ISSN:2394-2371
CODEN (USA):IJPTIL

RESEARCH PAPER

Antibacteial activity of *Aegle marmelos* against clinical isolates

Sunil Kumar[1]*, Sudarshan Singh Lakhawat[2]
[1]Amity Institute of Biotechnology, Amity University Rajasthan, Jaipur 303002, Rajasthan, India.

*Corresponding Author: **Dr. Sunil Kumar**

ABSTRACT

Aegle marmelos is a widely used plant in India that has several medicinal advantages for avoiding infections. It has been used for centuries to cure a range of maladies, including pneumonia, uterine abnormalities, fever, colic, headache, and other ethno-veterinary conditions. The objective of this study is to assess the antibacterial activity of the n-hexane, chloroform, methanol, ethanol and water extract of leaves from *Aegle marmelos* belonging to the against two Gram-positive bacteria, including Staphylococcus aureus and bacillus thuringiensis, as well as three Gram- negative bacteria, e coli. Using standard techniques, the extract's phytochemical characteristics including tannin, alkaloids, steroid, saponin, phenol, flavonoids, triterpenes glycosides and carbohydrate and minimum bactericidal and inhibitory concentrations (MBC and MIC) against the test isolates were also evaluated. Agar disc diffusion method were used for in-vitro antibacterial Screening. The purpose of this study is to evaluate the antibacterial activity of an n-hexane, chloroform, methanol, ethanol, and water extract of leaves from *Aegle marmelos* against two Gram-positive bacteria, Staphylococcus aureus and Bacillus thuringiensis, as well as three Gram-negative bacteria, E. coli. The extract's phytochemical properties, such as tannin, alkaloids, steroids, saponin, phenol, flavonoids, triterpenes, glycosides, and carbohydrates, as well as minimum bactericidal and inhibitory concentrations (MBC and MIC) against the test isolates, were assessed using established procedures. The agar disc diffusion technique was utilised for in-vitro antibacterial screening. Methanolic extract showed promising efficacy against the mentioned bacterial stains, with MICs ranging from 16.0 to 32.0 mg/m. Antibacterial activity was assessed by observing the zone of inhibition in well diffusion. This study found that the plant extract of Aegle marmelos Linn. exhibited high antimicrobial activity against the evaluated clinical isolates.

Keywords: - *Aegle marmelos extract; Clinical Isolates; aqueous; MIC.*

INTRODUCTION

Bael, *Aegle marmelos* (L.) Corrêa, is a medicinally valuable tree species [1] among the 250,000 live terrestrial plant species on the planet. Bael is also known as begal-quince, golden apple, and stone apple in India[2], and is considered a holy tree in Hindu communities. Bael trees are commonly grown around Lord Shiva temples and are regularly worshipped by devotees [3]. Bael was one of the most valued herbs used in ayurvedic

***CORRESPONDING AUTHOR**
Dr Sunil Kumar
Assistant Professor, Amity Institute of Biotechnology, Amity University Rajasthan, Jaipur, Rajasthan, India.
E.Mail: skumar6@jpr.amity.edu
Article Published: July-Sept. 2023
CITE THIS ARTICLE AS
Kumar S, Lakhawat SS. Antibacteial activity of *Aegle marmelos* against clinical isolates. Int J Pharm Technol Biotechnol. 2023; 10(3):21-29.

treatment by ancient Indian and South Asian populations [4]. Bael has been utilised as a medicinal and dietary item from 5000 B.C., as documented in historical sources. [5] The growing incidence of antibiotic resistance among clinical isolates has become a major problem in healthcare settings globally. Antibiotic-resistant bacteria are a major danger to the successful treatment of infectious illnesses, increasing morbidity, mortality, and healthcare expenditures. As a result, there is an urgent need to investigate alternate ways for fighting bacterial infections [6, 9].

Natural products derived from medicinal plants have long been recognized as potential sources of novel antimicrobial agents. Natural products derived from medicinal plants have long been recognized as potential sources of novel antimicrobial agents. These plant-based compounds offer a diverse array of chemical structures and mechanisms of action that can target bacterial pathogens through different pathways, potentially minimizing the development of resistance. Aegle marmelos , a widely distributed plant species in tropical and subtropical regions, has attracted considerable attention due to its traditional use in various folk medicinal systems for the treatment of infectious diseases [10].

Several investigations have found antibacterial activity in Aegle marmelos extracts against a wide range of bacterial infections. Phytochemical investigations of Aegle Maemelos indicated the presence of bioactive substances such as alkaloids, flavonoids, phenolics, and essential oils with antibacterial properties. These chemicals inhibit both Gram-positive and Gram-negative bacteria, including clinically relevant pathogens including Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Enterococcus faecalis.

The main objective of this dissertation is to investigate the antibacterial activity of Aegle marmeloscc extract against clinical isolates in vivo. The specific objectives are as follows:

To evaluate the *in vitro* antifungal activity of *Aegle marmelos* leaf extracts and fractions on the clinical isolates of dermatophytic fungi like *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Microsporum canis*, *Microsporum gypseum* and *Epidermophyton floccosum*..

METHODOLOGY

Collection of plant material

Leaves and stems of Aegle marmelos were collected from sarsai, in khaga Local village Area of uttar Pradesh, rajasthan INDIA. The plant was certified by a professor in Plant Science and Biotechnology Department, amity university Jaipur, rajasthan.

Preparation of Extracts

The plant material, including leaves and stems are carefully harvested, ensuring the selection of healthy and mature plants. The plant material is washed thoroughly with distilled water to remove any dirt or impurities. It is air-dried in a well-ventilated area away from direct sunlight to preserve the integrity of the bioactive compounds for 2 weeks. Once dried, the plant material is ground into a fine powder using a suitable grinder.

Dry powder was extracted separately with n-Hexane, Chloroform, Ethyl acetate, Methanol and water in the increasing polarity for 72 Hrs. with each solvent and then extract was filtered with filter paper The solvents were evaporated under reduced pressure to obtain a semi solid mass.

The extraction of bioactive compounds from Aegle marmelos is performed using an appropriate solvent extraction method. The choice of solvent will be based on previous studies and the solubility of the targeted bioactive compounds. Common solvents for plant extractions include n-Hexane, Chloroform, Ethyl acetate, Methanol and water the increasing polarity

Clinical Isolates

Microorganisms used were standard strains obtained from the Department of biotechnology, department of microbiology, amity university Rajasthan. They are two Gram-positive bacteria, including Staphylococcus aureus and bacillus vulgaris, as well as two Gram- negative bacteria, e coli and Klebsiella pneumoniae. The test organisms were further sub cultured at 370C for 24 hours. The cultures of bacteria were maintained in their appropriate agar slants at 40C throughout the study and used as stock cultures[9, 10].

ANTIMICROBIAL ACTIVITY

The antimicrobial activity of various plant extracts was tested using the Micro broth dilution Assay, as described in NCCLS document M-27A (NCCLS) 2011 [13]. The fungus and bacterial strains employed as inoculum were cultivated overnight on Sabouraud dextrose agar and nutrient agar at 37°C, respectively. The lowest dose that blocked any visual development was called the minimum inhibitory concentration (MIC).

TEST OF ANTIBACTERIAL ACTIVITY

The bactericidal activity of AC extract was tested using the disc diffusion technique. The extracts at varied concentrations were dissolved in 0.01% DMSO. Paper discs were soaked in extract solution for 15 minutes before drying under laminar air flow for 2 hours. Paper discs containing AC and PB extract, respectively, were put on the medium infected with SA and then incubated at 37°C for 24 hours. All the extracts were prepared in a concentration ranging from 0.750 to 48.0 µg/ml. Proper

controls were used for each experiment. The bacterial strains used as inoculum were grown at 37 o C to get OD 0.6 at 600 nm and used for susceptibility testing. The lowest concentration, which inhibited any visual growth, was considered to be minimum inhibitory concentration (MIC). The antibacterial activity of the Aegle marmelos extract was determined using the agar diffusion technique. The extract was tested against clinical isolates of bacteria to determine its efficacy in suppressing bacterial growth. The agar diffusion method, commonly known as the disc diffusion method, was employed to determine the antibacterial activity of Aegle marmelos plant extract against clinical isolates. This is a simple and commonly used approach for determining bacterial sensitivity to different antimicrobials. The following measures were conducted to assess antibacterial activity [9, 10].

RESULTS

Antibacterial Activity of *Aegle marmelos* Extract

The antibacterial activity of the Aegle marmelos extract was assessed against a panel of clinically significant bacterial isolates using the disk diffusion method. The zone of inhibition (ZOI) was measured as an indicator of the inhibitory effect of the extract on bacterial growth. The results obtained from the experiments are summarized in Table 1.

To exhibit antibacterial activity of *Aegle marmelos* extracts in n-hexane, chloroform, ethyl acetate, methanol, and water were shown in Table 1.

Table 1: Zone of inhibition in antibacterial activity of *Aegle marmelos*.

S. no	Name of sample	Sample concentration (microgram/ml)	Staphylococcus pneumoniae (mm)	Bacillus vulgaris (mm)	e-coli (mm)	Klebsiella Pneumoniae (mm)	control
1.	$n-hexane$	70 100 150 200 350	5 9 10 11 12	9 10 11 12 14	– – – – –	5 6 7 8 9	8 8 8 8 8
2.	$chloroform$	70 100 150 200 350	8 9 12 13 16	7 11 12 16 18	– – – – –	7 9 14 15 17	8 8 8 8 8
3.	$ethyl\ acetate$	70 100 150 200 350	– – – – –	– – – – –	– – – – –	– – – – –	8 8 8 8 8
4.	$methanol$	70 100 150 200 350	7 10 15 18 24	18 23 29 31 32	9 10 11 12 19	20 23 25 27 29	8 8 8 8 8

5.	*water*	70 100 150 200 350	– – – – –	– – – – –	– – – – –	— _ _ _ _	8 8 8 8 8

In vitro antibacterial activity of Aegle marmelos, the n-hexane, chloroform, ethyl acetate, methanol and water extract of Aegle marmelos Linn. was given in Table – 1.

Results showed that n-Hexane and Chloroforms exerted anti bacterial activity at all concentration in staphylococcus pneumoniae, klebsiella pneumoniae and bacillus vulgaris per disc, ethyl acetate extract not showed anti bacterial activity of all tested bacteria. Methanolic extract of Aegle marmelos showed four bacterial strains like e-coli, staphylococcus aureus, klebisella pneumoniae and streptococcus pneumoniae positive in all concentration. Water extract negative test for all five bacterial strain. N-Hexane, chloroform and methanol extract of Aegle marmelos 350 µg/ml concentration showed large zone of inhibition in disc diffusion was found out.

Minimum Inhibitory concentration (MIC)-

Anti bacterial activity of total four bacteria, two Gram positive and two Gram negative bacteria staphylococcus pneumoniae, klebsiella pneumoniae and bacillus vulgaris and ecoli were used for evaluation of n-Hexane, chloroform, ethyl acetate, methanol and water extracts of Aegle marmelos leaves. The result of minimum inhibition concentration (MIC) showed in Table No. 2.

Table 2: The minimum inhibitory concentration (MIC) of isolates.

ISOLATES	**MIC(mg/ml)**
staphylococcus pneumonia	3.1
klebsiella pneumonia	6.2
bacillus vulgaris	1.6
E-coli	3.5

The MIC values for the Aegle marmelos extract against the tested isolates ranged from 1.5 mg/mL to 6.2 mg/mL. E-coli and bacillus vulgaris demonstrated the lowest MIC values of 1.5 mg/mL and 1.6 mg/mL, respectively, indicating a higher potency of the extract against these isolates. Klebsiella pneumoniae showed the highest MIC value of 6.2 mg/mL, indicating a relatively lower inhibitory effect of the extract against this particular isolate.

These findings suggest that the ***A. marmelos*** extract has a potential inhibitory effect on the growth of

the tested clinical isolates. The observed variation in the inhibitory effects and MIC values could be attributed to variations in the susceptibility of the bacterial strains or differences in the composition and concentration of bioactive compounds present in the extract

The minimum inhibitory concentration (MIC) represents the lowest concentration of an antimicrobial agent required to inhibit the growth of a microorganism. It is a critical measure of the potency of the antimicrobial agent against a specific bacterial strain or isolate.

MIC allows to assess the effectiveness of the extract in inhibiting the growth of the tested clinical isolates. The MIC values can provide valuable insights into the concentration at which the extract exerts its maximum inhibitory effect.

To determine the MIC of the Aegle marmelos extract, a standard method such as the broth dilution method or agar dilution method can be employed. The process typically involves preparing a series of dilutions of the extract in a growth medium, inoculating the medium with a standardized concentration of the bacterial isolate, and incubating the plates or tubes for a defined period.

After incubation, the MIC is determined as the lowest concentration of the extract that completely inhibits visible growth of the bacterial isolate. This can be identified by the absence of turbidity or visible colonies in the growth medium. It is important to note that MIC values can vary among different bacterial isolates and even within the same species due to variations in bacterial susceptibility and the presence of resistance mechanisms. Therefore, it is common to determine MIC values for multiple bacterial isolates to assess the overall effectiveness of the Aegle marmelos extract.

Phytochemical screening extract:

The phytochemical screening of AC and PB extract were conducted to determine the presence of secondary metabolites such as flavonoids, alkaloids, tannins, polyphenols, quinones, steroids/triterpenoids, saponins, monoterpenes and sesquiterpenes.

Table 3: Phytochemical composition of extract of Aegle marmelos.

PHYTOCHEMICAL	AVAILABILITY
Carbohydrate	+
Glycosides	+
Saponins	+
Steroids	+
Flavonoids	+
Anthracene derivatives	-
Terpenoids	+
Alkaloids	+
Tannins	+

Abbreviations : (+)present (-)absent

DISCUSSION

The present study aimed to investigate the antibacterial activity of Aegle marmelos extract against clinical isolates in vivo. The results obtained from the disk diffusion method and determination of the Minimum Inhibitory Concentration (MIC) provide valuable insights into the potency and effectiveness of the extract against the tested bacterial isolates.

The disk diffusion method revealed varying degrees of antibacterial activity exhibited by the Aegle marmelos extract against the clinical isolates. The formation of clear zones of inhibition around the extract disks indicates the inhibitory effect of the extract on bacterial growth. The observed differences in the zone sizes can be attributed to variations in the susceptibility of the bacterial strains, differences in the concentration of bioactive compounds within the extract, or variations in the release and diffusion of these compounds from the disks.

Furthermore, the determination of MIC values allowed for a quantitative assessment of the inhibitory potency of the Aegle marmelos extract. The MIC represents the lowest concentration of the extract required to inhibit bacterial growth. The obtained MIC values ranged from 1.5 mg/mL to 6.2 mg/mL, indicating varying levels of susceptibility of the tested bacterial isolates to the extract.

E-coli demonstrated the lowest MIC value of 1.5 mg/mL, suggesting higher susceptibility to the extract, while klebsiella pneumoniae displayed the highest MIC value of 6.2 mg/mL, indicating a relatively lower sensitivity to the extract. These variations in MIC values could be attributed to multiple factors, including the specific mechanisms of bacterial resistance, variations in the bioactive compound profile of the extract, and variations in the cell envelope permeability of the tested bacterial strains.

The phytochemical screening of the Aegle marmelos extract revealed the presence of various bioactive compounds, such as alkaloids, flavonoids, tannins, and saponins. These compounds are known to possess antimicrobial properties and could contribute to the observed antibacterial activity of the extract. Further analysis and isolation of individual compounds within the extract could help identify the key components responsible for the antibacterial effects.

The results of this study support the traditional use of Aegle marmelos in folk medicine as an antibacterial agent. The extract exhibited significant inhibitory activity against the tested clinical isolates, indicating its potential as a natural alternative to conventional antibiotics. However, further investigations are needed to elucidate the precise mechanisms of action of the extract and to evaluate its safety and efficacy in more comprehensive in vivo and clinical studies.

The findings of this study contribute to the existing body of knowledge on Aegle marmelos and its antibacterial properties. The extract's broad-spectrum activity against both Gram-positive and Gram-negative bacteria suggests its potential applicability in combating various bacterial infections. Additionally, the identification and characterization of bioactive compounds within the extract could pave the way for the development of novel antimicrobial agents based on Aegle marmelos

CONCLUSION

The present study investigated the antibacterial activity of Aegle marmelos extract against clinical isolates in vivo. Through the implementation of the disk diffusion method and determination of the Minimum Inhibitory Concentration (MIC), valuable insights were obtained regarding the potency and effectiveness of the extract in inhibiting bacterial growth.

The results of the disk diffusion method revealed varying degrees of antibacterial activity exhibited by the Aegle marmelos extract against the tested clinical isolates. The formation of clear zones of inhibition around the extract disks indicates its inhibitory effect on bacterial growth. These findings provide evidence supporting the traditional use of Aegle marmelos as an antibacterial agent in folk medicine.

Furthermore, the determination of MIC values allowed for a quantitative assessment of the inhibitory potency of the extract. The obtained MIC values ranged from 1.6 mg/mL to 6.4 mg/mL, indicating varying levels of susceptibility of the tested bacterial isolates to the extract. This suggests that the extract possesses the ability to inhibit bacterial growth at relatively low concentrations.

The phytochemical screening of the Aegle marmelos extract revealed the presence of various bioactive compounds, such as alkaloids, flavonoids, tannins, and saponins. These compounds are known for their antimicrobial properties and could contribute to the observed antibacterial activity of the extract.

Based on the findings of this study, it can Aegle marmelos extract possesses significant antibacterial potential against a range of clinical isolates. The extract's broad-spectrum activity against both Gram-positive and Gram-negative bacteria highlights its versatility as a natural antimicrobial agent. However, further research is warranted to fully understand the underlying mechanisms of action and optimize the extraction process to ensure consistent and reproducible results.

References

1. R. Chanda, “Phytochemical and pharmacological activity of Aegle marmelos as a potential medicinal plant: an overview,” The Internet Journal of Pharmacology, vol. 6, no. 1, p. 3, 2008..

2. S. K. Roy and R. N. Sing, "Bael fruit (Aegle marmelos): a potential fruit for processing," Economic Botany, vol. 33, no. 2, pp. 203–212, 1979
3. P. C. Sharma, V. Bhatia, N. Bansal, and A. Sharma, "A review on bael tree," Indian Journal of Natural Products and Resources (IJNPR), vol. 6, pp. 171–178, 2007.
4. M. S. Uddin, M. S. Islam, M. A. Alam, and M. M. Hossain, "Study on physico-morphological characteristics of 14 bael (aeglemarmelos corr.) genotypes grown at chapainawabganj, Bangladesh," IJMFM & AP, vol. 2, pp. 29–33, 2016.
5. B. C. Y. Collard and D. J. Mackill, "Marker-assisted selection: an approach for precision plant breeding in the twenty-first century," Philosophical Transactions of the Royal Society B: Biological Sciences, vol. 363, no. 1491, pp. 557–572, 2008
6. J. Anandkumar and B. Mandal, "Removal of Cr (VI) from aqueous solution using Bael fruit (Aegle marmelos correa) shell as an adsorbent," Journal of Hazardous Materials, vol. 168, no. 2-3, pp. 633–640, 2009.
7. P. K. Mandal and A. K. Mukherjee, "Studies on bael (Aegle marmelos) seed glycoproteins," Carbohydrate Research, vol. 90, pp. 233–241, 1981
8. A. Roy, S. B. Bhattacharya, A. K. Mukherjee, and C. V. N. Rao, "The Structure of degraded Bael (Aegle marmelos) gum," Carbohydrate Research, vol. 50, pp. 87–96, 1976
9. Singh DD. Assessment of antimicrobial activity of hundreds extract of twenty Indian medicinal plants. *Biomedical Research 2018; 29 (9): 1797-1814.*
10. D D Singh and Singh V. Analysis of Molecular resistance mechanisms of Itraconazole in Candida albicans clinical isolates from India. Intern J of pharmac scie rev and res.2011;1 (11):75-79.