academicJournals

Vol. 7(27), pp. 3442-3447, 5 July, 2013
DOI: 10.5897/AJMR10.210
ISSN 1996-0808 ©2013 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Antibacterial activity of methanol and ethanol leaf extracts of *Antidesma venosum* and *Lannea barteri*

Adegoke, S. A.*, Agada, F. D. and Ogundipe, L. O.

Department of Microbiology, Faculty of Natural Sciences, Kogi State University, Anyigba, Kogi State, Nigeria.

Accepted 19 November, 2010

Methanol and ethanol cold percolation leaf extracts of *Antidesma venosum* and *Lannea barteri* were screened for their *in vitro* antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Salmonella typhi*, *Streptococcus lactis and Shigella* sp. using the agar well diffusion method. Methanol extracts gave higher filtrate of 16.57 and 9.44 g for *A. venosum* and *L. barteri*, respectively, while ethanol extract gave 13.52 and 7.10 g, respectively, from 500 g ground leaves extracted with 500 ml of each solvent. Both solvent extracts of *A. venosum* showed activity against all the clinical test organisms, with zone of inhibition range of 11 to 21 mm diameter except against *S. aureus. S. lactis* and *Shigella* sp. were the least susceptible to both solvent extracts of *L. barteri*. The minimum bactericidal concentration (MBC) range for *A. venosum* methanol leaf extracts against the clinical test organisms was 6.25 to 50 mg/ml, while for ethanol leaf extract, MBC range was 6.25 to 12.5 mg/ml. MBC range for *L. barteri* methanol and ethanol leaf extracts were 6.25 to 50 mg/ml and 6.25 to 12.5 mg/ml, respectively. The MBC killing rate of *A. venosum* methanol leaf extract shows that *S. typhi* was the most rapidly killed at a rate of 1.88 x 10^6 CFU/min, while *S. aureus* was the most rapidly killed by *L. barteri* methanol leaf extract MBC at a rate of 1.45 x 10^6 CFU/min.

Key words: Antibacterial, extract, inhibitory, bactericidal, susceptibility.

INTRODUCTION

Plant ability to grow on different nature of soils rich in microorganisms are as a result of their potential to produce wide range of selective anti-bacterial compounds either in a constitutive or an inducible manner to wade off potential microbial invaders (Cammune et al., 1992). Medicinal plants have been used for centuries as remedies for human diseases because of their therapeutic values. Hence, the quest for plants with medicinal properties ranging from antibiotics to antitumor continues to receive attention. Thus, plants have provided western medicine with an abundance of drugs and treatment for a variety of health problems (Lewis and Elvin-Lewis, 1977; Bruneton, 1999). The development of microbial resistance to the available antibiotics and the increasing acceptance of traditional medicine as an alternative form of health care have led researchers to investigate the antimicrobial activity of medicinal plants (Bisignano et al., 1996; Lis-Balchin and Deans, 1996; Maoz and Neeman, 1998; Hammer et al., 1999). Species used in traditional medicines continue to be the most reliable sources for the discovery of useful compounds. In addition, screening of plants growing under various stresses has provided yet another source of compounds with potential antimicrobial activities (Ben et al., 1992; Hanawa et al., 1992; Konger and Manion, 1994; Brockaert et al., 1997; Mohammed and Sehgel, 1997; Dubery et al., 1999; Pernas et al., 2000).

As part of the unabated search for antimicrobial compounds of plant origin and in view of the fact that there are still plants whose medicinal uses have not been ascertained, this study was carried out on the antibacterial activity of methanol and ethanol leaf extracts of *Antidesma venosum* and *Lannea barteri*. They are the plants of choice because of their traditional uses for the treatment of typhoid, gastrointestinal disorders and

*Corresponding author. E-mail:detunji_outreach@yahoo.co.uk.

genitourinary tract infection in Anyigba community of Kogi State, Nigeria.

A. venosum belongs to the family Euphorbiaceae. Members of this family are found in all parts of the world with greatest diversity in the tropics, particularly in the Indomalaysian and South American regions (Peter et al., 1992). The Euphorbiaceae are good sources of food, drugs, rubber, arrow poison and other products (Subhash, 1988). Many are used as fish and arrow poison and many species are poisonous (Walter et al., 1999). Some are ornamentals, while some are purgatives (Subhash, 1988; Peter et al., 1992).

L. barteri belongs to the family Anacardiaceae. Members are abundantly found in Satpuda, Vindhyachal, Aravali rangers, Khurchal valleys, Abhuimar and Patalkot areas of central India (Acharya, 2000). They are used as medicinal plants in the urban area of Chindwara town in India (Acharya, 2000). They are also used in Iran for curing infectious maladies. Members are also widely distributed in Nigeria.

MATERIALS AND METHODS

Plant source and identification

The plant stem with leaves and reproductive structures were collected from different locations in Anyigba, Dekina Local Government Area, Kogi State, Nigeria. The plants were identified in the herbarium, Department of Biological Sciences, University of Ilorin, Kwara State, Nigeria.

Plant treatment

The plant leaves were washed with distilled water and dried at room temperature in the Microbiology Laboratory, Kogi State University, Anyigba, until the leaves became crispy and of constant weight. The dried leaves were ground using sterile pestle and mortar.

Preparation of crude extracts

Plant extracts were prepared using the modified method of Alade and Irobi (1993). 500 g of the powered dried leaves were soaked separately in 500 ml of 90% methanol and 98% ethanol for 72 h in the dark. It was then agitated at 200 rpm for 1 h on a mechanical shaker. The resulting suspension was filtered using sterile Whatman filter paper No 1. The filtrate obtained was evaporated to dryness using a rotary evaporation (Falodun et al., 2006) and weighed on chemical balance.

Source of clinical test organisms

The test organisms were collected from the culture collections of the Federal Medical Centre, Owo and the Public Health Laboratory, Akure, both in Ondo State, Nigeria.

Culture media

The medium used for the activation of the test organisms was nutrient broth, while the Mueller Hinton agar was used for the antimicrobial sensitivity test. They were prepared according to the manufacturers' instruction.

Standardization of inoculums

Rajakaruna et al. (2002) method was used for the standardization. A loopful of each of the test organisms was suspended in 3 ml of nutrient broth. All cultures were incubated at 37°C for 18 h, and then diluted with 1/10 of the concentration to yield a culture density of approximately 10^8 CFU/ml.

Preparation of extract concentrations

To prepare 100 mg/ml concentration, 1 g of the filtrate was reconstituted in 2 ml of each solvent (methanol and ethanol). 50 mg/ml concentration was prepared by adding equal volume of solvent and 100 mg/ml concentration earlier prepared, that is, double fold dilution. Double fold dilution was carried out on the 50 mg/ml concentration to give 25 mg/ml, while it was repeated for 25 mg/ml to get 12.5 mg/ml concentration.

Antibacterial activity of leaf extracts

Zone of inhibition

The modified agar ditch diffusion method of Perez et al. (1990) was used. 0.2 ml of the standardized microbial suspension of the test organisms were mixed with 20 ml of molten Mueller Hinton agar at 40°C. The seeded agar was poured aseptically into sterile Petri dishes with a depth of about 6 mm and allowed to solidify. The solidified agar was punched with a 6 mm diameter sterile cork borer to create seven wells on the agar. The wells were filled with 0.1 ml of the concentrations of the leaf extracts (100, 50, 25 and 12.5 mg/ml). Distilled water was used to fill one of the wells which served as the solvent control, while gentamycin sulphate (1 mg/ml) was used as the positive control. Tests were carried out in duplicates and plates were incubated at 37°C for 24 h. The antibacterial activity was evaluated by measuring the inhibition zone diameter (IZD) in millimeter (mm).

Determination of minimum inhibitory concentration (MIC)

Nutrient broth was used to prepare the different concentrations. 4 ml of each concentration was introduced into sterile test plates. 1 ml of the standardized bacterial suspension was added to the test content. The control tubes were inoculated with sterile distilled water. All the tubes were incubated at 37°C for 24 h. The minimum bactericidal concentration (MCB) was taken to the lowest concentration that did not permit any visible growth when compared with the control (Rojas et al., 2006).

Determination of minimum bactericidal concentration

All the MIC tubes with no visible growth were plated out on Mueller Hinton agar and incubated at 37°C for 24 h. The MCB was taken to be the lowest concentration that did not produce colonies on Mueller Hinton agar (Prescott et al., 2005).

Determination of the MCB killing rate

The MCB killing rate was determined by the modified method of Olowosulu et al. (2003). 1 ml of the MCB was mixed with 3 ml of sterile nutrient broth and incubated with 1 ml of standardized culture

Table 1. Filtrate from methanol and ethanol extracts.

Plant	Methanol extract (500 g : 500 ml) (g)	Ethanol extract (500 g : 500 ml) (g)
A. venosum	16.57	13.52
L. barteri	9.44	7.10

Table 2. Antibacterial sensitivity (mm) of methanol leaf extract.

Test organism	*A. venosum*				*L. barteri*				Gentamycin (1 µg/ml)
	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	
S. aureus	6*	6	6	6	20	16	13	10	12
E. coli	21	17	15	13	21	17	14	10	12
P. vulgaris	21	18	16	14	20	18	16	14	25
S. typhi	20	18	16	13	15	13	9	6	18
S. lactis	20	17	15	14	10	6	6	6	15
Shigella sp.	21	18	15	12	10	6	6	6	20

*Values are in mm.

Table 3. Antibacterial sensitivity (mm) of ethanol leaf extract.

Test organism	*A. venosum*				*L. barteri*				Gentamycin (1 µg/ml)
	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	
S. aureus	6	6	6	6	20	16	11	9	12
E. coli	20	17	15	14	20	16	12	8	12
P. vulgaris	21	18	15	14	20	17	14	11	25
S. typhi	20	16	15	10	16	13	11	6	18
S. lactis	21	17	15	13	10	6	6	6	15
Shigella sp.	21	18	15	13	6	6	6	6	20

*Values are in mm.

of the test organism. 1 ml of the bacterial suspension was added to 4 ml of normal saline to serve as control. The tubes were incubated at 37°C. 0.1 ml of the incubated tubes was removed at regular intervals between 0 and 180 min. Each withdrawn sample was dispensed and serially diluted 10 fold to a dilution of 10^{-5} with normal saline. 0.1 aliquot of the final dilution was plated in duplicate on sterile Mueller Hinton agar and incubated at 37°C for 24 h. The number of colony forming units (CFU) on each plate was counted. The total number of viable bacterial in the original sample was computed and the graph of survival of the bacterial against time was plotted.

RESULTS

Preparation of crude extracts

The plant extracts prepared by the modification of Alabi and Irobi method (1993) showed that methanol gave a higher filtrate than ethanol for both plants as shown in Table 1.

Antibacterial activities of leaf extracts

The result of the different concentrations of the leaf extracts tested against the test organism on Mueller Hinton agar is shown in Table 2. All the concentrations for both methanol and ethanol leaf extract of *A. venosum* were active against the test organism except against *S. aureus*, while the methanol and the ethanol leaf extracts of *L. barteri* were active against *S. aureus*

A. venosum had more antibacterial activity with a wider zone of inhibition than *L. barteri* (Table 2). *Proteus vulgaris* was most sensitive to both leaf extracts. *S. aureus* appeared not to be sensitive to the leaf extract of *A. venosum*, while *Streptococcus lactis* and *Shigella* sp. were least sensitive to *L. barteri* (Table 2). Table 3 shows the antibacterial sensitivity of ethanol leaf extract. The sensitivity pattern was similar to that of the methanol leaf extract (Table 2) with *P. vulgaris* been most sensitive to both plant extracts but *S. aureus* was not sensitive to the ethanol leaf extract of *A. venosum*. *Shigella* sp. was not sensitive to the ethanol leaf extract of *Lannea barteri* as shown in Table 3.

The MIC (minimum inhibitory concentration) of the methanol and ethanol leaf extract is shown in Table 4.

Table 4. MIC of methanol and ethanol leaf extract of *A. venosum* and *L. barteri*.

Test organism	Methanol leaf extracts (mg/ml)		Ethanol leaf extracts (mg/ml)	
	A. venosum	***L. barteri***	***A. venosum***	***L. barteri***
S. aureu	Nil	6.25	Nil	6.3
E. coli	6.25	6.25	6.25	12.5
P. vulgaris	6.25	6.25	6.25	6.25
S. typhi	6.25	50	12.5	25
S. lactis	6.25	100	12.5	100
Shigella sp.	12.5	100	6.25	Nil

Table 5. MBC of methanol and ethanol leaf extract of *A. venosum* and *L. barteri*.

Test organism	Methanol leaf extracts (mg/ml)		Ethanol leaf extracts (mg/ml)	
	A. venosum	***L. barteri***	***A. venosum***	***L. barteri***
S. aureu	Nil	12.5	Nil	12.5
E. coli	6.25	12.5	6.25	12.5
P. vulgaris	6.25	6.25	6.25	6.25
S. typhi	12.5	50	12.5	25
S. lactis	50	Nil	12.5	Nil
Shigella sp.	25	Nil	12.5	Nil

The methanol leaf extract of *A. venosum* inhibited most of the test organisms at 6.3 mg/ml except for *Shigella* sp. with 12.5 mg/ml MIC as shown in Table 4. The ethanol leaf extract of *L. barteri* had a lower MIC of 6.3 mg/ml as against 12.5 mg/ml of methanol.

DISCUSSION

This study showed the antibacterial potential of the methanol and ethanol leaf extracts of *A.* venosum and *L. barteri*. The results of the study corroborate several investigations that established flowering plants as potential source of antimicrobial substances (Sandhu and Heinrich, 2005; Gupta et al., 2005). Methanol was found to be a better extracting solvent than ethanol. Methanol yielded a higher filtrate for both plants (Table 1) and had a wider inhibition zone diameter (Tables 2 and 3). This result is at variance with the report of Obi and Onuoha (2000) that ethanol is the best solvent for the extraction of most medicinal plant active principles. *A. venosum* and *L. barteri* extracts inhibited the growth of both the gram positive and negative bacterial test isolates. This is an indication of a possible broad-spectrum mode of action for the extracts. In addition, the *A.* vensosum extracts were effective against the gram negative bacteria which are notable for exhibiting resistance. However, *P. vulgaris* was the most sensitive to the methanol and ethanol plant extracts of *A. venosum* and *L. barteri* as shown in Table 3.

The inhibition zone diameter varied for the two plant extracts with the same concentration and extracting solvent as indicated in Tables 3 and 4; an indication of varied antibacterial activities of the plant extracts. This may be due to the different phytochemical present in the plants.

The antibacterial properties of plants are desirable tools in the control of undesirable microorganisms, especially in the treatment of infections (Aboaba et al., 2006). Therefore, the use of *A. venosum* and *L. barteri* by the herbal healers for treatment of typhoid fever, gastro-intestinal disorder and urinogenital tract infections is in the right direction. Some of the clinical test isolates used for this study and which showed susceptibility to these plant extracts have been implicated in ailment they are used in treating. *Escherichia coli* have been implicated in urinary tract infections (UTI) and gastrointestinal disorder (Adeyemo et al., 1994; Ebie et al., 2001). *Salmonella typhi* is an etiologic agent of typhoid fever, while *Shigella* sp. is known for causing gastrointestinal disorder.

Methanol leaf extract of *A. venosum* inhibited most of the clinical isolates at 6.3 mg/ml except for *Shigella* sp. which had MIC value of 12.5 mg/ml (Table 4). *S. aureus* that was not sensitive to methanol and ethanol leaf extract of *A. venosum* was susceptible to the extract of *L. barteri* with MIC of 6.3 mg/ml. The MIC and MBC of *A. venosum* and *L. barteri* leaf extracts were the same for *P. vulgaris* and *S. typhi,* an indication of a possible bactericidal effect of the extracts on the clinical isolates. The extracts with lower MIC value than the MBC, suggest that the extracts are bacteriostatic at the lower concentration but bactericidal at higher concentrations.

The killing rate of the *A. venosum* methanol leaf extract MBC in Table 5 shows a correlation equation indicating *S. typhi* as the most rapidly killed of the test clinical isolates

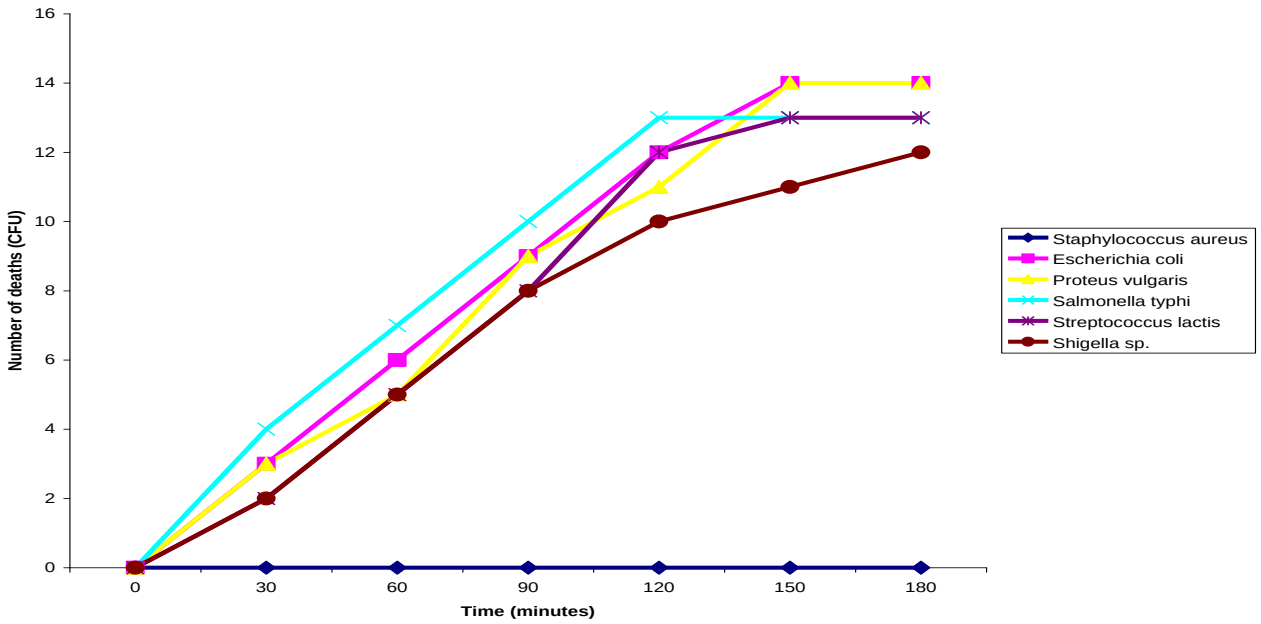

Figure 1. Killing rate of *A. venosum* methanol leaf extract MBC.

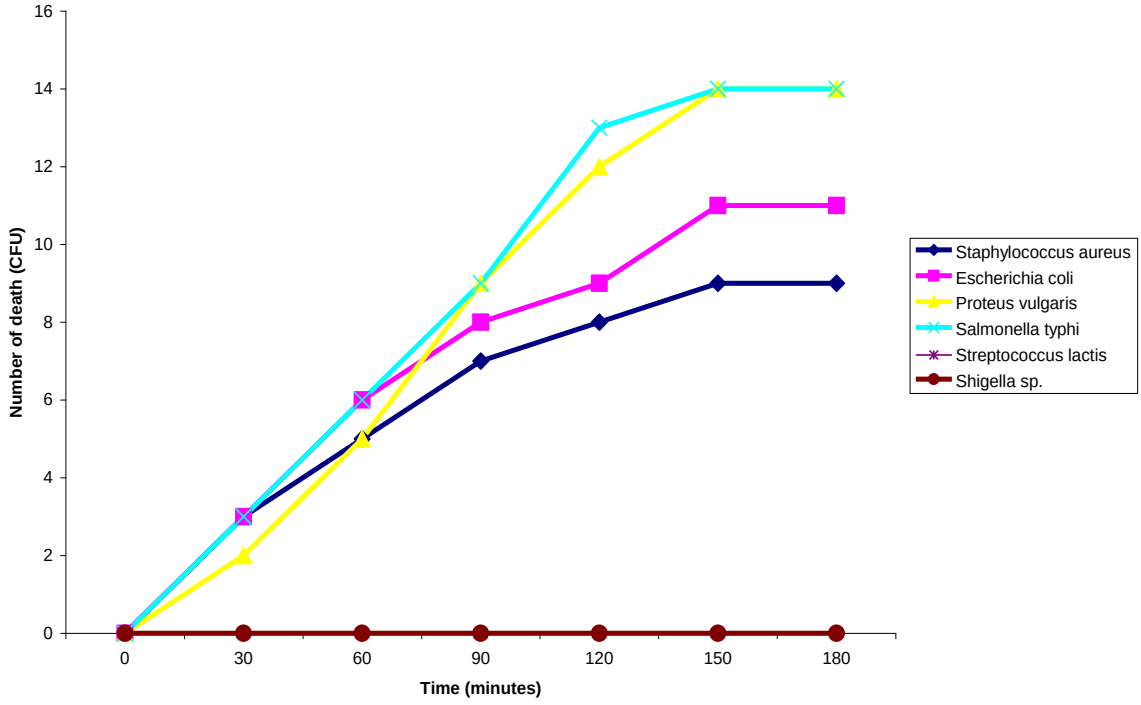

Figure 2. Killing rate of *L. barteri* methanol leaf extract MBC.

at a rate of 1.88 × 10^6 CFU/min (Figure 1), while *S. aureus* was the most rapidly killed by *L. barteri* (Figure 2). This result showed the potential of *A. venosum* in the control and cure of infection in which *S. typhi* is a causa-tive agent as in the case of typhoid in which the herbal healer in Anyigba community use *A. venosum* in treating.

REFERENCES

Aboaba OO, Smith SI, Olude FO (2006). Antibacterial effect of edible plant extract on *Escherichia coli* 0157:H7. Pakistan J. Nutr. 5(4):326.

Acharya D (2000). Medicinal Plants in urban area of Chhindwara town. http:www.selfgrowth.com.

Adeyemo AA, Gbadegesin RA, Onyemenen IN, Ekwezor CC (1999). Urinary tract pathogens and antimicrobial sensitivity in children in

Ibadan, Nigerian Ann. Trop. Pediatrics, 14: 271-274.
Ben YS, Rodov V, Kim JJ, Carmeli S (1992). Preformed and induced antifungal materials of Citrus fruits in relation to the enhancement of decay resistance by heat and ultraviolet treatments. J. Agric. Food Chem. 40: 1217-1221.
Bisignano G, Germano MP, Nostro A, Sanogo R (1996). Drugs used in Africa as dyes: Antimicrobial Activities. Phytother. Res. 9: 346-350.
Broekaert WF, Cammue BPA, Debolle MFC, Thevissen K, Desamblanx GW, Osborn RW (1997). Antimicrobial peptides from plants. Crit. Rev. Plant Sci. 16: 297-323.
Bruneton J (1999). Pharmacognosy, Phytochemistry, Medicinal Plants. 2nd Edition, Lavoisier Publishing, France. p. 1119.
Cammue BPA, De Boue MFC, Terras FRG, Proost P, Damme JV, Rees SB, Vanderleyden J, Broekaert WF (1992). Isolation and characterization of a novel class of plant antimicrobial peptide from *Mirablilis jalapa.* Seeds. J. Biol Chem. 267: 2228-2233.
Dubery IA, Louw AE, Van-Heerden FR (1999). Synthesis and evaluation of 4-(3-methy1-2- butenoxy) isonitrosacetophenone, a radiation- induced stress metabolite in citrus. Phytochemistry, 50: 983-989.
Ebie M, Kandakai-Olukemi YJ, Ayanbadejo OJ, Tanyigna KB (2001). Urinary Tract Infections in a Nigerian Military Hospital. Niger. J. Microbiol. 15(1): 31-37.
Falodun A, Okunrobo LO, Uzoanmake N (2006). Phytochemical Screening and anti-inflammatory Evaluation of methanolic and aqueous extract of *Euphorbia heterophylla* linn (Euphorbiaceae). Afr. J. Biotechnol. 5(6): 529-531.
Gupta MP, Solis PN, Calderon AI, Gulonneau-Sinclair F, Correa M, Galc C, Guerra C, Espinosa A, Alvenda GI, Robles G, and Ocampo R. (2005) Medical ethnobotany of the Tribes of Bocas del Toro, Panama. J. Ethnopharmacol. 96: 389-401.
Hammer KA, Carson CF, Riley TV (1999). Antimicrobial activity of essential oils and other plant extracts. J. Appl. Microbiol. 86: 985-1990.
Hanawa F, Tahara S, Mizutani T (1992). Antifungal Stress compounds from *Veratrum grandiflorum* leaves treated with Cupric Chloride. Phytochemistry, 31: 3005-3007.
Kruger BM, Manion PD (1994). Antifungal Compounds in aspen: Effect of water stress. Can J. Bot. 72: 454-460.
Lewis WH, Elvin-Lewis MPF (1977). Medical Botany. Plants Affecting Man's Health. John Wiley and Sons, New York. p. 515.
Lis-Balchin M, Deans SG (1996). Antimicrobial effects of hydrophilic extracts of *Pelargonium* species (Geraniaceae). Lett. Appl. Microbiol. 23:205-207.
Maoz M, Neeman I (1998). Antimicrobial effects of aqueous plant extracts on the fung *Microsporum canis* and *Trichophyton rubrum* and on three bacterial species. Lett. Appl. Microbiol. 26: 61-63.
Mohammed F, Sehgal OP (1997). Characteristics of pathogenesis related proteins induced in *Phaseolus vulgaris* following viral infection. J. Phytopathol. 145: 49-58.
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, pp. 271-286.
Olowosulu AK, Ibrahim YKE (2003). Studies on the antimicrobial properties of *Baphia nitida* lodd extract and *Cymbopogon citrates* staff oil in creams and ointments. J. Phytomed. Therapeutics, 8(11): 1-7.
Perez C, Pauli M, Bazevque P (1990). An antibiotic assay by the agar well diffusion method. *Acta Biolgoiae etmedicine experimentalis*. 15: 113-115.
Rajakaruna N, Harries CS, Towers GHH (2002). Antimicrobial activity of plants collected from serpentine outcrops in Sri-lanka, J. Pharm. Biol. 40(3): 235-244.12.
Rojas JJ, Ochoa VJ, Ocampo SA, Munoz JF (2006). Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: A possible alternative in the treatment of non-nosocomial infections.BMC Complementary and Alt. Med. 6: 2.
Sandhu DS, Heinrich M (2005).The use of health foods, spices and other botanicals in the Sikh community in London. Phytother. Res. 19: 633-642.
Subharsh CD (1988). Systematic Botany, 4th edition. pp. 320: 360-361.