

EVALUATION OF IN -VITRO ANTIBACTERIAL POTENTIAL OF RIPE FRUITS OF *AEGLE MARMELOS*

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ABSTRACT

Aegle marmelos belongs to the family Rutaceae, commonly known as bael. Ripe fruits were extracted with 50% methanol using separating funnel. Pellets of dried extract were obtained and different doses (25%, 50%, 75% and 100%) were prepared by dissolving pellets into doubled distilled water. Extracts were tested against three gram positive bacteria (*S.aureus*, *B.subtilis*, *S.epidermidis*) and three gram negative bacteria (*E.coli*, *Sh.flexneri*, *P.aeruginosa*) by agar disc diffusion method. Maximum inhibition was found with dose having 100% concentration of dried extract. It has shown significant antibacterial activity against tested gram positive and gram negative bacteria when compared with standard antibiotics. Study suggests that the ripe fruits of *Aegle marmelos* are promising in the development of phytomedicine for antimicrobial properties.

Keywords: *Aegle marmelos*, Antibacterial effect, Methanolic extract.

INTRODUCTION

Antibiotics are one of our most important weapons in fighting bacterial infections since their introduction. However, over the past few decades commonly used antibiotics have become less effective against certain illnesses not only because many of them produce toxic reactions but also due to emergence of drug resistant bacteria. It is essential to investigate newer drugs with lesser resistance.¹

Since ancient times, plants have been utilized as an important source of medicines as they are a reservoir of chemical agents with antimicrobial properties.²

The plant kingdom represents a rich storehouse of medicinal organic compounds, and could serve as lead for the development of novel agents having good efficacy in various pathological disorders in the coming years.³ At present nearly more than 30% of the modern pharmacological drugs are derived directly or indirectly from plants and in homeopathic or ayurvedic medicines, medicinal plants, their parts and extracts dominate the scenes.⁴ It has been reported that the plants are the cheapest and safer alternative sources of antimicrobials^{4,5,6}. *Aegle marmelos* (L.) Correa (Rutaceae), commonly known as Bael, is a sacred tree for Hindu Religion, native to northern India. But it is widely distributed throughout the Indian peninsula and in Ceylon, Burma, Thailand and Indo-China⁷. Leaves, fruits, stem and roots of this plant have been used in ethno medicines for several medicinal properties like astringent, antidiarrheal, antidysentric, demulcent, antipyretic, aphrodisiac and an antidote to snake venom⁸. Antifungal activity has been shown by essential oil isolated from the leaves of the *Aegle marmelos*⁹. The leaves are having astringent, laxative, and expectorant effects and are also useful in the treatment of ophthalmia, deafness, inflammations, cataract, diabetes, diarrhea, dysentery, heart palpitation, and asthma¹⁰. Leaves are claimed to possess contraceptive property as well¹¹. Fresh aqueous and alcoholic leaf extracts of *Aegle marmelos* were found to have cardio tonic effects in mammals^{12, 13}. Hypoglycemic and antioxidant activity of *Aegle marmelos* leaves against alloxan induced diabetic rats have been found to be useful in the long term management of diabetes¹⁴. The ethanolic extract of leaf possesses anti spermatogenic activity¹⁵ and aqueous extract of the leaf has antimotility action on spermatozoa in rats¹⁶. Hepatoprotective activity of leaves has also been evaluated with positive results¹⁷. Both fruit and leaves of *Aegle maemelos* have radio protective activity^{18, 19}. Fruit extract exhibits antihyperlipidaemic effect in streptozotocin-induced diabetic rat²⁰.

Unripe fruit extract of *Aegle marmelos* has shown gastro protective and anti diarrhoeal properties²¹. Like leaves, fruits have also shown hypoglycemic effect²². Modulation of doxorubicin-induced toxicity has been reported with *Aegle marmelos* extract²³.

Although various parts of the *Aegle marmelos* has been explored for their potential medicinal properties, activities related to ripe fruit still remains unexplored. The present study was designed to investigate the antibacterial properties of different concentration of 50% methanolic extract of ripe fruit of *Aegle marmelos*.

MATERIALS AND METHODS

Preparation of Extract

The ripe fruits of *Aegle marmelos* were purchased from local market at Bhopal (M.P.) India. A voucher specimen has been kept in the herbarium of Research Centre of the Jawaharlal Nehru Cancer Hospital and Research Centre for future reference. The fruits were sliced and seeds were removed, they were then dried in shade and powdered. A sample of 50 grams fruit powder was extracted with 50% methanol in a separating funnel. The extract was dried in water bath at 60°C. The powder was treated with petroleum ether for 3 hours for defatting. Pellets of the drug were obtained and the required dose for the treatment was prepared by dissolving the pellets in double distilled water.

Microorganisms used

The test organisms (*Psuedomonas aureoginosae*, *Staphylococcus aureus*, *Staphylococcus epidermis*, *Shigella flexineri*, *Bacillus subtilis* & *E.Coli*) were obtained from the Department of Research, JN Cancer Hospital and Research Center, Bhopal (M.P.).

Assay for antibacterial activity

Kirby-Bauer Method (Disc diffusion method) was followed to test the antibacterial activity of different concentration of plants extract. The paper discs were dipped in different concentrations of the extract. After that impregnate the discs on agar plate, it diffuses their drug and in case of drug sensitive bacteria make a clear area that is known as Inhibitory area or Zone of inhibition.

The medium used for the activation of the microorganisms was nutrient broth. The nutrient agar media was used for the antimicrobial test. All the culture media were prepared and treated according to the manufacturer guidelines (Hi Media Laboratories Ltd., Mumbai, India).

Discs of Whattmann filter paper no.1 (diameter 0.5mm) were made by the help of punching machine. The discs were then autoclaved in a Petri dish for sterilization. The discs were dried and preserved in Petri dishes at 2-8°C.

All of the required apparatus and materials were sterilized in autoclave and placed in a laminar airflow cabinet under pathogen free conditions. Test organisms were collected from department's

microbial standard stock. By streaking with loop, microorganisms were inoculated in nutrient broth and incubated at 35 °C for 12 hr. Nutrient agar media was prepared and poured in Petri plates and kept for drying. Swab (cotton) was dipped in broth having microbial growth and gently squeezed against the inside of the tube to remove excess fluid. The dried surface of agar plate was inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated two more times, and rotated the plate 60° each time to ensure an even distribution of inoculums. The plate tops was replaced and allowed for 3 to 5 minutes, but no longer than 15 minutes, for any excess surface moisture to be absorbed before applying the test and antibiotics disks. Disks were dipped in drug of (*Aegle marmelos*) different concentration (25%, 50%, 75% & 100%) and air dried in laminar air flow before this step. Appropriate disks (Drug and Antibiotics) were placed evenly (no closer than 24 mm

from center to center) on the surface of the separate agar plates by using a sterile forceps. The plates were then inverted and placed in an incubator at 35°C within 15 minutes after disks were applied. After 6-8 hours of incubation, each plate examined and measured the diameters of the zones of complete inhibition, including the diameter of the disk.

Data was analyzed by ANOVA.

RESULTS AND DISCUSSION

The results were summarized in Table 1. Among the extracts tested 100 % methanolic extract of *Aegle Marmelos* have showed good results against *Pseudomonas aeureoginosa*, *Shigella flexneri*, *E.coli*, *Bacillus subtilis*, *Staphylococcus epidermidis* and *Staphylococcus aureus*.

Table 1: Antibacterial activity of *Aegle marmelos* against the following micro-organisms

S. No.	Micro-organism	Concentration of Drug Zone of Inhibition			
		25%	50%	75%	100%
1.	<i>Bacillus subtilis</i>	8mm	8mm	13mm	16mm
2.	<i>Shigella flexneri</i>	6mm	15mm	13mm	16mm
3.	<i>Staphylococcus epidermidis</i>	13mm	13mm	15mm	15mm
4.	<i>Staphylococcus aureus</i>	10mm	11mm	15mm	16mm
5.	<i>E. coli</i>	6mm	9mm	11mm	9mm
6.	<i>Psuedomonas aeruginosa</i>	7mm	8mm	9mm	9.5mm

Table 2: Antibacterial activity of standard antibiotic against different gram positive bacteria

Name of microorganisms	Name Standard antibiotics [zone of inhibition(mm)]			
	TE	OF	AZ	PC
<i>S.aureus</i>	15	16	16	14
<i>B.subtilis</i>	14	16	18	14
<i>S.epidermidis</i>	14	18	17	17

TE- Tetracycline, OF- Ofloxacin, AZ- Azithromycin & PC- Piperacillin

Table 2 shows the antibacterial activity of standard antibiotics against gram positive bacteria *S.aureus*, *B.subtilis*, *S.epidermidis*.

Table 3: Antibacterial activity of standard antibiotic against different gram negative bacteria

Name of microorganisms	Name Standard antibiotics [zone of inhibition(mm)]			
	FU	GM	CX	NF
<i>E.coli</i>	12	16	8.0	16
<i>Sh.flexneri</i>	18	18	12	21
<i>P.aeruginosa</i>	14	13	18	20

FU- Nitrofurantoin, GM- Gentamicin, CX- Cefotaxime & NF- Norfloxacin

Table 3 shows the antibacterial activity of standard antibiotics against gram negative bacteria *E.coli*, *Sh.flexneri*, *P. aeruginosa*

Among the four concentrations used in the study, the dose having 100% concentration was considered as the effective one. Because it exhibited maximum zone of inhibition against all pathogens in comparison with the other concentration of the test drug (25%, 50% and 75%). Comparison of the different concentrations of methanolic extracts of *Aegle Marmelos* with reference to Standard antibiotics suggest that inhibitory potential of the dose having 100% concentration is quite interesting.

CONCLUSION

Our present study showed that dose having 100% concentration of dried extract of *Aegle Marmelos* caused antibacterial activity against *Pseudomonas aeruginosa*, *Shigella flexneri*, *E.coli*, *Bacillus subtilis*, *Staphylococcus epidermidis* and *Staphylococcus aureus*.

The antibacterial, antifungal and antiviral activity of *Aegle Marmelos* has also been reported by various workers in different microorganisms which support our finding. Although work is

required to be commenced on the broad range of microbial strain and use of specific isolated constituents for further studies.

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