American Journal of Bioscience and Bioengineering
2023; 11(5): 75-78
http://www.sciencepublishinggroup.com/j/bio
doi: 10.11648/j.bio.20231105.13
ISSN: 2328-5885 (Print); ISSN: 2328-5893 (Online)

Antimicrobial Activity of *Aegle marmelos* L. Leaf Extract

Shahanaz Khatun*, Nasrin Ferdous

Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi, Bangladesh

Email address:
dr.khatun@ru.ac.bd (Shahanaz Khatun)

*Corresponding author

To cite this article:
Shahanaz Khatun, Nasrin Ferdous. Antimicrobial Activity of *Aegle marmelos* L. Leaf Extract. *American Journal of Bioscience and Bioengineering*. Vol. 11, No. 5, 2023, pp. 75-78. doi: 10.11648/j.bio.20231105.13

Received: June 20, 2023; **Accepted**: July 13, 2023; **Published**: September 27, 2023

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 µg/ 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, Thiland 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-inflamatory [15], antimalarial [16] and antihyperlipidemic [17] activities. The leaf contains terpinoids 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, Penicilium 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, 10^6cfu/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 ± 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				
Bacillus megatorium	5±0.1*	6±0.1*	8±0.1*	31±0.1
Bacillus subtilis	5±0.1*	8±0.3*	10±0.4*	28±0.2
Staphylococcus pneumonia	6±0.1*	7±0.3*	15±0.3*	29±0.2
Gram negative				
Salmonella typhi	7±0.3*	8±0.1*	12±0.6*	30±0.1
Vibrio parahaemolyticus	7±0.4*	9±0.3*	19±0.2*	29±0.3
Escherichia colli	8±0.1*	10±0.2*	18±0.1*	29±0.4
Shigella Dysenteriae	6±0.4*	7±0.1*	13±0.4*	28±0.1
Shigella sonnei	6±0.1*	8±0.3*	12±0.4*	30±0.4

Values are mean ± 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				
Bacillus megatorium	6±0.1*	7±0.3*	9±0.1*	30±0.3
Bacillus subtilis	7±0.3*	9±0.2*	11±0.1*	29±0.1
Staphylococcus pneumonia	7±0.1*	9±0.1*	12 ±0.1*	30±0.5
Gram negative				
Salmonella typhi	8±0.2*	9±0.4*	13±0.1*	29±0.4
Vibrio parahaemolyticus	6±0.1*	8±0.2*	17±0.1*	30±0.2
Escherichia colli	6±0.3*	8±0.1*	16±0.1*	29±0.1
Shigella Dysenteriae	7±0.4*	8±0.1*	15±0.2*	29±0.1
Shigella sonnei	6±0.2*	7±0.4*	14±0.3*	28±0.2

Values are mean ± 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 theses 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)
T. mentagrophytes	6±0.1*	9±0.1*	16±0.3*	30±0.1
M. canis	5±0.1*	10±0.5*	17±0.1*	30±0.1
T. rubrum	7±0.2*	9±0.1*	16±0.3*	29±0.1
E. floccosum	6±0.4*	8±0.2*	16±0.3*	30±0.3
Penicilium sp	6±0.1*	7±0.3*	10±0.1*	29±0.1
Fusarium species	5±0.3*	6±0.1*	9±0.2*	30±0.1
Aspergillus niger	7±0.5*	7±0.1*	10±0.1*	29±0.2
Mucor	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)
T. mentogrophytes	8±0.1*	10±0.3*	18±0.1*	29±0.1
M. canis	6±0.2*	9±0.1*	16±0.5*	30±0.1
T. rubrum	7±0.5*	9±0.1*	14±0.4*	29±0.2
E. floccosum	8±0.4*	10±0.1*	18±0.1*	30±0.3
Penicilium sp	5±0.1*	7±0.4*	10±0.3*	29±0.1
Fusarium species	6±0.1*	8±0.1*	10±0.1*	30±0.4
Aspergillus niger	5±0.4*	7±0.2*	9±0.2*	25±0.1
Mucor	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.

Acknowledgments

The authors are grateful to the Chairman of the Department of Biochemistry and Molecular Biology, Rajshahi University, Rajshahi-6205, Bangladesh to give the lab facility to complete this research work.

References

[1] PANKKAJALAKSHMI, V. V & VENUGOPAL, T. V. (1994) Antidermatophytic activity of neem (Azadiracta indica) leaves in vitro, Indian Journal of Pharmacology, 26: 141-143.

[2] VADAPUDI, V. & CHANDRASHEKAR, N. K. (2010) In Vitro bioactivity of Indian Medicinal Plant Calotropis Procera (Ait.), Journal of Global Pharma Technology. 2 (2): 43-45.

[3] ALOM, M. A., HABIB, M. R., NIKKON, F., RAHMAN, M & KARIM, M. R. (2008) Antimicrobial activity of akanda (Calotropis gigantean L.) on some pathogenicbacteria, Bangladesh Journal of Scientific and Industrial Research, 43 (3): 397-404.

[4] FARRUKH, U., SHAREEF, H., MAHMUD, S., ALI, S. A. and RIZWANI, G. H. (2008) Antimicrobial activities of Coccinia grandis L., Pakistan Journal of Botani, 40 (3): 1259-1262.

[5] RAHMAN, M. M., SEIKH, M. M. I., SHARMIN, S. A., ISLAM, M. S., RAHMAN, M. A., RAHMAN, M. M. & ALAM, M. F. (2009) Antimicrobial activity of leaf juice and extracts of Moringa oleifera Lam. Against some human pathogenic bacteria. CMU. Journal of Natural Science, 8 (2): 219-227.

[6] HO, C. H., NORYATI, I., SULAIMAN, S. F. and ROSMA, A. (2010) In vitro antibacterial and antioxidant activities of Orthosiphon stamineus Benth. extracts against food-borne bacteria, Food Chemistry, 122: 1168-1172.

[7] DALAHAYA, C., RAINFORD, L., NIHOLSON, A., MITCHEL, S., LINDO, J & AHMAD, M. (2009) Antibacterial and antifungal analysis of crude extracts from the leaves of Callistemon viminalis, Journal of Medical and Biological sciences, 3 (1): 1-7.

[8] KRITZINGER, Q., LAIL, N. & AVELING, T. A. S. (2005) Antimicrobial activity of cowpea (Vigna unguiculata) leaf extracts, South African Journal of botany, 71 (1): 45-48.

[9] DEWANJEE, S., KUNDU, M., MAITI, A., MAJUMDAR, R., MAJUMDAR, A. & MANDAL, S. C. (2007) In Vitro evaluation of antimicrobial activity of crude extract from plants Diospyros peregrina, Coccinia grandis and Swietenia macrophylla. Tropical Journal of Pharmaceuticcal Research, 6: 773-778.

[10] HOOKER, J. D. (1975) The flora of British India, Reeve, United Kingdom, Vol. 1, pp 516–517.

[11] ISLAM, R., HOSSAIN, M., KARIM, M. R. & JOARDER, O. I. (1995) Regeneration of Aegle marmelos (L.) Corr., plantlets in vitro from callus cultures of embryonic tissues, Current Science, 69: 494–5.

[12] CHOPRA, R. (1982) Indigenous drugs of India Calcutta. Academic publishers, pp 71-76.

[13] ARUMUGAMA, S., KAVIMANIB, S., KADALMANIC, B., AHMEED, A. B. A., AKBARSHA, M. A. & RAO, M. V. (2008) Antidiabetic activity of leaf and callus extracts of Aegle marmelos in rabbit, Science Asia, 34: 317-321.

[14] JAGETIA, G. C., VENKATESH, P. & BALLIGA, M. S. (2005) Aegle marmelos (L) Correa inhibits the proliferation of transplanted Ehrlich ascites carcinoma in mice, Biological and Pharmaceutical Bulletin, 28: 58-63.

[15] ARUL, V., MIYAZAKI, S. & DHANANJAYAN, R. (2005) Studies on the antiinflammatory, antipyretic and analgesic properties of the leaves of Aegle marmelos Correa., Journal of Ethnopharmacology, 96: 159-163.

[16] MISRA, P., PAL, N. L., GURU, P. Y., KATIYAR, J. C. & TANDON, J. S. (1991) Antimalarial activity of traditional plant against erythrocyte stages of Plasmodium berghei International Journal of Pharmacology. 19: 29-34.

[17] RAJADURAI, M. & PRINCE, P. S. (2005) Comparative effects of Aegle marmelos extract and alpha-tocopherol on serum lipids, lipid peroxides and cardiac enzyme levels in rats with isoproterenol-induced myocardial infarction, Singapore Medical Journal, 46: 78-82.

[18] RANA, B. K., SINGH, U. P. & TANEJA, A. (1997) Antifungal activity and kinetics of inhibition by essential oil isolated from leaves of Aegle marmelos, Journal of Ethnopharmacology, 57: 29-34.

[19] VEERAPPAN, A. K. S. & RENGANATHAN, D. (2000) Cardiotonic effect of Aegle marmelos Corr. On amphibian heart in situ preparation, Proc. 6th Internet World Congress for Biomed. Sci.

[20] PRAKASH, C. G., DUTTA, B., PANT, D., JOSHI, P. & LOHAR, D. R. (2009) In vitro antibacterial activity of Artemisia annua Linn, Growing in India, International journal of Green Pharmacy, 4: 255-258.

[21] VANDER, B. D. A. & VLIETNCK, B. (1991) Screening methods of antibacterial and antiviral agents from higher plants. IN: Assay for Bioactivity (K. Hostiettman Academic Press London), pp 47-69.

[22] ASHRADUZZAMAN, M., MOHAMMAD, A. A., KHATUN, S. & ABSAR, N. (2016) Antimicribial activity of V. unguiculata L. Walp seed oil, International journal of biotechnology for wellness industries. 5: 70-75.

[23] SIVARAJ, R., BALAKRISHNAN, A., THENMOHI, M. & VENCKATESH, R. (2011) Antimicrobial activity of Aegle marmelos, Ruta graveolens, Opuntia dellini, Euphorbia royleana and Euphorbia antiquorum, Journal of Pharmacy Research, 4 (5): 1507-1508.

[24] KHATUN, S., KHAN, MMH., ASHRADUZZAMAN, M., PERVIN, F., BARI, L. & ABSAR, N. (2009) Antibacterial activity and cytotoxicity of three lectins purified from Moringa oleifera Lam. Leaves, Journal of Biosciences, 17: 89-94.

[25] PERUMALSAMY, R., IGNACHIMUTHU, S. & SEN, A. (1988) Screening of 34 Indian Medicinal plants for antimicrobial properities, Journal of Ethnopharmacology, 62: 173-81.

[26] AHMAD, I., MEHMOOD, Z., MOHAMMAD, P. & AHMED, S. (2000) Antimicrobial potency and synergistic activity of five traditionally used Indian medicinal plants, Journal of Medicinal Aromatic Plant Science, 22: 173-176.

[27] RANI, S. A. & MURTY, S. U. (2006) Antifungal potential of flower head extract of Spilanthes acmella Linn., African Journal of Biomedical Research, 9: 67-69.

[28] KHATUN, S., PERVIN, F., KARIM, M. R., ASHRADUZZAMAN, M. & ROSMA, A. (2012) Phytochemical screening and antimicrobial activity of Coccinia cordifolia L. plant Pakistan Journal of Pharmaceutical Sciences, 25 (4): 757-761.