Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 3, 2015

Short Communication

ANTIBACTERIAL ACTIVITY OF *AEGLE MARMELOS* (L) CORREA

SURENDER S. YADAV, KULDEEP DAHIYA, SHOWKAT A. GANIE, SURENDER K. GULIA

Department of Botany, Maharshi Dayanand University, Rohtak (Haryana), India.
Email: shahidshowkat90@gmail.com

Received: 02 Oct 2014 Revised and Accepted: 24 Oct 2014

ABSTRACT

Objective: To investigate the antibacterial property of *Aegle marmelos* (L.) Correa leaf extracts.

Methods: The antibacterial property of *A. marmelos* crude methanolic and chloroform leaf extracts was evaluated through agar disc diffusion method. Two Gram-positive (*Bacillus subtilis*, *Staphylococcus aureus)* and three Gram-negative bacteria (*Pseudomonas aeruginosa*, *Klebsiella pneumonia, Escherichia coli)* were used for the study.

Results: The zone of inhibitions obtained from the agar diffusion disc method showed that methanolic and chloroform leaf extracts exhibited maximum (15 mm) activity against *P. aeruginosa* and 18 mm against *S. aureus,* respectively. Both extracts were partially active against *B. subtilis and K. pneumonia.* The activity was maximum at higher concentration (100mg/ml) and decreased gradually with the decrease in the concentration of the extracts against all the test pathogens.

Conclusions: From this study, it can be concluded that *A. marmelos* extracts may be a potential antibacterial agent against both Gram-positive and Gram-negative bacteria. The antibacterial property may be attributed to different phytoconstituents present in the crude methanolic and chloroform extracts.

Keywords*: Aegle marmelos,* Antibacterial property, Disc diffusion, Traditional medicine.

Sky-high prices of medicines, side effects of chemical drugs and costly allopathic treatment have compelled men to consider an alternative source of medicines. Besides, the indiscriminate use of antibiotics has led to many bacterial pathogens rapidly becoming resistant to a number of originally discovered antimicrobial drugs. This is indeed very important because *Candida albicans, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* are some of the important human pathogens that have developed resistance to antimicrobials [1]. Thus, there is an ongoing search for new antibiotics, and medicinal plants may offer a new source of antimicrobial agents. This includes medicinal plant therapy, Ayurveda, Unani, Siddha, Acupuncture, Acupressure and the like. It is estimated that nearly 80% of the medical care are provided by traditional systems of medicine and major part of these therapies involve the use of plant extracts or their active principles [2]. Plants have been used as a natural source of medicine since thousands of years throughout the globe. Angiospermous plant species are treasure house of effective chemicals. Over the last 20 years, a large number of plant species have been evaluated for antimicrobial activity. Indian flora offers great possibilities for the discovery of new compounds with important medicinal applications in combating infection and strengthening the immune system. The antimicrobial compounds found in plants may prevent bacterial infections by different mechanisms than the commercial antibiotics and therefore may have clinical value in treating resistant microorganisms [3].

Aegle marmelos is a native plant of India, locally known as Bael or Bael Patther. It is a subtropical plant and grows up to an altitude of 1,200 m from sea level. It is widely distributed plant and found in India, Ceylon, China, Nepal, Sri Lanka, Myanmar, Pakistan, Bangladesh, Nepal, Vietnam, Laos, Cambodia, Thailand, Indonesia, Malaysia, Tibet, Sri Lanka, Java, Philippines and Fiji. In India, it is found in Sub-Himalayan tracts from Jhelum eastwards to West Bengal, in central and south India. It is found almost in all the states of India [4]. It is found growing in Uttar Pradesh, Bihar, Chattisgarh, Uttaranchal, Jharkhand, Madhya Pradesh, Deccan Plateau and along the East Coast [5]. The plant is generally grown as a temple garden plant and its leaves are used to pray to Lord Shiva. It belongs to the Rutaceae family and commonly known as wood apple. It is an important medicinal plant with several ethnomedicinal applications in traditional and folk medicine systems. Traditionally, this plant is used in the treatment of diarrhea and dysentery. Leaves of this plant are used to cause infertility/abortion. Recently, the plant is screened for its curative properties by scientific techniques. Based on the therapeutic potential of the plant species and scanty work on its pharmacological activities, the present study was designed to evaluate its antibacterial activity against the most common human pathogens.

The leaves of *Aegle marmelos* were collected from M. D. U campus, near the Herbal garden, Rohtak during 2014. The plant material was identified by the Department of Botany, Maharshi Dayanand University, Rohtak (Haryana) with available literature in the laboratory. The leaves were thoroughly washed, dried in shade and ground into powdered form for further analysis.

Extract was prepared by Soxhlet extraction method [6]. A thimble was prepared by using 0.5 mm Whatman No. 1 filter paper. About 100 gm of pulverized material was uniformly packed into a thimble and run in Soxhlet extractor. It was extracted with methanol and chloroform for the period of about 48 hours or 22 cycles or till the solvent in the siphon tube of extractor became colourless. Soxhlet apparatus comprises of extractor, condenser and a round bottom flask. It is heated on the heating mantle and evaporated solvent goes to extractor. Here, it is cooled by the water moving in the condenser and then solvent moves back to the round bottom flask with soluble bioactive components. After that solvent was evaporated to get the crude plant extracts. Extract was kept in a refrigerator at 4^0C for antibacterial activity.

Different bacterial strains were used for testing antibacterial activity included *Pseudomonas aeruginosa* (MTCC-424), *Klebsiella pneumonia* (MTCC-109), *Escherichia coli* (MTCC-1652), *Staphylococcus aureus* (MTCC-3160) and *Bacillus subtilis* (MTCC-2393). The test organisms used in the study were collected from MTCC (Microbial Type Culture Collection and Gene Bank), IMTECH (Institute of Microbial Technology), Chandigarh, India. The bacteria were maintained on nutrient agar slants, sub-cultured periodically and preserved at 4°C prior to use.

In vitro antibacterial activity of *Aegle marmelos* leaf extracts was tested by disc diffusion method [7].

For susceptibility testing, methanolic and chloroform leaf extracts were dissolved in suitable solvent (DMSO) separately. Different concentrations (100, 50, 25, 12.5, 6.25 and 3.25mg/ml) of extracts were prepared by serial dilution method. Empty sterile discs having diameter of 6 mm were impregnated with 25µl of each serial dilution of extract solution. These impregnated discs containing different concentrations of extract were incubated for 15 minutes for proper diffusion of extract. On the other hand, aseptically packed colonies from the pure culture were mixed (emulsify) in nutrient broth (7µl/ml broth). This broth was inoculated on the entire surface of the nutrient agar plate with the help of a cotton swab moistened with this culture. Then, wait for about 5-6 minutes after inoculation to allow the liquid culture to soak into the agar surface. With the help of sterile forceps, discs containing herbal extracts were placed on the inoculated surface of the agar plate. These plates were incubated for 24 hours at 37°C and the zone of inhibition was measured in millimeters and taken as antibacterial activity. The sensitivity was done in triplicate.

The findings of the present study clearly indicate that both the leaf extracts inhibited the growth of test pathogens in more or less extent. However, the methanolic leaf extract showed better antibacterial activity**.** Maximum activity (15 mm) was observed against *Pseudomonas aeruginosa* at 100 mg/ml concentration followed in descending order by *E. coli* (14 mm), *Bacillus subtilis* & *Staphylococcus aureus* (9 mm) and *Klebsiella pneumoniae* (7.4 mm) (Table 1 & Fig. 1). The activity was maximum at 100mg/ml concentration and decreased gradually against all the test pathogens. Therefore the methanolic leaf extract showed dose dependent antibacterial activity.

Table 1: Antibacterial activity of *Aegle marmelos* methanolic leaf extract

Zone of inhibition (mm)							
Test organism	100	50	25	12.5	6.25	3.125	C
P. aeruginosa	15.0±1.6	13.5±2	12.0±1	9. 2±0.9	6.3±0.4	6.0±1.0	23.0±1
B. subtilis	9.3±1.6	8.2±0.9	8.3±1	6.5±1.1	6.2±0.4	6.2±0.9	24.0±1.3
E. coli	14.0±2.4	13.0±2.9	12.0±1.3	10.0±0.6	6.5±0.9	6.3±0.6	15.0±1.0
K. pneumonia	7.4±0.4	6.4±1.1	6.3±1.0	6.3±0.7	6.2±0.6	6.0±1.0	29.0±1.0
S. aureus	9.0±0.9	8.2±0.9	8.3±0.7	6.5±0.7	6.3±0.9	6.2±1.0	30.0±1.6

Values are given in Mean ± SD (n=3); C: Control (Cefotaxime)

Table 2: Antibacterial activity of *Aegle marmelos* chloroform leaf extract.

	Zone of inhibition (mm)						
Test organism	100	50	25	12.5	6.25	3.125	C
P. aeruginosa	8.0±1.4	-	-	-	-	-	13.0±1.8
B. subtilis	12.2±1.0	-	-	-	-	-	21.0±1.6
K. pneumonia	17.0±1.7	-	-	-	-	-	29.0±1.4
S. aureus	18.3±1.9	15.0±0.9	-	-	-	-	30.0±1.3

Values are given in Mean ± SD (n=3); C: Control (Cefotaxime)

Chloroform leaf extracts exhibited maximum activity (18 mm) against *S. aureus* at 100 mg/ml concentration, followed in descending order by 17 mm in *K. pneumonia*, 12 mm in *B. s subtilis* and 8 mm in *P. aeruginosa* (table 2 and fig. 1). Chloroform leaf extract was found inactive against *E. coli. S. aureus* was the most sensitive test organism to the chloroform leaf extract of *A. marmelos.* The CLE was active against pathogens at higher concentrations only.

The findings of the present study reveal that *Pseudomonas aeruginosa* and E. coli were comparatively more sensitive test organisms to methanolic leaf extract of *A. marmelos*. Both the methanolic leaf extract (at higher concentration) and the commercial antibiotic, cefotaxime were found to be equally effective against *P. aeruginosa.* Our results are consistent with the reports of [8-9]. They also found the methanolic extract of *A. marmelos* more active to *P. aeruginosa* and *E. coli*.

The better antibacterial activity in the methanolic extract may be due to the presence of more phytochemicals, extracted in methanol from *A. marmelos* leaf. Earlier claims by Ajaiyeoba [10] also indicated that polar solvent extracts are more active than extract of non-polar solvents such as chloroform.

Plants have a wide range of compound *viz.* alkaloids, steroids, saponins, tannins, phenols, flavonoids, quinines, terpenes, terpenoids, glycosides, sugars, amino acids etc. which are responsible for bioactivity. These medicinally bioactive components exert antimicrobial action through distinct mechanisms. Tannins cause inhibition in the cell wall synthesis by forming irreversible complexes with proline rich protein [11]. The saponins have the ability to cause leakage of proteins and certain enzymes from the cell [12]. Terpenoids are responsible for dissolution of the cell wall of the microorganism by weakening the membranous tissue [13]. Flavonoids which have been found to be effective antimicrobial substances against a wide array of microorganisms *in vitro* are known to be synthesized in response to microbial infection by plants. They have the ability to bind with extracellular and soluble proteins and complexes with bacterial cell walls. Steroids are renowned for their antibacterial activity specifically associated with membrane lipids and cause leakage from liposomes [14].

It is clear from the present study that chloroform extract was most effective against *Staphylococcus aureus* which is in consistent with the results obtained by Kothari *et al*. [15] in *A. marmelos. S. aureus* was found to be most sensitive test organism to chloroform leaf extract. It may be due to its Gram-positive nature. Generally Gram-positive bacteria are more susceptible to commercial antibiotics, crude extracts and isolated compounds from natural sources, which may be related to cell wall structure. According to Tortora *et al*. [16] the cell wall of Gram-negative bacteria acts as a barrier to a number of substances, including antibiotics.

In the present study it was also found that the same plant extract (methanolic/chloroform) was effective against both Gram-positive as well as Gram- negative bacteria. According to Kostova and Dinchev [17] it may be explained by the presence of a wide spectrum of bactericidal substances, or the action of toxins produced by the plant.

Both the plant extracts worked in a dose dependent manner, as the concentration of the extract was decreased the activity also decreased. This is due to susceptibility of the pathogen towards concentration of the extracts, after which the extract damages that microbe which is not tolerable for it [18].

CONCLUSION

Based on the findings, it may be concluded that greater activity resides in methanolic leaf extract compared to chloroform. Methanolic leaf extract has a maximum inhibitory effect against *E. coli* and *Psedumonas*

aeruginosa. Both methanolic and chloroform extracts showed dose dependent antibacterial activity. Antibacterial activity is both pathogen and solvent dependent. The results of this study are very encouraging and indicate that it should be studied more extensively to explore its full phytochemical profile. The use of plant extracts with known antimicrobial properties can be of critical significance in modern therapy. However, further study on chemical constituents and their mechanisms in exhibiting certain biological activities is needed to understand the complex pharmacological effects of plant species.

ACKNOWLEDGEMENT

Financial assistance from the University Grants Commission (UGC), New Delhi and Haryana State Council for Science and Technology (HSCST), Panchkula is thankfully acknowledged.

CONFLICT OF INTEREST

There is no conflict of interest

REFERENCES

1. Barbour EK, Al-Sharif M, Sagherian VK, Habre AN, Talhouk RS, Talhouk SN. Screening of selected indigenous plants of Lebanon for antimicrobial activity. J Ethnopharmacol 2004;93:1-7.
2. Ramesh A, Hyma B. Traditional indian medicine in practice in an Indian metropolitan city. In: Geographical aspects of health and diseases in India. New Delhi: Akhtar and Learmonth Concept Publishing Co; 1985.
3. Eloff JN. Antibacterial activity of 27 Southern African members of the combretaceae. South Afr J Sci 1999;95:148-52.
4. Dhankhar S, Ruhil S, Balhara M, Dhankhar S, Chhillar AK. *Aegle marmelos* (Linn.) Correa: a potential source of phytomedicine. J Med Plants Res 2011;5(9):1497-1507.
5. Singh S. Standardization of processing technology of bael (*Aegle marmelos* Correa). Thesis, Doctor of philosophy in horticulture, College of Agriculture CCS, HAU, Hisar; 2000.
6. Okeke MI, Iroegbu CU, Eze EN, Okoli AS, Esimone CO. Evaluation of extracts of the root of *Londolphia owerrience* for antibacterial activity. J Ethnopharmacol 2001;78:119-27.
7. Mukherjee PK, Giri SN, Pal M, Saha BP. Antibacterial efficiency of *Nelumbo nucifera* (Nympheaceae) rhizome extract. Ind Drugs 1996;32:274-6.
8. Venkatesan D, Karrunakarn CM, Selva SK, Palani PTS. Identification of phytochemical constituents of *Aegle marmelos* responsible for antimicrobial activity against selected pathogenic organisms. Ethnobot Leaflets 2009;13:1362-72.
9. Mujeeb F, Bajpai P, Pathak N. Phytochemical evaluation, antimicrobial activity and determination of bioactive components from leaves of *Aegle marmelos.* Bio Med Res Int 2014;1-11.
10. Ajaiyeoba E. Phytochemical and antibacterial properties of *Parkia biglobosa* and *Parkia bicolor* leaf extracts. Afr J Biomed Res 2002;5(1):125-9.
11. Mamtha B, Kavitha K, Srinivasan KK, Shivananda PG. An *in vitro* study of the effect of *Centella asiatica* (Indian pennywort) on enteric pathogens. Ind J Pharmacol 2004;36(1):41.
12. Zablotowicz RM, Hoagland RE, Wagner SC. Effect of saponins on the growth and activity of rhizosphere bacteria. Adv Exp Med Biol 1996;405:83-95.
13. Hemandez NE, Tereschuk ML, Abdala LR. Antimicrobial activity of flavonoids of medicinal plants from Taf´ı del Valle (*Tucum´an Argentina*). J Ethnopharmacol 2000;73(1-2):317-22.
14. Epand RF, Savage PB, Epand RM. Bacterial lipid composition and the antimicrobial efficacy of cationic steroid compounds (Ceragenins). Biochim Biophys Acta 2007;1768(10):2500-09.
15. Kothari S, Mishra V, Bharat S, Tonpay SD. Antimicrobial activity and phytochemical screening of serial extracts from leaves of *Aegle marmelos* (linn.). Acta Pol Pharm Drug Res 2011;68(5):687-92.
16. Tortora GJ, Funke BR, Case CL. *Microbiology: An Introduction*. Benjamin Cummings, San Francisco; 2001. p. 88.
17. Kostova I, Dinchev D. Saponins in *Tribulus terrestris* –chemistry and bioactivity. Phytochem Rev 2005;4:111-37.
18. Ordonez RM, Ordonez AA, Sayago JE, Moreno MIN, Isla MI. Antimicrobial activity of glycosidase inhibitory protein isolated from *Cyphomandra betacea* Sendt. fruit. Peptides 2006;27:1187-91.