Int J Pharma Res Health Sci. 2016; 4 (4): 1315-1319
DOI:10.21276/ijprhs.2016.04.13
Gawade AE *et al.* CODEN (USA)-IJPRUR, e-ISSN: 2348-6465

PHS Scientific House
PHARMAHEALTHSCIENCES

International Journal of Pharma Research and Health Sciences

Available online at www.pharmahealthsciences.net

Original Article

Preliminary Phytochemical Analysis of Seeds and Leaves of *Aegle Marmelos* Extracts and In-Vitro Assessment of their Antibacterial Activity

Borse Pankajkumar Shantaram [1], Bale Sudhir Ramachandra [2], Gawade Avinash Eknath [3, *]

[1] Department of Microbiology, B.P.H.E Society's, Ahmednagar College, Ahmednagar, Maharashtra, India.
[2] Department of Botany, B.P.H.E Society's, Ahmednagar College, Ahmednagar, Maharashtra, India.
[3] Department of Microbiology, B.P.H.E Society's, Ahmednagar College, Ahmednagar, Maharashtra, India.

ARTICLE INFO

Received: 01 Aug 2016
Accepted: 27 Aug 2016

ABSTRACT

Aqueous, methanol, chloroform and petroleum ether extracts of leaves and seed powder of *Aegle marmelos* obtained through soxhlet extraction process were qualitatively tested for presence of active principles and in-vitro antibacterial activity. Terpenoids were found to be present in all leaf extracts while tannins were absent. In all seed extracts, flavonoids and terpenoids were absent while phenols and alkaloids were present except in chloroform. Antibacterial activity was tested by agar well diffusion method. All leaf extracts, concentrated and diluted (1:3) showed substantial antibacterial activity against *Escherichia coli, Staphylococcus aureus, Salmonella typhi and Pseudomonas aeruginosa. Pseudomonas aeruginosa* was not inhibited by diluted chloroform extract. Seed extracts also showed antibacterial properties in concentrated as well as diluted form. Aqueous extract and chloroform extract in diluted form did not showed any action on *Pseudomonas aeruginosa.* Potential applications of *Aegle marmelos* in treatment of skin infections are discussed.

Key Words: *Aegle marmelos*, soxhlet extraction, active principles, antibacterial.

1. INTRODUCTION

Aegle marmelos (L.), a native plant of India, belonging to family Rutaceae, commonly known as wood apple is one of the important plant in ayurvedic and traditional folk medicine. It is grown as garden plant found throughout India from sub-Himalayan forest to Burma. Its fruits, roots, barks, leaves, rind and flowers are mainly used for medicinal purpose[1]. Along with ayurvedic medicinal system *Aegle*

Corresponding author *
Gawade Avinash Eknath,
Department of Microbiology,
B.P.H.E Society's, Ahmednagar College,
Ahmednagar 414001, Maharashtra, India
E-mail: vishwatmak36@gmail.com

marmelos has also been described by traditional folk and Chinese medicine system[2]. In recent years, *Aegle marmelos* has been extensively investigated, its pharmacologically active components isolated, purified, characterized and shown to possess number of medicinal properties viz, hepatoprotective, anticancer, antiulcer, radioprotective, analgesic, antidiabetic, antioxidant, anti-inflammatory, antifertility etc [3-10]. Apart from pharmacological activities of *Aegle marmelos* described above, ayurveda has described its main use in treatment of gastrointestinal disorders such as diarrhea, and dysentery particularly fruits being of medical interest[11]. Numbers of authors have investigated its antibacterial and antifungal activity [12- 14.]

Present study was undertaken to qualitatively assay the presence of active principles and antibacterial activity of *Aegle marmelos* against the representative bacterial pathogens that causes diarrhea (*Escherichia coli* and *Salmonella typhimurium*) and skin infections (*Pseudomonas aeruginosa* and *Staphylococcus aureus*).

Determination of antibacterial activity of different seed extracts of *Aegle marmelos* and discussion on role of active components in treatment of skin infections are some of unique features of present study.

2. MATERIALS AND METHODS

2.1 Plant material:

Seeds and leaves of *Aegle marmelos* selected for present study were obtained from botanical garden of college of horticulture, Shahada (District-Dhule). Authentication of plant material was done at department of Botany, Ahmednagar College, Ahmednagar. Upon bringing to laboratory plant material was thoroughly washed with distilled water and air dried

2.2 Preparation of extracts:

Air dried material (leaves and seeds) were ground into fine powder using ball mill and stored at dry place until use. Active principles were extracted by warm extraction using soxhlet apparatus. For this, 50 grams of dry powder was packed in Whatman filter paper no.1 and subjected to extraction process using solvent for 25-30 cycles. Extracts were prepared in methanol, chloroform and petroleum ether. Aqueous extract was prepared by dissolving the plant material in warm water for 24 hrs (with constant stirring) followed by filtration. Extracts obtained in different solvents were concentrated, dried, dissolved in 20 ml of dimethyl sulfoxide (DMSO) and stored in refrigerator at 4^{o}C until further use.

2.3 Preliminary phytochemical analysis:

Active principles were qualitatively determined followed by the methods of Trease and Evans, 1989[15].

2.3.1 Alkaloids:

Dragendorff test: To 2-3 ml of filtrate, few drops of Dragendorff's reagent was added and observed for the formation of brown precipitate.

2.3.2 Tannins:

$FeCl_3$ test:To 2-3 ml of extract, 5% of $FeCl_3$ solution was added and observed for deep blue black color formation.

2.3.3 Flavonoids:

Sulphuric acid test: To 2-3 ml of extract 66 % and 75 % sulphuric acid was added and observed for formation of yellow colored precipitate.

2.3.4 Terpenoids:

Salkowaski's test: To 2-3 ml of extract, 2 ml of chloroform and 3 ml of H_2SO_4 was added and observed for formation of reddish brown color.

2.3.5 Phenolic compounds:

Folin-C test: To 2-3 ml of extract, 2 ml of Folin-C reagent along with aqueous sodium bicarbonate solution was added and observed for gray-blue color.

2.4 Microorganisms used:

Throughout studies, four bacterial strains *Escherichia coli* (ATCC 8739), *Salmonella typhimurium* (ATCC 13311), *Staphylococcus aureus* (ATCC 6538) and *Pseudomonas aeruginosa* (ATCC 9027) were used. Bacterial strains were maintained on Cysteine tryptone medium agar slants.

2.5 In-vitro antibacterial activity:

Inoculum for each test bacterium was prepared by growing it overnight in soybean casein digest (SCD) broth. Optical density (O.D) of the culture was adjusted to obtain 10^8 cells per ml of broth. Antibacterial activity was performed by disc diffusion method according to Cavanagh 1972 [16] and Hewitt W, 2004 [17]. For this, Mueller Hinton agar medium was seeded with 1 ml of test bacterium and plates were poured and solidified. Disc (diameter 8mm, made from whatman The inhibition zone was measured using zone reader (Hi-media, India) in nearest of millimeter as mean of three independent measurements and expressed as (mean ± standard deviation). paper no. 1) loaded with 20 µl of extract were placed on seeded agar, kept for pre-diffusion in refrigerator and incubated at 37^{o}C for 24 hrs. Disc loaded with 20 µl DMSO was used as control. All experiments were carried out in triplicate. Same procedure was repeated for two fold and three fold diluted extracts. Antibacterial activity was measured in terms of zone of inhibition formed around the disc after incubation. The inhibition zone was measured using zone reader (Hi-media, India) in nearest of millimeter as mean of three independent measurements and expressed as (mean ± standard deviation).

3. RESULTS AND DISCUSSION

Alkaloids were present in all fractions of leaf and seed extract except aqueous and chloroform respectively. Tannins were absent in all leaf extracts. Results of analysis of active principles are summarized in table 1.

The results of assay were expressed in terms of standard deviation of mean zone of inhibition. The results of antibacterial activity of leaves of *Aegle marmelos* are summarized in table 2, while comparative activities of concentrated extracts are depicted in figure 1. In case of leaf extracts, all extracts tested inhibited the growth of test

bacteria except that of diluted (1:3) chloroform extract. Petroleum ether extract showed maximum inhibitory activity on all microorganisms while least was seen in chloroform (fig 3 to 6). All seed extracts exhibited antibacterial activity except diluted chloroform and aqueous extract. Among all seed extracts petroleum ether showed maximum inhibition while that of in chloroform extract was minimum (fig. 7 to 10). The results are shown in table 3, while comparative activity is depicted in figure 2.

Aegle marmelos has been used in ayurvedic as well as other traditional folk system such as Chinese and Tibetan system. In ayurveda, its principle use has been for the treatment of common disorders like dysentery and diarrhea. Diarrhea has been one of the most common diseases globally, especially in developing countries, infants and children being most susceptible groups. Mortality rate of diarrhea is quite high, causing 1.5 million deaths of children globally[18].Bacterial pathogens like *E.coli*, *S.typhi* and *Shigella dysentriae* are common cause of diarrhea and dysentery.

Present study carried out to assess invitro potential of *Aegle marmelos* leaves and seed extracts against *E.coli* and *S.typhi* proves its traditional use in Indian medicinal system. Antibacterial activity of *Aegle marmelos* has been carried out by number of investigator using different test microorganisms. Brijesh et.al. 2009[19] studied antidiarrheal activity of *Aegle marmelos* unripe fruit and reported antibacterial, antigiardial and antirotaviral nature of crude extracts. Same study, using different systems extensively carried out antidiarrheal assays validating the established use *Aegle marmelos* as therapeutic agent.

Significant inhibition of growth of *S. aureus* indicates that leaf and seed extracts could be used in treatment of skin infections. *S.aureus* is known to cause the common skin and pyogenic infection in humans[20]. In our study, petroleum ether extract of both leaves and seeds showed maximum activity against all tested microorganisms. It shows that active antibacterial phytochemical constituents are extracted in petroleum ether. Alkaloids such as aegeline, skimmianine, marmeline which are soluble in petroleum ether are known to inhibit bacterial growth. In routine practice, the ayurvedic medicines from *Aegle marmelos* are formulated in aqueous base and alcoholic base[1]. For instance, it is integral component of *Dasmula*, a decoction used for enema therapy.[21] Presence of significant activity in petroleum ether extracts against *P.aeruginosa* and *S.aureus* indicates that, these compounds could be explored for their use in topical agents to treat skin infections. Further, many studies aimed at testing toxicity and safety of *Aegle marmelos* have almost proved that it is safe for applications in human medicine[22-23].

4. CONCLUSION

Present study undertaken to assess invitro antibacterial activity of leaves and seeds of *Aegle marmelos*, revealed that high concentration of antimicrobial compounds are present in different extracts of plant material. Significant inhibition at three fold concentration by almost all extracts indicates that that IC_{50} value of the crude preparation is low (data not shown), the property which would make it possible to use these purified compounds as lead candidates in the process of drug discovery. Extracts in different solvents could be used for purification of individual active component, determining their IC_{50} values and explore them to make formulations that could be used as therapeutic agents.

Table 1: Antibacterial activity of leaf extracts of *Aegle marmelos*.

Micro Organism	Zone of Inhibition of different extracts (mm)												
	Aqueous			Methanol			Chloroform			Petroleum ether			DMSO
	1:1	1:2	1:3	1:1	1:2	1:3	1:1	1:2	1:3	1:1	1:2	1:3	--
E.coli	14	10	08	24	22	19	14	12	10	24	22	18	--
S.aureus	17	13	11	25	23	18	16	13	08	19	16	15	--
S.typhi	19	12	10	20	15	10	16	14	12	24	21	19	--
P.aeruginosa	18	13	11	16	15	12	16	11	--	27	23	19	--

Table 2: Antibacterial activity of seed extracts of *Aegle marmelos*.

Micro Organism	Zone of Inhibition of different extracts (mm)												
	Aqueous			Methanol			Chloroform			Petroleum ether			DMSO
	1:1	1:2	1:3	1:1	1:2	1:3	1:1	1:2	1:3	1:1	1:2	1:3	--
E.coli	18	12	09	22	16	14	16	14	08	27	24	18	--
S.aureus	18	13	11	28	22	20	18	13	10	27	22	17	--
S.typhi	16	14	10	25	16	15	16	15	13	23	19	15	--
P.aeruginosa	11	09	--	25	22	18	15	10	--	18	15	13	--

Table 3: Phytochemical analysis of active constituents of *Aegle marmelos*

	Aqueous		Methanol		Chloroform		Petroleum ether	
	Leaves	Seeds	Leaves	Seeds	Leaves	Seeds	Leaves	Seeds
Alkaloids	-	+	+	+	+	-	+	+
Flavonoids	-	-	-	-	-	-	-	-
Terpenoids	+	-	+	-	+	-	+	-
Phenol	-	+	+	+	-	-	+	+
Tannins	-	+	-	-	-	+	-	+

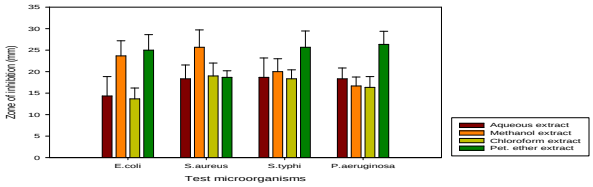

Fig 1: Comparative antibacterial activity of different leaf extracts (1:1 concentration) of *Aegle marmelos*

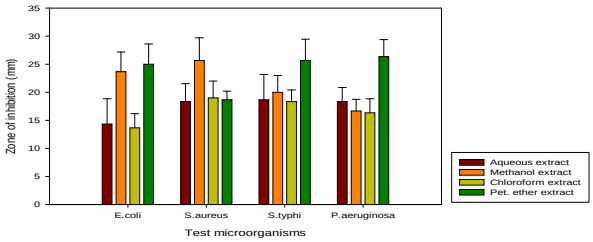

Fig 2: Comparative antibacterial activity of different seed extracts (1:1 concentration) of *Aegle marmelos*

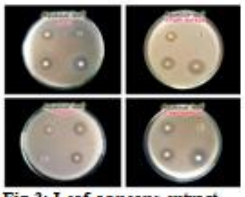
Fig 3: Leaf aqueous extract.

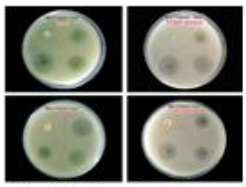
Fig 4: Leaf methanol extract

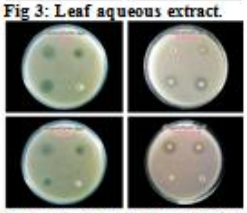
Fig 5: Leaf chloroform extract

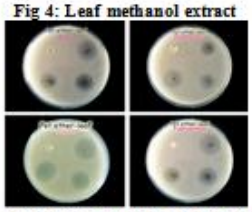
Fig 6: Leaf petroleum ether extract

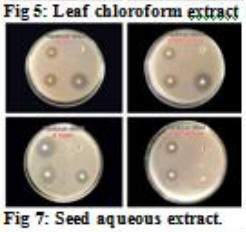
Fig 7: Seed aqueous extract.

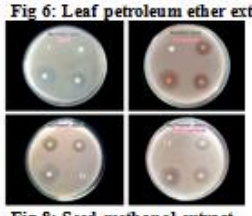
Fig 8: Seed methanol extract.

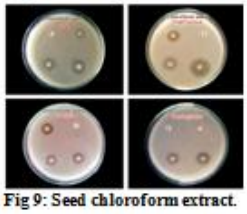
Fig 9: Seed chloroform extract.

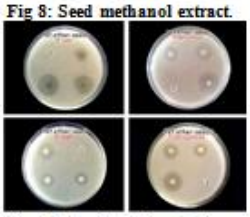
Fig 10: Seed petroleum ether extract.

5. ACKNOWLEDGEMENT

Authors acknowledge The Principal, B.P.H.E Society's, Ahmednagar College, Ahmednagar and The Head, Department of Microbiology, Ahmednagar College for providing facility to carry out present research work.

6. REFERENCES

1. Swami Sadashiv Tirth, Uniyal R.C. (ed), The Ayurvedic Encyclopedia, USA: Ayurveda Holistic Centre press; 2005. p.79-80
2. Sekar D, Gaurav Kumar, Karthik L and Bhaskara Rao KV, A review on pharmacological and phytochemical properties of *Aegle marmelos* (L.) Corr. Serr. (Rutaceae), Asian Journal of Plant Science and Research 2011; 1 (2): 8-17
3. Kala C.P., Ethnobotany and ethnoconversion of *Aegle marmelos* (L.) corr, Indian journal of Traditional knowledge 2006; 5(4):537-540.
4. Anandharajan R, Jaiganesh S, Shankernarayanan NP, Viswakarma RA and Balkrishnan A. In vitro glucose uptake activity of *Aegles marmelos* and *Syzygium cumini* by activation of Glut-4, PI3 kinase and PPARgamma in L6 myotubes. Phytomedicine 2006; 13(6):434-441.
5. Jagetia GC, Venkatesh P, Baliga MS. *Aegle marmelos* (L.) Correa inhibits the proliferation of transplanted Ehrlich ascites carcinoma in mice. Biol Pharm Bull 2005; 28(1):58-64.
6. Jagetia GC, Venkatesh P, Baliga MS. Evaluation of the radioprotective effect of bael leaf (*Aegle marmelos*) extract in mice. Int J Radiat.Biol, 2004; 80(4):281-290.
7. Kesari AN, Gupta RK, Singh SK, Diwakar S, Watal G. Hypoglycemic and antihyperglycemic activity of *Aegle marmelos* seed extract in normal and diabetic rats. J Ethnopharmacol 2006; 107(3):374-379.
8. Agrawal SS, Ashok Kumar, Gullaiya S, Dubey V, Nagar A, et.al, Antifertility activity of methanolic bark extract of *Aegle marmelos*(l.) in male wistar rats, DARU Journal of Pharmaceutical Sciences 2012; 20:94, 1-10
9. Sabu MC, Kuttan R. Antidiabetic activity of Aegle marmelos and its relationship with its antioxidant properties. Indian J Physiol Pharmacol 2004; 48(1):81-88.
10. Arul V, Miyazaki S, Dhananjayan R. Studies on the anti-inflammatory, antipyretic and analgesic properties of the leaves of *Aegle marmelos* Corr. J Ethnopharmacol 2005; 96(1-2):159-163.
11. Todd Caldecott. Ayurveda: The divine Science of Life, China: Mosby Elsevier; 2006. p. 185-186.
12. Dabur R, Gupta A, Mandal TK, Singh DD, Bajpai V et.al, Antimicrobial activity of some Indian medicinal plants, Afr. J. Trad. CAM 2007; 4 (3): 313 - 318
13. Dabur R., Singh. H., Chhillar AK., Ali M. and Sharma GL., Antifungal potential of Indian medicinal plants. Fitoterapia, 2004; 75(3-4):389-391
14. M. Poonkothai and M. Saravanan, Antibacterial activity of *Aegle marmelos* against leaf, bark and fruit extracts, Ancient Science of Life, 2008; 17(8), 15-18
15. Trease G.E., Evans W.C., Textbook of Pharmacognosy, 12th edn, London: Balliere, Tindall; 1989.
16. Kavanagh F. Analytical Microbiology, Volume 1, New York. : Academic press; 1972.
17. Hewitt William. Microbiological Assay for pharmaceutical analysis:A rational approach. New York: Interpharm CRC Press; 2004.
18. Lopez AD, Mathers CD, Measuring the global burden of disease and epidemiological transitions: 2002-2030, Annals Trop Med Parasitol, 2006; 100:481-499.
19. S Brijesh, Daswani P, Pundarikakshudu T, Noshir Antia and Birdi T, Studies on the antidiarrhoeal activity of *Aegle marmelos* unripe fruit: Validating its traditional usage, BMC Complementary and Alternative Medicine 2009; 9(47): 1-12
20. Mims C.A., Medical Microbiology, New York: Mosby Elsevier; 2003. p-387
21. Sharma A. Panchakarma therapy in ayurvedic medicine. In: Mishra LC, editor. Scientific basis for ayurveda therapy. New York: CRC Press; 2004. p.54.
22. Veerappan A, Miyazaki S, Kadarkaraisamy M, Ranganathan D., Acute and subacute toxicity studies of

Aegle marmelos Corr., an Indian medicinal plant. Phytomedicine; 2007; 14(2-3):209-215.
23. Aritajat S, Kaweewat K, Manosroi J, Manosroi A., Dominant lethal test in rats treated with some plant extracts. Southeast Asian Journal of Tropical Medicine and Public Health, 2000, 31(suppl 1):171–173

Conflict of Interest: None
Source of Funding: Nil