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***In Vitro* Evaluation of Antibacterial Activity of Phytochemical Extracts from Leaves of *Aegle marmelos* (L.) Corr. (Rutaceae)**

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ABSTRACT

The present study was carried out to screen and evaluate the antimicrobial activity of leaf extracts from *Aegle marmelos* (L.) Corr. Petroleum ether, Dichloromethane, Chloroform, Ethanol and Aqueous extract of the leaves were tested against selected Gram positive and Gram negative bacteria. Results depict that phytochemical extracts of *A. marmelos* exhibited significant anti-bacterial activity. However, the inhibitory activity was found to be both organism and solvent dependent. Ethanol and chloroform leaf extracts of *Aegle marmelos* were found to be more active towards the bacterial species tested. The leaf extracts inhibited the growth of both Gram-positive and Gram-negative bacterial species. Further, the aqueous leaf extract was moderately active followed by dichloromethane extract. However, petroleum ether extract was not effective against any of the organisms tested. Growth of *Lactobacillus bulgaris* and *Bacillus cereus* was not inhibited by any of the tested leaf extracts of *A. marmelos*. The study shows that ethanol and chloroform leaf extracts of *A. marmelos* can be used as a potential source of antimicrobial agents.

KEYWORDS: *Aegle marmelos*; Medicinal Plants; Antibacterial Agents; phytochemicals; Disc Diffusion Assay (DDA).

INTRODUCTION

Medicinal plants form the backbone of traditional system of medicine in India. Pharmacological studies have acknowledged the value of medicinal plants as potential source of bioactive compounds (Prusti et al., 2008). Phytochemicals from medicinal plants serve as lead compounds in drug discovery and design (Chakravarthy and Gode, 1985; Ebi and Ofoefule, 2000). Medicinal plants are rich source of novel drugs that forms the ingredients in traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates, bioactive principles and lead compounds in synthetic drugs (Ncube, 2008). WHO (2005), pointed out that more than 80% of world's population depends on plants to meet their primary health care needs. However, overexploitation of the selected medicinal plant species lead to the reduction in number of plants in the wild and inclusion of their name in the red data book (Ahmedullah and Nayar 1999).

In recent years, multiple drug/ chemical resistance in both human and plant pathogenic microorganisms has been developed due to indiscriminate use of synthetic drugs. This drives the need to screen medicinal plants for

novel bioactive compounds as they are biodegradable, safe and have fewer side effects (Prusti et al., 2008).

Aegle marmelos (L.) Corr., belongs to the family Rutaceae, and is popularly known as Bael tree (Eng.) (Gamble, 1935; Mathew, 1983). In Hindu mythology leaves and wood of *Aegle marmelos* are used to worship Lord Shiva. This is a sacred tree amongst the Hindus. This tree is commonly found in Hindu sacred grooves. It is considered sacrilegious to destroy it; enormous quantities of the leaves are gathered for use during ritual ceremonies. In ancient time it is frequently alluded to as an emblem of fertility (Jain and Sastry, 1979).

Hindu physicians regard the unripe or half ripe fruit as astringent, digestive, and stomachic, and prescribe it for diarrhoea and dysentery (Bakhr, 1997). The ripe fruit is aromatic, cooling and is used as laxative. The root bark is used as a remedy in hypochondriasis, melancholia and palpitation of the heart. The fresh juice of the leaves is taken with honey as a laxative and febrifuge; it is used in asthmatic complaints. Addition of black pepper in anasarca is used to treat costiveness and jaundice; moreover, in external inflammations it is given to correct the supposed derangement of the humours. Small unripe fruit is consumed with fennel seeds and ginger in decoction for piles (Kamalakkannan and Prince, 2005). The fruit is used as a remedy for diarrhoea. Two tolas of bark juice is given with a cummin in milk to increase the quality of seminal fluid.

The tribals in Salem, Dharmapuri, Vellore regions, Tamilnadu, India offer leaves in the month of July/ August, to god to overcome sterility problem and subsequent year the couples are blessed with the child. Therefore, *A. marmelos* is considered as an emblem of fertility. Beverages prepared with fruit pulp are used to relieve body heat. Cologne is obtained by distillation from flowers. The wood is used for carving, small-scale turnery, tool and knife handles, pestles and combs, taking a fine polish. The ripen fruit, tamarind and sugar in mixture is used as laxative to overcome constipation and body heating problems (Jain and Sastry, 1979). Mature but still unripe fruits are made into jam. A firm jelly is made from the pulp alone or better still, combined with guava to modify the astringent flavor. The pulp is also pickled (Bakhr, 1997). The shell of hard fruits has been fashioned into pill- and snuff boxes, sometimes decorated with gold and silver. The gum from seeds is used as household glue and as an adhesive by jewelers. It is used to wash the silver ornaments and shields.

The fruit pulp is used as detergent for washing clothes. The fruit pulp is used as shampoo. The dried pulp is also used in local based hair cosmetics along with mehandi and amla. The yellow dye obtained from the fruits is traditionally used in the textile painting and printing works (Siva, 2007). In the present study we have evaluated the antimicrobial potential of *A. marmelos* *in vitro*.

MATERIALS AND METHODS

Collection of Plant Material

Mature leaves of *A. marmelos* were collected from Vellore, Tamilnadu, India during Apr 2008. The Flora of Presidency of Madras (Gamble, 1935) and The Flora of Tamil Nadu Carnatic (Matthew, 1983) were used for identification and authentication of the plants. Collected material was washed thoroughly in running tap water, rinsed in distilled water and shade dried in open air and grounded into powder.

Preparation of Phytochemical Extracts

The powder was extracted by maceration in different solvents used in the study using the cold percolation method. The plant extracts were concentrated using rotary evaporator (Buchi, Switzerland) and stored at 4 °C until used in the assay.

Test Organisms

Eight strains of Gram-positive bacteria - *Micrococcus glutamicus*, *Lactobacillus bulgaris*, *Streptococcus*

faecalis, *Staphylococcus aureus*, *Bacillus stearothermophilus*, *Staphylococcus pyogenes*, *Micrococcus luteus*, *Bacillus cereus* and two strains of Gram negative bacteria - *Escherichia coli* and *Pseudomonas aeruginosa* were used to evaluate the antibacterial activity (Table 1). All bacterial cultures were maintained in NA slants/ plates; stored at 4 °C and periodically sub-cultured.

Antimicrobial Activity Test

Antimicrobial activity was tested using a modified disc diffusion assay (DDA) method originally described by Bauer (1966) and Ncube et al (2008). Plant extracts were dissolved in 20% DMSO treated water. The inoculums for each microorganism were prepared from broth cultures (10^5 CFU/ml). A loop of culture from the NA slant stock was cultured in LB medium overnight and spread with a sterile swab into Petri-plates. Sterile disc (6 mm dia, Hi-media, Mumbai, India) impregnated with the plant extracts (1 mg/ml, and 5 mg/ml) were placed on the cultured plates and incubated for 24 h at 37 °C. The solvent loaded disc without extracts in it served as control in the study. The results were recorded by measuring the zones of growth inhibition. Clear inhibition zones around discs indicated the presence of antimicrobial activity. All data on antimicrobial activity were average of triplicate.

RESULTS AND DISCUSSION

Plants are known to have beneficial therapeutic effects documented in Traditional Indian System of Medicine. Much work has been done on ethnomedicinal plants in India. Interest in a large number of traditional natural products has increased. It has been suggested that phytochemical extracts from plants holds promise to be used in allopathic medicine as they are potential sources of antiviral, antitumoral and antimicrobial agents (Nair et al., 2005, Ramya et al., 2008a). The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world. Plants have been reported to possess antimicrobial, antifungal and other activities. This has been elucidated by various workers (Sasidharan, 1998; Sudharameshwari, 2007; Ramya et al., 2008 a,b).

In the present study ethanolic and chloroform leaf extracts of *Aegle marmelos*, showed maximum inhibition against Gram-positive and Gram-negative bacteria. In aqueous extract, moderate activity was observed followed by dichloromethane extract. Petroleum ether extract was not effective against any of the organisms tested. Among the different microorganisms tested, maximum inhibition was found in *Micrococcus glutamicus* and *E. coli*. In case of *Bacillus cereus* and *Lactobacillus bulgaris* growth was not inhibited by any of these extracts.

CONCLUSION

In the present study antibacterial activity of *A. marmelos* extracts towards drug resistant/ clinically significant microbes are reported and it was observed that the active constituents of plant material seep-out in organic solvents to display biological activity. The phytochemical extracts were not active against the probiotic organisms *Bacillus* and *Lactobacillus*, indicating that it is not going to affect the gut micro flora. Further, phytochemical studies for identification and elucidation of active constituents in the plant materials tested is expected to serve as lead in the development of novel bioactive antimicrobial compounds.

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Table 1. Bacterial strains used in the present study.

S. No	Bacterial strain	Gram (+/-)
1	<i>Escherichia coli</i>	-
2	<i>Pseudomonas denitrificans</i>	-
3	<i>Micrococcus glutamicus</i>	+
4	<i>Lactobacillus bulgaris</i>	+
5	<i>Streptococcus faecalis</i>	+
6	<i>Staphylococcus aureus</i>	+

7	<i>Bacillus stearothermophilus</i>	+
8	<i>Staphylococcus pyogenes</i>	+
9	<i>Micrococcus luteus</i>	+
10	<i>Bacillus cereus</i>	+

Table 2. Anti-microbial activity of *Aegle marmelos* extracts.

S. No	Strains used	Zone of inhibition (mm)					
		Control	P	D	C	E	A
1	<i>E. coli</i>	NI	NI	-	18	19	09
2	<i>M. glutamicus</i>	NI	NI	09	20	12	-
3	<i>L. bulgaris</i>	NI	NI	NI	NI	NI	NI
4	<i>S. faecalis</i>	NI	NI	14	13	12	13
5	<i>S. aureus</i>	NI	NI	-	14	11	10
6	<i>B. st'philus</i>	NI	NI	11	14	18	12
7	<i>S. pyogenes</i>	NI	NI	11	15	13	11
8	<i>P. denitrificans</i>	NI	NI	-	15	12	12
9	<i>M. luteus</i>	NI	NI	12	15	10	12
10	<i>B. cereus</i>	NI	NI	NI	NI	NI	NI

E = Ethanolic extract P = Petroleum ether extract
A = Aqueous extract C = Chloroform extract
NI = No inhibition. D = Dichloromethane extract