

Evaluation of Antimicrobial Potential of *Aegle Marmelos* Fruit Extract against Selected Microorganisms

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Abstract:

Medicinal plants are the potential source of natural products and naturally derived compounds. These compounds show several useful properties like antioxidant, antimicrobial, antipyretic etc. The aim of this study is to evaluate the antimicrobial properties of *Aegle Marmelos*. In this study, antimicrobial activities of different extract of *Aegle Marmelos* fruit were evaluated against different microbial strains like *Escherichia coli* (MTCC-443), *Bacillus subtilis* (MTCC-441), *Pseudomonas aeruginosa* (MTCC-4673), *Staphylococcus aureus* (MTCC-3160), *Aspergillus brasiliensis* (MTCC-1344) and *Candida albicans* (MTCC-227) by agar well diffusion method & MIC determination by broth dilution method. The extract showed a broad spectrum of antimicrobial activities by inhibiting the growth of respective microorganism in Agar well diffusion assay. The present study supports the immense medicinal properties of *Aegle Marmelos* fruit. It may be insight for future researches in area of new drug development of herbal origins.

Key words: *Aegle Marmelos*, Antimicrobial activity, MIC, Zone of inhibition, *Aegle Marmelos* fruit.

INTRODUCTION:

The world has rich wealth of medicinal plants. Without the plant kingdom humans cannot survive on this earth because the plant products and their active constituents play an important role in our survival. Human is using numerous plants and plant derived products to cure and get relief from various physical and mental illness. The plants are used in Chinese, Ayurveda, Siddha, Unani and Tibetan medicines. Ancient literature such as Rigveda, Yajurveda, Atharvaveda, Charak Samhita and Sushrut Samhita also describes the use of plants for the treatment of various health problems. [1]

The green medicines are healthier and safer than synthetic ones. [2] A number of herbal medicines are used for the management of various diseases. Though the recovery is slow, the therapeutic use of medicinal plants is becoming popular because of its less side effects and combat antibiotic resistant microorganisms. [3]

The most frequently used type of herbal preparations is churna, a powdery preparation of medicinal plants that may be single or in combinations. The combinations of medicinal plants may increase the antimicrobial spectrum and potency of the preparations. More than 1500 herbal preparations are available in market as dietary supplements or ethnic traditional medicines in India. The search for eternal health and longevity for remedies to relieve pain and discomfort drove early man to explore his immediate natural surroundings to the use of many plants, animal products and minerals etc, for the development of a variety of therapeutic agents. Today, there is a renewed interest in traditional medicine and an increasing demand for more drugs from plants sources. [4]

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. [5-12] Modern medicine is rooted in ethno botanical traditions using indigenous flora to treat symptoms of human diseases or to improve specific aspects of the body conditions. Today a great number of modern drugs are still derived from natural sources and 25 per cent of all prescriptions contain one or more active ingredients from plants. [13] WHO has estimated that 80 per cent of the population of developing countries still relies on traditional medicines mostly plant drugs for their primary health care needs and ensure patient safety by upgrading the skills and knowledge of traditional medicine providers. [14]

More than 6000 plants are mentioned in the traditional systems of medicine. [15] *Aegle marmelos* (Bael) is one of them which play a vital role in day to day usage.

Bael (*Aegle marmelos*) is one of the most important tree species used in various indigenous systems of medicine in India, China, Burma and Sri Lanka. [16] Bael (*Aegle marmelos* (Linn), family Rutaceae, also known as Bale fruit tree, is a moderate sized, slender, aromatic tree, 6.0 -7.5 m in height, and 90 to 120 cm in girth, with a somewhat fluted bole of 3.0-4.5 meter growing wild throughout the deciduous forests of India, ascending to an altitude of 1200 meter in the western Himalayas and also occurring in Andaman island. Bael is the only member of the monotypic genus *Aegle*. [17]

Almost every part of the tree viz. stems, barks, roots, leaves, flowers & fruits at all stages of maturity have medicinal virtues and have been used in various Ayurvedic medicines since long time for the treatment of specific disorders such as respiratory disorders, constipation, ulcer, diarrhoea, dysentery and many others. It is also an

important environmental protector as leaves and bark act as a sink by absorbing dust and foul and poisonous gases from surrounding atmosphere and makes them clean. [18] Due to its endless uses, *A.marmelos* is also known as *Mahaphala* or Great fruit. [19] Thus, plants are the most important source for the new drug development because of the growing recognition that the natural products are non-toxic, have fewer side effects and are available at affordable price [20]. Although it has several potential but still more area is to be explored therefore the study is planned to identify the antimicrobial potential of *Aegle Marmelos* fruit.

MATERIAL & METHODS:

1 Collection of sample:

The fruits of *A. marmelos* were collected from local market.

2 Chemicals and solvents:

The different solvents such as acetone, ethanol, ethanol 70%, methanol, chloroform, petroleum ether and aqueous were used for the extraction of active agents from fruit of plant.

3 Media used:

The media which are used for study are: Nutrient Agar (NA), Nutrient Broth (NB), Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB).

4 Preparation of extracts: The plant materials (fruit) was collected, washed with water and shade dried at room temperature, crushed and broken using a pestle and mortar, and then stored in a cool and dry place. The materials (20g) were mixed with 100ml of different solvents such as acetone, ethanol, ethanol 70%, methanol, chloroform, and petroleum ether and aqueous in conical flask and kept on a rotary shaker for 12 hours at 30°C. Thereafter, it was filtered with the help of Whatman No. 1 filter paper. The filtrate was allowed to evaporate until completely dry. Extracts were stored in amber colored storage vials in refrigerator at 4°C until used for experiment.

Formulation of extract: Each extract of 100mg/ml was dissolved in the solvents separately and mixed with the help of vortex mixture just before antimicrobial testing.

5 Microbial Culture: Different microorganisms were used such as *Escherichia coli* (MTCC-443), *Bacillus subtilis* (MTCC-441), *Pseudomonas aeruginosa* (MTCC-4673), *Staphylococcus aureus* (MTCC-3160), *Aspergillus brasiliensis* (MTCC-1344) and *Candida albicans* (MTCC-227). These micro-organisms were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), and Chandigarh. The bacterial isolates were first sub cultured on a nutrient agar and incubated at 37°C for 24 hrs while the fungal isolates were sub cultured on a potato dextrose agar for 72 hrs at 25°C. The MTCC cultures were characterized and identified on the basis of gram staining.

6. Screening for antimicrobial activity: For the determination of antibacterial activity the agar well diffusion method [21- 22] was used for solvent extracts.

(A) Agar Well Diffusion Method:

Nutrient agar/Potato dextrose agar plates were prepared and after 10-15 min. when medium was solidified it was swabbed with 400µl of different microbial cultures then wells of 6mm were cut and the cut wells were then filled with 50µl of each (i.e. Acetone, Ethanol, Ethanol 70%, Methanol, Chloroform, Petroleum ether and Aqueous) extracts fruit and the plates were kept for incubation at 37°C for 24 hrs for bacteria and at 25°C for 48-78 hrs for fungus. After incubation period, the plates were observed for the zone of inhibition of growth. The zones were measured with a transparent ruler and the result recorded in millimeters.

(B) Minimum inhibitory concentration (MIC):

The extract which will show antibacterial activity in agar well assay, will be subjected to MIC assay. The antimicrobial MIC studies will be carried out by broth dilution method [23]

RESULTS:

In the present study, fruits of the *A.marmelos* (Bael) were collected from Local Market. The different types of solvents (acetone, ethanol, ethanol 70%, methanol, chloroform, petroleum ether and aqueous) were used for the extraction of active agents from fruit. These extract were used to check antimicrobial potential against different tested pathogens by agar well diffusion assay method. [21; 22]

Table 1 shows the result of antibacterial activity of *Aegle Marmelos*. In this it is observed that chloroform extracts of fruits, showed the inhibitory activity against all the four pathogens. It showed maximum zones of inhibition against *S.aureus* (22.5mm) then *E.coli*, *B.subtilis* and *P.aeruginosa* (14.5 mm, 11.5 mm, and 8.3 mm). Whereas, ethanol, methanol & petroleum ether extracts showed inhibitory effect against *E.coli*, *S.aureus* and *B.subtilis*. Acetone extract showed zone of inhibition against *B.subtilis* and *E.coli*. Ethanol 70% extract showed zone of inhibition against *E.coli* and *P.aeruginosa*. In case of aqueous extract it did not show non significant activity.

Table 2 shows the result of antifungal activity of *Aegle Marmelos* fruit extract. It is observed that chloroform extract showed maximum zones of inhibition (25.5mm) against *A.brasiliensis* as compared to *C.albicans* (0.8 mm). Acetone extract showed highest zone of inhibition against *C.albicans* as compared to *A.brasiliensis*. The methanol extract showed higher zone of inhibition against *A.brasiliensis* as compared to *C.albicans*. Ethanol, petroleum ether and ethanol 70% extracts showed zone of inhibition only against *C.albicans*. Aqueous extract it did not show significant zone of inhibition.

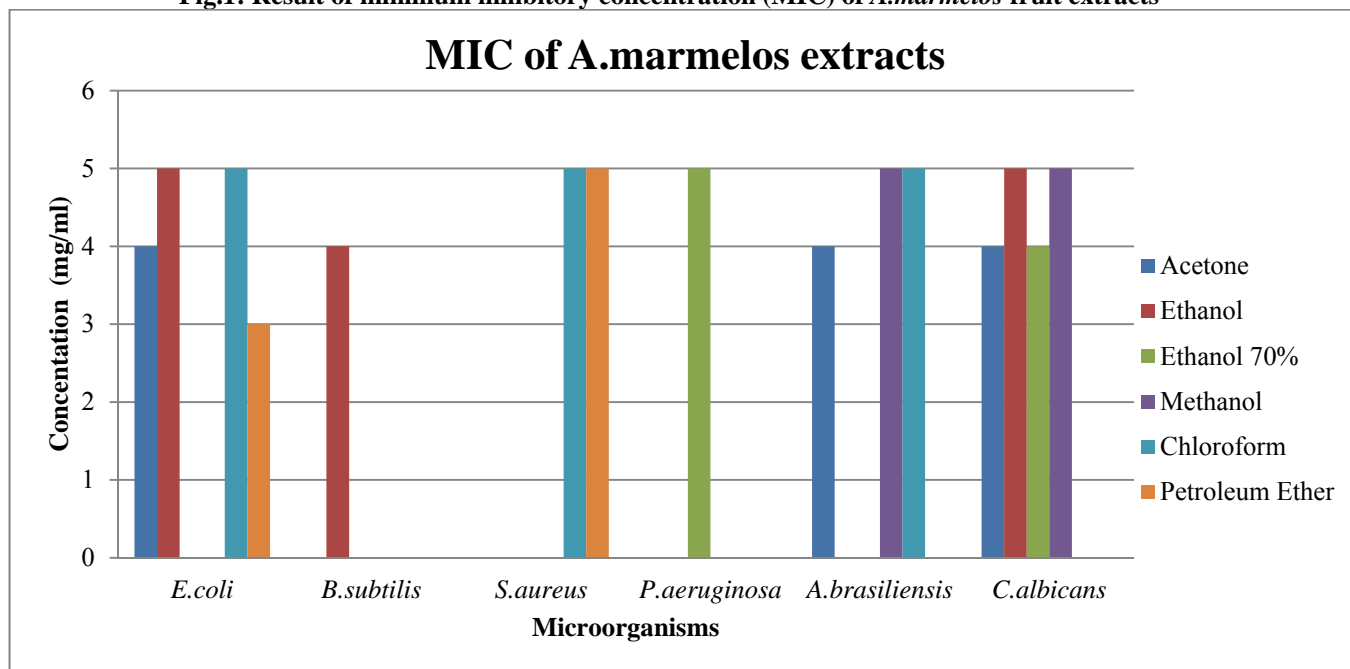
Table 1: Antibacterial activity of *A. marmelos* Fruit extracts.

S.No.	Name of the solvents	<i>E.coli</i>		<i>B.subtilis</i>		<i>S.aureus</i>		<i>P.aeruginosa</i>	
		Control	Test	Control	Test	Control	Test	Control	Test
1	Acetone	-	16.5	-	4.3	-	-	-	-
2	Ethanol	-	13	-	18.5	-	6.4	-	-
3	Ethanol 70%	-	4	-	-	-	-	-	12.4
4	Methanol	-	3.5	-	8.5	-	4.3	-	-
5	Chloroform	-	14.5	-	11.5	-	22.5	-	8.3
6	Petroleum Ether	-	16	-	18	-	17	-	-
7	Aqueous	-	3.5	-	4.2	-	2.4	-	-

Table 2: Antifungal activity of *A. marmelos* fruit extracts.

S.No	Name of the solvents	<i>A.brasiliensis</i>		<i>C.albicans</i>	
		Control	Test	Control	Test
1	Acetone	-	9.8	-	17.5
2	Ethanol	-	-	-	16
3	Ethanol 70%	-	-	-	14.5
4	Methanol	-	14.5	-	13.5
5	Chloroform	-	25.5	-	0.8
6	Petroleum Ether	-	-	-	7.8
7	Aqueous	-	3	-	3.5

Fig.1: Result of minimum inhibitory concentration (MIC) of *A.marmelos* fruit extracts



Determination of minimum inhibitory concentration (MIC) of *A.marmelos* fruit extracts

The extract which showed antibacterial activity in agar well assay were subjected to MIC assay. The antimicrobial MIC studies were carried out by broth dilution method [23]. For the antimicrobial assays of *A.marmelos* fruits extracts with solvents acetone, ethanol, ethanol 70%,methanol, chloroform, petroleum ether were used and they showed

both antifungal and antibacterial activities at different concentration 1-5mg/ml against different pathogens like *Escherichia coli* (MTCC-443), *Bacillus subtilis* (MTCC-441), *Pseudomonas aeruginosa* (MTCC-4673), *Staphylococcus aureus* (MTCC-3160), *Aspergillus brasiliensis* (MTCC-1344) and *Candida albicans* (MTCC227).The result is indicated in Fig -1

DISCUSSION:

In this study *A.marmelos* fruit were chosen for extract preparation. Total seven different solvents were used. These extracts were tested against 4 different bacterial and 2 fungal strains. Antimicrobial assay of all these extracts were performed for the detection of zone of inhibition. Out of 7 samples, 4 were reported for antibacterial activity (one showed strong response, nine moderate and thirteen mild antibacterial potential) and 05 were reported for antifungal activity (five showed strong, five moderate and fifteen mild response antifungal potential).

Ethanol, chloroform, petroleum ether and methanol extracts of fruit showed activity against *B.subtilis*, *S.aureus* and *E.coli* as compared to *P.aeruginosa*. In previous research Maisuthisakul *et al.* (2007) [24-25] reported that the methanol extract of fruit contains high antimicrobial activity. This study indicated that the major antioxidants in bael fruit are phenolics, flavonoids, carotenoids, and vitamin C. [26, 27] so; our study also supports the previous results that bael fruit pulps contain high phenolic content which may be responsible for the antibacterial activity.

Moreover total 5 extracts showed the significant inhibition against selected fungal strains. All the extracts of *A.marmelos* (fruit) were effective against *Candida albicans* as compared to *A.brasiliensis* in our study. Few previous researchers like Parihar *et al.*, (2013) [28] have reported that antifungal activity exhibited by ethanol/methanol extract of *A.marmelos* fruit against *Aspergillus niger*, *Penicillium chrysogenum*, *Candida albicans*, *Fusarium solani*, which was due to the presence of phytochemicals. Moreover H.R. Gheisari *et al.*, 2011 also reported that the activity against *Aspergillus Niger*, *Aspergillus fumigatus*, *Candida albicans* and *Staphylococcus aureus*. [29] So, our findings support the same result and it has been indicated that the antifungal activity may due to the presence of different phytochemicals.

CONCLUSION

Our findings suggest that, Ayurvedic herbal preparations extracts have great potential as antimicrobial agents and they can be used in the treatment of infectious diseases. The organic solvent extraction was suitable to verify the antimicrobial properties of medicinal plants and they support many investigators. The present study justifies the uses of different fruit extract in the traditional system of medicine to treat various infectious disease caused by the microbes. Day by day we face new and complex health related problem, and we are getting addicted of modern medicine or the synthetic medicine, which definitely gives the fast result but brings several new problem with them like side effect and adverse effect. Bioactive compounds of bael fruit in this study contain relatively high content of dietary fiber, ascorbic acid, total phenolics, total flavonoids, total carotenoids, and are responsible for

antibacterial activities. Moreover herbal compound like *Aegle marmelos* (Bael) is much more valuable and safe compared to chemicals. Looking upon the wide prospect of Bael tree, government/NGO should either cultivate it or try to preserve it for the proper utilization and to discover the new and effective herbal medicines. The present findings support the applicability of *A.marmelos* in traditional system for its claimed uses and can be recommended by the scientific community as an accessible alternative to synthetic antibiotics. This study is a preliminary evaluation of antibacterial activity of fruit of *A. marmelos*.

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