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# ANTIBACTERIAL EFFECTS OF SOME CAMEROONIAN MEDICINAL PLANTS AGAINST COMMON PATHOGENIC BACTERIA.

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## Abstract

We screened forty crude extracts of twenty Cameroonian medicinal plants commonly used to treat bacterial infections for broad spectrum antibacterial activity, as a more affordable alternative against resistant organisms. The extracts were screened on common pathogenic gram negative and gram positive bacteria initially by the disc diffusion method followed by the tube dilution method. Using discs containing 30µg of extract, *Escherichia coli* showed sensitivity to 23 extracts with diameter of zone of inhibition ranging from 7 – 19mm, fifteen of which were up to or > 10mm. *Pseudomonas aeruginosa* was sensitive to 11 extracts, whereas *Salmonella typhimurium* and *Staphylococcus aureus* were not sensitive to any of the extracts. Based on the zones of inhibition the activity of the extracts were equivalent to 30 to 138 % efficacy of the standard antibiotic discs. The lowest Minimum Inhibitory Concentration (MIC) recorded was 2 mg/ml for *Euphorbia hirta* against S. *aureus* and *P. aeruginosa* and the lowest Minimum Bactericidal Concentration (MBC) was 6 mg/ml for six extracts from *Ageratum conyzoides*, *Aframomum citratum*, *Euphorbia hirta*, *Momordica charantia, Mangifera indica* and *Khaya senegalensis* against three bacterial species. Three extracts had broad spectrum bacteriostatic activity (MICs ≤ 4 mg/ml) while in terms of MBCs none of the extracts showed broad spectrum bactericidal activity. We conclude that most of the tested plants used as traditional antibacterials have a bacteriostatic effect on gram-negative pathogenic bacteria.

**Keywords:** plant extracts, broad spectrum, bacteriostatic, bactericidal

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## Introduction

Bacterial infections are common particularly in the tropics where Cameroon is located and constitute a significant part of the disease burden (Kumar and Clark, 1990). Chemotherapy is the main approach in the treatment of bacterial infections. A major problem encountered with antibiotics in clinical use is resistance of bacteria to antibiotics, which may lead to treatment failure (Mckeegan et al, 2002). Other problems with various antibiotics include toxicity, high cost and low efficacy. These problems therefore necessitate a constant search for new antibacterials.

Medicinal plants are widely used in African communities to treat bacterial infections (Sofowora, 1993). Also a significant proportion of pharmaceutical products in current use are derived from plants (Cowan, 1999 and Raskin et al, 2002). A large number of phytochemicals belonging to several chemical classes have been shown to have inhibitory effects on all types of microorganisms *in vitro* (Cowan, 1999); and some plant extracts have shown activity on both gram negative and gram positive organisms (Nascimento et al, 2000). Presently there is no single plant-derived antibacterial chemical entity used clinically (Gibbons, 2004). Medicinal plants generally contain a number of compounds which may be a potential natural antibacterial combination and which may serve as an alternative effective, cheap and safe antibacterial for treatment of common bacterial infections. However, for some of these plants their effect in terms of bacteriostatic and bactericidal action and spectrum of organisms affected remains to be established. This knowledge would enable more rational exploitation of such plants both in traditional medicine and in the empirical development of new antibacterials. We therefore studied medicinal plants commonly used to treat bacterial infections in Cameroon, on selected common pathogenic bacteria to establish the type of effect produced and to identify plants with broad spectrum activity.

## Materials and Methods

### Ethnobotanical survey

Twenty plants used in villages around the town of Buea, in Cameroon, for treatment of bacterial infections ranging from enteric infections such as diarrhoea, respiratory infections, urogenital infections, skin infections, wounds, ulcers and typhoid fever were identified in collaboration with traditional medicine practitioners, local people and botanists of the Limbe Biodiversity and Conservation Centre, Cameroon, where samples of the plants have been kept in the herbarium. The parts used were collected for the study.

### Preparation of Extracts

The plant materials were dried in the shade, chopped, ground and macerated in (i) Methanol:methylene chloride (1:1) for 48 hours or (ii) Ethylacetate (48 hours) followed by Methanol (48 hours ), then filtered and the filtrate concentrated by rotary evaporation.

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**Antibacterial Sensitivity Test**
**(a) Organisms**

Two isolates of each bacterial species (both gram positive and gram negative) were obtained from different clinical specimens from health centres within the study area by standard culture techniques and further identified by biochemical techniques using API 20E (BioMerieux SA) kit as described (Ndip et al, 2005).The isolates were maintained during the study period by subculturing every 48 hours on nutrient agar. The required suspension of bacteria was prepared equivalent to McFarland standard 1 ($1x10^8$ CFUs/ml) in 0.85% NaCl $_{(aq)}$ and crosschecked and adjusted by the standard plate count method (Black, 1996).

**(b) Disc Method**

Five millimeter discs containing 30 µg of extract were prepared as described by Cheesborough (1984), and placed on cultured pathogenic bacteria on agar plates (Columbia agar base) and incubated at 37°C. The plates were checked for bacterial growth after a minimum of 16 hours and occasionally till 24 hours. The diameter of the zone of inhibition was then measured. The sensitivity of *Escherichia coli* to 40 extracts was determined. This was repeated with *Pseudomonas aeruginosa, Salmonella typhimurium* and *Staphylococcus aureus* using the extracts to which *E. coli* was sensitive. Commercial discs of Ampicillin (25µg), Cotrimoxazole (25µg), Chloramphenicol (30µg) and Amikacin (30µg) were used as positive control.The experiment was done twice for each extract.

**(c) Tube dilution method**

This was performed as described by Cheesbrough (2000) with some modification. Stock solutions were prepared by dissolving the extracts in dimethylsulfoxide (DMSO) followed by distilled water to give a final stock concentration of 30 mg/ml. Each microorganism was incubated with an extract in duplicate tubes containing a total volume of 10 ml, comprising 6 ml of peptone water sugar medium, 1ml of bacterial suspension containing 1 x $10^8$ CFUs and 3 ml of extract solution. The final concentration of extract was in the range 0.1 to 10 mg/ml, (final DMSO concentration = 5%). The tubes were tightly sealed and incubated at 37°C for 24hours (Nkuo-Akenji et al, 2001). Control tubes without extract were constituted similarly. Ampicillin (50 -100 µg/ml) was included as positive control. The Minimum Inhibitory Concentration (MIC) was the lowest concentration of extract with no visible bacterial growth (no turbidity and or colour change of indicator). To determine the Minimum Bactericidal Concentration (MBC), 0.2 ml of the contents of the MIC tubes were diluted 10 fold in fresh medium and incubated at 37°C for 24 hours and the lowest concentration of the MIC tubes with no visible bacterial growth was recorded as the MBC.

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### Statistical Analysis

The experiments were set up in duplicate and repeated once for each of the two isolates per bacterial species. The mean diameters of the zones of inhibition, MICs and MBCs were calculated from the values of the two experiments for each isolate and reported as final results. The four mean values of the zone diameters for each extract per bacterial species were compared with the values of the controls using the Mann-Whitney test.

## Results

### Antibacterial activity by Disc Method

Of the 40 extracts investigated using discs with 30µg of extract on pathogenic bacteria, *E. coli* showed sensitivity to twenty-three extracts with diameters of zone of inhibition ranging from 7 – 19 mm, fifteen of which were either as high as or > 10 mm as shown on Table 1 (i.e. 30 to 83 % efficacy of Ampicillin and 28 to 78 % efficacy of Cotrimoxazole). *Ageratum conyzoides* gave the highest zone of inhibition (average of 19 mm) against *E. coli* but it was significantly lower than the zone diameters of Ampicillin and cotrimoxazole ($p < 0.05$). Seventeen extracts showed no inhibition of *E. coli* growth i.e. methanol extract of *A. conyzoides*; ethylacetate extracts of *Aloe vera*, *Cymbopogon citratus, Euphorbia hirta*, *Musa paradisiaca*, *Ocimum gratissimum* and *Terminalia superba*; the methanol and ethylacetate extracts of *Biden pilosa*, *Costus afer* and *Mangifera indica*; and the methanol:methylene chloride extract of *Costus afer* and *Stachytapheta cayenensis*. *P. aeruginosa* was sensitive to eleven extracts (Table 1), equivalent to 35 to 90 % efficacy of Amikacin and 54 to 138 % efficacy of Chloramphenicol. *Carica papaya* produced the highest zone of inhibition (average of 18 mm) against *P. aeruginosa* which was equivalent to chloramphenicol ($p = 0.05$) but significantly lower than that of Amikacin ($p < 0.05$).
*S. typhimurium* and *S. aureus* were not sensitive to any of the extracts(Table 2). The control discs i.e. Ampicillin (25µg), Cotrimoxazole (25µg), Chloramphenicol ( 30µg) and Amikacin (30µg) gave zones of inhibition of 21- 25, 21 - 27, 9 – 14 and 20- 22 mm respectively.

### Minimum Inhibitory Concentrations of Extracts (MICs)

The MICs of twenty-two extracts were determined in the range 0.1 to 10 mg/ml on two gram negative and one gram positive bacteria and the results are shown on Table 3. The MICs ranged from 2 to10 mg/ml with the lowest MIC of 2 mg/ml recorded for *E. hirta* (EHWE) extract on *S. aureus.* The lowest MIC for *E. coli* was 2.5 mg/ml recorded for *A. conyzoides* (ACWE). In terms of MICs, three extracts i.e the ethylacetate extracts of *Aframomum citratum* (APFE) and *Euphorbia hirta* (EHWE) and the methanol extract of *Scoparia dulcis* (SDWM) had broad-spectrum bacteriostatic activity with MICs equal to or less than 4 mg/ml. DMSO had no effect on bacterial growth at 5% final concentration.

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**Table1:** Antibiogram results showing sensitivity of *E. coli* and *P. aeruginosa* to 30µg of plant extract

| Plant Name (Family) | Plant Part | Extract Code* | Diameter of zone of inhibition (mm) | |
|---|---|---|---|---|
| | | | *E. coli* | *P.aeruginosa* |
| | | AMP‡ | 23 | N |
| | | COT‡ | 25 | N |
| | | AMI‡ | N | 20 |
| | | CHL‡ | N | 13 |
| *Aframomum citratum* (Zingiberaceae) | Fruit | APFE | 7 | 0 |
| | | APFM | 6 – 8 | 0 |
| *Ageratum conyzoides* (Asteraceae) | Whole plant | ACWE | 19 | 8 -13 Ψ |
| *Aloe vera* (Liliaceae) | Leaf skin | AVLM | 8 | 0 |
| *Carica papaya* (Caricaceae) | Leaves | CPLX | 8 – 10 | 10-18 |
| | Seeds | CPEE | 10 | 0 |
| | Seeds | CPEM | 7 – 10 | 7 – 9 |
| *Cylicodiscus gabunensis* (Mimosaceae) | Stem bark | C.G. | 10 | 0 |
| *Cymbopogon citrates* (Gramineae) | Leaves | CCLM | 6 – 7 | 0 |
| *Euphorbia hirta* (Euphorbiaceae) | Whole plant | EHWM | 8 – 10 | 7 |
| *Khaya senegalensis* (Meliaceae) | Leaves | TKSf | 7 - 9 | 0 |
| | Seeds | TKSg | 11-13 | 7 – 8 |
| *Momordica charantia* (Cucurbitaceae) | Whole plant | MCLE | 7 – 10 | 0 |
| | | MCLM | 9 – 10 | 9 |
| *Musa paradisiaca* (Musaceae) | Fruit skin | MPFM | 9 – 10 | 0 |
| *Ocimum gratissimum* (Labiatae) | Leaf and leaf stem | OGLM | 8 – 10 | 9 |
| *Psidium guajava* (Myrtaceae) | Leaves | PGLX | 7 | 0 |
| *Peperonia fernandopoioana* (Piperaceae) | Whole herb | P.F. | 6 –10 | 7 – 9 |
| *Peperonia vulcanica* (Piperaceae) | Whole herb | P.V. | 11-13 | 7 – 12 |
| *Rauwolfia vomitoria* (Apocynaceae) | Root | RVRM | 8 | 0 |
| *Scoparia dulcis* (Scrophulariaceae) | Whole plant | SDWE | 10 – 12 | 8 – 10 |
| | | SDWM | 10 | 7 |
| *Terminalia superba* (Combretaceae) | Leaves | TSLM | 6 – 7 | 0 |

***** The last letter of the four blockletters code represents the solvent used in the extraction process:- M = Methanol; E = Ethylacetate; X = Methanol:methylene chloride; except the following: Methylene chloride for TKSg, TKSf and C.G.; Methanol for P.F. and P.V.

‡Control discs: AMP = Ampicillin, COT= Cotrimoxazole, AMI = Amikacin and CHL = Chloramphenicol. N = not applicable for this microorganism

Ψ Non-uniform zones of inhibition are reported as a range, range values represent means of the lowest and highest zone diameters measured per extract respectively.

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**Table 2:** Summary of Bacteria and number of Plant extracts to which they showed Intermediate to High sensitivity on culture Plate

| Bacteria | Type | Number of Active Extracts ( Intermediate to High Sensitivity ) | Diameter of Zone of Inhibition (range in mm) |
|---|---|---|---|
| *Escherichia coli* | Gram negative | 15 | 7 –19 |
| *Pseudomonas aeruginosa* | Gram negative | 8 | 7 – 18 |
| *Salmonella typhimurium* | Gram negative | 0 | 0 |
| *Staphylococcus aureus* | Gram positive | 0 | 0 |

Zone of Inhibition = or > 10mm was considered sensitive, compared to standard antibiotics. *E. coli* and *P. aeruginosa* showed intermediate to high sensitivity to 30µg of the extracts.

**Table 3:** MICs and MBCs of Extracts against Bacteria in mg/ml

| N$^{O}$ | Extract code | *E. coli* | | *P. aeruginosa* | | *S. aureus* | |
|---|---|---|---|---|---|---|---|
| | | MIC | *MBC* | MIC | *MBC* | MIC | *MBC* |
| 1 | ACWE | 2.5 | *10* | 4 | *6* | 6 | *>10* |
| 2 | APFE | 4 | *6* | 4 | *6* | 4 | *>10* |
| 3 | BPWE | 6 | *>10* | 4 | *> 10* | 4 | *>10* |
| 4 | BPWM | > 10 | *>10* | 6 | *> 10* | 4 | *>10* |
| 5 | C.G. | 7.5 | *>10* | 4 | *> 10* | 6 | *>10* |
| 6 | CPLX | 10 | *>10* | 8 | *> 10* | 6 | *>10* |
| 7 | CPEE | 10 | *> 10* | > 10 | *> 10* | 6 | *>10* |
| 8 | EHWE | 4 | *> 10* | 2 | *> 10* | 2 | *6* |
| 9 | EHWM | 7.5 | *7.5* | 6 | *> 10* | 6 | *>10* |
| 10 | MCLE | 5 | *7.5* | 4 | *6* | 6 | *>10* |
| 11 | MCLM | 4 | *> 10* | 6 | *8* | >10 | *>10* |
| 12 | MIBM | 5 | *> 10* | 4 | *> 10* | 4 | *>10* |
| 13 | MILE | 4 | *6* | 6 | *> 10* | 4 | *>10* |
| 14 | MPFM | > 10 | *> 10* | 6 | *> 10* | 6 | *>10* |
| 15 | OGLM | 5 | *10* | 6 | *> 10* | 6 | *>10* |
| 16 | PGLX | 7.5 | *>10* | 6 | *> 10* | 4 | *8* |
| 17 | P.F. | 7.5 | *7.5* | 4 | *> 10* | 4 | *>10* |
| 18 | P.V. | 7.5 | *10* | 4 | *> 10* | 4 | *>10* |
| 19 | SDWE | 5 | *10* | 4 | *> 10* | 6 | *>10* |
| 20 | SDWM | 4 | *> 10* | 4 | *> 10* | 4 | *>10* |
| 21 | TKSg | 10 | *> 10* | 4 | *> 10* | >10 | *>10* |
| 22 | TKSf | 6 | *8* | 4 | *6* | 4 | *>10* |

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**Minimum Bactericidal Concentrations of Extracts (MBCs)**

The MBCs (lowest concentration that killed the bacteria) showed that 10, 2 and 5 extracts were bactericidal against *E. coli, S. aureus* and *P. aeruginosa* respectively (Table 3). The lowest MBC recorded was 6 mg/ml in all three microorganisms produced by six extracts namely *A. conyzoides* (ACWE), *A. citratum* (APFE), *E. hirta* (EHWE), *Momordica charantia* (MCLE), *Mangifera indica* (MILE) and *Khaya senegalensis* (TKSf). None of the extracts had broad-spectrum bactericidal activity. Most of the bactericidal extracts were ethylacetate extracts suggesting that the active components could be non-polar compounds. Considering the MICs and MBCs within the concentration range in which the extracts were tested (0.1 – 10 mg/ml), the overall classification of the activity of the extracts is as shown on Table 4

## Discussion

We studied the antibacterial activity of some medicinal plants traditionally used in village communities around the town of Buea in the Southwest province of Cameroon, to treat diseases caused by bacteria. In our ethnobotanical survey involving some traditional medicine practitioners and people in the community, the plant parts listed in Table 1 are commonly used in the community to treat bacterial infections in Cameroon ranging from enteric diseases such as diarrhoea, respiratory infections associated with coughing, urogenital infections including sexually transmitted diseases, skin infections, wounds and ulcers; and bacterial fevers such as typhoid. These plants are used similarly in many African communities (Iwu, 1993).

**Table 4:** Summary of Activity of Extracts in the Concentration range of 0.1 - 10 mg/ml by tube method

| Microorganism | No. of Extracts and Type of Activity | | | Total |
|---|---|---|---|---|
| | No activity | Bacteriostatic only | Bactericidal | |
| *E. coli* | 2 | 10 | 10 | 22 |
| *S. aereus* | 2 | 18 | 2 | 22 |
| *P. aeruginosa* | 1 | 16 | 5 | 22 |

Discs containing 30µg of extract were used in the sensitivity test because this amount was comparable to the 25 -30 µg of antibiotics in control discs. The zone of inhibition for standard antibacterial discs ranges from <9 to 20 mm, 10 to 18 mm and >12 - 29 mm for resistant, intermediate and sensitive organisms respectively (Baker and Silverton, 1985). We observed zones of inhibition ranging from 7 – 19 mm for twenty three plant extracts against *E. coli* fifteen of which were either as high as or > 10 mm (Table 1), eight of which gave a range of 7 – 18 mm against *P. aeruginosa* (Table 2). The

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values obtained overlap with those of standard antibacterials, overall fifteen of them greater than or equal to 10mm.This shows that the sensitivity of the bacteria to most of the active extracts could be considered as intermediate, with high sensitivity to one extract, ACWE, from *A. conyzoides*. Also, *Carica papaya* leaf extract (CPLX) showed a varied inhibition of 77 to 138% against *P. aeruginosa* when compared to Chloramphenicol. Several factors including the chemical nature of the drug or substance influence results of the disc diffusion method (Barry and Thornsberry, 1991). These factors may account for two observations; firstly, for the variation in the diameter of the zones of inhibition with non-uniform zones observed for some discs; hence the diameter was reported as a range (Table 1). Secondly, these factors may explain why some extracts which showed no activity when implanted in the disc, were active when added to the bacterial suspension in the tubes. This suggests that the tube method is preferable when testing fairly complex mixtures such as plant crude extracts. The results obtained by the tube dilution method show that most of the extracts are bacteriostatic with a few having broad spectrum bacteriostatic activity, while a smaller number were bactericidal, and none having broad spectrum bactericidal activity (Table 4). This suggests that most of these plants, when used traditionally as antibacterials, inhibit bacterial growth without necessarily killing the bacteria. Overall, our results show that these medicinal plants have selective activity, being bacteriostatic or bactericidal on either gram positive or gram negative bacteria. Also, *A. conyzoides* and *E. hirta* have considerable antibacterial activity while *A. citratum, E. hirta* and *S. dulcis* have broad spectrum bacteriostatic activity.

Similar studies of extracts from some other plants in the study area including *C. papaya* and *C. citratus*, on single organisms have demonstrated antibacterial activity (Nkuo-Akenji et al, 2001 and Akoachere, 2002). The values we recorded from both methods suggest the extracts have weak antibacterial activity; probably because the extracts being crude contain very small amounts of the bioactive compound(s). However, as with some drugs, some of these plant extracts may be more potent *in vivo* due to metabolic transformation of its components into highly active intermediates or interaction with the immune system.An aqueous extract of *M. indica* has been shown to enhance some antibody subtypes (Garcia, 2003) while *O. gratissimum* appears to improve the phagocytic function of reticuloendothelial cells in mice (Atal, 1986).

It is necessary to fractionate the most active bactericidal extracts to better assess the activity of their components. Some of the plant extracts tested in this work may contain compounds with selective action against certain bacteria, this may account for the traditional use as medicinal plants. The phytochemical profiles of the more active and bacteriostatic plants show that *A. conyzoides* contains essential oils, phenolic compounds, coumarins and ageratochromone compounds; *E. hirta* contains tannins, an alkaloid, flavonoids, terpenes, ellagic acid and gallic while the *Aframomum* family contain mainly essential oils (Oliver-Bever, 1986 and Iwu, 1993). *S. dulcis* contains coumarins, phenols, saponins, tannins, aminoacids, flavonoids and terpenoids ( Ratnasooriya et al, 2005). Some of these compounds are likely to be active against certain bacteria, this may account for the traditional use as medicinal plants. Traditional medicine practitioners often use two or more plants in concoctions to treat infections caused by a variety of bacteria. Since identification of the causative bacterium is usually not done, the combined effect of

the antibacterial components of the plants in the concoction may account for the acclaimed effectiveness of such combinations in producing broad spectrum activity. Nkuo-Akenji et al (2001) demonstrated that a combination of extracts was more active against *Salmonella spp.* than the individual extracts. The *in vivo* effects of the active extracts need to be investigated to fully establish the effectiveness of these medicinal plants.

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## References

1. Akoachere, J-F T.K., Ndip, R.N., Chenwi, E.B., Ndip L.M., Njock, T.E. and Anong, D.N. (2002). Antibacterial effect of *Zingiber officinale* and *Garcinia kola* on respiratory tract pathogens. E. Afr. Med. J. **79(11):** 588 - 592.
2. Atal, C.L., Sharma, M.L., Kaul, A., Khajuria, A. (1986). Immunomodulating agents of plant origin I: preliminary screening. J. Ethnopharmacol. **18 (2):**133 -141.
3. Baker, F.J. and Silverton, R.E. (1985). Introduction to Medical Laboratory Technology, sixth edition, Butterworths, pp 297 – 300.
4. Barry, A.L. and Thornsberry, C. (1991). Susceptibility tests: diffusion test procedures. Manual of Clinical Microbiology, 5th edition. American Society for Microbiology, Balows A. (Ed). Washington, D.C., **4:** 1117 – 1125..
5. Black, J. C.(1996). Growth and culturing of bacteria. Microbiology: Principles and Applications, 3rd edition, Prentice Hall, New Jersey, pp140-142.
6. Cheesbrough, M. (1984).Medical laboratory Manual for Tropical Countries, Butterworth- Heinemann, Oxford, UK, pp 200.
7. Cheesbrough, M. (2000). District Laboratory Practice in Tropical Countries, Part II Cambridge University Press, pp 401 -402.
8. Cowan, M. M. (1999). Plant products as antimicrobial agents. Clin. Microbiol. Rev. **12(4):** 564-582.
9. Garcia, D., Leiro, J., Delgado, R., Sanmartin, M.L., Ubeira, F.M. (2003). *Mangifera indica* L. extract (Vimang) and mangiferin modulate mouse humoral immune responses. Phytother. Res.**17 (17):**1182 – 7.
10. Gibbons, S. (2004). Anti-staphylococcal plant natural products. Nat. Prod. Rep. **21(2):** 263 – 277.
11. Iwu, M.M.(1993). Handbook of African Medicinal Plants, CRC Press, Boca Raton, Florida.

12. Kumar, P. and Clark, M. (1990). Clinical medicine: A textbook for medical students and doctors, second edition, ELBS, pp 1 – 104.
13. Mckeegan, K. S., Borges-Walmsley, M. I. and Walmsley, A. R. (2002). Microbial and viral drug resistance mechanisms: A Trends guide to Infectious Diseases, a supplement to Trends Microbiol.**10(10):** S8 – S13
14. Nascimento, G.G.F., Locatelli, J., Freitas, P.C. and Silva, G.L. (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Braz. J. Microbiol.**31:** 247- 256
15. Ndip,R.N., Dilonga, H.M., Ndip, L.M., Akoachere, J.F.K. and Nkuo-Akenji, T. (2005). *Pseudomonas aeruginosa* isolates recovered from clinical samples in Buea, Cameroon: current status on biotyping and antibiogram. Trop. Med. Int. Health **10(1):** 74 – 81.
16. Nkuo-Akenji, T., Ndip, R., McThomas, A., Fru, E.C. (2001). Anti-Salmonella activity of medicinal plants from Cameroon. Cent. Afr. J. Med. **47(6):** 155 – 158
17. Oliver-Bever, B.(1986). Medicinal plants in tropical West Africa, Cambridge: pp 259.
18. Raskin, I., Ribnicky, D.M., Komarnytsky, S., Ilic, N., Poulev, A., Borisjuk, N., Brinker, A., Moreno, D.A., Ripoll, C., Yakoby, N., O'Neal, J.M., Cornwell, T., Pastor, I. and Fridlender, B. (2002). Plants and human health in the twenty-first century. Trends Biotechnol. **20(12):** 522 - 531
19. Ratnasooriya, W.D., Jayakody, J.R.A.C., Premakumara,G.A.S., and Ediriweera, E.R.H.S.S. (2005). Antioxidant activity of water extract of *Scoparia dulcis*. Fitoterapia **76:** 220-222
20. Sofowora, A. (1993). Medicinal Plants and Traditional Medicine in Africa, Spectrum Books Limited, Ibadan, Nigeria, pp 9 – 25.