IJPSR (2018), Volume 9, Issue 8 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 07 October, 2017; received in revised form, 13 February, 2018; accepted, 19 February, 2018; published 01 August, 2018

ANTIMICROBIAL SUSCEPTIBILITY TEST ANALYSIS OF *AEGLE MARMELOS* BARK EXTRACTS

Rama Dahiya [1], Rishabh Charan Choudhary [1], Rajesh Singh Tomar [2] and Vikas Shrivastava [* 2]

Department of Applied Science and Agriculture [1], Suresh Gyan Vihar University, Jaipur - 302017, Rajasthan, India.
Amity Institute of Biotechnology [2], Amity University, Gwalior - 474005, Madhya Pradesh, India.

Keywords:

Aegle marmelos, Bark extracts, Bael, Antimicrobial activity, Minimum Inhibitory concentration

Correspondence to Author:
Vikas Shrivastava

Associate Professor,
Amity Institute of Biotechnology,
Amity University, Gwalior - 474005,
Madhya Pradesh, India.

E-mail: vshrivastava@gwa.amity.edu

ABSTRACT: India is a land of medicinal flora, there are large numbers of plants with miraculous medicinal values has been used traditionally to cure several diseases from decades in Ayurveda. So the present study was designated to evaluate the antimicrobial activities of *Aegle marmelos* (bale) bark extracts. In this study different bark extracts were prepared using various solvents, further these extracts were analyzed against selected 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 and determination MIC by broth dilution method. The extracts showed a broad spectrum of antimicrobial activities by inhibiting the growth of respective microorganism in agar well diffusion assay. Extracts of acetone and ethanol 70% showed highest activity against *Escherichia coli* whereas methanol and choloroform extracts showed significant activity against *Bacillus subtilis.* The present study supports the immense medicinal properties of *Aegle marmelos* bark. The study may be insight for future researches in area of new drug development of herbal origins.

INTRODUCTION: Traditional plants have been used for medicinal purposes conventionally in several parts of world including countries in the Indian sub-continent like India, Pakistan and Bangladesh. In recent years medicinal plants have been more focused in the developing countries because of herbal medicine, these have been reported safe without any adverse effect especially when compared with synthetic drugs. Herbal medicines represent one of the most important fields of traditional medicine system in all over the world.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.9(8).3515-18

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.9(8).3515-18

For promotion of herbal medicine as potential source for new drugs. It is necessary to study medicinal plants in wider prospect which will improve folklore reputation in a more intensified way [1]. *Aegle marmelos* is one of the potent medicinal plant of India. It belongs to Rutaceae family, commonly known as Bael tree, is a deciduous tree, 7 - 8 m in height with trifoliate aromatic leaves and bisexual flowers, indigenous to India, Myanmar and Sri Lanka, often planted in the vicinity of Shiva temple [2, 3]. *A. marmelos* has been found in writings dating back to 800 B.C. It is cultivated throughout India, mainly in temple gardens, because of its status as a sacred tree, also in Pakistan and Northern Malaysia, the drier areas of Java, and to a limited extent on Northern Luzon in the Philippine Islands where it first fruited in 1914.

Owing to its eco-friendly nature, *A. marmelos* is being placed among plant species group called "climate purifiers" which release a higher percentage of oxygen in sunlight as compared to other plants [4]. Although several studies have been conducted to evaluate the potential of different parts of *Aegle marmelos* but still bark is less explored so the study is planned to evaluate the antimicrobial effect of *A. marmelos* bark extract against chosen microorganisms.

MATERIALS AND METHODS:

Collection of Sample: The barks of *A. marmelos* were collected from nearby region of Kangra, Himachal Pradesh.

Chemicals and Solvents: The different solvents such as acetone, ethanol, ethanol 70%, methanol, chloroform, petroleum ether and aqueous *etc*.

Media Used: The media which are used for study are:

1. Nutrient Agar and Broth
2. Potato Dextrose Agar and Broth

Preparation of Extracts: The bark of *A. marmelos* was collected, washed with water and shade dried at room temperature, then crushed and broken using a pestle and mortar and stored. The powder materials (10g each) were mixed with 50 ml of each solvents such as acetone, ethanol, ethanol 70%, methanol, chloroform, petroleum ether and aqueous in conical flask and kept on a rotary shaker for 12 h at 30 ºC. 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 [5].

Microbial Culture: Several organisms 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 procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh. The bacterial isolates were first sub-cultured on a nutrient medium and incubated at 37 °C for 24 h while the fungal isolates were sub-cultured on a potato dextrose medium for 72 h at 25 °C. The MTCC cultures were characterized and identified on the basis of gram staining.

Preparation of Inoculums: Nutrient medium and Potato dextrose medium (agar and broth) were used for growth and diluting the microorganism suspensions. Bacterial strains were grown to exponential phase in Nutrient broth at 37 °C for 18 h and adjusted to a final density of 10^8 CFU/ml by diluting fresh cultures and comparing with McFarland density whereas fungal cultures were aseptically inoculated on Petri dishes containing autoclaved, cooled and settled PDA medium. The Petri dishes were incubated at standard conditions for five days. The colonies from dish were suspended in sterilized PDB and incubated until its turbidity with 0.5 McFarland Standard.

Formulation of Extract: Each extract of 200 mg/ml was dissolved in the solvents separately and mixed with the help of vertex mixture just before antimicrobial testing. Pure solvents were used as control.

Screening for Antimicrobial Activity: For the determination of antimicrobial activity the agar well diffusion method [6, 7] were used. Further Minimum inhibitory concentration assay was done by broth tube dilution method [8].

RESULTS AND DISCUSSION: In the present study, bark extract of *A. marmelos* (Bael) were prepared using different types of solvents (acetone, ethanol, ethanol 70%, methanol, chloroform, petroleum ether and aqueous) for extraction of active ingredient. These extract were used to check antimicrobial potential against different standard pathogens by agar well diffusion assay method.

Antibacterial Activity: The results of antibacterial study showed (**Table 1**) the maximum zone of inhibition by acetone extract against *S. aureus*, *E. coli* and *B. subtilis* (15 mm, 17 mm and 10.2 mm respectively). Chloroform extract also showed significant zone of inhibition against *P. aeruginosa* and *B. subtilis.* Whereas, methanol and 70% ethenol extracts showed significant inhibitory effect against *B. subtilis* and *E. coli* respectively. Rest all extracts did not showed any significant inhibitory effect against selected bacteria.

TABLE 1: ANTIBACTERIAL ACTIVITY OF *A. MARMELOS* BARK EXTRACTS

S. no.	Name of the solvents (Used in extract preparation)	Zone of Inhibition (in mm)			
		E. coli	*B. subtilis*	*S. aureus*	*P. aeruginosa*
1	Acetone	17	10.2	15	-
2	Ethanol	9	5.2	-	2
3	Ethanol 70%	12.2	6	-	2.1
4	Methanol	4	11.5	3.6	-
5	Chloroform	-	12.5	1.2	15.7
6	Petroleum Ether	3.3	-	3.8	-
7	Aqueous	2.6	3	1.6	-

Antifungal Activity of *A. marmelos* against different Pathogens: Antifungal activity of *A. marmelos* extract was determined against two fungi *viz*., *Aspergillus brasiliensis* & *Candida albicans*. **Table 2** shows the results of different bark extracts of the plant. Ethanol extracts of *A. marmelos bark* showed maximum activity against *C. albicans* (16.2 mm) as compared to *A. brasiliensis* (12.4 mm). Methanol and acetone extracts showed significant zone of inhibition against *C. albicans* and *A. brasiliensis*. Whereas ethanol 70% and acetone extract showed significant inhibitory effect against *A. brasiliensis* as compared to *C. albicans*. Rest all extracts did not showed significant inhibition against chosen fungi.

TABLE 2: ANTIFUNGAL ACTIVITY OF *A. MARMELOS* BARK EXTRACTS

S. no.	Name of the solvents (Used in extract preparation)	Zone of Inhibition (in mm)	
		A. brasiliensis	*C. albicans*
1	Acetone	5.3	14
2	Ethanol	12.4	16.2
3	Ethanol 70%	14	6.1
4	Methanol	14.6	13.3
5	Chloroform	-	8
6	Petroleum Ether	-	-
7	Aqueous	4.2	3.7

Minimum Inhibition Concentration (MIC): The bark extract of different solvent, which showed antimicrobial activity in agar well assay were subjected to MIC assay. The antimicrobial MIC studies were carried out by broth dilution method [8, 23]. For this assays of *A. marmelos* bark extracts of different solvents like acetone, ethanol, ethanol 70%, methanol, chloroform were used and they showed both antifungal and antibacterial activities at different concentration 1 - 5 mg/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 results are shown in **Table 3**.

TABLE 3: MINIMUM INHIBITION CONCENTRATION

S. no.	Name of Microorganisms	MIC value of different solvent based extract (mg/ml)				
		Acetone	Ethanol	Ethanol 70%	Methanol	Chloroform
1	*E. coli*	5	-	4	-	-
2	*B. subtilus*	-	-	-	5	5
3	*S. saureus*	4	-	-	-	-
4	*P. aurginosa*	-	-	-	-	5
5	*A. brasiliensis*	-	5	4	4	-
6	*C. albicans*	4	5	-	5	5

DISCUSSION: In this study *A. marmelos* bark extract in seven different solvents were prepared. Total 7 extracts were tested against 4 different bacterial pathogens and 2 fungal pathogens. Antimicrobial assay of all these extracts were performed for the detection of zone of inhibition. Out of 7 samples, 5 were reported for antibacterial activity (two showed strong response, 02 moderate and 01 mild antibacterial potential) and 04 were reported for antifungal activity (02 showed strong, 02 moderate response antifungal potential). *A. marmelos* is considered as potent medicinal plant due to the high phytochemical content. It has been used from ancient time for the treatment of various infectious diseases and been extensible reported to suppress the broad range of pathogenic flowra. Several *in-vitro* studies proved the medicinal potential of *A. marmelos* extracts towards the pathogenic microorganisms including bacteria and fungi. The different part of the plants has shown potent antimicrobial activity in various studies [9, 10, 11, 12].

Although no separate report is available on bark extracts but various crude extracts of *A. marmelos* such as leaves, roots and fruits have been reported to be active against many bacterial strains [13, 14, 15, 16, 17]. Literature indicates that *A. marmelos* contain several phytoconstituents mainly marmenol, marmin, marmelosin, marmelide, psoralen, alloimperatorin, rutaretin, scopoletin, aegelin, marmelin, fagarine, anhydromarmelin, limonene, â-phellandrene, betulinic acid, marmesin, imperatorin, marmelosin, luvangentin and auroptene [18].

Yadav *et al*. have determined the contents of tannin (0.985%) and riboflavin (0.005%) [19, 20]. The presence of these phytochemicals may be the reason for antimicrobial activity shown in the study.

CONCLUSION: Now day modern medicine system is also based in ethno medicine. In this traditional system, indigenous flora is used to treat human diseases or to improve specific aspects of the body conditions. Ancient plants are rich source of novel compounds that forms the ingredients in traditional as well as modern systems of medicine, nutraceuticals, food supplements and pharmaceutical intermediates *etc*. These plants have bioactive principles, which could be used as precursor of synthetic drugs. Today a large number of advanced drugs are still derived from natural sources. The approach of green medicines is healthier and more harmless or safer than synthetic ones. In this study results have indicated high medicinal potential of *Aegle marmelos*, which may be due to the presence of different bioactive compounds. This study may provide the insight for the use of bioactive compounds of *Aegle marmelos* as precursor for semisynthetic/synthetic medicine.

ACKNOWLEDGEMENT: We are thankful to the management of Suresh Gyan Vihar University, Jaipur and Amity University Madhya Pradesh, Gwalior for their valuable support.

CONFLICT OF INTEREST: Nil

REFERENCES:

1. Shaikh AM, Shrivastava B, Apte KG and Navale SD: Medicinal plants as potential source of anticancer agents: A Review. Journal of Pharmacognosy and Phyto-chemistry 2016; 5(2): 291-295.
2. CSIR: The wealth of India. National Institute of Science communication and Information Resources 1985; I(A): 86.
3. Chopra RN, Chopra IC, Handa KI and Kapur LD: Chopra's Indigenous Drugs of India. Academic Press; New Delhi 1982; 342-345.
4. Malviya R, Kumar A, Singh A and Kulkarni GT: Pharmacological screening, Ayurvedic values and commercial utility of *Aegle marmelos.* Int. J. Drug Dev. and Res 2012; 4(1): 28-37.
5. Shrivastava V, Bhardwaj U, Sharma V, Mahajan N and Sharma V: Antimicrobial activities of Asafoetida resin extracts (A Potential Indian Spice). Journal of Pharmacy Research 2012; 5(10): 5022-5024.
6. Perez C, Paul M and Bazerque P: An antibiotic assay by the agar well diffusion method. Acta Biol. Med. Exp 1990; 15: 113-115.
7. Nair R and Chanda S: Antibacterial activity of *Punica granatum* in different solvents. Ind. J. Pharm. Sci 2005; 67: 239-243.
8. Balouiri B, Sadiki M and Ibnsouda SK: Methods for *in-vitro* evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis 2016; 6(2): 71-79.
9. Sasidharan VK, Krishnakumar T and Manjula CB: Antimicrobial activity of nine common plants in Kerala, India, PJS 1998; 127(1): 59-67.
10. Sudharameshwari K and Radhika J: Antibacterial screening *of Aegle marmelos*, *Lawsonia inermis* and *Albizzia libbeck.* Afr J. Traditional, Complementary and Alternative Medicines 2007; 4: 205-210.
11. Ramya S, Govindaraji V, Kannan NK and Jayakumararaj R: *In-vitro* evaluation of antibacterial activity using crude extracts of *Catharanthus roseus* Linn. (G.) Don. Ethnobotanical Leaflets 2008; 12: 1013-1018.
12. Ramya S, Kalayansundaram M, Kalaivani T and Jayakumararaj R: (Phytochemical screening and antibacterial activity of leaf extracts of *Pterocarpus marsupium* Roxb. (Fabaceae)). Ethnobotanical Leaflets 2008; 12: 1051-1056.
13. Rojas R, Bustamante B, Bauer J, Fernández I, Albán J and Lock O: (Antimicrobial activity of selected Peruvian medicinal plants). J Ethnopharmacol 2003; 88(2):199-204.
14. Duraipandiyan V, Ayyanar M and Ignacimuthu S: Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. BMC Complement Altern Med 2006; 6(1): 35.
15. Parekh J and Chanda SV: *In-vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal plants. Turk J Biol 2007; 31(1): 53-58.
16. Dhaiya R, Tomar RS and Shrivastava V: Evaluation of Antimicrobial Potential of *Aegle Marmelos* Fruit Extract against Selected Microorganisms. J. Pharm. Sci. and Res 2015; 7(9): 681-684.
17. Dhaiya R, Tomar RS and Shrivastava V: Evaluation of Antimicrobial Potential of *Aegle marmelos* root extract against selected microorganisms. International Journal of Pharma and Bio Sciences 2016; 7(3): 1175-1178.
18. Bansal Y and Bansal G: Analytical methods for standardization of *Aegle marmelos*: A review. J Pharm Educ Res 2011; 2(2): 37- 44.
19. Yadav N, Tyagi G, Jangir DK and Mehrotra R: Rapid determination of polyphenol, Vitamins, organic acids and sugars in *Aegle marmelos* using reverse phase-high performance liquid chromatography. J Pharm Res 2011; 4(3): 717-719.
20. Prakash D, Upadhyay G, Pushpangadan P and Gupta C: Antioxidant and free radical scavenging activities of some fruits. J Complement Integr Med 2011; 8(1).

How to cite this article:
Dahiya R, Choudhary RC, Tomar RS and Shrivastava V: Antimicrobial susceptibility test analysis of *Aegle marmelos* bark extracts. Int J Pharm Sci & Res 2018; 9(8): 3515-18. doi: 10.13040/IJPSR.0975-8232.9(8).3515-18.