Research Article

Antibacterial activity of *Solenostemon monostachyus* and *Ocimum gratissium* extracts

Ikpesu Thomas Ohwofasa[1]*, Opara Christina Ngozi[2]

[1]*Department of Biology Federal University Otuoke, Nigeria*

[2]*Department of Microbiology, Federal University Otuoke Nigeria*

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Abstract

Objective: The in vitro antimicrobial screening of *Solenostemon monostachyus* and *Ocimum gratissium* against gram negative bacteria was investigated. **Materials and methods**: The plants were cultivated for this research, while *Salmonella typhii*, *Escherichi coli* and *Eertrobacter aerogenes* that were freshly isolated from clinical samples were studied. The leaves of both plants were analyzed quantitatively for the present of phytochemicals. The sensitivity of these bacteria isolates was performed using disk diffusion method using ofloxacin as a sensitivity drug. The antimicrobial activity was determined by measuring the diameter zones of inhibition (mm), and for the sensitive measurement (inhibitory zones ≥18) and resistant measurement (inhibitory zones ≤15). **Results**: The diameter zones of inhibition revealed that ethanol extract had the highest followed by methanol and aqueous extract. However, the synergy of the two plants extract had higher diameter zone of inhibition than individual plants extract. **Conclusion**: This study had shown the potency of the plants extract; therefore, more attention should be given to medicinal plants as they have shown to be clinically effective. Similarly, the plant's extract can be used as research baseline to find out active natural products that may provide a leads in the formulation of new pharmaceuticals product to tackle resistance bacteria strains.

Keywords: Antimicrobial activity, *Solenostemon monostachyus*, *Ocimum gratissium*, Gram negative bacteria, ofloxacin

Introduction

The uses of medicinal plants for the treatments of ailments have been in vogue for a very long time. Plant products have been in used from the pre-historic times till present day, though the usage differs. All plants are useful, but for the fact that a lot of screening involves in identifying a particular plant that is anti- to certain ailment or infections involve long screening, has made it difficult to explore the efficacy of many plants (Matasyoh et al., 2000).

Herbal medicine remains the mainstay of about 75-80% of the world population, mainly in the developing countries like Nigeria for primary health services (Kamboj, 2006). This is primarily because of the faith that herbal drugs are without any negative effects, besides being cheap and readily available. Many plants extract are useful for therapeutic purposes or active components for the synthesis of useful drugs. Plants contain chemical compounds for protection of biological entities, including resistance against insect bites, fungi, bacteria and other pathogenic organisms (Ekundayo and Ezeogu, 2006). There are numerous active compounds are known to science community that are useful in safe guarding organisms. The plant extracts work on the human system in a similar way as conventional drugs do (Murray and Pizzorno, 2000). So herbal medicines can be helpful and have detrimental side effects just like approved drugs. Pharmacologist makes use of ethnobotany to investigate for pharmacologically active substances in nature, and has in this way discovered hundreds of helpful compounds that are refined for human and animal treatments. Worldwide, herbal medicine received a boost when the WHO encouraged developing countries to use traditional plant medicine to fulfill needs unmet by modern systems (Winslow and Kroll, 1998).

**Address for Corresponding Author:*
Ikpesu Thomas Ohwofasa
Department of Biology Federal University Otuoke, Nigeria
E-mail : tomohwofasa@yahoo.com
Cell number: +23480323121441

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Solenostemon monostachyus is an essential herb that is common in west and central Africa. It is an annual plant that is readily available, and does not selective in nutritional requirement. It is luscious, aromatic and grows between 80 -100 cm in height), with the leaf shown to contain B-piueue, B-caryophyllene octem-3-01, oct-1-eu-3-01 (Mve-Mba et al., 1994). The decoction of the plant can be used as a diuretic and the treatment of hypertension (Koffi et al., 2009). Recent finding revealed the biological activities of the plant such as antiulcer (Amazu et al., 2015) and antioxidant (Datte et al., 2010).

Ocimum gratissimum is a home grown shrub use as medicinal plant to treat many diseases, because of its bioactive constituents of this plant; alkaloids, tannins, flaronoids, saponins and phenolic compounds. Oil from the leaves has been shown to inhibit bacterial fungal activities and serves as antiseptic a useful food preservative and treatment leishmaniasis (Elujoba , 2000).

Salmonella typhii is a rod-shaped gram-negative bacteria belonging to the Enterobacteriaceae family. When matured on ordinary agar, they show spherical smooth colonies about two to four millimeters in diameter (Elujoba, 2000). However, on hekben enteric agar, colonies are bluish-green with black centers and are motile and facultative anaerobe. They are oxidase and KCN negative, catacase citrate and Hzs positive (Ryan et al., 2004). They are responsible for disease condition such as typhoid fever, paratyphoid fever, and food poisoning.

Escherichia coli is the most commonly member of enterobacteriaceae in the normal colonic flora and the most familiar causes of opportunistic infections. They are facultative, ferment glucose and are well known in reduction of nitrates to nitrites and are oxidase negative bacterium (Sheridan, 1994). The bacterium was originally thought to be a harmless member of the colon flora, but is now linked with different disease and infections such as meaningeal, gastrointestinal, urinary tract, wound, peritonitis, cholecystitis, septic wounds, bedsores and bacterenia infections (Connie et al., 2014). *Eertrobacter aerogenes* are gram-negative, facultative anaerobic, rod-shaped, non-spare-forming bacteria. They are pathogenic and cause opportunistic infection in immunocompromised hosts especially in a poorly ventilated area. Infect principally the urinary and respiratory tracts (Murray et al., 1998).

The use of plants for curing different ailment has been in existence for a very long time and forms the basis of contemporary medicine. Hence, this study was therefore conducted to test the efficacy of leaf extracts of *S. monostachyus* and *O. gratissimum* against *Salmonella typhii*, *E.coli* and *Eertrobacter aerogenes* isolated from clinical samples.

Materials and methods

Cultivation of *S. monostachyus* and *O. gratissimum*

A well-drained, sunny location within the school premise was selected for the cultivation of the herbs. 20- by 20- foot garden was used to grow the herbs and were propagated together. The soil was tested to determine the soil pH and nutrient levels. The soil was within pH range (6.0 - 6.8) .Geophysics Department of the University assist in ascertain the physicochemical parameter of the soil. The seeds of *S. monostachyus* and *O. gratissimum* were purchased from the market and cultivated within 2 hours. The plants were monitored and grew into maturity within three months

Collection and preparation of plant extracts

The fresh leaves of *O. gratissium* and *S. monostachyus* were collected from the garden and dried separately at room temperature (22+0.15)^{o}C for 21 days. They were grounded using Nakai blender and filtered through a 40-mesh screen and extracted for 7 hours using the Soxhlet apparatus (Onunkwo, 2004) with slight modification.

Fifty (50) gram of each plant leaves powder was extracted separately using ethanol, methanol and water. Five hundred (500) ml of each solvent (ethanol, methanol and water) were measured individually using a volumetric flask and was dispensed into the flat bottom flask, and subjected to a vigorous shaking in a sonication bath for 3 hours. The solvent was separated, concentrated in vacuum at 30^{o}C. After total evaporation of the solvent, the extract for each extract was weighed and preserved aseptically at 4^{o}C.

Phytochemical Determination

The phytochemical compositions of the plants were analysis using the methods described by Trease and Evans (1989). The leaves of both plants were analyzed quantitatively for the present of; steroids, resin, flavonoid, tanins, saponins, alkaloid and phenolics**.**

Preparation of Test Concentration

Four (4) different graded concentrations (10mg/ml, 20 mg/ml, 40 mg/ml and 80 mg/ml) of the extracts were aseptically prepared using distilled water and then subjected to antibacterial activity assays.

Collection of Test Organisms

Salmonella typhii, *Escherichi coli* and *Eertrobacter aerogenes* that were freshly isolated from clinical samples were used for this investigation. They were obtained from the Department of Microbiology, Federal Medical Centre (FMC), Yenagoa Bayelsa State Nigeria. The Bacterial

strains were grown and maintained on Muller-Hinton Agar medium, slants at 4°C in incubator.

Antibiotic Sensitivity Test

The sensitivity of the bacteria isolated from the clinical samples was performed using disk diffusion method as described by Perez et al. (1990) using ofloxacin as a sensitivity drug with little alteration. Briefly, 1 ml of each bacterium isolates were seeded into each of the Petri dishes containing Mueller-Hinton agar (MHA) and were allowed to stand for 45 minutes to allow the pre-diffusion of the inoculated organisms. The disc that has ofloxacin was placed on the surfaces of the Muller-Hinton agar plates with a sterilized forceps and lightly pressed to allow even contact and these were then incubated for 24hrs at 35°C. The antimicrobial activity was determined by measuring the diameter of zones of inhibition (mm) produced. For the sensitive measurement (Inhibitory zones ≥ 18) and resistant measurement (Inhibitory zones ≤ 15) was determined following the manufacturer's standard zone size manual.

Antibacterial bioassay procedure

The screening test of antimicrobial activity to the different extracts and the plants synergy was done using by using the disk diffusion method (Perez et al., 1990). An inoculums suspension was swabbed homogeneously to solidified 20 mL Mueller-Hinton Agar and allowed to dry for 3 mins. Sterile cork borer was used to make holes of 8 mm in diameter in the seeded agar. Aliquot of 50 µL from each graded plant's crude extract concentrations (50mg/ml, 100 mg/ml, 200 mg/ml and 400 mg/ml) were added into each well on the seeded medium and allowed to stand on the bench for 1 hr for proper diffusion and thereafter incubated at 37°C for 24 hrs. The antimicrobial activity was determined by measuring the diameter of zones of inhibition (mm) produced. Control using 50 µL Phosphate Buffered Saline (PBS) was also run following the same procedure as the treatments. All the screenings were performed in triplicate.

Assessment of the Synergistic effect of the leaves extracts

The test above showed that the zone of maximum inhibition increased with increase in the concentrations of the plant's extract. Thus, 400mg/ml of each of *S. monostachus* and *O. gratissium* were used for synergic examination, using methanol, ethanol and aqueous extraction. Therefore, equal volume (400mg/ml) of each of extraction type (ethanol, methanol and aqueous) of the plants leaves were homogenized, and 0.5ml of the homogenate was put into each well and follows the procedure of the antibacterial bioassay to test its sensitivity. The synergic assessment was also replicated.

Activity Index (A.I) of the crude plant's extract

The procedure for the calculation of the activity index of the extract followed the procedure described by Vedpriya et al. (2010) where A.I. is the ratio of mean of zone of inhibition of the extract to the mean of zone of inhibition of standard antibiotic drug i.e.

$$\text{A. I.} = \frac{\text{Mean of zone of inhibition of the extract}}{\text{Mean of zone of inhibition of standard antibiotic drug}}$$

Statistical analysis

The results of the antibacterial activity of different plants leaves extract treatments to various bacteria strains were expressed as mean ± standard error (SE). The difference between the control and the various treatments and within the treatments were analyses using the student's t- test at 95% confidence level (Chao-Hsun et al., 2010). and one-way analysis of variance SPSS (14.0 version), SPSS Inc, Chicago, USA, P values of 0.05 or less were considered statistically significant.

Results

The phytochemical analysis of compositions of the leaves extracts of *S.monostachyus* and *O. gratissimum* showed the presence of the following phyto-constituents; Steroids, Resin, flavonoid s, Saponins, Alkaloid, Phenolics and Tanin. However, tanin was not detected in *S.monostachyus*. The extent of concentrations of the chemicals in these plant leaves extract differs (Table 1).

Table 1. Phytochemical compositions of the leaf extracts of *S.monostachyus* and *O. gratissimum*

Phytochemicals	*S. monostachyus*	*O. gratissimum*
Steroids	*	*
Resins	*	*
Flavonoids	**	*
Saponins	**	*
Alkaloids	**	**
Phenolics	*	**
Tannins	≠	**

*Present in moderately concentration; **Present in high concentrations; ≠ Not detected

The results of antibacterial activity of the leaves extracts of the individual plants are given in table 2 and 3, while the equal ratio by volume of *S.monostachyus* and *O. gratissimum* extracted with only ethanol was shown in table 4. There was no significant different ($p > 0.05$) in the diammeter zone of inhibition in all the treaments for both plants irrespective of the concentrations of the plants, extraction solvents and the bacteria isolates.

The antibacterial activities of *S. monostachyus* and *O.*

Table 2. Antibacterial activities of the leaves of *S. monostachyus* extracted with aqueous, ethanol and methanol solutions at different concentrations on some bacteria species (Mean diammeter zone of inhibition (mm) ± SE)

Aqueous			
Extract (mg/ml)	***S.typhii***	***E. coli***	***E. aerogenes***
100	5.70 ± 0.23[a]	6.10 ± 0.14[a]	7.20 ± 0.35[a]
200	6.60 ± 0.27[a]	6.90 ± 0.22[a]	7.20 ± 0.21[a]
400	7.90 ± 0.15[a]	8.10 ± 0.31[a]	8.40 ± 0.51[a]
Ethanol			
100	8.60 ± 0.20[a]	8.80 ± 0.43[a]	8.10 ± 0.52[a]
200	9.20 ± 0.17[a]	8.90 ± 0.19[a]	8.50 ± 0.17[a]
400	16.90 ± 0.22[a]	15.70 ± 0.37[a]	15.20 ± 0.42[a]
Methanol			
100	5.50 ± 0.10[a]	5.80 ± 0.15[a]	6.10 ± 0.31[a]
200	5.70 ± 0.35[a]	6.50 ± 0.18[a]	7.10 ± 0.28[a]
400	8.10 ± 0.41[a]	7.80 ± 0.22[a]	9.60 ± 0.19[a]
Ofloxacin (Control)	27.00 ± 0.20	26.50 ± 0.15	24.00 ± 0.10

Mean with different superscript in the row are significantly different * ($p < 0.05$)

Table 3. Antibacterial activities of the leaves of *O. gratissimum* extracted with aqueous, ethanol and methanol solutions at different concentrations on some bacteria species (Mean diammeter zone of inhibition (mm) ± SE)

Aqueous			
Extract (mg/ml)	***S. typhii***	***E. coli***	***E. aerogenes***
100	5.20 ± 1.03[a]	5.70 ± 0.20[a]	5.80 ± 0.12[a]
200	5.80 ± 0.20[a]	5.90 ± 0.09[a]	6.10 ± 0.11[a]
400	6.20 ± 0.10[a]	6.40 ± 0.10[a]	6.00 ± 0.20[a]
Ethanol			
100	6.70 ± 0.16[a]	7.10 ± 0.20[a]	7.40 ± 0.30[a]
200	11.50 ± 0.30[a]	9.50 ± 0.30[a]	9.20 ± 0.20[a]
400	18.10 ± 0.10[a]	17.20 ± 0.10[a]	17.10 ± 0.14[a]
Methanol			
100	6.10 ± 0.30[a]	6.30 ± 0.20[a]	6.80 ± 0.20[a]
200	8.50 ± 0.21[a]	8.90 ± 0.20[a]	9.30 ± 0.11[a]
400	11.30 ± 0.21[a]	9.10 ± 0.06[a]	11.00± 0.20[a]
Ofloxacin (Control)	27.00 ± 0.20	26.50 ± 0.15	24.00 ± 0.10

Mean with different superscript in the row are significantly different * ($p < 0.05$)

gratissimum revealed that the extracts showed antibacterial activities on all the bacteria isolates studied and varies with type of extract. Ethanol extract had the highest antibacterial activity followed by methanol extract and aqueous extract (Table 2 and 3). Equally, the synergic treatment (concoction) showed antibacterial activity than individual treatment (Table 2, 3 and 4). The zone of inhibition in all the treatments is directly proportional to the concentration of the extract and the activity index of these plants extract revealed that the higher the concentrations of the extract the higher the A.I. in all the species of bacteria irrespective of the extraction methods (Figure 1-6). However, ethanol extraction method showed more prospect with the highest A.I. of 0.55 and 0.59 in *S. monostachyus* and *O. gratissimum* respectively and was recorded in *S. typhii* (Figure 2 and 5)

The combination of the plants extracts (synergy) is shown in table 4. The zone of inhibition in the synergic treatments revealed better inhibition zone than when treated separately. Similar to individual treatment, the synergic studied showed the zone of inhibition increased with

increase in the concentration of the extracts and the ethanol extract of the synergic treatment had the highest zone of inhibition follow by methanol and least in the aqueous solution.

Table 4. Synergic assessment of the antibacterial activities of the leaves of *S. monostachyus* and *O. gratissimum* leaves on some bacteria species extracted with methanol, ethanol and aqueous solutions (Mean diammeter zone of inhibition (mm) ± SE)

Extract (400 mg/ml)	*S. typhii*	*E. coli*	*E. aerogenes*
MSO	10.40 ± 1.30[a]	15.50 ± 0.60[a]	14.10 ± 0.32[a]
ESO	21.30 ± 2.10[a]	23.10 ± 0.19[a]	19.60 ± 0.29[a]
ASO	12.50 ± 0.50[a]	16.50 ± 0.30[a]	18.50 ± 0.32[a]
Ofloxacin (Control)	27.00 ± 0.20[a]	26.50 ± 0.15[a]	24.00 ± 0.10[a]

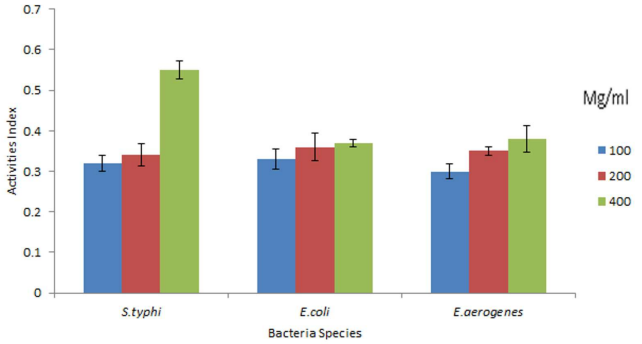

Figure 1. The activities index (A.I) of the leaves of ***S.*** *monostachyus* extracted with methanol at different concentrations in bacteria isolates (Ofloxacin as the standard antibiotic drug)

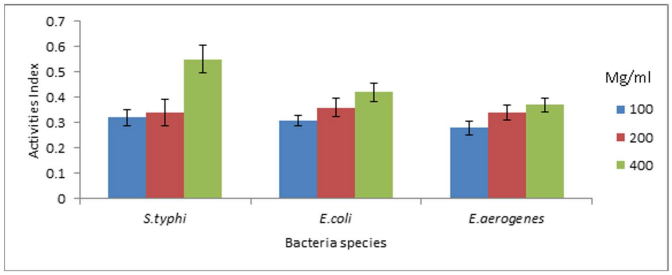

Figure 2. The activities index (A.I) of the leaves of *S. monostachyus* extracted with ethanol at different concentrations in bacteria isolates (Ofloxacin as the standard antibiotic drug)

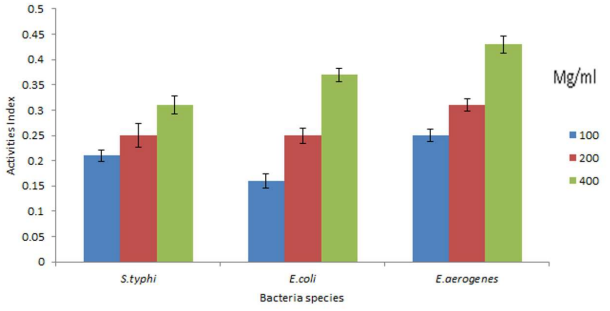

Figure 3. The activities index (A.I) of the leaves of **S**. *monostachyus* extracted at different concentrations (aqueous extract) in bacteria isolates (Ofloxacin as the standard antibiotic drug)

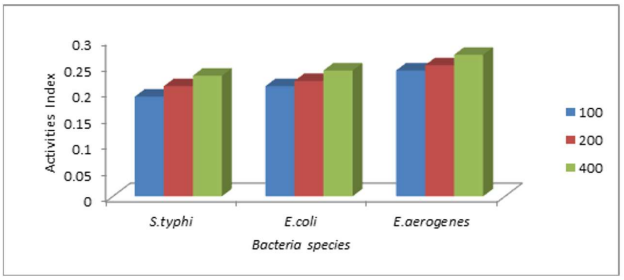

Figure 4. The activities index (A.I) of the leaves of *O. grattissium* extracted with methanol at different concentrations in bacteria isolates (Ofloxacin as the standard antibiotic drug)

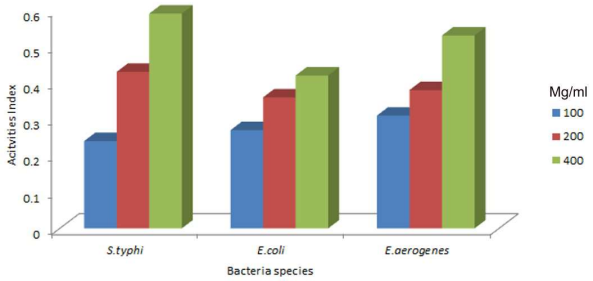

Figure 5. The activities index (A.I) of the leaves of *O. grattissium* extracted with ethanol at different concentrations in the species of bacteria (Ofloxacin as the standard antibiotic drug)

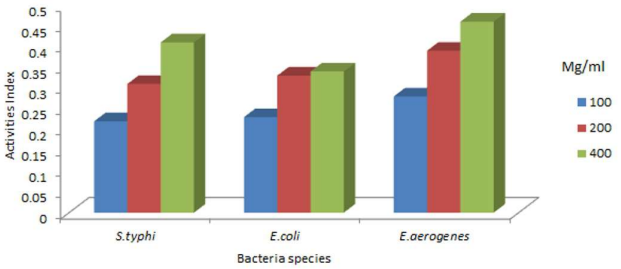

Figure 6. The activities index (A.I) of the leaves of *O. grattissium* extracted at different concentrations (aqueous extract) in the species of bacteria (Ofloxacin as the standard antibiotic drug).

Discussion

The prevalence of antibiotic resistance bacteria had call for extra investigation for alternative cure to the stubborn antibiotic resistant pathogen (Westh et al., 2004). In this investigation, the phytochemical analysis of *S. monostachyus* and *O. gratissimum* revealed the presence of steroids, resin, saponins, alkaloid, phenolics, flavonoid and tanins (not detected in *S. monostachyus*). Though the extent of concentrations of the compound in this plant's leaves extract differs, their presence indicates that these plants can be used as antimicrobial agents (Doherty et al., 2010).

This study had shown that the crude extracts of some medicinal plants have good inhibitory effect against bacterial species. Though, the efficacies of *S. monostachyus* and *O gratissimum* depend on the extraction method and also directly proportional to the concentrations of the extracts. Undoubtedly, the leaves extracts of both plants inhibit the activities of all the investigated bacteria. Similar findings on antibacterial activity of some plant were reported by other researchers (Rahman et al., 2011).

Methanol and ethanol extracts of *S. monostachyus* and *O gratissimum* showed comparatively better activities index with ethanol more promising, while aqueous extract has poor antibacterial activity. The propensity of the test bacteria to methanol and ethanol extracts is not new, this is due to the fact that the active phytochemical constituents of the leaf extract can easily dissolve in ethanol (organic solvent) than in water (inorganic solvent).Previous investigations have shown that methanol and ethanol are better solvent for extraction than water (Obi and Onuoha, 2000).

The synergic treatment had more inhibition than individual dose, thus more effective in treating bacterial infection. The high inhibition may be as a result of complexes that result from the mixture of the two plants. Similar observation was reported by Zafar et al. (2010) who investigated the combination of the extract of *Salvadora persica* extracts, tetracycline and penicillin against a bacterium species.

This investigation had shown that medicinal plants are indispensable for pharmacological research and drug production. They contain phytochemicals that are inexplicable in drugs development in this contemporary period. It can be deduced that both extracts demonstrated antibacterial activities against all the investigated bacteria species and the synergic treatment showed more inhibition than individual dose and had higher activity index.

In general, more attention should be given to medicinal plants as they shown to be clinically effective and safer alternatives to the synthetic drugs. Similarly, the plant's extract can be used as research baseline to find out active natural products that may provide a leads in the formulation of new pharmaceuticals product to tackle resistance bacteria strains. It becomes imperative that plant's extracts need to be formulated into tablet or capsules for accurate dose, great precision, and protection from atmospheric condition such as moisture light and air

Conflict of Interests

I would like to undertake that the research was self-sponsored. The authors hereby declare that, there is no conflict of interests regarding the publication of this article.

References

Amazu LU, Okokon JE, Nwidu LL. 2015. Antiulcergenic Activity of Stem Extract and fractions of *Homalium Letestui*. International Journal of Pharmacognosy, 2(5):242-247 .

Chao-Hsun Y, Yu-Chun H, Yu-fen C, Ming-Hsiang C. 2010. Foam properties ,detergent abilities and long term preservative efficacy of the saponins from *Sapindus mukorossi*. Journal of Food Drug Analysis, 18:155–160.

Connie RM, Donald C, George M. 2014.Textbook of Diagnostic Microbiology, Elsevier Health Sciences, pp. 1104.

Datte JY, Kpahe F, Offoumou AM. 2010. Acute toxicity and antioxidant activity of hydroethanolic extract of *S.monostachyus* P. Beauv.leaves. Journal of complementary and Integrative Medicine, 7(4):37- 45.

Doherty L, Sheen MR, Vlachos, A, Choesmel V, O'Donohue MF, Clinton C, Schneider HE, Sieff CA, Newburger PE, Ball SE, Niewiadomska E, Matysiak M, Glader B, Arceci RJ, Farrar JE, Atsidaftos E, Lipton JM, Gleizes PE, Gazda HT. 2010. Ribosomal protein genes RPS10 and RPS26 are commonly mutated in Diamond-Blackfan anemia. American Journal of Human Genetics, 86(2):222-228.

Ekundayo EO, Ezeogu LI .2006. Evaluation of antimicrobial activities of extracts of five plants used in traditional medicine in Nigeria. International Journal of Tropical Medicine, 1:93-96.

Elujoba AA .2000. Studies on the antidiarrhoea activity of *Ocimum gratissimum*. University of Ile Ife Press.

Kamboj VP. 2000. Herbal Medicine. Current Science, 78:9–35.

Koffi N, Marie-Solange T, Emma A-A, Noel ZG. 2009. Ethnobotanical Study of Plants Used to Treat Arterial Hypertension, in Traditional Medicine, by Abbey and Krobo Populations of Agboville (Côte-d'Ivoire). European Journal of Scientific Research, 35:85-98.

Matasyoh LG, Matasyoh JC, Wachira FN, Kinyua MG, Muigai Thairu, AW, Mukiama TK . 2000. Chemical composition and antimicrobial activity of the essential oil of *Ocimum gratissimum* L. growing in Eastern Kenya. African Journal of Biotechnology, 76(6):760–765.

Murray P, Ken S, Michael A.1998. Medical Microbiology. 3rd Edition, Mosby Incorporation, London. Pp. 234-239.

Murray MT, Pizzorno JE Jr .2000. Botanical medicine-

amodern perspective. In Text Book of Natural Medicine Vol 1(eds. Pizzorno JE Jr., Murray M T) Churchill Livingstone, pp. 267-79.

Mve-Mba CE, Monut C, Lamaty G, Zollo PH, Tchoumbougnang HD. 1994. Aromatic plants of tropical central Africa. Part XIX. Volatile components from leaves of two lamiaceae from Cameroon: *Leucas deflexa* and *Solenostemon monostachyus* (P. Beauv.) Briq. Flavour and Fragance Journal, 9(6):315-317.

Obi VI, Onuoha C. 2000. Extraction and characterization method of plants and plant products. In: Biological and Agricultural Techniques. Ogbulie, J. L, and O. J. Ojiako (eds).Webs media publications, Owerri.

Onunkwo GC, Egeonu HC, Adikwu MU, Ojile JE, Olowosulu AK.2004. Some physical properties of tabletted seed of *Garcinia kola* (Heckel). Chemical and Pharmaceutical Bulletin, 52 (6):649–653.

Perez C, Paul M, Bazerque P. 1990. Antibiotic assay by agar-well diffusion method. Acta Medicine and Experimental Biology, 15:113-115.

Rahman S, Abd-Ellatif S, Deraz S, Khalil A. 2011. Antibacterial activity of some wild medicinal plants collected from western Mediterranean coast, Egypt: Natural alternatives for infectious disease treatment. African Journal of Biotechnology, 10(52):10733-10743.

Ryan K, Ray C, Sherris J. 2004. Medical Microbiology: An Introduction to Infectious Diseases, 4th edition.

Sheridan MA.1994. Regulation of lipid metabolism in poikilothermic vertebrates. Comparative Biochemistry and Physiology, 107:495-508.

Trease GE, Evans WC.1989. Pharmacognosy, 13th edition: ELBS Oxford University Press, London, UK, pp. 245-263.

Vedpriya A, Sanjay Y, Sandeep K, Yadar J P. 2010. Antimicrobial activity of *Cassia occidentalis* against various human pathogenic microbes Life Science Medical Research, 9:1–10.

Westh H, Zinn CS, Rosahi VT. 2004. An international multicenter study of antimicrobial consumption and resistance in *Staphylococcusaureus* isolates from 15 hospitals in 14 countries. Microbial and Drug Resistance, 10:169 –179.

Winslow LC, Kroll DJ. 1998. Herbs as medicine. Archive of International Medicine, 158:2192- 2199.

Zafar A, Shaukat SK, Mohnaaz K. 2010. Synergistic effect of *Salvadora persica* extracts, tetracycline and penicillin against *Staphylococcus aureus*. Africa Journal of Basic and Applied Science, 2:25–29.