, viruses and fungi [1, 2]. These diseases if not treated appropriately using suitable herbs or drugs could degenerate into severe medical health complications. However, in Nigeria, as indeed most parts of Africa, indigenous knowledge of traditional medical practices is passed on orally and thus memory plays a very prominent role in the training of a practitioner [3]. The therapeutic value of plants used in traditional medicine are derived from the presence of phytochemical principles [4], which are found in parts of the plants such as alkaloids, tannins and phenols [5].

Entada africana Guill. & Perr. is a small tree of 4 to 10 meters in height and 90 cm in girth, with local names as 'Dorot' in Arabic and 'Ogurobe' in Yoruba, Nigeria [6]. It belongs to the phylum Magnoliophytas and family Fabaceae. The stem bark is said to have abortive effects, while a root decoction is a stimulating agent and tonic [7]. The plant is said to have antidote effects against various toxic agents because of its emetic properties [7]. Mbatchesou *et al.*, [6] stated that healing and fever reducing beverages are prepared from leaves, stem bark, roots, and shoots. Mbatchesou *et al.*, [6] also stated that the ethanolic extract of the stem bark of *E. africana* inhibited the growth of some bacteria such as *S. typhi*. Nevertheless, the bark of the root and stem yields a long fiber used for cordage, commonly for roof binding and grass matting as well a low-quality gum [7]. In Northern Nigeria and Ghana, an infusion of the stem bark is taken as a tonic for stomach ache [7]. The leaves constitute a good wound dressing, preventing suppuration [7, 8].

Sarcocephalus latifolus also called *Nauclea latifolia* is a straggling shrub or small tree of about 10 ft high. It belongs to the family Rubiaceae, native to savannah forest and fringe tropical forests of West Africa [9]. It is known locally in Nigeria as 'Egbo egbesi' in Yoruba, 'Ubulu inu' in Igbo, and 'Tabasiya' in Hausa [10]. It is called 'African quinine' in Northern Nigeria in which a cold infusion of the stem bark is taken as a diuretic and anthelmintic [11]. The Fulanis in Nigeria uses the leaf extract to regularly deworm animals [12]. Other local uses of *Nauclea latifolia* includes in the treatment of malaria, hypertension, diarrhea, tuberculosis, dysentery and also as a laxative [13]. It is also used in the treatment of gastrointestinal tract disorders, sleeping sickness and prolong menstrual flow [14, 15]. The bark is said to be useful in the treatment of wounds, cough, and gonorrhea in Nigeria [16].

However, bacteria are prokaryotic microorganisms numbered in quantum in which some are pathogenic and others beneficial to man. *Staphylococcus aureus* is a gram-positive coccal bacterium which is a successful pathogen due to a combination of nasal carriage and bacterial immuno-evasive strategies and causes a range of illnesses, from minor skin infections, such as pimples, impetigo, boils, cellulitis folliculitis, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, and host of others [17, 18]. *Salmonella typhi* is a gram-negative bacterium that is deadly and causes typhoid fever [19]. It is a strong pathogen for humans due to its resistance to the innate immune response system [20]. *Escherichia coli* is a gram-negative bacterium with some virulent strains typically causing a bout of diarrhea that is unpleasant in healthy adults and is often lethal to children in the developing world [21]. *Bacillus subtilis* is a gram-positive bacterium which is known to cause disease in severely immunocompromised patients, and rarely causes food poisoning [22, 23].

Therefore, the purpose of this study was to evaluate the phytochemical constituents and the possible antibacterial activities of *E. africana* Guill. & Perr. and *S. latifolus* so as to enhance assured traditional usage and hence give a spotlight for the usage in developing effective antibacterial drugs.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation: The plants stem barks were collected from Idu, Industrial Area of the Federal Capital Territory, Abuja, and were characterized by Mrs. Jamilat at the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. The plant materials were washed separately under running tap water, followed by distilled water. The washed plant leaves were air-dried for two weeks and later pulverized into fine powder and stored. Voucher specimens for *E. africana* (NIPRD/H/5743) and *S. latifolus* (NIPRD/H/5739) were deposited in the herbarium of NIPRD. The pathogenic bacteria isolates (*S. aureus* ATCC 25923, *S. typhi* ATCC 700931, *E. coli* ATCC 8739 and *B. subtilis* ATCC 6633) were gotten from the Microbiology Laboratory of the Department of Microbiology, Anambra State University. The bacteria isolates were recharacterized and reidentified using biochemical and microbiological procedures of Cowan and Steel [24].

2.2 Methods for Phytochemical Screening

Phytochemical screenings of the stem bark extract were carried out in order to determine the presence of selected phytochemicals following standard methods. Tannins, alkaloids, sterols were determined by the method described by Sabri *et al.* [25]. Phenol (Ferric Chloride Test) and saponins were determined

respectively by the method of Prashant et al. [26] and Sofowora [27]. Flavonoids was determined by the method of Trease and Evans [28], glycosides was by the method of Mbatchou et al. [6], resins by the method of Bello et al. [29], anthraquinone (Borntrager's test) by the method of Onwukaeme et al. [30], terpenes (Salkowski test) by the method of Edeoga et al. [31].

2.3 Antibacterial Test

2.3.1 Preparation of concentrations of solvents soluble fractions of extracts: Stock solutions of 10 mg/ml concentrations of solvent soluble fractions of extracts of the plants were prepared by dissolving 5 mg of each fraction extract in 0.5 ml of dimethyl sulfoxide (DMSO). From the stock solution, concentrations of 2 mg/ml were made. Concentrations of Ciprofloxacin standard antibiotic were similarly prepared as those of the plant extracts. They were stored in sterilized vials and kept at the lower compartment of a refrigerator until required for use [6]. Mueller-Hinton agar was prepared as described by Sahoo et al. [32] and Uma et al. [33].

2.3.2 Antibacterial sensitivity test: The bacteria was cultured on nutrient agar and incubated at 37 °C for 24 hours. The bacteria were repeatedly cultured to obtain pure isolates. Morphological and biochemical reactions were carried out to ascertain proper identification. They were inoculated into nutrient agar slant and stored at 4 °C using agar diffusion method [34]. Mueller-Hinton agar plates were inoculated with standard test inocula by direct streaking, and plates were properly labeled. A sterile cork borer (5 mm in diameter) was used to make wells in the plates for the extracts. An aliquot (0.1 ml) of the fractions was dispensed into each well. The plates were left to allow diffusion of extracts before being placed in the incubator at 37 °C for 24 hours. The relative susceptibility of the organism to the extracts was indicated by zones of inhibition produced after incubation.

2.3.3 Determination of minimum inhibitory concentration

Ten (10) mg/ml of extract solution was serially diluted in Muller-Hinton broth to give decreasing concentrations of 5 mg/ml, and 2.5 mg/ml. An aliquot (0.1 ml) of broth culture left overnight of test microorganism (concentration 1.5×10^8 cfu/ml) in sterile normal saline was introduced into each extract dilution. The mixtures in sterile test tubes were incubated (37 °C, 24 h) and observed for growth or inhibition. Ciprofloxacin (a synthetic broad spectrum antibacterial drug) was used as controls and sterile normal saline as negative control. The minimum inhibitory concentration was the lowest concentration of the extract solution that inhibited microbial growth.

2.3.4 Minimum bactericidal concentration

A loopful of the test mixture was removed from each MIC tube that showed no growth, inoculated onto antibiotic-free Mueller-Hinton agar plate, incubated (37 °C, 24 h) and inspected for presence of colonies indicating growth. The minimal bactericidal concentration is the lowest concentration of extract that showed no bacterial.

3. RESULTS AND DISCUSSION

The result of the phytochemical analysis presented in Table 1 revealed that eight out of ten phytochemicals screened were present in the stem bark of *E. africana*. They included tannins, alkaloids, terpenes, and resins in high quantity. This was consistent to those reported by Mbatchou et al. [6]. Sterols, anthraquinone, phenols, and saponins were also present but in low amount. *E. africana* is associated with the classes of phytochemicals present in them which have been reported to consist of antimicrobial therapeutic properties by Gislene et al. [35]. However, the phytochemical constituent of the stem bark of *S. latifolius* (Table 1) include alkaloids and saponins in high amount, flavonoids, glycosides, anthraquinone, terpenes, phenols, resins and tannins in low amount.

Table 1: Phytochemical screening of the ethanolic extract of the stem bark of *E. africana* and *S. latifolus*

Phytochemicals	Stem bark	
	<i>E. africana</i>	<i>S. latifolus</i>
Tannins	++	+
Alkaloids	++	++
Flavonoids	-	+
Glycosides	-	+
Anthraquinone	+	+
Terpenes	++	+
Sterols	+	-
Phenols	+	+
Resins	++	+
Saponins	+	++

***key:** ++ = Highly present, + = Slightly present, - = Not detected

Antibacterial activities were expressed as zone of inhibition in (mm) (Table 2). An observable zone of inhibition of growth of each microorganism served as a measure for concluding that a bacterium is sensitive or not. The result of the analysis however revealed that the ethanolic extract of the stem bark of *E. africana* significantly exhibited anti-microbial activity on *S. typhi* (12.00±0.02) and *B. subtilis* (8.02±0.05). Antimicrobial activity of the ethanolic extract of the stem bark of *E. africana* was also observed on *S. aureus* (0.80±0.01) and *E.coli* (0.90±0.00) which could be considered as not significant. While the ethanolic extract of *S. latifolus* inhibited the growth of *E. coli* (11.12±0.01) and *B. subtilis* (9.01±0.01) with insignificant activities on *S. aureus* (1.10±0.02) and *S. typhi* (2.20±0.00).

Table 2: Zones of inhibition (mm) of extracts (10mg/ml) against bacterial isolates

Test Bacteria	Ethanolic extract of the stem bark of the sample		
	<i>E. africana</i>	<i>S. latifolus</i>	Ciprofloxacin
<i>S. aureus</i>	0.80±0.01	1.10±0.02	7.03±0.01
<i>S. typhi</i>	12.00±0.02	2.20±0.00	15.00±0.00
<i>E.coli</i>	0.90±0.00	11.12±0.01	10.10±0.02
<i>B. subtilis</i>	8.02±0.05	9.01±0.01	12.00±0.01

Values = mean±SD of triplicate determination.

The MIC and MBC results were presented in (Table 3) and (Table 4) respectively. Aliquot of the ethanolic extract 10 mg/ml, 5 mg/ml and 2.5 mg/ml were used for all the bacterial isolates. MBC tests were carried out to establish whether the extracts were bactericidal or bacteriostatic. MBC, in general was found to be 10 mg/ml for ethanolic extracts of both plants stem bark. The MIC and MBC for Ciprofloxacin was effective at 10 mg/ml and 5 mg/ml.

Table 3: Minimum inhibition Concentration (MIC) of the stem bark extracts bacteria isolates

Test Bacteria	Ethanolic extract of the stem bark of <i>E. africana</i>			Ethanolic extract of the stem bark of <i>S. latifolus</i>			Ciprofloxacin		
	10 mg/ml	5 mg/ml	2.5 mg/ml	10 mg/ml	5 mg/ml	2.5 mg/ml	10 mg/ml	5 mg/ml	2.5 mg/ml
<i>S. aureus</i>	++	++	+++	+	+	++	-	-	+
<i>S. typhi</i>	-	-	+	-	+	++	-	-	-
<i>E.coli</i>	+	++	++	-	-	+	-	-	-
<i>B. subtilis</i>	-	+	++	-	+	+	-	-	-

***key:** - = No colony growth, + = Scanty colony growth, ++ = Moderate colony growth, +++ = Heavy colony growth.

Table 4: Minimum bactericidal Concentration (MBC) of the stem bark extracts on bacteria isolates

Test Bacteria	Ethanolic extract of the stem bark of <i>E. africana</i>			Ethanolic extract of the stem bark of <i>S. latifolus</i>			Ciproflaxacin		
	10 mg/ml	5 mg/ml	2.5 mg/ml	10 mg/ml	5 mg/ml	2.5 mg/ml	10 mg/ml	5 mg/ml	2.5 mg/ml
<i>S. aureus</i>	++	+++	++++	+	+	+++	-	+	++
<i>S. typhi</i>	-	+	+	-	++	+++	-	-	++
<i>E.coli</i>	+	++	+++	-	+	++	-	+	+
<i>B. subtilis</i>	-	+	++	-	+	+			

*key: - = No colony growth, + = Scanty colony growth, ++ = Moderate colony growth, +++ = Heavy colony growth.

The phytochemical results revealed that the plants contained bioactive agents which are connected with antimicrobial properties in plants [36]. Flavonoids are reported to have antibacterial, anti-inflammatory, anticancer, antifungal, antiallergic, and diuretic properties [1]. Tannins are known to possess general antimicrobial and antioxidant activities [37]. Other compounds like saponins and anthraquinone have been implicated as bioactive antibacterial agent of plants [27, 38, 39]. Alkaloids are medicinal agents for their analgesic antispasmodic and antibacterial effects [1]. The high presence of resins in the stem bark sample of *E. africana* confirms the report of Orwa et al. [7] that it yields low-quality gum. Terpenes have also been reported to be active against bacteria [40].

However, it could be deduced that the ethanolic extract of *E. africana* inhibited the growth of *S. typhi* which was consistent as those reported by Mbatchou et al. [6]. *E. africana* also has anti-bacterial activity on *B. subtilis*. This could be attributed to the presence of tannins which have shown potential antiviral [41], antibacterial [42] and antiparasitic effects [43]. The ethanolic extract of *S. latifolus* inhibited the growth of *E. coli* and *B. subtilis* which was in conformity with what was demonstrated by Okiei et al. [44] that ethanolic extract of *S. latifolus* showed antibacterial property. Isah et al. [45] reported also that *S. latifolus* inhibited the growth of *S. typhi*, *B. subtilis*, *E. coli*, and *S. aureus*. From Tables 3 and 4, it was observed that the MIC and MBC for the organisms were at 10mg/ml.

4. CONCLUSION

The study revealed that the stem bark of *E. africana* and *S. latifolus* contained different phytochemicals. The stem bark of *E. africana* significantly inhibited the growth of *S. typhi* and *B. subtilis* while *S. latifolus* significantly inhibited the growth of *E.coli* and *B. subtilis*. This medicinal property of *E. africana* and *S. latifolus* could serve as an antidote for the treatment of bacterial infections and as well serve as resource for useful drugs.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

197 REFERENCES

- 198 1. Odeh IC, Tor-Anyiin TA. Phytochemical and Antimicrobial Evaluation of Leaf-extracts of
199 *Pterocarpus santalinoides*. European Journal of Medicinal Plants. 2014; 4(1): 105-115.
- 200 2. Aibinu A. Medicinal Plants as Antimicrobials. In: Olugbemi T, editor. Outlines and Pictures of
201 Medicinal Plants from Nigeria. Lagos: University of Lagos Press; 2008.
- 202 3. Giertych MJ, Karolewski P, de Temmerman LO. Foliage age and pollution alter content of
203 phenolic compounds and chemical elements in *Pinus nigra* needles. Water Air Soil Pollut. 1999;
204 110:363-377.
- 205 4. Igoli JO, Igwue IC, Igoli NP. Traditional medicinal practices among the Igede people of Nigeria. J.
206 Herbs Spices Med. Plants. 2004; 10:1-10.
- 207 5. Bako SP, Bakfur MJ, John I, Bala EI. Ethnomedicinal and Phytochemical Profile of Some
208 Savanna Plant Species in Nigeria. International Journal of Botany. 2005; 1:147-150.
- 209 6. Mbatchou VC, Ayebila AJ, Apea OB. Antibacterial activity of phytochemicals from *Acacia nilotica*,
210 *Entada africana* and *Mimosa pigra* L. on *Salmonella typhi*. Journal of Animal and Plant Sciences.
211 2011; 10(1):1248-1258.
- 212 7. Orwa C, Mutua A, Kindt R, Jamnadass R, Simons A. Agroforestry Database: A tree reference
213 and selection guide version 4.0. 2009.
214 Available: <http://www.worldagroforestry.org/af/treedb/>.
- 215 8. Oliver-Beyer B. Medicinal plants in tropical West Africa. Cambridge: Cambridge University Press;
216 1986.
- 217 9. Irvine FR. Woody plants of Ghana. London: Oxford University Press; 1961.
- 218 10. Arise RO, Akintola AA, Olarinoye JB, Balogun EA. Effects of Aqueous Extract of *Nauclea latifolia*
219 stem on lipid profile level of some enzymes of Rat liver and kidney. Int. j. pharmacol. 2012; 1(1):1-
220 7.
- 221 11. Ademola IO, Fagbemi BO, Idowu SO. Anthelmintic Efficacy of *Nauclea Latifolia* Extract against
222 Gastrointestinal Nematodes of Sheep: *In vitro* and *In vivo* Studies. Afr. J. Trad. CAM. 2007; 4
223 (2):148–156.
- 224 12. Adebawale EA. Some ethnoveterinary and traditional management practices in livestock
225 production. In Proceeding of a workshop on indigineous knowledge in Agriculture and
226 Development, Ibadan, Nigeria; 1993.
- 227 13. Okiemy-Andissa N, Miguel ML, Etou AW, Ouamba JM, Gbeassor M, Abena AA. Analgesic Effect
228 of aqueous and hydroalcoholic extracts of three Congolese Medicinal Plants: *Hyptis snavolens*,
229 *Nauclea latifolia* and *Ocimum gratissimum*. Pak. J. Biol. Sci. 2004; 7:1613-1615.
- 230 14. Egbung GE, Atangwho IJ, Iwara IA, Odey MO and Ebong PE. Chemical composition of root and
231 stem bark extracts of *Nauclea latifolia*. Applied Science Research, 2013; 5 (3):193-196.
- 232 15. Ruben D, Elana MS, Wagner FD. Journal of Material Chemistry, 2003; (33):1125-1144.
- 233 16. Madibunyi IT. Anti hepatotoxic of ethanolic extract of *Nauclea Latifolia* root bark. Journal of herbs,
234 spices and medicinal plant. 1995; 3(2):23-25.
- 235 17. Kluytmans J, van Belkum A, Verbrugh H. Nasal carriage of *Staphylococcus aureus*:
236 epidemiology, underlying mechanisms, and associated risks. Clin. Microbiol. Rev. 1997; 10 (3):
237 505–20.
- 238 18. Cole AM, Tahk S, Oren A, Yoshioka D, Kim YH, Park A, Ganz T. Determinants of *Staphylococcus*
239 *aureus* nasal carriage. Clin Diagn Lab Immunol. 2001; 8 (6):1064–9.
- 240 19. Weng D, Shian L, Guy P, George FM, Debra JR, Valerie B, et al. Comparative genomics of
241 *Salmonella enteric serovar typhi* strains Ty2 and CT18. J. Bacteriol. 2003; 185(7): 2330-2337.
- 242 20. Falkow S, Monack DM, Mueller A. Persistence Bacterial Infections: The interface of the pathogen
243 and the host immune system. Nature Review of microbiology, 2004; 2:747-765.
- 244 21. Nataro JP, Kapar JB. Diarrheagenic *Escherichia coli*. Clin. Microbiol. Rev. 1998; 11 (1):142-201.
- 245 22. Ogioni MR, Pozzi G, Valensin PE, Galieni P, Bigazzi C. Recurrent septicemia in an
246 immunocompromised patient due to probiotic strains of *B. subtilis*. J. Clin. Microbiology. 1998;
247 36(1):325-6.
- 248 23. Ryan KJ, Ray CG editors. Sherris Medical Microbiology. 4th ed. McGraw Hill; 2004.
- 249 24. Cowan ST, Steel KJ. In Manual for identification of Medical Bacteria. 3rd ed. Cambridge
250 University Press. 2004; 50-140

25. Sabri FZ, Belarbi M, Sabri S, Alsayadi, MMS. Phytochemical Screening and Identification of some Compounds from Mallow. J. Nat. Prod. Plant Resource. 2012; 2(4):512-516.
26. Prashant T, Bimlesh K, Mandeep K, Gurpreet K, Harleen K. Phytochemical Screening and Extraction: A Review. Internationale Pharmaceutica Scientia. 2011; 1(1): 98-106.
27. Sofowora A. Medicinal plants and Traditional Medicine in Africa. Nigeria: Spectrum Books Limited, Ibadan; 1993.
28. Trease GE, Evans WC. Textbook of Pharmacognosy. 14th ed; Balliere Tindall London. 2002; 81-90.
29. Bello OM, Zack, AM, Adikwu JG. The comparative studies of phytochemical screening of *Piliostigma thoningii* root and leave extracts. Asian journal of Plant Science and Research, 2013; 3(6): 74-77.
30. Onwukaeme DN, Ikuegbvweha TB, Asonye CC. Evaluation of phytochemical constituents, antibacterial activities and effect of exudates of *Pycanthus angolensis* Weld Warb (Myristicaceae) on corneal ulcers in rabbits. Trop. J. Pharm. Res. 2007; 6:725-730.
31. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr.J. Biotechnol. 2005; 4: 685-688.
32. Sahoo S, Kar DM, Mohapatra S, Rout SP, Dash SK. Antibacterial activity of *Hybanthus enneaspermus* against selected UTI pathogens. Indian Journal of Pharmaceutical Sciences. 2006; 68:653-655.
33. Uma B, Prabhakar K, Rajendran S. *In vitro* Antimicrobial Activity and Phytochemical Analysis of *Ficus religiosa* L. and *Ficus bengalensis* L. against Diarrhoeal Enterotoxigenic E. coli. Ethnobotanical Leaflets. 2009; 13: 472-74.
34. Cruickshanks R, (JP), Oluguid BPM, Swam RHA. Medical Microbiology: A guide to laboratory diagnosis and control of infections. 12th ed. Edinburg: Churchill Livingstone; 1980.
35. Gislene GF, Locatelli J, Freitas PC, Silva GL. Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. Brazilian Journal of Microbiology. 2000; 31(4):20-33.
36. Adegoke AA, Adebayo-Tayo BC. Phytochemical composition and antimicrobial effects of *Corchorous olitorius* leaf extracts on four bacterial isolates. Journal of Medicinal Plants Research. 2009; 3(3):155-159.
37. Rievere C, Van Nguyen JH, Pieters L, Dejaegher B, Heyden YV, Minh CV, Quetin-Leclercq J. Polyphenols isolated from antiradical extracts of *Mallotus metcalfeanus*. Phytochemistry. 2009; 70:86-94.
38. Mandal P, Sinha SP, and Mandal NC. Antimicrobial activity of Saponins from *Acacia auriculiformis*. Fitoterapia. 2005; 76(5):462-565.
39. Manjunatha BK. Antibacterial activity of *Pterocarpus santalinus*. Ind. J. Pharm. Sci., 2006; 68(1):115-116.
40. Geyid A, Abebe D, Debella A, Makonnen Z, Abera F, Tekla F, Kebede T, Urga K, Yersaw K, Biza T, Mariam BH, Guta M. Screening of medicinal plants of Ethiopia for their anti-microbial properties and chemical profiles. Journal of Ethnopharmacology. 2005; 97:421-427.
41. Lü L, Liu SW, Jiang SB, Wu SG. Tannin inhibits HIV-1 entry by targeting gp41. Acta Pharmacol. Sin. 2004; 25(2):213-8.
42. Akiyama H, Fujii K, Yamasaki O, Oono T, Iwatsuki K. Antibacterial action of several tannins against *Staphylococcus aureus*. J. Antimicrob. Chemother. 2001; 48(4):487-491.
43. Kolodziej H, Kiderlen AF. Antileishmanial activity and Immune modulatory effects of tannins and related compounds on *Leishmania* parasitised RAW 264.7 cells. Phytochemistry. 2005; 66(17): 2056-2071.
44. Okiei WM, Ogunlesi EA, Osibote MK, Binutu, Ademoye MA. Comparative studies of the antimicrobial activity of components of different polarities from the leaves of *Nauclea latifolia*. Res. J. Med. Plant. 2011; 5: 321-329.
45. Isah Y, Ndukwe IG, Joseph OA. Isolation and bioactivity of pentacyclic triterpenoid (Betunilic acid) from the bark of *Sarcocephalus latifolius*. Journal of Natural Sciences Research. 2012; 2(4):13-23.