Science Focus, 15(1) 2010 pp 19– 25
Faculty of Pure and Applied Sciences, LAUTECH, Printed in Nigeria

Phytochemical and antibacterial screening of Basil (*Ocimum gratissimum)* leaf extract

Jimoh, T[1]; Oyeleke, S.B[2]; Dauda, B.E.N[1] and Oladiran, T.O[2].

[1]Department of Chemistry, Federal University of Technology, Minna; [2]Department of Microbiology, Federal University of Technology, Minna.

Abstract

Leaf extract of Basil (*Ocimum gratissimum*) was screened for antibacterial activities. Agar diffusion method was used, and the result showed that *Salmonella typhi, Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli* were all susceptible to the leaf extract of Basil (*Ocimum gratissimum)*. The acetone extract of the plant leaf inhibited all the organisms. *Pseudomonas aeruginosa* was the most inhibited by the extract, while *Escherichia coli* was the least inhibited. The Minimum Inhibitory Concentration (MIC) and the Minimum Bacteriocidal Concentration (MBC) of extract ranged from 1250µg/mL - 5000µg/mL respectively. The phytochemical screening of the extract revealed the presence of tannins, saponins, alkaloids, terpenes, steroids and flavonoids. Thin layer chromatography (TLC) of acetone and aqueous extracts of the leaf revealed two major spots while that of petroleum ether and methanol revealed three major spots respectively. The TLC purified fractions of the extract had no inhibitory activities on tested organisms. The efficacy of the leaf of *Ocimum gratissimum* in the treatment of diseases related to these pathogens was commendable as against the commercially available metronidozole used for similar disease related to these pathogens.

Keywords: *Ocimum gratissimum*, antibacterial, phytochemical

Introduction

Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoids, which have been found *in vitro* to have antibacterial properties (Lewis and Ausubel, 2006). These metabolites appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers (Varma and Dubey, 1999). A number of phytotherapy manuals have mentioned various medicinal plants for treating infectious diseases due to their availability, fewer side effects and reduced toxicity (Lee *et al*., 2007). There are several reports on the antibacterial activity of different herbal extracts (Bonjar, 2004; De Boer *et al*., 2005; Islam *et al*., 2008).For instance, the antibacterial activity of the essential oil as well as eugenol purified from *Ocimum gratissimum* to treat pneumonia, diarrhea and conjunctivitis has also been reported earlier (Nakamura *et al*., 1999). Many plants have been found to cure urinary tract infections, gastrointestinal disorders, respiratory diseases and cutaneous infections (Somchit *et al*., 2003). Owing to their popular use as remedies for many infectious diseases, searches for plants containing antibacterial substances are frequent (Betoni, *et al*., 2006).

Basil *Ocimum gratissimum* is a very effective medicinal plant that has been used to cure different diseases. It is called *basilic* in France, *basilikum* in Germa, *baslienkrant* by Indians, *Tulsi* by Italians and *albanaca* in Spainish. Basil (*Ocimum gratissimum*) of the order *Tubifloacae* belongs to the family *Labitae*. The family is a large one consisting of 200 genera and 3,300 species (Evans, 2002). It is referred to by different local names, peculiar to each locality. It is called Daidoya among the Hausas, Nehonwu among the Igbos and '*Efirin'* among the Yorubas. The plant grows to between 30 – 130cm tall, it has a light green sulky leaves and a white flower. The leaves are oppositely arranged and are usually greenish all round measuring to about 10 cm long (Mann *et al*., 2003).

Correspondence author: *E-mail:* teejayola01@yahoo.com

In Nigeria, Basil has been used by people as treatment for cough, diarrhoea and typhoid fever (Mann *et al.*, 2003). Studies have established that basil essential oil contain a substance that have a strong antioxidant that can prevent premature ageing, age related problems and even cancer (Kabir *et al.*, 2005). Winston, (1999) reported that basil was found effective and restrict growth of numerous bacteria like *Staphylococcus enterococcus* and *Pseudomonas species* that has become resistant to commonly used antibiotic drugs. Robert and Nkere, (2005) reported the efficiency of Basil *Ocimum gratissimum* in the therapy of haemorrhoids, nose bleeding, skin rashes, hormonal problems and Diabetes mellitus. Previous studies showed that the essential oils of Basil *Ocimum gratissimum* species grown in Rwanda, i.e. *Ocimum canum*, *Ocimum santrum*, *Ocimum trichodon* and *Ocimum urticifolium*, display antibacterial activity (Janssen *et al.*, 1989). *Ocimum* oil was also active against some species of bacteria (Juurilink, 2000). The fresh seed of the plant when pounded and mixed with honey may be taken as blood tonic (Kabir *et al.*, 2005). Akinpelu, (1999) observed that fresh shoots of the plant are used to clear stuffy nose by inhaling the vapour. Plant extracts of many higher plants have equally been reported to exhibit antibacterial properties under laboratory trails (Satish *et al.*, 1999; Okigbo and Ogbonnaya, 2006; Shariff *et al.*, 2006; Bouamama *et al.*, 2006; Ergene *et al.*, 2006; Kiran and Raveesha, 2006; Mohana and Raveesha, 2006). Nicholas and Iroegbu (2005) demonstrated the antibacterial activity of *Nauciea latifolia* root extract against *Pseudomonas aeruginosa*, *Corynebacterium diphtheria, Streptobacillus, Niesseriae* and *Salmonella species*. Ekhaise and Aluyi, (2005) reported the antimicrobial and antiviral activity of *Cocos mucifera*.

Due to the emergence of antibiotic resistant pathogens in hospitals and homes, plants are being looked upon as an excellent alternate to combat the further spread of multidrug resistant microorganisms. The search for suitable medicinal plants with potent active principles against these pathogens becomes imperative. The objective of this study therefore is to identify the phytochemical components of Basil (*Ocimum gratissimum*) and determine the antibacterial activity of the extracts of the leaves on selected pathogenic organisms.

Materials and methods

Collection and identification of plant leaves

Fresh leaves of Basil (*Ocimum gratissimum*) were collected from Bosso Estate area of Niger State and authenticated by Professor Z.I.E Ezenwa of School of Agriculture and Agricultural Technology, Federal University of Technology, Minna, Niger State.

Processing of plant

The identified leaves of *Ocimum gratissimum* were removed from the stem, washed and air dried over a period of two weeks at room temperature (28±2^{0}C). The air – dried plant material were then crushed using laboratory mortar and pestle before it was milled into fine powder with a blender. The powder were collected into sterile cellophane bags and labelled appropriately. The samples were kept in a cool dry place till further use.

Extraction of plant material and concentrations of extracts

The powdered sample (40 g) was extracted with 400 mL of petroleum ether for 6 h using Soxhlet extraction method. The extract was filtered with filter paper and the filtrate was evaporated to dryness on a steam bath. The dried petroleum ether crude extract was stored in sterile container at 4^{0}C until required for use. The procedure was repeated using other solvent such as acetone, methanol and distilled water. Each extract was concentrated by evaporating to dryness using a steam bath. 0.5 g, 0.4 g, 0.3 g, 0.2 g and 0.1 g of each of the extract was reconstituted into 5mL of distilled water to give the following concentrations 100 mg/mL, 80 mg/mL, 60 mg/mL, 40 mg/mL, 20 mg/mL. (Oyeleke *et al., 2008).*

Screening of test organism

Clinical samples of *Eschericha coli, Salmonella typhi, Pseudomonas aerugionsa* and *Staphylococcus aureus* were collected from General Hospital, Minna. The bacterial isolates were cultured on nutrient agar and incubated at 37^{0}C for 24 h. The microorganisms were repeatedly sub-cultured in order to obtain pure isolates. Morphological and biochemical reactions were carried out to ascertain proper identification. They were inoculated into nutrient agar slants and stored at 4^{0}C.

Phytochemical screening of plant extracts

The *Ocimum gratissimum* leaf petroleum ether, acetone, aqueous and methanol extracts were subjected to phytochemical screening using standard methods (Sofowora, 1994, Oyewale *et al.*, 2001 and Oyeleke *et al.*, 2009).

Screening of crude extracts for antibacterial activity

The agar diffusion method as reported by (Aboaba *et al.*, 2006) was used. Sterile nutrient agar plates were prepared. 1mL of test organism was added to 9 mL of the nutrient agar, each plate was properly labelled. A sterile cork borer (4 mm) was used to make wells in each plate for these extracts. The base of each dish was filled with sterile molten nutrient agar to seal the bottom and left for sometime to allow it to gel. 0.2 mL of the extract was dispensed into each well. The plates were left to allow diffusion of the extract before being placed in the incubator at 37^0C for 24 h. The zones of inhibition produced after incubation was measured and recorded.

Thin layer chromatography (TLC) preparation

Mini thin layer chromatography plates were prepared using microscopic slides. The slide were cleaned with acetone and washed with hot water to remove all stains, dirt and oil marks. 2 g of silica gel (Merek AR60) was mixed with 4ml of distilled water and ground in a mortar until it began to thicken. The slurry was then poured on the slide and spread evenly with a glass spreader. It was allowed to set for 5 min and then dried at 110^0C in the oven for at least 15 min to activate the plates and allowed to cool by exposing the plates to the atmosphere for 30 min excessive silica gel was carefully removed from the slide using a razor blade, preparation of the macro plates involved the same procedure, using 20 x 10 cm glass plates. 25 g of silica gel was mixed with sterile distilled water. (Oyeleke *et al.*, 2008)

Chromatographic separation of the methanol extracts of Basil *Ocimum gratissimum*

Ocimum gratissimum leaves on the preparative macro plates involved the use of a solution of dried extract which was made by dissolving 1g of extract in 3 mL of the solvent. A capillary tube was used to make a concentrated bind of the solution on the TLC plate about 2 cm from the base of the plate the mobile phase used was a mixture of ethylacetate, methanol and triethanoiamine in a ratio 16:2:2. This was put in a glass tank. The tank was closed and allowed to stand for about 10minutes so that the think becomes saturated with solvent. The plate was inserted into the tank with the origin spot towards the bottom of the tank. The spot was higher than the level of the solvent. The glass tank was covered tightly and the solvent allowed to ascending until it gets close to the top. The plates were removed and dried in the oven. Distance moved by the solvent and that moved by the extract were measured. (Oyeleke *et al.*, 2008).

Determination of antibacterial activity of fractions separated from the preparation macro TLC plates

The activity of reconstituted extract from the resultant bands was determined against the test organisms using standard culture. 0.01g of each extract was weighed and dissolved in 5 mL of water to give a concentration of 100 µg/mL. Sterile nutrient agar was prepared and the standard culture inoculated wells were made and filed with extract. The plates were incubated for 24 h at 37^0C after which zones of inhibition were observed. (Oyeleke *et al.*, 2008).

Determination of minimum inhibitory concentration (MIC)

The agar diffusion method as reported by Aboaba *et al.*, (2006) was used. 0.2, 0.1, 0.05 and 0.025 g of the extracts were dissolved in 5mL of sterile distilled molten nutrient agar, mixed thoroughly and poured into pre-labelled sterile petri-dishes to make a final concentration of 2000, 1000, 500 and 250 µg/mL respectively. A loopful of the standardized bacteria culture was used to inoculate the plates which were incubated at 37^0C for 24 h. Growth of organisms on each concentration was examined to determine the minimum concentration that inhibits growth of test organism.

Determination of minimum bactericidal concentration (MBC)

Minimum Bactericidal Concentration was determined by sub-culturing the test dilutions on to a fresh solid medium and incubated further for 24 h. The highest dilution that yielded no bacterial growth on solid medium was taken a MBC (Suffredini *et al.*, 2004; Aboaba *et al.*,2006 ; Doughari *et al.*, 2007)

Results and discussion

The results of phytochemical screening of the petroleum ether, acetone, methanol and aqueous extracts of *Ocimum gratissimum* (dried leaves) are shown in Table 1.

Table 1: Phytochemical screening results of crude extract of *Ocimum gratissimum*

Constituent	Petroleum ether	acetone	methanol	Aqueous
Saponins	-	++	++	++
Tannins	++	++	+++	+++
Flavonoids	++	-	-	++
Steroids	++	-	-	-
Cardiac glycosides	-	-	-	-
Alkaloids	-	-	+++	-
Phlobatannins	-	-	-	+++
Anthraquinones	-	-	-	-
Carbohydrates	-	++	++	-
Balsam	-	-	-	+
Terpenes	++	-	-	-

Key: -= *absent* + = *low* ++ = *moderate* +++ = *high.*

The phytochemical screening of the plants studies showed that the leaves were rich in alkaloids, steroids, tannins, saponins, flavonoids and terpenes. This is in agreement with Robert and Nkere, (2005) and Chhetri *et al.*, (2008) who have independently reported the presence of these components in the leaves of *Ocimum santrum*,member of the family of *Labitae* to which the plant used in this study belong. Also, Kabir *et al.*, (2005) also reported that the phytochemical analysis of the crude extracts of *Ocimum* species revealed the presence of alkaloids, saponins, tannins and steroids. The presence of tannins in all the extracts reveals its importance in herbal medicine in treating wounds and to arrest bleedings (Juhee *et al.*, 2004). This also confirms the use of *Ocimum gratissimum* for wounds in circumcision (Joshua, 2006).

The antibacterial activity of the petroleum ether, acetone, methanol and aqueous extracts of *Ocimum gratissimum* are shown in Table 2.

Table 2: Antibacterial activity of leaves of *Ocimum gratissimum* against test organisms
Zones of inhibition (mm)

Organisms	Petroleum ether extract	aceton e extract	methanol extract	Aqueous Extract	Metronid azole
Salmonella typhi	6	11	16	-	9
Pseudomonas aeruginosa	-	11	16	8	10
Staphylococcus aureus	-	16	-	6	5
Escherichia coli	14	1	-	-	8

The crude extracts concentration (100mg/L) showed antibacterial activity against the test organisms with acetone extract having the highest inhibitory activity against *Staphylococcus aureus* with 16.0 mm, followed by methanol extract with 16.0 mm against *Salmonella typhi Pseudomonas aeruginosa.* Petroleum ether and aqueous extract showed a weak or no antibacterial activity. The strong activity of the plant of *Ocimum gratissimum* suggests that it may be used for treatment of infection caused by these patogens. Standard antibiotics (Metronidazole) used as control in this experiment revealed; *Salmonella typhi* (9.0mm), *Pseudomonas aeruginosa* (10.0mm), *Staphylococcus aureus* (5.0mm) ,and *Escherichia coli* (8.0mm).The findings from this study further support Winston, (1999) and Mann *et al.*,(2003) who had earlier reported that basil was found effective and restrict growth of numerous bacteria like *Staphylococcus enterococcus* and *Pseudomonas species* that has become resistant to commonly used antibiotic drugs. Thus, the antibacterial activity of the extracts on the test organisms may be due to the presence of the above phytochemical components

The MIC and MBC value obtained in this work showed that different concentrations were effective against some of the organisms as shown in Table 3 and 4.

Table 3: Minimum Inhibitory Concentration (MIC) of Extract on test organisms (MIC value in µg/ml)

Organisms	Petroleum ether extract	acetone extract	methanol extract	Aqueous Extract
Salmonella typhi	1250	5000	2500	-
Pseudomonas aeruginosa	-	2500	1250	1250
Staphylococcus aureus	-	1250	-	1200
Escherichia coli	2500	-	-	-

Table 4: Minimum Bactericidal Concentration (MBC) of Extracts on test organisms MBC (valve in µg/ml)

Organisms	Petroleum ether extract	acetone extract	methanol extract	Aqueous Extract
Salmonella typhi	2500	5000	5000	-
Pseudomonas aeruginosa	-	5000	2500	2500
Staphylococcus aureus	-	1250	-	1500
Escherichia coli	3500	-	-	-

The MIC and MBC value obtained from acetone extract ranged from 1250.00 µg/mL-5000.00 µg/mL, methanol extract 1250.00 µg/mL - 2500.00 µg/mL, petroleum ether extract ranged from 1250.00 µg/mL - 2500.00 µg/mL, while aqueous leaf extract also ranged from 1200.00 µg/mL - 1250.00 µg/mL. According to Salvat *et al.*, (2004), plant extracts with MIC and MBC less than/or around 0.5 mg/mL (500 µg/mL) indicate good antibacterial activity. Based on this fact, it can be concluded that the extracts of *Ocimum gratissimum* exhibited good antibacterial activity against *Escherichia coli*, *Salmonella typhi*,and *Pseudomonas aeruginosa.* The individual fractions obtained from the thin layer chromatography (TLC) showed less antibacterial activities against the test organisms. The lower antibacterial activities of the pure fractions of the extracts than the crude ones might have been as a result of the synergic action of the active components in the plant thereby agreeing with the views of Harborne (1984) and Oyeleke *et al.*,(2008) who reported that the activities of plant extracts could sometimes change after fractionation with the obtained pure component eventually lacking in the activity of the original crude extracts.

Conclusion

The study have shown that the leaves of *Ocimum gratissimum* possess antibacterial activity against *Escherichia coli*, *Pseudomonas aueruginosa, Salmonella typhi* and *Staphylococcus aureus* .This may help to discover new chemical classes of antibiotics that may be used as topical treatment of disorders resulting from the tested pathogens. It is equally suggested that more research be conducted that will further isolate, elucidate and characterize the active components and their mechanism of action.

References

Aboaba O. O., Smith, S. I., and Olude, F. O. (2006). Antibacterial effect of Edible Plant Extract on *Escherichia coli* 0157:H7. *Pakistan Journal of Nutrition.* **5**(4):325-327.

Akinpelu, D. A. (1999). Antimicrobial activity of *Ocimum gratissimum* leaves. Fitoterapia – *The Journal for the Study of Medical Plants*. **70**(4) pp.432

Betoni, J.E.C., Mantovani, R.P., Barbosa, L.N., Di-Stasi, L.C. and Fernandes, A.(2006). Synergism between plant extract and antimicrobial drugs used on *Staphylococcus aureus* diseases. *Mem. Inst. Oswaldo Cruz,* 101:387 390.

Bonjar, S. (2004). Evaluation of antibacterial Properties of Some Medicinal Plants Used in Iran. *J. Ethnopharmacol.* **20**, 94, 301-305.

Bouamama, H., Noel, T., Villard, J., Benharref, A. and Jana, M. (2006) Antimicrobial activities of the leaf extracts of two Moroccan *Cistus L.* species. *Journal of Ethnopharmacology* **104**: 104-107.

Chhetri,H.P., Yogol,N.S., Sherchan,J., Anupa K.C. and Mansoor,S.(2008).Phytochemical and antimicrobial evaluations of some medicinal plants of Nepal. *Kathmandu University Journal of Science, Engineering and Technology.***1**:49-54.

De Boer, H.J., Kool, A., Broberg, A., Mziray, W.R., Hedberg, I. and Levenfors, J.J.(2005). Antifungal and antibacterial activity of Some Herbal Remedies from Tanzania. *J. Ethnopharmacol,* **96**,461-469.

Doughari, J. H., Pukuma, M.S. and De,N.(2007). Antibacterial effects of *Balanites aegyptiaca* and *Moringa oleifera* Lam. on *Salmonella typhi. African Journal of Biotechnology*, **6**(19). 2212-2215.

Ekhaise, F. O, and Aluyi , H. S. A. (2005). The Antibacterial effect of *Ocimum gratissimum* (Basil) Extract on antibiotic Resistant Strains of *Staphylococcus auresus. Bioscience Research Communications.* **16** (2), 7-10.

Ergene, A., Guler, P., Tan, S., Mirici, S., Hamzaoglu, E. and Duran, A. (2006) Antibacterial and antifungal activity of *Heracleum sphondylium subsp. artvinense. African Journal of Biotechnology* **5**: 1087-1089.

Evans, W.C.(2002). Trease and Evan's Pharmacognosy. 5th ed., Haarcourt Brace And Company, pp 336.

Harbone, J. B. (1984). Phytochemical methods. A Guide to Modern Technique of Plant Analysis. Second Edition. Chapman and Hall, London. **1**(19): 37 – 168.

Islam, B., Khan, S.N., Haque, I., Alam, M., Mushfiq, M. And Khan, A.U.(2008). Novel Anti-adherence Activity of Mulberry Leaves: Inhibition of *Streptococcus mutans* Biofilm by 1-Deoxynojirimycin Isolated from *Morus alba. J. Antimicrob. Chemother*. (in press).

Janssen,A. M., Scheffer J. J., Ntezurubanza L. and Baerheim Svendsen A. (1989). Antimicrobial activities of some *Ocimum* species grain in Rwanda. *Journal of Ethnopharmacology.* **26**: 57 – 63.

Joshua,A.A.(2006). Medicinal Plants among the People of Ekiti. *Journal of Ethnopharmacology* **19** : 216-217.

Juhee, B., Ahm, U., Grlin, I. and Mustapha, A. (2004). Antimicrobial and antioxidant activities of natural extract in vitro and in ground beef. *Journal of Food Protection,* **67**: 148-155.

Juurlink, B. H. J. (2000). Therapeutic Potential of Dietary Phase 2 Enzyme Inducers in Ameliorating Disease that have an Underlying Inflammating Component. C. A. N. *J. Pharmacol.* **79**: 266 - 282.

Kabir, O. A., Oladapo, O., Okwara, C. E., Christopher, C. I. and Kehinde , F. A. (2005). Screening of six medical plants used in South- West Nigerian unorthodox medicine for Anti-methicillin resistant. *Complimentary and Alternative Medicine 5(6)*:1-21.

Kiran, B. and Raveesha, K.A., (2006). Antifungal activity of seed extract of *Psoralea corylifolia* L. Plant Disease Research, **20**: 213-215.

Lee, S.B., Cha, K.H., Kim, S.N., Altantsetseg, S., Shatar, S., Sarangerel, O. and Nho, C.W.(2007).The Antimicrobial Activity of Essential Oil from *Dracocephalum foetidum* against Pathogenic Microorganisms. *J. Microbiol.* 45, 53-57.

Lewis, K. and Ausubel, F.M.(2006). Prospects of plant derived antibacterials. *Nat. Biotechnol.* **24**:1504-1507.

Mann, A., Gbate, M and Umar, A.N.(2003).Medicinal and Economic Plants of Nupe land. 1st Edn; Jube-Evans Books and publications, Bida, Nigeria, pp.139

Mohana, D.C. and Raveesha, K.A. (2006). Antibacterial activity of *Caesalpinia coriaria* (Jacq.) Willd. Against

plant pathogenic *Xanthomonas pathovars*: an eco-friendly approach. *Journal of Agricultural Technology.* **2:** 317-327.

Nakamura, C.V., Ueda-Nakamura, T., Bando, E., Melo, A.F., Cortez, D.A. and Dias Filho, B.P.(1999).Antibacterial activity of *Ocimum gratissimum* L. Essential Oil. *Mem. Inst. Oswaldo Cruz,* **94**:675-678.

Nicholas, S. and Iroegbu, C. U. (2005). Invitro Antibacterial Activity of *Ocimum gratissimum* Leave Extracts, *Journal of Biotechnology* **4**(9):946 – 952.

Okigbo, R.N. and Ogbonnaya, U.O. (2006). Antifungal effects of two tropical plant leaf extracts (*Ocimum gratissimum* and *Aframomum melegueta*) on post harvest yam (Dioscorea spp.) rot. *African Journal of Biotechnology* **5:** 727-731.

Oyeleke, S. B., Dauda, B. E. N., and Boye, O. A. (2008). Antibacterial Activity of *Ficus capensis*. *African Journal of Biotechnology Science, Education and Technology* 7 (10):1414 – 1417.

Oyeleke, S.B., Dauda, B.E.N. and Damasus, I. (2009). Comparative analysis of the Antibacterial activity of *Citrus aurantifolia* on some selected Pathogens, *Journal of science, education and Technology*, **2**(1). 343-345.

Oyewale,A., Musa,A.A. and Amupitan,J.O.(2001). Chemical composition and Biological activity of *Cochlospermum tinctorium*. (A.RICH). *J.Sci.Eng.Technol.*, **8**: 3300-3311.

Robert, C. U. and Nkere. C. K. (2005). Evaluation of the Antibacterial Properties of *Ocimum gratissimum* Stem bark Extracts. *International Journal of Molecular Medicine Advances in Sciences* **1***(2)*:182-189.

Salvat, A., Antonacci, L., Fortunato, R.H., Suarez, E.Y. and Godo,H.M.(2004). Antimicrobial activity in methanolic extracts of several plant species from Northern Argentina. *Phytomedicine* **11**: 230-234.

Satish. S., Raveesha, K.A. and Janardhana, G.R. (1999). Antibacterial activity of plant extracts on phytopathogenic *Xanthomonas campestris pathovars*. *Letter in Applied Microbiology* **28:** 145–147.

Shariff, N., Sudarshana, M. S., Umesha, S. and Hariprasad, P. (2006) Antimicrobial activity of *Rauvolfia tetraphylla* and *Physalis minima* leaf and callus extracts. *African Journal of Biotechnology* **5**: 946-950.

Sofowora, A. (1994). Medicinal plants and traditional medicine in Africa, University of Ife press Nigeria; pp.23.

Somchit, M.N., Reezal, I., Nur, I.E. and Mutalib, A.R.(2003). *In vitro* Antimicrobial Activity of Ethanol and Water Extracts of *Cassia alata*. *J. Ethnopharmacol.* **84**:1-4.

Suffredini, I.A., Paciencia, M.L., Nepomuceno, D.C., Younes, R.N. and Varella, A.D.(2006). Antibacterial and Cytotoxic Activity of Brazilian Plant Extracts Clusiaceae. *Mem. Inst. Oswaldo Cruz*,**101**:287-290.

Verma, J. and Dubey, N.K. (1999). Prospectives of botanical and microbial products as pesticides of tomorrow. *Current Science* **76**: 172-179.

Winston , J. (1999). Health promoting properties of common herbs. *America Journal of Clinical Nutrition* **70** : 491-499.