

Phytochemical Screening and Antibacterial Activity of *Aegle Marmelos* Leaf Extracts



Ved Ram Sharma

Research Scholar,
Deptt. of Chemistry,
Gandhi Faiz-e-Aam College,
Shahjahanpur, Uttar Pradesh



Mohsin Hasan Khan

Designation,
Deptt. of Chemistry,
Gandhi Faiz-e-Aam College,
Shahjahanpur, Uttar Pradesh

Abstract

Phytochemical investigation was carried out on the chloroform extracts of the leaves of *Aegle marmelos*. Preliminary phytochemical studies revealed the presence of flavonoids, alkaloids, glycosides, steroids, phenols, saponins, terpenoid, cardiac glycosides and tannins as the chemical class present in the extracts. During antimicrobial analysis *Pseudomonas aeruginosa* and *Enterobacter aerogens* both showed significant sensitivity for all the extracts. The minimum activity was reported against *Escherichia coli*. This outcome indicates that the leaves can be useful for treating different diseases because the therapeutic activity of a plant is due to the presence of particular class of compounds and thus can serve as potential sources of useful drugs in future.

Keywords: *Aegle marmelos* Leaves Extracts, Phytochemical Screening, Antibacterial Activity.

Introduction

During last two decades, it has made massive investments on pharmacological, clinical and chemical researches all over the world in an effort to discover still more potent plant drugs. About 250,000 living plant species contain a much greater diversity of bioactive compounds than any chemical library made by humans but only few plant species have been systematically investigated for the presence of bioactive compounds (Wakdikar, 2004). Plant's secondary metabolites have been of interest to man for a long time due to their pharmacological relevance (Arora et al., 2003). The use of drugs derived from plants has been utilized as a source of many potent and powerful drugs for thousands of years all over the world (Lewis and Elvin-Lewis, 1977). Even in modern times, plant-based systems continue to play an essential role in health care and in the recent past increasing research evidence is getting accumulated, which clearly indicate the positive role of plant extracts for health care (Shabnam Javed et al., 2012). The bael fruit sometimes referred as Shreephal and worshiped in many hindu houses. The leaves are used for a diabetic. The infusion of leaves can be used against peptic ulcer. Leaves are also useful for the treatment of jaundice, leucorrhea, wounds, deafness, conjunctivitis. Raw leaves can be used to cure gastric problems and irritation in the bowel. Oil prepared from leaves is proved to stop insect infestation. The leaves are also used in pediatric disorder. Extract from leaves is used in the anti-fungal activity. Aqueous extract of *Aegle marmelos* leaves, was evaluated for hypoglycemic and antioxidant effect by Upadhyay et al., (2004). Maheshwari et al., (2009) studied on ethnolic extract of dried fruit pulp of *Aegle marmelos* against various intestinal pathogens i.e. *Shigella boydii*, *S. sonnei* and *S. flexneri* and proposed that certain phytochemicals including phenols, tannins and flavonoids were effective against all. It was also confirmed by Kaur et al., (2009) by getting treat *E. coli* with *Aegle marmelos* fruit extract. Citarasu et al, (2003) also experimented *Aegle marmelos* on certain pathogenic bacteria like *Salmonella typhi*, *Pseudomonas aeruginosa*, *Aeromonas hydrophyla* and *Vibrio* sp., and concluded its positive bactericidal effects. Yadav et al., (2018) studied leaf extracts of *Aegle marmelos* against many bacterial strains and found that *Pseudomonas aeruginosa* and *Staphylococcus aureus* were most sensitive against acetone and chloroform extract.

Aim of The Study

The present investigation was performed for phytochemical analysis and to test the antimicrobial activity of leaf extract of bael plant against selected pathogens.

Materials and Methods**Plant Material**

The leaves of *Aegle marmelos* were collected from local area of Shahjahanpur district of Uttar Pradesh (India) and were authenticated by Botany Department, Gandhi Faiz-e-Aam College, Shahjahanpur.

Bacterial Cultures

Enterobacter aerogens (NCIM-2340), *Pseudomonas aeruginosa* (NCIM-5210) and *Escherichia coli* (NCIM-2064).

Solvents and Media

Chloroform, Nutrient Agar

Preparation of Extract

10 gms of powdered leaves were used for solvent extraction via Soxhlet apparatus following standard protocol (Nag et al, 2012). After the complete process, the collected extracts were subjected for evaporation at room temperature. The dried extracts were stored at 4°C for future analysis.

Phytochemical Screening

Phytochemical analysis were carried out for the chloroform extract as per the standard methods for Alkaloids, Amino acids, Anthocyanin, Carbohydrates, Flavonoids, Fatty Acid, Glycosides, Phytosterols, Proteins, Phenols, Saponin, Steroid, Tannin and Terpenoids.

Agar Well Diffusion Method

Extracts were tested for the anti-bacterial potential by Agar well diffusion method (Irshad et al, 2012). Initially, autoclaved nutrient media were poured in the Petri plates under laminar air flow and after solidification of media the bacterial suspension (24 hrs old) swab over the media. The wells were prepared using cork borer. Test sample was dissolved in DMSO in different concentrations such as 25, 50, 100 µg/ml and 40 µl dissolved test sample from each concentration was loaded to the wells and incubated for 24 hrs at 37°C. DMSO (Di Methyl Sulfoxide) was used as a negative control whereas antibiotic amoxicillin disc (10µg) used as positive control.

Results and Discussion

The results of qualitative phytochemical analysis of *Aegle marmelos* is shown in Table 1. The chloroform leaf extract of *Aegle marmelos* show the presence of alkaloids, amino acids, anthocyanin, carbohydrates, fatty acids, flavonoids, glycosides, proteins, phenols, saponin, tannin and terpenoids. Out of 14 phytochemical compound analyzed major 12 components are present. In the present study clearly indicates that the presence of many number of phytochemicals are present. Because of the presence of these bioactive chemicals in the leaves, it has the medicinal property to cure almost all common human ailments. In present study, the antimicrobial activity of leaf extracts of *Aegle marmelos* was also carried out. Table-2 shown the antimicrobial activity of leaf extract against *Enterobacter aerogens*, *Escherichia coli* and *Pseudomonas aeruginosa*. The maximum zone of inhibition was observed against *Pseudomonas aeruginosa* (18mm) and *Enterobacter aerogens* (18mm) at 100 µg/ml of chloroform and acetone respectively. Zone of inhibition was followed by 50 µg/ml chloroform extract against *Pseudomonas aeruginosa* (16mm). The observed results suggest that *Aegle marmelos* have significant antimicrobial activity. In the present study of *Aegle marmelos*, antimicrobial activity against *Pseudomonas aeruginosa* and *Enterobacter aerogens* was found significantly maximum by all the extract. Antimicrobial activity of extracts increases as the concentration increases. The observation revealed that among all the test organisms *E. coli* was least sensitive.

Conclusion

The data supports the hypothesis that *Aegle marmelos* leaves has an inhibitory effect on the growth of certain pathogens and may be used effectively against various microbial infections and may be employed as a source to develop new antimicrobial agents. This may be due to the presence of phenols, alkaloids, anthocyanin, xanthoproteins, flavanoids and sterols in the extract.

Table 1: Phytochemical Analysis of Chloroform Leaf Extract of *Aegle marmelos*

S.No.	Phytochemicals	Presence Or Absence
1	Alkaloids	+
2	Amino acids	+
3	Anthocyanin	+
4	Carbohydrates	+
5	Fatty acids	+
6	Flavonoids	+
7	glycosides	+
8	Phytosterol	-
9	Proteins	+
10	Phenols	+
11	Saponin	+
12	Steroids	-
13	Tannin	+
14	Terpenoids	+

Table 2: Effect of leaf extract on growth of bacteria *in vitro*

Bacteria	Chloroform extract(µg/ml)			DMSO (Negative control)	Amoxycillin (Positive control)
	25	50	100		
<i>Pseudomonas aeruginosa</i>	10	16	18	-	20
<i>Enterobacter aerogens</i>	5	5	18	-	16
<i>Escherichia coli</i>	-	5	11	-	6

References

1. Wakdikar, S. (2004). Health care challenge: Indian experiences and new prescriptions. *J Biotech*, 7(3):217-223.
2. Arora S., Kaur K, Kaur S. Indian medicinal plants as a reservoir of protective phytochemicals, Teratogenesis, carcinogenesis, and mutagenesis, ISSN. 2003, 0270-3211.
3. Lewis, H. W. and Elvin-Lewis M. P. F. (1977). *Medical Botany: Plants Affecting Man's Health*. John Wiley and Sons Inc., New York, USA.
4. Shabnam, J., Ahmad A.S., Muhammad S. H., Aysha U., Rauf A. and Sobia M. (2011). Nutritional, phytochemical potential and pharmacological evaluation of *Nigella Sativa* (Kalonji) and *Trachyspermum Ammi* (Ajwain). *Journal of Medicinal Plants Research* Vol. 6(5), pp. 768-775, 9 February, 2012.
5. Upadhya, S., Shanbhag, K. K., Suneetha, G., Naidu, B. M, and Upadhya, S. 2004. A study of hypoglycemic and antioxidant activity of *Aegle marmelos* in alloxan induced diabetic rats. *Ind. J. Physiol. Pharmacol*, 48(4):476-80.
6. Maheshwari, V. L., Joshi, P. V. and Patil, R. H. (2009). In vitro anti diarrhoeal activity and toxicity profile of *Aegle marmelos* Correa ex. Roxb. dried fruit pulp. *Natural Product Radiance*.
7. Kaur, S, Kaur, P, Walia, A. and Kumar, S. (2009). Antigenotoxic Activity of Polyphenolic Rich Extracts from *Aegle marmelos* (L.) Correa in Human Blood Lymphocytes and *E.coli* PQ 37. *Rec. Nat. Prod.*, 3(1): 68-75.
8. Citarasu, T., Rajajeyasekar, R., Venkatmalingam, K., Dhandapani, P. S and Peter Marian, M. (2003). Effect of wood apple *Aegle marmelos*, Correa (Diacotyledons, Sapindales, Rutaceae) Extract as an antibacterial agent on pathogens infecting prawn (*Penaeus indicus*) larviculture. *Indian Journal of Marine Sciences*,32(2):156-161.
9. Yadav, S., Gupta, P. and Rastogi D. (2018). Antimicrobial activity of *Aegle marmelos* leaf extracts. *IJCRT*, 6(2) : 879-881.
10. Nag, S., Paul, A. and Dutta, R. (2012). Phytochemical analysis of methanolic extracts of leaves of some medicinal plants. *Int J Sci Res Publ*,3(4): 1-5.
11. Irshad, S., Mahmood, M. and Parveen, F. S. (2012). In-Vitro Anti-Bacterial Activities of Three Medicinal Plants Using Agar Well Diffusion Method. *Research Journal of Biology*,2(1):1-8.