



Antibacterial activity of *AegleMarmelos* against fruit extracts

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ABSTRACT

The antibacterial activity of the ethanol, and aqueous extracts from the fruit of *A. marmelos* was studied using disc diffusion method against five pathogenic bacterial strains, three Gram-positive bacteria (*B. cereus*, *S. epidermidis*, *S. aureus*) and two Gram-negative bacteria (*E. aerogens*, *K. pneumoniae*). The maximum 14.3mm zone of inhibition was observed at the concentration of 40mg/ml for aqueous extract. This may be due to the presence of the compounds Cuminaldehyde and Eugenol. In the case of ethanol, the maximum antibacterial activity was seen against *E. aerogens* followed by *S. epidermidis* and *K. pneumoniae*. At the highest concentration of 160 mg/ml, it showed 18.3mm zone of inhibition. The antibiotic susceptibility showed that among all the bacterial strains *S. aureus* was found more susceptible to ampicillin followed by *K. pneumoniae*. Results suggest that the ethanolic extract has significant antibacterial activity against tested bacteria. The present study justifies the claimed uses of *A. marmelos* in the traditional system of medicine to treat various infectious diseases.

Keywords: *Aeglemarmelos*, cytotoxicity, Antiviral Activity, Influenza A.

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INTRODUCTION

The plants have an immense potential to be used as a source of antimicrobial agents. Traditionally for the treatment of human infectious diseases, crude plant extracts were being used as herbal medicine. [1] In the developing countries, about 60 to 90% of populations use plant-derived medicine. Due to the presence of a variety of phytochemicals including tannins, terpenoids, alkaloids, and flavonoids plants possess *in vitro* antimicrobial properties. The use of plants and their products as medicines can be traced as far back as the beginning of human civilization. The use of crude extracts of different parts of plants and phytochemicals, of known antimicrobial properties, can be of great significance in the therapeutic treatments. Various plants have been used because of their antimicrobial

traits, which are due to the secondary metabolites synthesized by the plants. [2] The screening of plant products for antimicrobial activity has shown that the higher plants represent a potential source of novel antibiotic prototypes. This has forced the scientist to search for new antimicrobial substances from various sources like the medicinal plants. The plant produces a wide variety of secondary metabolites which are used either directly as precursors or as lead compounds in the pharmaceutical industry. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant microbial pathogens.

Bael (*Aeglemarmelos*) (Linn.) belonging to family rutaceae has been known to be one of the most important medicinal plants of India since Charak (1500 B.C). More than 100 phytochemical compounds have been isolated from its various parts of the plant viz. phenols, flavonoids, alkaloids, cardiac glycosides, saponins, terpenoids, steroids, and tannins. These compounds are well known to possess biological and pharmacological activity against various chronic diseases such as cancer, cardiovascular and gastrointestinal disorders. [3]

Furthermore antioxidant, antiulcer, antidiabetic, anticancer, antihyper lipidaemic, anti-inflammatory, antimicrobial, antispermatic effects have also been reported in various animal models by the crude extracts of this plant. Every part of *Aeglemarmelos* plant such as its fruits, stem, bark and leaves possess the medicinal property and are used for treating various eye and skin infections.

Fruit is considered to be one of the highest accumulative parts of the plant containing bioactive compounds which are synthesized as secondary metabolites. Considering the vast potentiality of this plant and a prospective source of biologically active components, we, therefore, aimed at evaluating the antibacterial activity of fruit of *Aeglemarmelos* using different solvents.^[4]

MATERIALS AND METHODS

Chemicals

Ethanol, Mueller –Hinton Agar, dimethyl sulphoxide (DMSO), Ampicillin and all other chemicals used were purchased from Merck and Himedia.

Collection and Authentication of Plant Material

The fruit pulp of *Aeglemarmelos* were collected from the herbal garden, Chennai and authenticated by Professor Dr. Jayaraman Taxonomist, Department of Botany, Tambaram, Chennai, Tamil nadu India. After authentication, the fruit pulp were collected by scooping the pulp using sterile scoop and blade, shade dried and then milled into coarse powder by a mechanical grinder.

Extract preparation

For antibacterial screening, the fruit extracts were prepared from *Aeglemarmelos* by following the in Soxhlet apparatus using 180 ml of distilled water and 90% ethanol. Every extraction was carried out for 24 hrs and the extract was then dried, weighed and stored in a refrigerator at 4°C.

Calculation of the percentage yields of the crude extract using different solvents

$$\% \text{ yield} = \frac{\text{weight of crude extract}}{\text{weight of raw material}} \times 100$$

Antibacterial activity

The extracts mentioned above were tested against five pathogenic bacterial strains, three Gram-positive bacteria (*B. cereus*, *S. epidermidis*, *S. aureus*) and two Gram-negative bacteria (*E. aerogens*, *K. pneumoniae*)⁵. The antibacterial screening was done using agar well diffusion method. For this 20 ml of sterile Mueller –Hinton Agar (Hi-media) was poured in sterile autoclaved petriplates. After solidification, the sterile cotton swab was dipped into the bacterial culture.^[6] The entire agar surface of each plate was evenly inoculated by swabbing. The seven uniform wells were prepared with the help of sterile 6mm diameter cork-borer. Each well was filled with the various concentrations of both the aqueous and methanol extracts (10, 20, 25, 30 and 40 mg/ml) respectively whereas, in case of aqueous: ethanol (40, 80, 100 and 120 mg/ml) concentrations were used and allowed for diffusion for 45 minutes.^[7] The plates were then incubated at 37°C for 24 hrs. Triplicate

plates were prepared for each treatment and the average zone of inhibition excluding well was recorded. 9% DMSO was used as negative control.

The turbidity of bacterial culture was maintained up to 1×10^8 CFU/ml. The antibacterial potential of extracts was compared with standard antibiotic Ampicillin (10µg/disc) with paper disc (Hi-media) method.

Statistical analysis

Statistical Analysis was carried out by using the SPSS software version 15.0 (SPSS Inc., Chicago, USA). For data analysis, Dunnett's t-test, was used, *P< 0.05, **P<0.01, ***P<0.001 was considered statistically significant.

RESULTS AND DISCUSSION

Percentage yield of crude extract using three different solvents

The % yield was found to be the maximum in aqueous extract i.e. 79.58% followed by ethanol 53.76%.

Effect of Antibacterial study

The pharmacological action of the plant cannot be ascertained by the result of phytochemical studies only. The antibacterial activity of the three extracts (aqueous, ethanol) obtained from the fruits of *Aeglemarmelos* was evaluated against pathogenic bacteria. The present investigation shows the efficacy of all the extracts against the selected pathogenic bacteria (Table 1&2). The aqueous extract showed highest antibacterial activity against *S. epidermidis* (14.3 mm) followed by *S. aureus* (13 mm) and *K. pneumonia* (10.6 mm). The maximum zone of inhibition was observed at the concentration of 40mg/ml. The presence of the compounds Cuminaldehyde and Eugenol may be responsible for the antibacterial activity of fruit extracts against various bacterial strains.^[8]

In the case of ethanol, the maximum antibacterial activity was seen against *E. aerogens* followed by *S. epidermidis* and *K. pneumonia*. At the highest concentration of 160 mg/ml, it showed 18.3mm zone of inhibition. The antibiotic susceptibility showed that among all the bacterial strains *S. aureus* was found more susceptible to ampicillin followed by *K. pneumonia*.

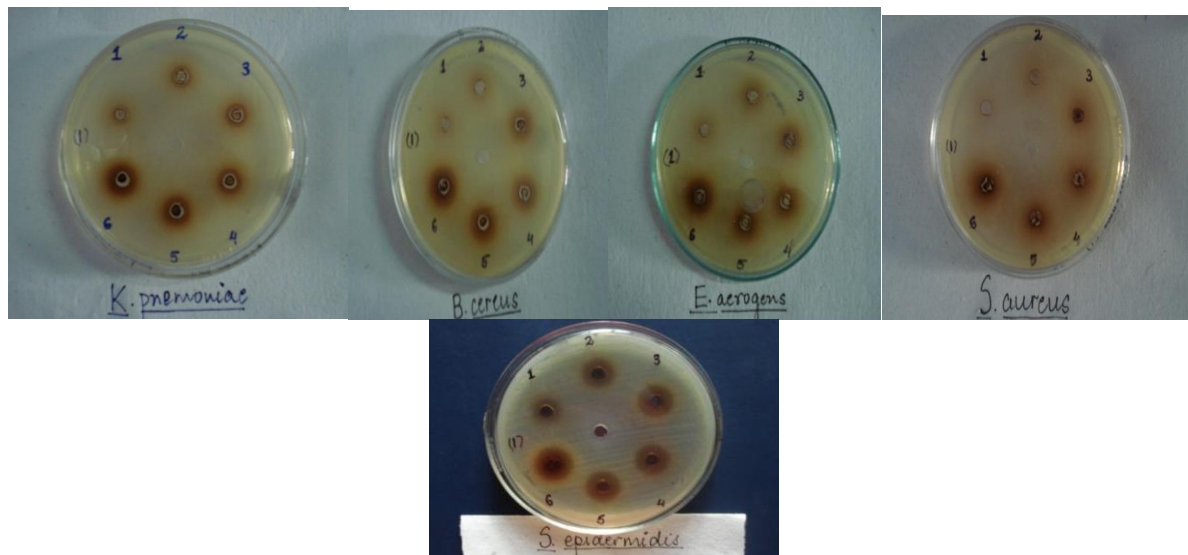
As reported the aqueous and ethanol extracts of medicinal plants showed antibacterial activities against some human pathogenic bacteria and observed that the ethanol extracts had wider range of activity on these organisms than the aqueous extracts, which indicates that the ethanol extracts of (*Aeglemarmelos*) selected plants may contain the active components.

Recently, a study conducted on the antibacterial potential of ripe, unripe fruits of *Aeglemarmelos* revealed that the MIC for the above extracts ranged from 200 mg/ml to 6.25 mg/ml. The high antibacterial activity in the ethanolic extract may be due to the presence of tannins, flavonoids, and terpenoids.^[9]

Table 1: Zone of Inhibition against different bacteria by the aqueous extract of Bael leaves

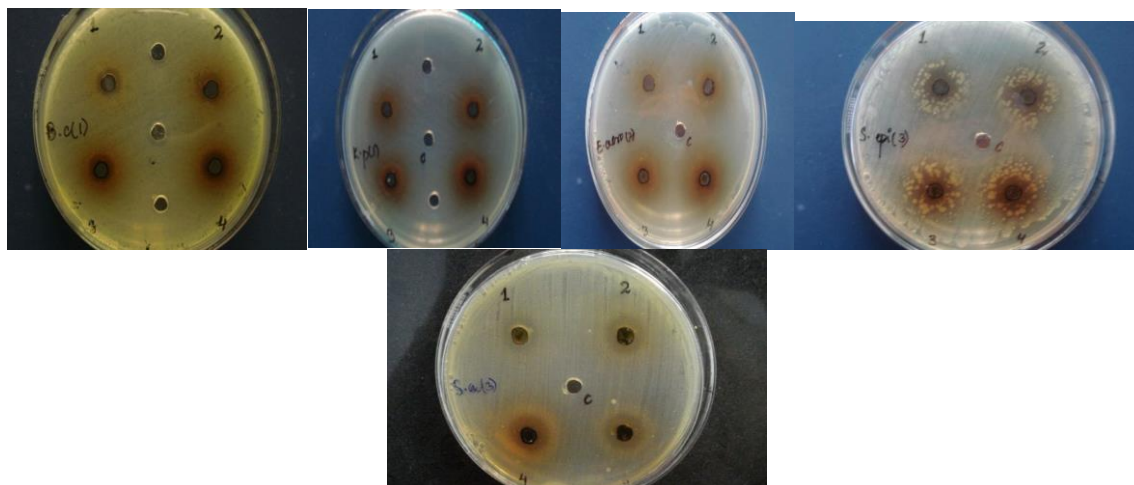
Conc.(mg/ml)	Aqueous extract						Standard Ampicillin (10µg/disc)	Control DMSO
	10	15	20	25	30	40		
<i>K.pneumoniae</i>	7.33±0.03	8.33±0.03	8.5± 0.02	6.5± 0.02	9.83±0.04	10.6± 0.03	21.3±0.088	0
<i>B. cereus</i>	7.33±0.03	8.33±0.03	9.00	9.00	9.00±0.05	9.33± 0.05**	10.3±0.088	0
<i>E. aerogens</i>	8.00	8.00	8.33±0.03	9.00	9.00	9.00**	11.3±0.088	0
<i>S. aureus</i>	8.66±0.03	9.66±0.03	10.6±0.03	11.6±0.03	12.6±0.03	13.0	40.6±0.120	0
<i>S. epidermidis</i>	9.66±0.03	11.0	12.0	13.0	13.6±0.03	14.3± 0.06*	20.6±0.145	0

Values are expressed as mean + SEM. and analyzed by One-way Analysis of variance (ANOVA) followed by Dunnett's t-test, *P< 0.05, **P<0.01, ***P<0.001.

**Figure 1:****Table 2: Zone of Inhibition by ethanol extract**

Conc.(mg/ml)	Ethanol extract			
	40	80	120	160
<i>pneumonia</i>	9.0±0.05	11.0±0.05	12.3±0.03	14.6±0.06*
<i>B. cereus</i>	9.0	10.0	11.0	11.3±0.03
<i>E. aerogens</i>	10.0	15.0	16.0	18.3±0.03**
<i>S. aureus</i>	10.3±0.03	11.6±0.03	12.0	13.0
<i>S. epidermidis</i>	10.3±0.03	12.3±0.03	13.3±0.06	14.6±0.03*

Values are expressed as mean + SEM. and analyzed by One-way Analysis of variance (ANOVA) followed by Dunnett's t-test, *P< 0.05, **P<0.01, ***P<0.001.

**Figure 2: Bacteria showing zones of inhibition by ethanol extract**

These medicinally bioactive components exert antimicrobial action through a different mechanism.^[10]

Tannins cause inhibition of the cell wall synthesis by forming irreversible complexes with proline rich protein. The saponins have the ability to cause leakage of proteins and certain enzymes from the cell. Terpenoids are responsible for the dissolution of the cell wall of micro-organism by weakening the membranous tissue.^[11] Flavonoids which have been found to be effective antimicrobial substances against a wide array of microorganism *in vitro* are known to be synthesized in response to microbial infection by plants.^[12]

They have the ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls. Furthermore, steroids are known for their antibacterial activity specifically associated with membrane lipids and cause leakage from liposomes.^[13]

The present study justifies the claimed uses of *A. marmelos* in the traditional system of medicine to treat various infectious diseases.^[14] The ethanolic extract of *Aegle marmelos* showed higher antibacterial activity to a group of bacterial pathogens. The functions of triterpenesaponin in plants for its antimicrobial, fungicidal, antibacterial, antiviral, analgesic, anti-inflammatory, antitumor, cytotoxic, immunostimulant, antihelminthic, expectorant and antitussive activities, have been known for many years.^[15]

CONCLUSION

Since ancient times, plants have been used by several communities to treat a large number of diseases, including infections. Numerous studies on the pharmacology of medicinal plants have been accomplished since they constitute a potential source for the production of new medicines and may enhance the effects of conventional antimicrobials, which will probably decrease costs and improve the treatment quality. Although this study investigated the preliminary screening of antibacterial activity, the results showed that the extracts from *Aegle marmelos* possess good antibacterial activity, conferring the great potentials of this plant used in folkloric medicine for the production of bioactive compounds and are useful for rationalizing the use of this medicinal plant in preliminary health care. Moreover, the phytochemicals identified through GC-MS analysis further supports the above observed antibacterial activity. It is, therefore, the above findings recommended the further investigation on isolation and purification of bioactive compounds responsible for the antibacterial activity.

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