

Antimicrobial Activity of *Aegle marmelos* L. Leaf Extract

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Abstract: Ethno medicine has been gaining popularity for years, yet there is still a vast amount of medicinal flora that remains undiscovered through research. The *in vitro* antimicrobial activity of methanol and water extract from the leaf of medicinal plant *Aegle marmelos* L. were investigated. The investigation was performed against three Gram positive bacteria (*Bacillus megaterium*, *Bacillus subtilis*, and *Staphylococcus pneumonia*) and five Gram negative (*Salmonella typhi*, *Vibrio parahaemolyticus*, *Escherichia coli*, *Shigella dysenteriae* and *Shigella sonnei*) bacteria and eight fungi (*T. mentagrophytes*, *M. canis*, *T. rubrum*, *E. floccosum*, *Penicilium sp*, *Fusarium species*, *Aspergillus niger* and *Mucor*) by disc diffusion assay method. The lowest concentration of every extract considered as the minimal inhibitory concentration (MIC) values were determined for both test organisms. Statistical significance was determined with one-way ANOVA and the level of significance was $P < 0.05$. The water extract of leaves at the concentration of 600 $\mu\text{g}/\text{disc}$ showed the higher activity against *S. typhi* (13 ± 0.1), and *S. dysenteriae* (15 ± 0.2) than methanol extract. Methanol extract showed the higher activity against *E. coli* (18 ± 0.1), *V. parahaemolyticus* (19 ± 0.2) and *S. pneumonia* (15 ± 0.3) than water extract. Methanol extract of *A. marmelos* leaf showed more susceptibility towards skin disease causing fungi like *T. mentagrophytes*, *M. canis*, *T. rubrum*, *E. floccosum*, than the non-skin disease causing fungi like *Penicilium sp*, *Fusarium species* and *Mucor*. The result implies that the both methanol and water extract of *Aegle marmelos*, L. has great potential for medicinal purposes due to its antimicrobial quality.

Keywords: Antimicrobial Activity, Medicinal Plant, *Aegle marmelos*, Bacteria, Fungi

1. Introduction

To cure a large number of diseases in human beings, medicinal plants can call the gifts of nature. For the management of various diseases, a large number of traditional herbal medicines are used. Therapeutic efficacy of many medicinal plants for several diseases has been described by practitioners of traditional medicine [1]. Many plants around the world have been extracted and purified to investigate their antimicrobial activity [2]. The plants such as *Calotropis gigantean* L. [3], *Coccinia grandis* L. [4], *Moringa oleifera* L. [5], *Orthosiphon stamineus* Benth [6], *Callistemon viminalis* [7], *Vigna unguiculata* L. [8] and *Swietenia macrophylla* [9] exhibits antimicrobial activity.

Aegle marmelos L. Correa, a tree species belonging to the family of Rotaceae, is commonly called bael tree. This is native to the Indo-Malayan region [10] and is cultivated in Bangladesh, India, Pakistan, Sri Lanka, Thailand and Burma

[11]. In India, *A. marmelos* L. has been used not only as nutritive food for human but also used as medicinal food for its various medicinal properties [12]. The leaf of this plant has antidiabetic [13], anticancer [14], anti-inflammatory [15], antimalarial [16] and antihyperlipidemic [17] activities. The leaf contains terpenoids which act as antifungal compounds [18]. Fruits of this plant are used in diarrhea, constipation, gastric troubles, dysentery, brain and heart tonic, antiviral, ulcer, intestinal, gonorrhea, epilepsy [19]. Plants secondary metabolites can either inhibit the growth or kill the pathogens and have least or no toxicity to host cells are considered to develop new microbial drugs [20]. In our laboratory we found that, *A. marmelos* leaves contain various secondary metabolites. In the present study, antimicrobial activities of methanol and water extract of *A. marmelos* leaves were determined.

2. Materials and Methods

2.1. Collection and Preparation of Samples

Fresh leaves of *A. marmelos* L. plant were collected from Rajshahi University campus, Bangladesh. The leaves were dried and powdered. For methanol, 40 g powdered samples were mixed by occasional shaking and starring in a shaker for 4 days and filtered through Whatman No 1 paper. With a rotary evaporator at 40°C, the solvents were removed to obtain dry extract. The extracts were stored at -20°C until used. For water extract, 40 g powdered sample was mixed in boiling water and filtered and the extract was dried by freeze drier and stored at -20 °C until used.

2.2. Test Microorganisms

Eight bacterial cultures were used in the study, among these were three Gram positive (*Bacillus megaterium*, *Bacillus subtilis* and *Staphylococcus pneumonia*) and four Gram negative (*Salmonella typhi*, *Vibrio parahaemolyticus*, *Escherichia coli*, *Shigella dysenteriae* and *Shigella sonnei*) bacteria and eight fungi (*T. mentagrophytes*, *M. canis*, *T. rubrum*, *E. floccosum*, *Penicillium sp*, *Fusarium species*, *Aspergillus niger* and *Mucor*). All these microorganisms were collected from the Institute of Biological Sciences (IBSC), University of Rajshahi, Bangladesh.

2.3. Antimicrobial Activity

The bacterial and fungal strains were cultured in nutrient Agar medium and Dextrose Agar medium respectively for 12 and 24hrs. The antibacterial activity of leaf extracts were tested by disc diffusion assay method [21]. The Nutrient Agar plates used for antibacterial tests were incubated at 37°C for 24 hrs whereas the Dextrose Agar plates for antifungal tests were incubated at 30°C for 72 hrs. In nutrient agar plates, an inoculums size, 10^6 cfu/ml for bacteria was used and on dextrose agar plates 2×10^5 spores were used for fungi. As positive control, Streptomycin is used for antibacterial tests whereas Doxycycline is used as positive control for antifungal tests. Antifungal and antibacterial activity was determined by measuring the diameter of the zone of inhibition surrounding fungal and bacterial growth.

2.4. Statistical Analysis

All data are expressed as mean \pm standard error of the means (SEM) from triplicate experiments. Statistical significance was determined with one-way ANOVA and the level of significance was $P < 0.05$.

3. Results and Discussion

The antibacterial activity of methanol and water extract of *A. marmelos* L. leaves were examined against tested bacteria was shown in table 1 and Table 2.

Table 1. In vitro antibacterial activity of Methanol extract of *A. marmelos* L. leaves and Streptomycin.

Test bacteria	Zone of inhibition (diameter in mm.)			
	Extract 200µg/disc (Ie 200)	Extract 400µg/disc (Ie 400)	Extract 600 µg/disc (Ie 600)	Streptomycin (µg/disc)
Gram positive				
<i>Bacillus megatorium</i>	5±0.1*	6±0.1*	8±0.1*	31±0.1
<i>Bacillus subtilis</i>	5±0.1*	8±0.3*	10±0.4*	28±0.2
<i>Staphylococcus pneumonia</i>	6±0.1*	7±0.3*	15±0.3*	29±0.2
Gram negative				
<i>Salmonella typhi</i>	7±0.3*	8±0.1*	12±0.6*	30±0.1
<i>Vibrio parahaemolyticus</i>	7±0.4*	9±0.3*	19±0.2*	29±0.3
<i>Escherichia colli</i>	8±0.1*	10±0.2*	18±0.1*	29±0.4
<i>Shigella Dysenteriae</i>	6±0.4*	7±0.1*	13±0.4*	28±0.1
<i>Shigella sonnei</i>	6±0.1*	8±0.3*	12±0.4*	30±0.4

Values are mean \pm S. E. M. of 3 replicates. * P value < 0.05 when compared with positive control Streptomycin.

Table 2. In vitro antibacterial activity of water extract of *A. marmelos* L. leaves and Streptomycin.

Test bacteria	Zone of inhibition (diameter in mm.)			
	Extract 200µg/disc (Ie 200)	Extract 400µg/disc (Ie 400)	Extract 600 µg/disc (Ie600)	Streptomycin (µg/disc)
Gram positive				
<i>Bacillus megatorium</i>	6±0.1*	7±0.3*	9±0.1*	30±0.3
<i>Bacillus subtilis</i>	7±0.3*	9±0.2*	11±0.1*	29±0.1
<i>Staphylococcus pneumonia</i>	7±0.1*	9±0.1*	12 ±0.1*	30±0.5
Gram negative				
<i>Salmonella typhi</i>	8±0.2*	9±0.4*	13±0.1*	29±0.4
<i>Vibrio parahaemolyticus</i>	6±0.1*	8±0.2*	17±0.1*	30±0.2
<i>Escherichia colli</i>	6±0.3*	8±0.1*	16±0.1*	29±0.1
<i>Shigella Dysenteriae</i>	7±0.4*	8±0.1*	15±0.2*	29±0.1
<i>Shigella sonnei</i>	6±0.2*	7±0.4*	14±0.3*	28±0.2

Values are mean \pm S. E. M. of 3 replicates. * P value < 0.05 when compared with positive control Streptomycin.

In this study, the water extract of leaf at the concentration of 600 µg/ disc showed the high activity against *S. typhi* (13±0.1), *S. sonnei* (14±0.3) and *S. dysenteriae* (15±0.2) from its methanol extract. *S. typhi* is responsible for typhi fever; *S. sonnei* and *S. dysenteriae* are responsible to cause dysentery in human. So, water extract can be used as alternative medicine for these diseases. It is reported that *V. unguiculata* also possess the antimicrobial activity against *S. typhi*, *S. sonnei* and *S. dysenteriae* [22]. Methanol extract showed the higher activity against *E. coli* (18±0.1), *V. parahaemolyticus* (19±0.2) and *S. pneumonia* (15±0.3) than water extract. *E. coli* and *V. parahaemolyticus* can cause a moderate to severe gastroenteritis in humans, so methanol extract can be used as medicine in gastroenteritis. It is reported that the ethanol extract of *A. marmelos* L. leaves and

purified lectins of *M. oleifera* leaves showed the high activity against *E. coli* [23, 24] and the methanol extract of *Orthosiphon stamineus* plant showed the high activity against *V. parahaemolyticus*. *S. pneumonia* is one of the most common causes of bacterial meningitis in adults [6], so methanol extract can be useful in the treatment of meningitis. In both methanol and water extract *Bacillus megaterium* and *Bacillus subtilis* showed the moderate activity but in high concentration they may show the high activity against these bacteria. In this study, both methanol and water extract showed antibacterial activity but, water extract is more potent than methanol extract.

The antifungal sensitivity tests for the methanol and water extracts of *A. marmelos* L. leaves at different doses was shown in table 3 and table 4.

Table 3. In vitro antifungal activities of methanol extract of *A. marmelos* L. leaves and Doxycycline.

Test fungi	Zone of inhibition (diameter in mm.)			
	Extract (50µg/disc)	Extract 100µg/disc)	Extract (200µg/disc)	Doxycycline (µg/disc)
<i>T. mentagrophytes</i>	6±0.1*	9±0.1*	16±0.3*	30±0.1
<i>M. canis</i>	5±0.1*	10±0.5*	17±0.1*	30±0.1
<i>T. rubrum</i>	7±0.2*	9±0.1*	16±0.3*	29±0.1
<i>E. floccosum</i>	6±0.4*	8±0.2*	16±0.3*	30±0.3
<i>Penicilium sp</i>	6±0.1*	7±0.3*	10±0.1*	29±0.1
<i>Fusarium species</i>	5±0.3*	6±0.1*	9±0.2*	30±0.1
<i>Aspergillus niger</i>	7±0.5*	7±0.1*	10±0.1*	29±0.2
<i>Mucor</i>	7±0.1*	8±0.2*	11±0.1*	29±0.2

Values are mean ± S. E. M. of 3 replicates. * P value < 0.05 when compared with positive control Doxycycline.

Table 4. In vitro antifungal activities of water extract of *A. marmelos* L. leaves and Doxycycline.

Test fungi	Zone of inhibition (diameter in mm.)			
	Extract (100µg/disc)	Extract (200µg/disc)	Extract (400µg/disc)	Doxycycline (µg/disc)
<i>T. mentagrophytes</i>	8±0.1*	10±0.3*	18±0.1*	29±0.1
<i>M. canis</i>	6±0.2*	9±0.1*	16±0.5*	30±0.1
<i>T. rubrum</i>	7±0.5*	9±0.1*	14±0.4*	29±0.2
<i>E. floccosum</i>	8±0.4*	10±0.1*	18±0.1*	30±0.3
<i>Penicilium sp</i>	5±0.1*	7±0.4*	10±0.3*	29±0.1
<i>Fusarium species</i>	6±0.1*	8±0.1*	10±0.1*	30±0.4
<i>Aspergillus niger</i>	5±0.4*	7±0.2*	9±0.2*	25±0.1
<i>Mucor</i>	6±0.1*	8±0.3*	10±0.1*	28±0.2

Values are mean ± S. E. M. of 3 replicates. * P value < 0.05 when compared with positive control Doxycycline.

Methanol extract showed maximum inhibition at 200 µg/mL against *T. mentagrophytes*, *M. canis*, *T. rubrum*, *E. floccosum*, *Penicilium sp*, *Fusarium species* and *Mucor* whereas Water extracts showed the maximum inhibition at 400 µg/mL against all the tasted fungi. In both methanol and water extract the fungi *T. mentagrophytes*, *M. canis*, *T. rubrum* and *E. floccosum* showed more activity than the fungi *Penicilium sp*, *Fusarium species* and *Mucor*. Methanol extract of *A. marmelos* leaf showed more susceptibility towards skin disease causing fungi like *T. mentagrophytes*, *M. canis*, *T. rubrum*, *E. floccosum*, than the non-skin disease causing fungi like *Penicilium sp*, *Fusarium species* and *Mucor*. So methanol extract of *A. marmelos* leaf may be useful to cure skin diseases. A similar study of plant extract against different fungal pathogens was reported in literature [25-28].

4. Conclusion

The results of this study clearly demonstrated that the extracts of *A. marmelos* L. leave exhibit antifungal and antibacterial activity which might be useful in preventing the progress of various diseases and can be useful in alternative system of medicine.

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