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ANTIMICROBIAL ACTIVITY OF THREE INDIAN MEDICINAL PLANTS – AN *IN VITRO* STUDY

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ABSTRACT
The alcoholic leaf extract of the three Indian medicinal plants *Annona squamosa*, *Aegle marmelos* and *Citrus limon* were screened for its antimicrobial activity using disc diffusion method. They were tested against four gram positive bacteria (*Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus, Bacillus subtilis*), three gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia*) and against three fungi (*Aspergillus niger, Aspergillus fumigates,Candida albicans*). It was observed that all the three alcoholic leaf extracts showed antibacterial and antifungal activity. The alcoholic leaf extract of *Annona squamosa* was found to be most active against *S. aureus, B. cereus, K. pneumonia, A. niger* and *A.fumigates*. The alcoholic leaf extract of *Aegle marmelos* was found to be active against *B. subtilis, E. coli, P.aeruginosa* and *C. albicans*. The alcoholic leaf extract of *Citrus limon* was most active against *S.epidermidis* and *E. coli*. The susceptibility of the microorganisms to the extracts of these plants was compared with each other and with the standard antibiotics ciprofloxacin and ketoconozole. The antimicrobial activities of the three alcoholic leaf extracts are discussed according to their phytochemical components. It is concluded that these three Indian medicinal plants may serve as a valuable source of compounds with therapeutic potential.

INTRODUCTION

Infectious diseases are the leading cause of death world-wide. For a long period of time plants have been valuable sources of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. The use of plant compounds for pharmaceutical purposes has gradually increased in India. Many works have been done which aim at knowing the different antimicrobial and phytochemical constituents of medicinal plants using them for the treatment of microbial infections as possible alternatives to chemically synthetic drugs to which many infectious microorganisms have become resistant. During the last ten years the pace of development of new antimicrobial drugs has slowed down while the prevalence of resistance has increased astronomically (Hugo and Russell, 1984). Literature reports and ethnobotanical records suggest that plants are the sleeping giants of pharmaceutical industry (Hostettmann and Hamdurger, 1991).

Natural products, either as pure compound or as standardized plant extracts, provide unlimited opportunities for new drug leads, because of the unmatched availability of chemical diversity. There is continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases (Rojas *et al.,* 2003). Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections.

The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Colombo and Bosisio, 1996).

In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents (Krishnaraju *et al.,* 2005). Thus it is anticipated that phytochemicals with adequate antibacterial efficacy can be used for the treatment of bacterial infections (Balandrin *et al.,* 1985). Since time immemorial, it has been suggested that ethanolic extract from plants used in allopathic medicines are potential sources of antiviral, antitumoral and antimicrobial agents (Negi *et al.,* 1993).

Annona squamosa Linn, commonly known as Sugar apple, belonging to the family Annonaceae, is said to show varied medicinal effects, including insecticide, antiovulatory and abortifacient. A bark is used to prevent diarrhoea, the root is used in the treatment of dysentery. A decoction of the leaves is used for cold and to clarify urine, to treat hysteria and fainting spells (Asolkar *et al.,* 1992). The fruits of *Annona* are haematinic, cooling, sedative, stimulant, expectorant, maturant tonic. The bark and leaves contain annonaine, an alkaloid (Vohora *et al.,* 1975) which is found to posses many of these properties.

Aegle marmelos, commonly known as bael, is a spiny tree belonging to the family Rutaceae. The leaves, roots, bark, seeds and fruits of *Aegle marmelos* are edible. The medicinal properties of this plant have been described in the Ayurveda. In fact, as per Charaka (1500 B.C.), no drug has been longer or

better known or appreciated by the inhabitants of India than the bael (Chemexcil, 1992). The leaves of bael are astringent, a laxative, and an expectorant and are useful in treating ophthalmia, deafness, inflammations, cataract, diabetes and asthmatic complaints. The leaves are bitter and are used as a remedy for ophthalmia, ulcers, dropsy, cholera and beri beri. Fresh aqueous and alcoholic leaf extracts of *Aegle marmelos* are reported to have a cardiotonic effect (Nadkarni, 1976). An aqueous decoction of the leaves has been shown to possess a significant hypoglycemic effect (Karunanayake *et al*., 1984). *Aegle* leaf extract has been reported to regenerate damaged pancreatic ß-cells in diabetic rats (Das *et al.,* 1996). It is found to be as effective as insulin in the restoration of blood glucose and body weight to normal levels (Seema *et al*., 1996).

Citrus limon originated in Southeast Asia, China, and the Malayan Archipelago. The fruit is rich in vitamin C which helps the body to fight off infections and also to prevent or treat scurvy (Chopra *et al.,* 1986). Lemon juice is also a very effective bactericide (Chiej, 1984). It is also a good antiperiodic and has been used as a substitute for quinine in treating malaria and other fevers, in the treatment of rheumatic conditions (Chevallier, 1996). The skin of the ripe fruit is carminative and stomachic (Chopra *et al.,* 1986). The essential oil from the skin of the fruit is strongly rubefacient and when taken internally in small doses has stimulating and carminative properties (Grieve, 1984). The stem is bitter, stomachic and tonic (Duke and Ayensu, 1985). An essential oil from the fruit rind is used in aromatherapy. They are rich in vitamin C, bioflavonoids, acids and volatile oils. They also contain coumarins such as bergapten which sensitizes the skin to sunlight. Main properties of *Citrus limon* are antiseptic, anti-rheumatic, antibacterial, antioxidant, reduces fever.

The purpose of the present study was to screen the alcoholic leaf extracts of these medicinal plants that could be useful for the development of new tools as antimicrobial agents.

MATERIALS AND METHODS

Plant material

Fresh leaves of *Annona squamosa, Aegle marmelos* and *Citrus limon* plants were collected locally during the month of November to January and were authenticated.

Preparation of crude extract

10g of air dried powder was macerated with 100 mL of absolute alcohol and stored for 72 hr in ice cold condition. After 72 hr the miscella was filtered using Whatmann No. 1 filter paper and the organic layer was allowed to evaporate. The resulted dark green extract was concentrated upto 100 mL on Rota vapour under reduced pressure. The concentrated crude extracts were lyophilized into paste (5 and 15 g respectively) and were taken for the screening of antimicrobial activity.

Microorganisms

In vitro antimicrobial studies were carried out against the strains purchased from American Type Culture Collection (ATCC). The four Gram-positive organisms studied were *Staphylococcous aureus* (ATCC 9144), *Staphylococcus epidermidi s*(ATCC 155), *Bacillus cereus* (ATCC H778), *Bacillus subtilis* (ATCC 6633) and the three Gram-negative organisms studied were *Escherichia coli*(ATCC 25922), *Pseudomonas aeruginosa* (ATCC 1688) *Klebsiella pneumonia* (ATCC 11298). The fungi strains studied were *Aspergillus niger* (ATCC 9029), *Aspergillus fumigates* (ATCC 46645) and *Candida albicans* (ATCC 10231).

Disc diffusion method

The disc diffusion method that followed by National Committee for Clinical Laboratory Standards (NCCL) protocol was used to evaluate the antimicrobial activities. For susceptibility testing, 100 mg/mL crude ethanolic extract was prepared in dimethyl sulfoxide (DMSO). Sterile Antibiotic Assay (AA) Discs (Whatman, 6mm) were impregnated with test compounds (25, 50 100 μL and μg of the reconstituted extract and were dried completely. The Discs were placed on the surface of the agar dispersion plates inoculated with the suspension (105 CFU/mL) of microbes that was matched to McFarland barium sulphate standard. The extracts were tested in triplicates. DMSO saturated assay disc and blank assay discs were used as negative controls. Standard antibiotic disc such as Ciprofloxacin (50 μg/disc) and Ketoconozole (50 μg/disc) were used as positive controls. The plates were incubated at 37ºC for 24 hr and 48 hr for bacteria and fungi. Inhibition zones were recorded as the diameter of growth-free zones, including the diameter of the disc, at the end of the incubation period.

RESULTS

The antimicrobial activity of three Indian medicinal plants *Annona squamosa*, *Aegle marmelos, Citrus limon*, were tested against four Gram-positive, three gram-negative and three fungi as reported in Figs. 1, 2 and 3.

It was observed that all the three alcoholic leaf extracts showed antibacterial and antifungal activity. Alcoholic leaf extract of *Annona squamosa* was found to be most active against *Staphylococcus aureus, Bacillus cereus, Klebsiella pneumonia, Aspergillus niger* and *Aspergillus fumigates*. Alcoholic leaf extract of *Aegle marmelos* was found be most active against *Bacillus subtilis, Escherichia coli, Pseudomonas aeroginosa* and *Candida albicans*. *Citrus limon* was found to be most active against *Staphylococcus epidermidis,* and *E. coli*. The activity of all the leaf extracts were found to be dose dependent *i.e.,* 100 μg/mL showed greater inhibition. The susceptibility of the microbes to the extracts of the plants was compared with standard antibiotics, ciprofloxacin and ketoconozole.

DISCUSSION

Medicinal plants have provided a source of inspiration for novel drugs compounds as since plant derived medicines have made large contributions to human health. The traditional healers make use of water primarily as solvent but alcoholic extracts of these plants were certainly much better and powerful. This may be due to the better solubility of the active components in organic solvents (Yoganarasimhan, 2000; Kumar *et al.,* 2009).

Annona squamosa was found to be more active against *S. aureus*, *B. cereus, S.epidermidis, K. pneumonia, A. niger*, *A.fumigates* and showed least activity against *C. albicans, B.*

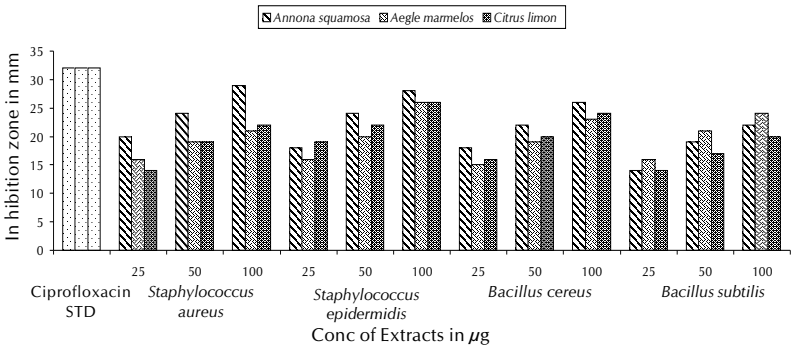

Figure 1: Screening of antibacterial activity of three medicinal plants against gram positive organisms

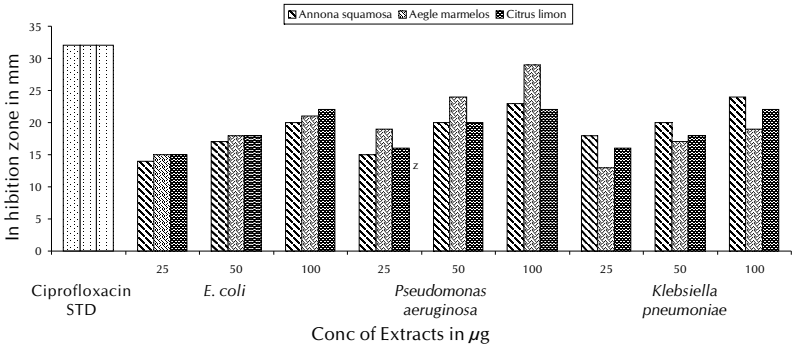

Figure 2: Screening of antibacterial activity of three medicinal plants against gram negative organisms

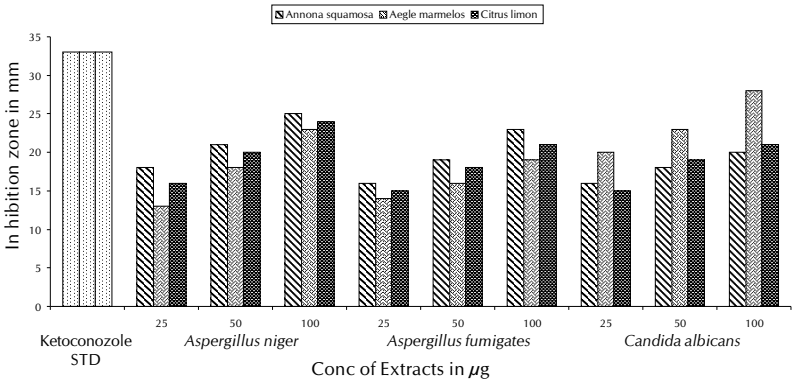

Figure 3: Screening of antibacterial activity of three medicinal plants

subtilis, E. Coli, P. aeroginosa. The biochemical components of *Annona squamosa* contains flavonoids which expose strong antibacterial activity. Volatile compounds of this plant were also studied for its antibacterial activity (Chavan *et al*., 2006). The family Annonaceae contains a large number of pharmacologically active substances, which are antibacterial (De Feo, 1992), antifungal (Lopez Abraham *et al*., 1979) and antiviral (Padma *et al*., 1998). They are used as counter medicine to treat a number of bacterial diseases (Hasrat, *et al.,* 1997; Wirat, 2000.) The active ingredients that contribute to its antimicrobial property are believed to be acetogenin an alkaloid, flavones from this species like 5- methoxy-7-hydroxyflavone and 6-hydroxydehydrouvaretin inhibited the growth of *B. subtilis* (Wirat, 2000).

Alcoholic leaf extract of *Aegle marmelos* was found to be active against *B. subtilis, E. coli, P. aeroginosa* and *C. albicans,* and the activity was found to be less against *S. aureus*, *B. cereus, S.epidermidis,K. pneumonia, A. niger*, *A.fumigates*. Futhermore, *E.coli,* which is a gram-negative bacterium, was also inhibited by bael leaf extract at low concentration. Although *E.coli* belongs to the normal flora of humans, an enterohaemorrhagic strain of *E.coli* has caused serious food poisoning, and preservatives to eliminate its growth are needed (Buchanan and Doyle,1997 and Gulcin *et al.,* 2003) the extract of *Aegle marmelos* might therefore be of use. *E.coli* was found to be more susceptible to *Aegle marmelos* and this may be due to the presence of tannins and alcohols which inhibits the growth of microorganisms. Thus it is evident that bael has antibacterial activity and the mechanism of action may be due to the blockage of protein synthesis either at transcription or translation level and its synthesis at membrane level. The antimicrobial activity of bael leaf extract may be due to the presence of cuminaldehyde and eugenol as these compounds have already shown their activity against various bacterial strains (Katayama and Nagai, 1960; Duke, 1992).

Alcoholic leaf extract of *Citrus limon* was found to be active against *S.epidermidis* and E.coli. Citric acid is a major organic acid and malic acid is a minor constituent as reported by Nii *et al.,* 1997. These organic acids present in higher concentration may be the causative factor for the antimicrobial activity against *S.epidermidis* and *E. coli*. Vinegar has been reported to exhibit antimicrobial activity against number of gram positive and gram negative bacteria (Entani *et al.,* 1998). The antimicrobial activity of citric acid could be due to the presence of high concentration of citric acid and malic acid. The antimicrobial activity due to citric acid is because of its undissociated structure and cytotoxic effect.

The results of the present study support the traditional usage of the assessed plants and suggest that some of the plant extracts possess compounds with antimicrobial properties. These plants are used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. It is observed from the present study that *Annona squamosa* is most active against *S. aureus*, *B. cereus, S.epidermidis, K. pneumonia, A. niger*, *A.fumigates* while *Aegle marmelos* is active against *B. subtilis, E. coli, P. aeroginosa* and *C. albicans* yet *Citrus limon* was found to be least inhibitory towards bacterial and fungal growth. However further work may be carried out to isolate the active principle and study the nature of the compound. Thus it may be concluded that the alcoholic leaf extracts of *Annona squamosa*, *Aegle marmelos* and *Citrus limon* possess significant *invitro* antimicrobial activity and these extracts can be subjected to isolation of therapeutic antimicrobials to carry out further

pharmacological evaluation.

REFERENCES

Asolkar, L. V., Kakkar, K. K. and Chakre, O. J. 1992. *Second supplement to Glossary of Indian Medicinal Plants with Active principles,* Part I (A-K). (1965 -1981) Publications and Informations Directorate, (CSIR) New Delhi. 2nd Edn, pp. 18 - 20.

Balandrin, M. F., Kjocke, A. J. and Wurtele, E. 1985. Natural plant chemicals: sources of industrial and mechanical materials. *Science.* **228:** 1154-1160.

Buchanan, R. L. and Doyle, M. P. 1997. Foodborne disease significance of *E.Coli* 0157:H7 and other enterohaemorrhagic *E. coli*. *Food Technol.* **51:** 69-76.

Chavan, M. J., Shinde, D. B. and Nirmal, S. A. 2006. Major volatile constituents of *Annona squamosa L*. bark, *Natural Product Research*. **20(8/9):** 754-757.

Chemexcil, 1992. Selected Medicinal plants of India. Basic chemicals, Pharmaceutical and Cosmetic Export Promotion Council, Bombay, India. pp. 205 - 207.

Chevallier, A. 1996. *The Encyclopedia of Medicinal Plants.* Dorling Kindersley. London. pp. 336.

Chiej, R. 1984. *Encyclopaedia of Medicinal Plants*. MacDonald. Brown. Pp. 448

Chopra, R. N., Nayar, S. L. and Chopra, I. C. 1986.*Glossary of Indian Medicinal Plants (*Including the Supplement). Council of Scientific and Industrial Research, CSIR Publications, New Delhi.

Colombo, M. L. and Bosisio, E. 1996. Pharmacological activities of *Chelidonium majus* L (papaverceae). *Pharmacol* **88:** 199-204.

Das, A. V., Padayatti, P. S. and Paulose, C. S. 1996. Effect of leaf extract of *Aegle marmelos* (L.)Correa ex Roxb on histological and ultrastructural changes in tissues of streptozotocin induced diabetic rats. *Indian J. Exp. Biol.* **34(4):** 341-345.

De Feo, V. 1992. Medicinal and magical plants in the northern Peruvian Andes. *Fitoterapia*. **63:** 417-440.

Duke, J. A. 1992. Handbook of biologically active phytochemicals and their activities, CRC press, Boca Raton, FL p. 183.

Duke, J. A. and Ayensu, E. E. 1985. Medicinal Plants of China 2 vols, Reference Publications, Algonac, ML p. 705.

Entani, E., Asai, M. and Ohta, M. 1998. Antibacterial action of vinegar against *Escherichia coli* O157: H7 and other foodborne bacterial pathogens in cooked foods. *J. Jpn. Soc. Nutr. Food. Sci*. **51**: 101-106.

Grieve, A. 1984. *Modern Herbal.* Penguin ISBN 0-14-046-440-9.

Gulcin, I., Oktay, M., Kirecci, E. and Kufrevioglu, I. O. 2003. Screening of antioxidant and antimicrobial activities of anise (*Pimpinella anisum L*) extracts. *Food Chem*. **83:** 371-382.

Hasrat, J. A., Pieters, L., Debacker, J. P., Vauquelin, G. and Vlietinck, A. J. 1997. Medicinal plants from suriname, screening of medicinal plants from suriname for 5-HT 1 A ligands: Bioactive isoquinoline alkaloids from the fruit of *Annona muricata*. *Phytomedicine.* **4:** 133-140.

Hostettmann, K. and Hamdurger, M. 1991. Medicinal Plants in Tropical West Africa. *Phytochem.***30 (12):** 3864-3874.

Hugo, W. B. and Russell, A. D. 1984. *Pharmaceutical Microbiology,* Blackwell Scientific Publications, Third edition pp.179-200.

Karunanayake, E. H., Welihinda, J., Sirimanne, S. R. and Gowri, S. 1984. Oral hypoglycemic activity of some medicinal plants of Srilanka. *J. Ethanopharmacol.* **11:** 223-231.

Katayama, T. and Nagai, I. 1960. Chemical significance of the volatile components of spices from the food preservative view point, IV – Structure and antibacterial activity of some terpenes, *Nippon Suisan Gakkaishi*. pp. 26-29.

Krishnaraju, A. V., Rao, T. V. N., Sundararaju, D., Vanisree, M., Tsay, H. S. and Subbaraju, G. V. 2005. Assessment of bioactivity of Indian medicinal plants using Brine Shrimp (*Artemia salina*) lethality assay. *Int. J. Appl. Sci. Eng.* **3(2):** 125-134.

Kumar, B. S. A., Lakshman, K., Tirupathi, M. S., Jayaveera, K. N., Narayanasamy, V. B., Sabemulla, K. and Nandeesh, R. 2009. Free radical scavenging and antibacterial activities of Armycard power (An Ayurvedic formulation). *European Bulletien of Drug Research.* **17:** 5-9.

Lopez Abraham, A. M., Rojas Hernandez, N. M. and Jimenez Misas, C. A. 1979. Plant extracts with cytostatic properties growing in cuba. *Revista Cubana De Medicina Tropical.* **31(2):** 97-104.

Nadkarni, A. K. 1976. Indian Materia Medica, 3rd Edn. Popular press, Mumbai, India.pp 1308 - 1315.

Negi, K. S., Tiwari, J. K. and Gaur, R. D. 1993. Notes on ethnobotany of five districts of Garhwal Himalya,Uttarpradesh, India. *Ethnobotany.* **5:** 73-81.

Nii, Y., Fukuta, K., Kiyokage, R., Sakai, K. and Yamamoto, S. 1997.

Invitro effects of citrus fruit juices on solublization of calcium from shirasuboshi (boiled and semidried whitebaits). *J. Jpn. Soc. Nutr. Food. Sci*. **50(6):** 439-443. (in japanese).

Padma, P., Pramod, N. P., Thyagarajan, S. P. and Khosa, R. L. 1998. Effect of the extract of *Annona muricata* and *Petunia nyctaginiflora* on Herpes simplex virus. *J. Ethnopharmocology.* **61(1):** 81-83.

Rojas, R., Bustamante, B., Bauer, J., Fernandez, I., Alban, J. and Lock, O. 2003. Antimicrobial activity of selected Peruvian medicinal plants. *J. Ethnopharmacol*. **88:** 199 – 204.

Seema, P. V., Sudha, B., Pandayatti, P. S., Abraham, A., Raghu K. G. and Paulose, C. S. 1996. Kinetic studies of purified malate dehydrogenase in liver of streptozotocin-diabatic rats and the effect of leaf extract of *Aegle Marmelos(L.)* Correa ex Roxb. *Indian J. Exp. Biol.* **34:** 600-602.

Vohora, S. B., Ishwar Kumar and Naqvi, S. A. H. 1975. Phytochemical, Pharmacological, antibacterial and anti-ovulatory studies on *Annona Squamosa*. *Planta Med.* **28(1):** 97-100.

Wirat, C. 2000. *Medicinal plants of Southeast Asia,* pp. 3-18. Kuala Lumpur: pelanduk publisher.

Yoganarasimhan, S. N. 2000. *Medicinal Plant of Indian Tamilnadu.* **II:** 64-65, 230.