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*Full Length Research Paper*

# Antimicrobial activities of *Vernonia tenoreana*

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**The antimicrobial activity of *Vernonia tenoreana* was investigated. Crude extracts and solvent fractions of the leaf and bark of *V. tenoreana* were obtained using methanol and distilled water (2:3, v:v), ethylacetate, chloroform, and n-hexane as solvents of extraction. The crude and solvent fractions showed broad spectrum activities against *Staphylococcus aureus, Streptococcus faecalis, Bacillus subtilis, B. cereus, Shigella dysenteriae, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Proteus vulgaris,* with zones of inhibition ranging from 1.7 to 18.3 mm. The leaf extracts showed better antimicrobial activities, as the minimum inhibitory concentration (MIC) values for most of the isolates was 10 mg/ml with a least value of 5 mg/ml recorded for *P. aeruginosa* (NCIB 950). On the contrary, in the case of bark extract, 15 mg/ml was the MIC value recorded for most of the test isolates with the least value of 10 mg/ml recorded for *S. aureus, B. subtilis*, and *K. pneumoniae*. Out of all the solvent fractions, the chloroform fractions exhibited the highest antimicrobial activities. The bark extracts exercised antifungal activities against *Candida albicans, Aspergillus niger* and *A. flavus,* while the leaf extract was inactive against all the fungal isolates. Phytochemical constituents revealed the presence of saponins, tannins, and anthraquinones in the bark extracts, while, tannins, anthraquinones, and cardenolides were present in the leaf extract.**

**Key words:** Antimicrobial, inhibition, extracts, microorganisms.

## INTRODUCTION

Plants play a vital role in the existence and survival of man. They supply foods and the much-required oxygen for breathing. Plants also provide fibres for diverse purposes and wood for housing and shelter. It is also known that they provide a tremendous reservoir of various chemical substances with potential therapeutic properties (Lewis and Elvin-Lewis, 1995). *Vernonia* is a large genus characterized by two or three whorls of pappus bristles on the achene, eligulate florets in generally oblong heads and many series of involucral bracts on the receptacle. They are in the family Asteraceae. Members of this family are important as weeds, ornaments and as green vegetables. They are referred to as the bitter genus. *V. tenoreana* is a distinct shrub in savanna with purple markings on the stem and petiole (Olorode, 1984). It is called Ewuro Igbo by the Yorubas of Nigeria. It grows as wild plant in some parts of the Yorubaland of Nigeria where it is not eaten. However, in some other parts of the Yorubaland, the leaf is eaten as cooked vegetable. The decoction of the leaf is used to relief intestinal ailment.

Traditionally, the use of plant preparation as sources of drugs are based on the experience and superstition passed from generation to generation, virtually by the word of mouth (Sofowora, 1993). Plants have provided a source of inspiration of novel drug compounds, as plant derived medicines have made large contributions to human health and well-being. Their role is two fold in the development of new drugs: (1) they may become base for the development of a medicine, i.e. natural blue print of the development of new drugs or (2) a phytomedicine to be used for the treatment of disease.

The isoquinoline alkaloid emetine obtained from the underground part of *Cephaelis ipecacuanha* and related species has been used for many years as an amoebicidal drug as well as for the treatment of abscesses due to the spread of *Escherichia histolytica* infections. Another important drug of plant origin with a long history of use is

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quinine. This alkaloid occurs naturally in the bark of *Cinchona* tree (Iwu et al., 1999). Apart from its continued usefulness in the treatment of malaria, it can also be used to relieve nocturnal leg cramps. Currently, the widely prescribed drugs are analogs of quinine such as chloroquine and nivaquine.

The major causes of human communicable diseases are the microorganisms and this has worsened dramatically within the last two decades. Man's wellbeing now depends on the production of more clinically useful antimicrobial drugs to curtail and/or eradicate pathogens responsible for these diseases. Multiple antibiotic resistant microorganisms are emerging and constituting major public health problem (Appelbaum, 1992; Huovinen et al., 1995). For many of these diseases, there is no cure or treatment yet, but literature reports and ethnobotanical records suggest that plants are the sleeping giants of the pharmaceutical industry (Hamburger and Hostettmann, 1991).

## MATERIALS AND METHODS

### Microorganisms

The bacteria used were collected from the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria. The clinical isolates were collected from samples taken directly from patients at the Obafemi Awolowo Teaching Hospital, in form of sputum, stool, septic sore, while some were collected from sewage, and water samples. The microorganisms used include: *Staphylococcus aureus* (NCIB 67), *Staphylococcus aureus* (NCIB 67), *Streptococcus faecalis* (NCIB84), *Bacillus subtilis* (NCIB3610), *Pseudomonas aeruginosa* (NCIB 950), *Escherichia coli* (NCIB 86), *Klebsiella pnuemoniae* (NCIB418), *Staphylococcus aureus* (LIO), *Streptococcus faecalis* (LIO), *Bacillus subtilis* (LIO), *Bacillus cereus* (LIO), *Proteus vulgaris* (LIO), *Shigella dysenteriae* (LIO), *Serratia marcesens* (LIO), *Pseudomonas aeruginosa* (LIO), *Escherichia coli* (LIO), *Candida albicans, Aspergillus niger, Aspergillus niger, Aspergillus flavus, Rhizopus stolonifer* and *Fusarium poae*.

### Collection of plant materials and extraction

*V. tenorreana* was collected on a mountain top behind the Agip Filing Station, Ajilosun, Ado-Ekiti, Ekiti State, Nigeria where it was found growing. The plant was authenticated by Mr. Adaramola, curator of the herbarium at the Obafemi Awolowo University, Ile-Ife and confirmed by comparison with herbarium specimens. Voucher specimen was then deposited at the herbarium for future reference.

Fresh leaves and bark of *V. tenoreana* collected between 10 a.m. and 12.00 noon were air-dried on the laboratory bench until well dried. The dried leaves and bark were ground into fine powder. Exactly 218 g of the dried leaf and 400 g of the dried bark were soaked in methanol/distilled water solvent mixture (2:3 v/v) and left on the laboratory bench for four days. On the fourth day, the solution was filtered and the filtrate dried, using the rotary evaporator, until all the methanol was removed while the aqueous part remaining was removed by freeze drying. Exactly 45.55 g and 35 g of crude extracts were obtained from leaf and bark of *V. tenoreana*, respectively.

30 g of each of the freeze-dried crude extracts of the leaves and bark of *V. tenoreana* was dissolved in 100 ml of sterile distilled water and extracted with n-hexane. The organic layer (n-hexane) was separated from the aqueous layer (in the separating funnel) and concentrated to dryness *in vacuo* using rotary evaporator and kept in a freezer in an air tight container. This is the n-hexane fraction. The resultant aqueous fraction was further extracted with chloroform to obtain the chloroform fraction. The ethyl acetate fraction was also obtained using the same procedure.

### Phytochemical screening

The extracts obtained were subjected to phytochemical screening (Harbone, 1984) to determine the presence of bioactive agents such as alkaloids, tannins, phlobatannin, anthraquinones and cardiac glycoside.

### Minimum inhibitory concentration (MIC)

The minimum inhibitory concentrations (MIC) of the extracts were determined using the agar well diffusion method. Different concentrations (25, 20, 15, 10 and 5 mg/ml) of the extracts were prepared and tested against the isolates that were susceptible to these extracts during the preliminary antimicrobial activity tests. A 4 mm cork borer was used to make the appropriate number of holes inside a solidified sterile nutrient agar in a sterile petridish containing the test organism. The holes were filled each with different concentrations of the crude extract solutions that had been prepared. The plates were incubated at 37°C for 24 h. Zones of inhibition around the wells indicate antimicrobial activity. The minimum concentration that inhibited the growth was taken as the MIC. Control experiment was set up using sterile distilled water.

## RESULTS AND DISCUSSION

The extracts from *V. tenoreana* leaf are active against a wide range of both Gram positive and Gram negative bacteria (Table 1). None of the fungi except *C. albicans* was susceptible to the extracts, which recorded 2.0 mm as zone of inhibition when treated with the n-hexane solvent fraction. The crude extract recorded as high as 18.3 mm zone of inhibition for *P. aeruginosa* at 25 mg/ml. The lowest inhibition value was 2.0 mm which was recorded using n-hexane solvent fractions activity against *C. albicans*. The first eight bacterial isolates shown on Table 1 and *K. pneumoniae* were however, susceptible to the antimicrobial activities of all the fractions.

There is a broad spectrum activity of *V. tenoreana* bark extracts (Table 2). The crude extracts exhibited good antifungal activity against *C. albicans, A. niger,* and *A. flavus* with the following zones of inhibition 11.30, 15.70 and 14.0 mm, respectively. The ethyl acetate solvent fraction recorded a zone of inhibition of 2.3 mm against *A. niger* whereas the chloroform and n-hexane fractions were inactive on all the test fungi.

*V. tenoreana* leaf extract has relatively low values of MIC for all the susceptible organisms. This has enhanced its high antimicrobial activities. *V. tenoreana* bark extracts had 10, 15 and 20 mg/ml as values for various organisms (Table 4).

The extracts used in this work exhibited a broad spectrum activity; however Gram positive bacteria have

**Table 1.** The antimicrobial activities of leaf extracts of *Vernonia tenoreana.*

| Organism | Zone of inhibition (mm) | | | |
|---|---|---|---|---|
| | ME | EAE | CE | HE |
| *Staphylococcus aureus* (NCIB 67) | 11.0 ± 0.1 de | 4.7 ± 0.6 de | 4.7 ± 1.2 ef | 3 ± 0.0 c |
| *Staphylococcus aureus* (LIO) | 11.3 ± 1.5 de | 3.3 ± 0.6 bc | 2.7 ± 0.6 bc | 2.7 ± 0.6 bc |
| *Streptococcus faecalis* (NCIB 84) | 13.3 ± 1.2 f | 7.3 ± 0.6 h | 4.3 ± 0.6 de | 3.0 ± 1.0 c |
| *Streptococcus faecalis* (LIO) | 10.0 ± 0.0 d | 7.3 ± 1.2 h | 3.3 ± 0.6 bcd | 2.3 ± 0.6 bc |
| *Shigella dysenteriae* (LIO) | 11.6 ± 1.5 de | 7.3 ± 0.6 h | 5.7 ± 0.6 f | 2.3 ± 0.6 bc |
| *Bacillus subtilis* (NCIB 3610) | 11.6 ± 1.5 de | 6.3 ± 0.6 gh | 4.6 ± 0.6 ef | 2.7 ± 0.6 bc |
| *Bacillus subtilis* (LIO) | 12 ± 0.0 ef | 3.7 ± 0.6 bcd | 3.7 ± 0.0 cde | 2.3 ± 0.6 bc |
| *Bacillus cereus* (LIO) | 10 ± 1.7 d | 5.3 ± 0.6 ef | 3.3 ± 0.6 bcd | 3.0 ± 1.0 c |
| *Escherichia coli* (NCIB 86) | 12.3 ± 0.6 ef | 6.7 ± 0.6 gh | 7.0 ± 1.0 g | I a |
| *Escherichia coli* (LIO) | 8.3 ± 0.6 c | 6.0 ± 0.0 fg | 7.0 ± 1.0 g | I a |
| *Pseudomonas aeruginosa* (NCIB 950) | 18.3 ± 0.6 h | 4.7 ± 0.6 de | I a | I a |
| *Pseudomonas aeruginosa* (LIO) | 12 ± 0.0 ef | 3.3 ± 0.6 bc | I a | I a |
| *Klebsiella pneumoniae* (NCIB 418) | 16.3 ± 1.5 g | 2.7 ± 0.6 bc | 2.7 ± 0.6 bc | 2.3 ± 0.6 bc |
| *Serratia marcesens* (LIO) | 11.3 ± 0.6 de | 4.0 ± 1.0 cd | 2.3 ± 0.6 b | I a |
| *Proteus vulgaris* (LIO) | 6.0 ± 0.0 b | 3.3 ± 0.6 bc | 3.7 ± 0.6 b | I a |
| *Candida albicans* (LIO) | I a | I a I | I a | 2.0 ± 0.0 b |
| *Aspergillus niger* | I a | I a | I a | I a |
| *Aspergillus flavus* | I a | I a | I a | I a |
| *Rhizopus stolonifer* | I a | I a | I a | I a |
| *Fusarium sp.* | I a | I a | I a | I a |

Values are means of triplicates, EAE = Ethyl acetate extract, ± = Standard deviation, HE = n-Hexane extract, Values along column with same superscript are not significantly different, LIO = Locally isolated organism, ME = Methanol extract , I = Inactive.

**Table 2.** Antimicrobial activities of the bark extracts of *V. tenoreana.*

| ORGANISM | ZONE OF INHIBITION (mm) | | | |
|---|---|---|---|---|
| | ME | EAE | CE | HE |
| *Staphylococcus aureus* (NCIB 67) | 15.7 ± 0.6 I | 7.7 ± 0.6 g | I a | 2 ± 0.0 b |
| *Staphylococcus aureus* (LIO) | 7.7 ± 0.6 de | 4.3 ± 1.2 cd | I a | 2.0 ± 0.0 b |
| *Streptococcus faecalis* (NCIB 84) | 7.7 ± 2.1 de | 5.7 ± 0.6 ef | 4.3 ± 1.5 c | 1.7 ± 0.3 b |
| *Streptococcus faecalis* (LIO) | 6.7 ± 0.6 cd | 4.0 ± 1.0 cd | 4.3 ± 1.2 c | 3.3 ± 0.6 d |
| *Shigella dysenteriae* ***(LIO)*** | 5.7 ± 0.6 g | 4.0 ± 1.0 cd | 2.3 ± 0.5 b | I a |
| *Bacillus subtilis* (NCIB 3610) | 11.7 ± 0.6 ef | 4.7 ± 0.6 f | I a | I a |
| *Bacillus subtilis* (LIO) | 8.3 ± 0.0 ef | 4.7 ± 0.6 de | I a | I a |
| *Bacillus cereus* (LIO) | 4.3 ± 0.6 b | 9.7 ± 0.6 h | I a | I a |
| *Escherichia coli* (NCIB 86) | 6.3 ± 0.6 cd | I a | 2.3 ± 0.6 b | 2.0 ± 0.0 bc |
| *Escherichia coli* (LIO) | 9.3 ± 0.6 f | I a | 2.3 ± 0.6 b | 3.0 ± 0.0 d |
| *Pseudomonas aeruginosa* (NCIB 950) | 7.7 ± 0.0 be | 3.7 ± 0.6 cd | 2.0 ± 1.2 b | 6.0 ± 0.0 e |
| *Pseudomonas aeruginosa* (LIO) | 7.2 ± 0.6 de | 4.7 ± 0.6 de | 3.6 ± 0.6 c | 5.7 ± 0.6 e |
| *Klebsiella pneumoniae* **(**NCIB418**)** | 13.3 ± 0.6 h | 3.3 ± 1.5 bc | I a | 2.3 ± 0.6 c |
| *Serratia marcesens* ***(LIO)*** | I a | 0.00 | I a | I a |
| *Proteus vulgaris* ***(LIO)*** | 8.3 ± 0.6 ef | 3.6 ± 0.6 cd | I a | I a |
| *Candida albicans* ***(LIO)*** | 11.3 ± 1.2 g | I a | I a | I a |
| *Aspergillus niger* | 15.7 ± 0.6 i | 2.3 ± 0.6 b | I a | I a |
| *Aspergillus flavus* | 14 ± 0.6 i | I a | I a | I a |
| *Rhizopus stolonifer* | I a | I a | I a | I a |
| *Fusarium sp.* | I a | I a | I a | I a |

Values along column with same superscript are not significantly different. I=Inactive, Values are mean of triplicates, LIO=locally Isolated Organism, ME = Methanol Extract, EAE = Ethyl acetate Extract, CE = Chloroform Extract, HE = n - hexane Extract.

**Table 3.** Minimum inhibitory concentration of *V. tenoreana* leaf extracts.

| ORGANISM | CONCENTRATION (mg/ml) | | | |
|---|---|---|---|---|
| | ME | EAE | CE | HE |
| *Staphylococcus aureus (NCIB67)* | 10 | 10 | 10 | 15 |
| *Staphylococcus aureus (LIO)* | 10 | 10 | 10 | 15 |
| *Streptococcus faecalis (NCIB84)* | 10 | 10 | 10 | 15 |
| *Streptococcus faecalis (LIO)* | 10 | 10 | 10 | 15 |
| *Shigella dysenteriae* | 15 | 15 | 10 | 15 |
| *Bacillus subtilis (NCIB3610)* | 10 | 10 | 15 | 15 |
| *Bacillus subtilis (LIO)* | 15 | 10 | 15 | 15 |
| *Bacillus cereus* | 10 | 10 | 15 | 15 |
| *Escherichia coli (NCIB86)* | 10 | 15 | 10 | ND |
| *Escherichia coli (LIO)* | 15 | 15 | 10 | ND |
| *Pseudomonas aeruginosa (NCIB950)* | 5 | 15 | ND | ND |
| *Pseudomonas aeruginosa (LIO)* | 10 | 15 | ND | ND |
| *Klebsiella pneumoniae(NCIB 418)* | 10 | 15 | 15 | 15 |
| *Serratia marcescens* | 15 | 10 | 15 | ND |
| *Proteus vulgaris* | 25 | 15 | 15 | ND |
| *Candida albicans* | ND | ND | ND | 15 |
| *Aspergillus niger* | ND | ND | ND | ND |
| *Aspergillus flavus* | ND | ND | ND | ND |

ME = Methanol Extract, EAE = Ethyl acetate Extract, CE = Chloroform Extract, HE = n –hexane Extract, ND = Not Determined

been shown to be more susceptible to antimicrobial action of the extract than the Gram-negative ones. The fungal isolates were resistant, which could be based on the structural built up of their cell wall. Bacteria are prokaryotes with thin cell wall and relatively simple genetic system, which enhance easy penetration of bioactive substances, leading to manipulation of genetic system as a result of bioactive interruption. Fungi, however, are eukaryotes with chitinous cell wall (nitrogen containing polysaccharides) which disallow easy penetration of the bioactive substances (Prescott et al., 1999). Again, Gram positive bacteria possess a permeable cell wall that usually does not restrict the penetration of antimicrobials as does the Gram negative bacteria (Lambert, 2002). The structural composition and arrangement of the cell wall of Gram negative bacteria is such that does not easily permit the penetration of the bioactive compounds (Russell, 2002).

Results obtained from Tables 1-2 showed that the ethyl acetate extracts among the solvent extracts exhibited the highest antimicrobial activity. This activity is followed by the chloroform fraction while the n-hexane fraction exhibited the least antimicrobial activities. This can be explained on two basis: (i) It may be that the active components are not well dissolved out of the extracts by the solvent or and if so, very small quantity are being extracted out which gives little or no effectivity against the extracts. (ii) It is most likely that the bioactive components have the polarity which is best extracted out by ethyl acetate among the solvent fractions. Experiments have shown the ability of solvents to extract out different antimicrobial substances, (Ahmad et al., 1998). n-Hexane fractions have been reported to exhibit very low activity against test organisms (Kumarasamy et al., 2004). Since it is known that different solvents extract different compounds, it therefore means some bioactive components can only be extracted by polar compounds, while some by less polar and yet some by non-polar compounds. It is therefore likely that n-hexane could not extract the bioactive components in these extracts as much as ethyl acetate. Considering the fact that the n-hexane fraction lacks some phytochemical components in comparison to the crude extract (Table 5), it may be that there is a form of synergism in the activities of the compounds hence the absence of some reduced the activity of the n-hexane fraction.

The antimicrobial activities of the crude extract of *V.tenoreana* leaf gave inhibitory activities of as high as 18.3 mm against *P. aeruginosa* (NCIB950) in the methanol extract (Table 1). This has compared favourably with past works as Ahmad and Beg (2001) who reported zones of inhibition of even less than 10 mm, when the plant extracts were tested against *E. coli, S. paratyphi* and *S. dysentariae.* The minimum inhibitory concentration (Tables 3-4), justifies the result of the antimicrobial activities (Tables 1-2).

Phytochemical screening of the crude extracts (Table 5) revealed the presence of the constituents such as saponins, tannins, alkaloids, phlobatannins, anthraquinones, cardenolides, steroidal nucleus, steroid ring and

**Table 4.** Minimum inhibitory concentration of *V. tenoreana* bark extracts.

| ORGANISM | CONCENTRATION (mg/ml) | | | |
|---|---|---|---|---|
| | ME | EAE | CE | HE |
| *Staphylococcus aureus (NCIB67)* | 10 | 10 | ND | 15 |
| *Staphylococcus aureus (LIO)* | 15 | 10 | ND | 15 |
| *Streptococcus faecalis (NCIB84)* | 15 | 10 | 15 | 15 |
| *Streptococcus faecalis (LIO)* | 15 | 10 | 15 | 15 |
| *Shigella dysenteriae* | 15 | 10 | 15 | ND |
| *Bacillus subtilis (NCIB3610)* | 10 | 15 | ND | ND |
| *Bacillus subtilis (LIO)* | 15 | 15 | ND | ND |
| *Bacillus cereus* | 20 | 10 | ND | ND |
| *Escherichia coli (NCIB86)* | 20 | ND | 15 | 15 |
| *Escherichia coli (LIO)* | 15 | ND | 15 | 15 |
| *Pseudomonas aeruginosa (NCIB950)* | 20 | 15 | 15 | 10 |
| *Pseudomonas aeruginosa (LIO)* | 20 | 15 | 15 | 15 |
| *Klebsiella pneumoniae(NCIB 418)* | 10 | 15 | ND | 15 |
| *Serratia marcescens* | ND | ND | ND | ND |
| *Proteus vulgaris* | 20 | 15 | ND | ND |
| *Candida albicans* | 15 | ND | ND | ND |
| *Aspergillus niger* | 15 | 15 | ND | ND |
| *Aspergillus flavus* | 15 | ND | ND | ND |

ME = Methanol Extract, EAE = Ethyl acetate Extract, CE = Chloroform Extract, HE = n –hexane Extract, ND = Not Determined

**Table 5.** Phytochemical components of *v. tenoreana* extracts.

| COMPONENTS | *V. tenoreana* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Leaf | | | | Bark | | | |
| | M | E | C | H | M | E | C | H |
| Alkaloid | - | - | - | + | - | - | - | + |
| Saponins | + | + | + | - | + | + | + | - |
| Tannins | + | + | + | + | + | + | + | + |
| Phlobatannins | - | - | - | - | - | - | - | - |
| Anthraquinones | + | + | + | + | + | + | + | + |
| Cardenolides | + | + | + | - | - | + | + | - |
| Steroidal nucleus | - | - | - | - | + | - | - | - |
| Steroidal ring | + | + | - | - | - | + | - | - |
| Desoxy sugar | - | - | + | - | - | - | + | - |

\+ = Present E = Ethylacetate
\- = Absent C = Chloroform
M = Methanol H = n-hexane

deoxy sugar. These bioactive compounds are reported to be responsible for antimicrobial activities in plants (Ghoshal et al., 1996; Iwu et al., 1999).

In conclusion, from this work, it has been established that extracts of *V. tenoreana* possess significant antimicrobial activities to certain pathogens with a broad spectrum result. Since the plants used in this study have proved to possess antimicrobial properties, and are locally available in this part of the world which is a developing country, they may become alternative sources of antimicrobial drugs that will complement existing antibiotics and/or provide novel/or lead compounds that may be employed in controlling some infections in our communities. However, the toxicological analysis of the active components is necessary in order to assess its tolerance in the human body when administered.

## REFERENCES

Ahmad I, Beg AZ (2001). Antimicrobial and phytochemical studies on 45 indian medicinal plants against multi- drug resistant human pathogens .J. Ethnopharmacol. 74(1): 13 –123

Ahmad I, Mehmood Z, Mohammed F (1998). Screening of some Indian medicinal plants for their antimicrobial properties. J. Ethnopharmacol. 62 : 183-193.

Appelbaum PC (1992) Antimicrobial resistance in *Streptococcus pneumoniae* an overview. *Clinical Infect Dis*. 15: 17-83.

Ghoshal S, Krishna, Prasad BN, Lakshmi V (1996). Antiamoebic activity of *Piper longum* fruits against *Entamoeba histolytica in vitro* and in vivo. J. Ethnopharmacol. 50:167 – 170.

Hamburger M, Hostettmann K (1991) Bioactivity in plants: The link between phytochemistry and medicine. Phytochemist. 30 (2): 3864-3874.

Harbone JB (1984) Phytochemical methods. A guide to modern techniques of plant analysis. 2nd ed. Chapman and Hall, N. Y. pp. 1-35.

Huovinen P, Sundstrom I, Swedberg G, Skold O (1995) Trimethoprim and Sulfonamide resistance. *Antimicrobial and Chemotherapy* 39 (2): 279-289.

Iwu MW, Duncan AR, Okunji CO (1999) New antimicrobials of plant origin. In:Perspectives on new crops and new uses. J. Janick (ed). ASHS Press, Alexandria, V.A.pp 457 – 462.

Kumarasamy Y, Cox PJ, Jaspars M, Nahar L, Sarker SD (2004) Comparative studies on biological activities of *Prunus padus* and *P. Spinosa*. *Fitoterapia* 75: 77-80.

Lambert PA (2002) Cellular impermeability and uptake of biocides and antibiotics in Gram positive bacteria and mycobacteria. J. Appl. Microbiol. 92: 465-545.

Lewis WH, Elvin-Lewis MP (1995) Medicinal plants as source of new therapeutics .*Ann. Mo. Bot. Gard*. 82:16-20.

Olorode O (1984) Taxonomy of West African Flowering Plants, 1st ed. England pp.98-100. Pathol. 56: 554-559.

Prescott LM, Harley Z P, Klein DA (1999) Microbiology 4th ed. USA McGrawHill, pp 679-684.

Russell AD (2002). Antibiotic and biocide resistance in bacteria: Introduction. J. Appl. Microbiol. symposium supplement, 92: 15-35.

Sofowora EA (1993) Medicinal plants and traditional medicine in Africa. 3rd ed. Spectrum books limited, Nigeria. pp 9-12.