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Antimicrobial Activity of *Aegle marmelos* Against Pathogenic Organism Compared with Control Drug

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Abstract

The aqueous and ethanolic extracts from the leaves of *Aegle marmelos* traditionally used in Indian system of Medicines were screened against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* by using disc diffusion test technique. *Bacillus subtilis* exhibit about 22mm inhibition zone were considered resistant. The zone of inhibition of the extract was compared with the standard antibiotics such as Penicillin. The study suggests that the plant is promising development of phytomedicine for antimicrobial properties.

Key words: *Aegle marmelos*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, Antimicrobial activity.

Introduction

Infectious diseases are the leading cause of death worldwide. The clinical efficiency of many existing antibiotics is being threatened by the emergence of multidrug resistant pathogens (Bandow et al., 2003). Bacterial pathogens have evolved numerous defense mechanism against antimicrobial agents and resistance to old and newly produced drug is on the rise. 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; Scazzocchio *et al.*, 2001). There are several reports in the literature regarding the antimicrobial activity of crude extracts prepared from plants (El-seedi *et al*., 2002; Rojas et al., 2003; Duraipandiyan *et al*., 2006; Parekh and Chanda, 2007a).

Plants produce a diverse range of bioactive molecules making them a rich source of different types of medicines (Stuffness and Douros, 1982). Higher plants as sources of medicinal compounds have continued to play a dominant role in the maintenance of human health care since ancient times. Over 50% of all modern clinical drugs are of natural product origin and natural product play and vital

role in modern drug development in the pharmaceutical industry (Baker *et al*., 1995).

Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it. The effects of plant extract on bacteria have been studied by a very large number of researches in different parts of the world (Ates and Erdogrul, 2003). Much work has been done on ethnomedicinal plants in India (Negi *et al.*, 1993). Interest in a large number of traditional natural products has increased (Taylor *et al*., 1996). It has been suggested that aqueous and Ethanolic extract from plants used in allopathic medicine are potential sources of antiviral, Anti tumural and antimicrobial agents (Chung *et al*., 1995). The selection of the crude plants extract for screening programmes has the potential of being more successful in initial steps than the screening of pure compounds isolated from natural products (Kusumoto *et al.,* 1995). In the present study was aimed to focus the antibacterial activity of *Aegle marmelos* were studied.

Materials and Methods

Plant material

Fresh plants and plants parts were collected randomly from the semi-arid region of in and around Chennai. And the plants were identified and authenticated by botanist, Tamil nadu medicinal aromatic plants co-operation limited, Government Siddha medicinal college campus Chennai, India. The fresh plant material were washed under running tap water, shade dried and then homogenized to fine powder and stored in airtight container.

Aqueous Extraction

For aqueous extraction, 10g of air dried powder was placed in distilled water and boiled for 6 hours. At intervals of 2 hours it was filtered through 8 layers of muslin cloth and centrifuged at 5000 x g for 15 minutes. The supernatant was collected after 6 hours, the supernatant was concentrated to make the final volume one-fourth of the original volume. Finally 10gram of material was extracted in 25 ml of Distill water giving a concentration of 40mg/0.1ml. It was then autoclaved at 121˚c and 15 lbs pressure and stored at 4˚c (Nair et al., 2005).

Solvent Extraction

Ten grams of air dried powder was placed in 100 ml of ethanol in a conical flask, plugged with cotton and then kept on a rotary shaker at 190-220 rpm for 24 hours. After 24 hours it was filtered through 8 layers of muslin cloth and centrifuged at 5000xg for 15 minutes. The supernatant was collected and the solvent was evaporated to make the final volume one-fourth of the original volume. It was stored at 4˚c in air tight bottles for further studies (Nair et al., 2005)

Test microorganism

The microbial strains are identified and strains were obtained from National Collection of industrial microorganism (NCIM) Pune. India. The bacterial strains studied are *Pseudomonas aeruginosa* (NCIM 2200), *Staphylococcus aureus* (NCIM 2079), *Escherichia coli* (NCIM 2065), and *Bacillus subtilis* (NCIM 1196).

Antibacterial Assay

A loop full of the strain was inoculated in 30 ml of nutrient broth in a conical flask and incubated on a rotary shaker for 24 hours to activate the strain. Muller Hinton Agar was prepared for the study. The assay was performed using Agar disk diffusion method. The media and the test bacterial cultures were poured into pertidishes (Hi media). The test strain (0.2ml) was inoculated into media (Inoculum size 10^8) cells/ml when the temperature reached 40-42˚c. Care was taken to ensure proper homogenization. The experiment was performed under strict aseptic condition. For the Agar disk diffusion method the test compounds (0.1ml) was introduced onto the disk (0.7 cm) (Hi media) and then allowed to dry. Thus the disk was completely saturated with the test compounds. Then the disk was introduced onto the upper layer of the medium with the bacteria. The plates were incubated overnight at 37˚c. Microbial water were used as the control. The control activity was deducted from the test and the result obtained was plotted.

Table 1. Minimum inhibitory concentration of *Aegle marmelos* and control drug Penicillin with cultures of *Escherichia coli, Pseudomonas aeruginosa Staphylococcus aureus* and *Bacillus subtilis.*

S. No	Name of the organism	Concentration (mg/ml)	Zone of Inhibition (mm)		
			Aegle marmelos		*Penicillin*
			Aqueous extract	Ethanolic extract	
1	*Escherichia coli*	0.5	8	9	8
		1.0	10	11	9
		1.5	11	12	11
		2.0	12	14	12
		2.5	15	16	14
2	*Pseudomonas aeruginosa*	0.5	9	10	7
		1.0	10	11	9
		1.5	11	13	11

		2.0	13	15	12
		2.5	14	16	13
3	*Staphylococcus aureus*	0.5	6	13	11
		1.0	7	14	13
		1.5	9	17	14
		2.0	12	19	16
		2.5	14	21	18
4	*Bacillus subtilis*	0.5	12	14	12
		1.0	14	16	14
		1.5	16	17	15
		2.0	17	19	17
		2.5	20	22	19

Results and Discussion

The antimicrobial activity of *Aegle marmelos* of aqueous and alcoholic extract against the microorganism against different concentration of 0.5 mg/ml, 1.0 mg/ml, 1.5 mg/ml, 2.0 mg/ml and 2.5 mg/ml concentration was employed assessed qualitatively by the presence (or) absence of inhibition zone, zone diameter, Minimum inhibitory concentration (MIC).

The antibacterial activity of *Aegle marmelos* extract of both solvents (Aqueous and ethanolic) against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* were tabulated in the table -1. The ethanolic extract showed considerably more activity than the aqueous extract. Maximum antibacterial activity was shown against *Bacillus subtilis* followed by *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*.

In Table 1 the ethanolic extract of *Aegle marmelos* against *Escherichia coli* exhibits maximum zone of inhibition about 9mm, 11mm, 12mm, 14mm and 16mm and aqueous extract exhibit about 8mm, 10mm, 11mm, 12mm, and 15mm zone of inhibition and control drug exhibit minimum zone of inhibition when compared ethanolic and aqueous extract about 8mm, 9mm, 11mm, 12mm and 14 mm And the same dilutions were subjected *Pseudomonas aeruginosa*, the maximum zone exhibited only ethanolic extract about 10mm, 11mm, 13mm, 15mm, and 16mm and aqueous extract exhibits about 9mm, 10mm, 12mm, 13mm, and 14mm and the control drug penicillin shows lowest zone of inhibition about 7mm, 9mm, 11mm, 12mm, and 13mm. The extract of *Aegle marmelos* were subjected against *Staphylococcus aureus*, the maximum zone of inhibition was obtained in only in the

ethanolic extract about 13mm, 14mm, 17mm, 19mm and 21mm and

Control drug penicillin exhibit lower zone of inhibition of about 11mm, 13mm, 14mm, 16mm and 18mm and aqueous extract exhibit lowest zone of inhibition about 6mm, 7mm, 9mm, 12mm, and 14mm. The same dilutions were subjected to Gram positive organism of *Bacillus subtilis* and the maximum zone of inhibition was found in the ethanolic extract of about 14mm, 16mm, 17mm, 19mm and 22mm and aqueous extract exhibit minimum zone of inhibition of about 12mm, 14mm, 16mm, 17mm and 20mm and control drug penicillin exhibit lowest zone of inhibition of about 12mm, 14mm, 15mm, 17mm and 19mm. Since ancient times, plants have been a veritable source of drugs. However, man tends to ignore the importance of herbal medicine. Different extract from traditional medicinal plants have been tested to identify the source of the therapeutic effects, as a result some natural products have been approved as new antibacterial drugs

Hence presence study was aimed to focus the antibacterial activity of *Aegle marmelos* were studied further investigations to obtain information on chemical composition, to purify and determine the structure of active principle has been in progress in our laboratory.

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