



## Research Article

ANTIBACTERIAL ACTIVITY OF ETHANOLIC LEAF EXTRACTS OF *AEGLE MARMELOS* (L)

CORR.

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**Abstract:** The present investigation aims to evaluate the antimicrobial activity of ethanolic leaf extracts of *Aegle marmelos* (L) Corr. on selected microbial strains. Results of the experiment depict that phytochemicals present in the ethanolic leaf extracts of *A. marmelos* exhibit considerable antibacterial activity. The inhibitory effect was both strain and dose dependent. Further, at concentration of 300µl and above, ethanolic leaf extracts of *A. marmelos* exhibited significant activity towards all the selected bacterial strains. However, *B. subtilis* and *E. coli* were more sensitive towards the treatment when compared to *S. aureus*, *P. aeruginosa* and *K. pneumonia*.

**Key words:** *Aegle marmelos*; Medicinal plants; Antibacterial agents; Phytochemicals

## INTRODUCTION

World Health Organization, pointed out that more than 80% of world's population in the rural/ remote area depend on wild plants to meet primary healthcare needs<sup>1</sup>. On the other hand, medicinal plants today remain as pillar of traditional healthcare systems of medicine in many countries including India. Besides, several pharmacological studies have acknowledged the magnitude of medicinal plants as potential natural source of bioactive compounds<sup>2</sup>. As of now, it is well established that phytochemicals obtained from the medicinal plants serve as lead molecules in modern medicines and nutraceuticals<sup>3</sup>. Furthermore, phytoconstituents from medicinal plants provide lead compounds in drug discovery<sup>4</sup>. Extensive investigations have been carried out on medicinal plants based on the ethanobotanical information. Isolation and characterizations of phytoconstituents from several medicinal plants have been reported by Indian scientists. Consequently, varied classes of compound viz., alkaloids, coumarins, terpenoids, fatty acids and aminoacids have been isolated, characterized and exploited on commercial scale. Similarly, extensive investigations have been carried out on different parts of the sacred medicinal plant -*Aegle marmelos*.

*Aegle marmelos* (Linn) Corr., commonly known as Bael, belongs to the family Rutaceae, is a moderate-sized, slender and aromatic tree with cosmopolitan distribution. However, phytogeographical information depicts that Bael tree is indigenous to India and is grown throughout

the sub-continent as well as most countries of South-east Asia. The history of Bael has been traced to Vedic period (2000-800 BC)<sup>5</sup>. Bael tree has great mythological significance and abounds in the vicinity of temples. Bael tree is held sacred by Hindus. Leaves of *A. marmelos* are used to worship Lord Shiva. It is considered as an emblem of fertility<sup>6</sup>. Vedic literature depicts that leaf extract is used to cure asthmatic complaints. Fresh leaf juice along with honey has been reported to serve as a laxative and febrifuge<sup>7</sup>. The potential use of leaf of *A. marmelos* in the aliment of backache, eye complaints, vomiting, cut and wound, animal injuries, ulcer, dropsy, beriberi, weakness of heart, cholera, diarrhoea, diabetics, nervous disorders, acute bronchitis and complications associated with child birth are reviewed and mentioned<sup>8</sup>. Leaves of *A. marmelos* contain alkaloids, phenylpropanoids, terpenoids and other miscellaneous compounds with potential hypoglycemic, anti-inflammatory, antimicrobial, anticancer, radioprotective, chemopreventive and anti-oxidative activity was already reported<sup>9</sup>. Further, it has been emphasized that anhydroaegeline obtained from *A. marmelos* could be used as marker to standardize the plant material with potential anti diabetic activity. Invariably, all part of the tree viz. stems, barks, roots, leaves, flowers and fruits have been described for their use in Ayurveda and Siddha for the treatment of respiratory disorders, constipation, ulcer, diarrhea, dysentery<sup>10</sup>.

However, only few reports pertinent to the antimicrobial potential of *A. marmelos* is available.

Pattnaik *et al* using disc diffusion method reported that essential oils of *A. marmelos* exhibit significant antibacterial and antifungal activities<sup>11</sup>. Similarly, Rana *et al.*, using spore germination assay demonstrated that essential oil in the leaves of *A. marmelos* exhibit significant antifungal activity<sup>12</sup>. Owing to its overwhelming medicinal use, in the present study, antimicrobial potential of ethanolic leaf extracts of *A. marmelos* has been investigated on selected microbial strains.

## MATERIALS AND METHODS

### Collection of Plant Material

Mature leaves of *A. marmelos* were collected from Periyakulam, Theni, Tamilnadu, India during Apr 2012. The Flora of Presidency of Madras<sup>13</sup> and The Flora of Tamil Nadu Carnatic<sup>14</sup> were used for identification and authentication of the plants. Collected material was washed thoroughly in running tap water, rinsed in distilled water and shade dried in open air and grounded into powder.

### Test Organisms

Three strains of Gram-positive and two strains of Gram negative bacteria were used to evaluate the antibacterial activity. All bacterial cultures were maintained in NA slants/ plates; stored at 4°C and periodically sub-cultured.

## ANTIMICROBIAL ACTIVITY

### Disc Diffusion Assay (DDA)

Ethanolic leaf extracts were dissolved in 20% DMSO treated water. The inoculums for each microorganism were prepared from broth cultures ( $10^5$  CFU/ml). A loop of culture from the NA slant stock was cultured in LB medium overnight and spread with a sterile swab into Petri-plates. Sterile disc (6 mm, Hi-media, Mumbai, India) impregnated with the ethanolic leaf extracts (1.0 mg/disc and 5.0 mg/disc) were placed on the cultured plates and incubated for 24 h at 37°C. The solvent loaded disc without extracts in it served as

control in the study. The results were recorded by measuring the zones of growth inhibition. Clear inhibition zones around discs indicated the presence of antimicrobial activity.

### Minimal Inhibitory Concentration (MIC)

Furthermore, to ascertain the level of minimal inhibitory concentration by the inhibition in growth, the selected microbial strains were grown in test tube containing 5ml of LB medium supplemented with varying concentrations of ethanolic leaf extracts for 6h with shaking. Test tube containing 5ml of LB medium without ethanolic leaf extracts served as control. Optical density of the culture broth was determined spectrophotometrically using UV-VIS spectrophotometer at 600 nm. All data on antimicrobial activity were average of triplicate.

## RESULTS

Preliminary data obtained from disc diffusion assay indicated that the ethanolic leaf extracts of *A. marmelos* exhibit significant antibacterial activity against all the selected microbial strains (Table 1). Further, to ascertain the minimal inhibitory concentration, cells of the selected bacterial strains were grown in LB medium with or without (control) leaf extracts of *A. marmelos* and OD was determined at 600nm after 6h of incubation (Fig. 1). The ethanolic leaf extracts showed effective inhibitory activity against selected bacterial strains at 100 µl concentration. The decreasing order of inhibition in growth at a concentration of 100 µl of ethanolic leaf extracts of *A. marmelos* was  $0.47 > 0.31 > 0.20 > 0.10 > 0.10$  (OD) for *P. aeruginosa*, *K. pneumonia*, *S. aureus*, *E. coli* and *B. subtilis* respectively. However, further increase in the concentration of ethanolic leaf extracts exhibited corresponding increase in the level of inhibition. Significant reduction in the growth of all the selected bacterial strains was observed at a concentration of 300µl and above. Further, the level of inhibition in growth was both strain and concentration dependent as indicated by the regression equation (y) and the slope ( $R^2$ ) (Fig. 2a-e).

**Table 1** Disc Diffusion Assay of *A. marmelos* ethanolic leaf extracts on growth of selected microbial strains

Microbial Strain	Inhibition of Growth
<i>B. subtilis</i>	+++
<i>E. coli</i>	+++
<i>S. aureus</i>	++
<i>P. aeruginosa</i>	+
<i>K. pneumonia</i>	++

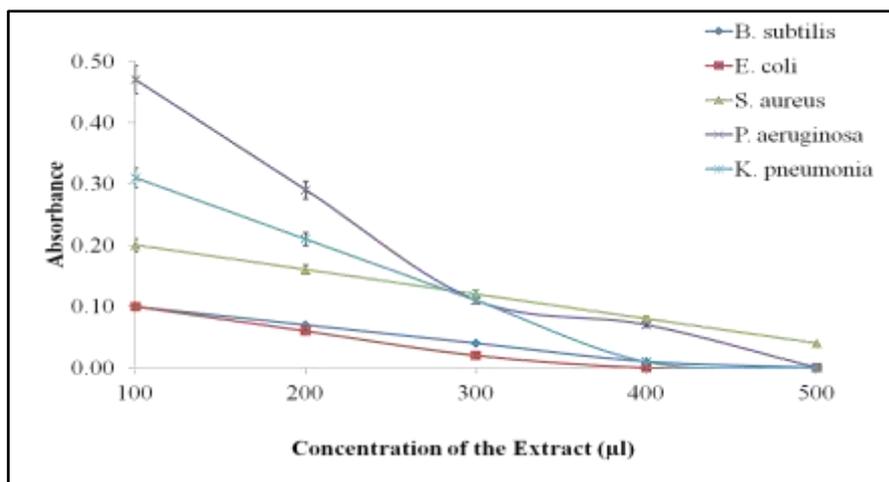


Fig. 1 Minimal Inhibitory Concentration (MIC) of *A. marmelos* ethanolic leaf extracts on growth of selected microbial strains

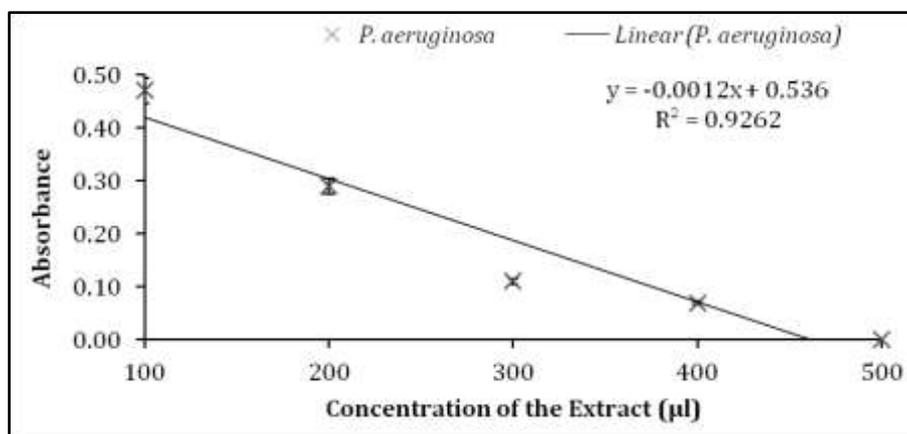


Fig. 2a Minimal Inhibitory Concentration (MIC) of *A. marmelos* ethanolic leaf extracts on growth of *P. aeruginosa*

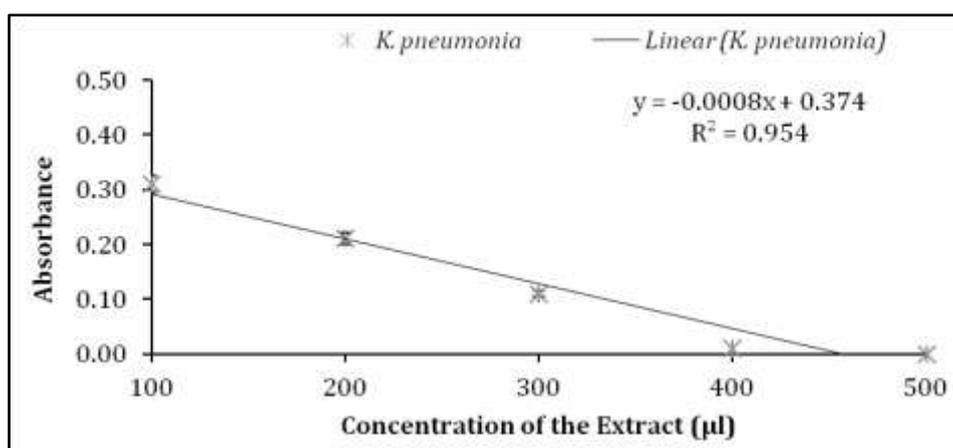


Fig. 2b Minimal Inhibitory Concentration (MIC) of *A. marmelos* ethanolic leaf extracts on growth of *K. pneumonia*

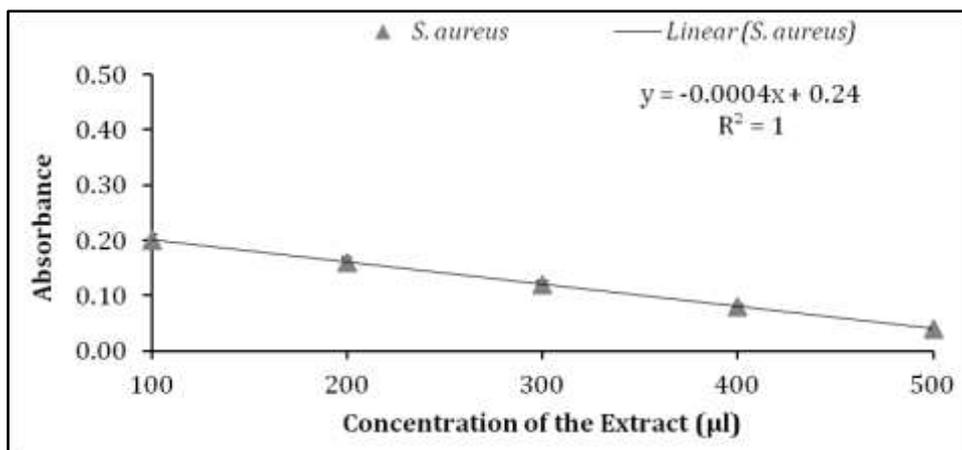


Fig. 2c Minimal Inhibitory Concentration (MIC) of *A. marmelos* ethanolic leaf extracts on growth of *S. aureus*

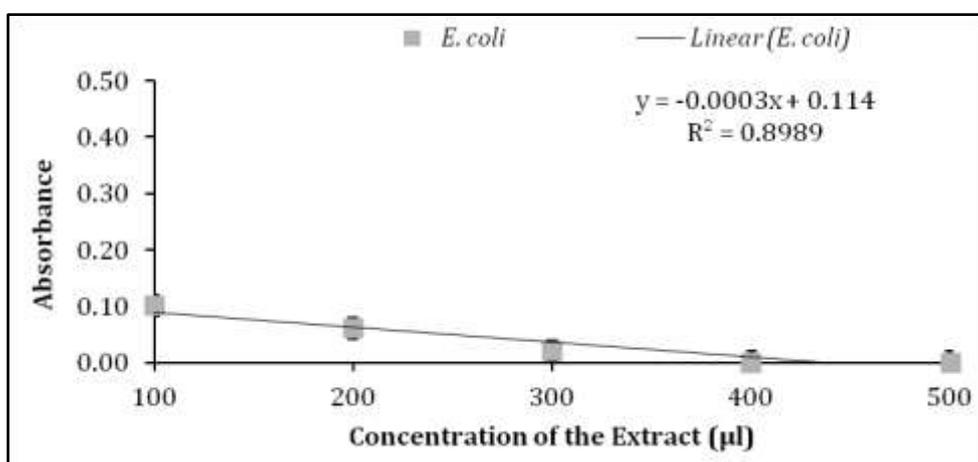


Fig. 2d Minimal Inhibitory Concentration (MIC) of *A. marmelos* ethanolic leaf extracts on growth of *E. coli*

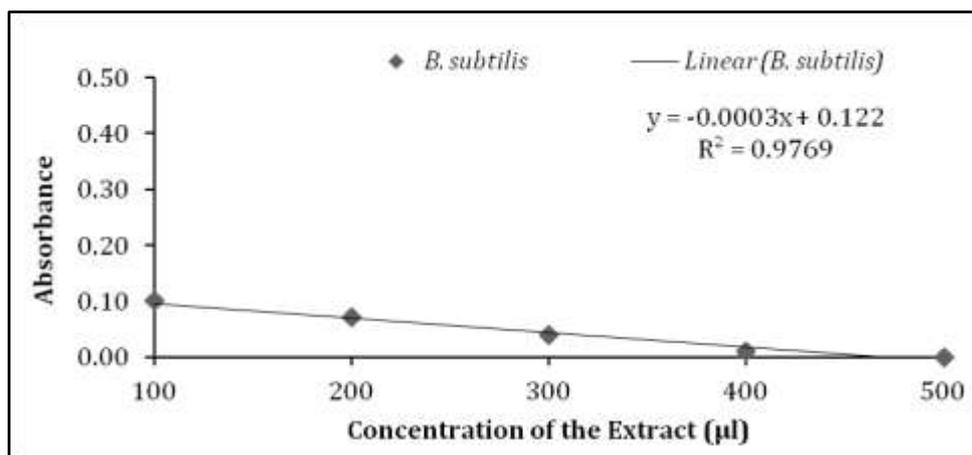


Fig. 2e Minimal Inhibitory Concentration (MIC) of *A. marmelos* ethanolic leaf extracts on growth of *B. subtilis*

**DISCUSSION**

Plants are known to have beneficial therapeutic effects documented in Traditional

Indian System of Medicine. Much work has been done on ethnomedicinal plants in India. Interest in a large number of traditional natural products has increased in recent times<sup>14, 15</sup>. The effect of plant

extracts on bacteria has been studied by a very large number of researchers across the world. Plants have been reported to possess antimicrobial, antifungal and other activities<sup>15,16,17,18</sup>.

Suresh *et al.*, evaluated the leaves and flower extract of *A. marmelos* against five clinical pathogens (*S. aureus*, *P. aeruginosa*, *Proteus mirabilis*, *E. coli*, *Salmonella typhi*) at different concentrations (50, 100 and 200 ppm) and reported that *E. coli* was the most susceptible towards the treatment than other selected strains<sup>19</sup>. Further, it was reported that presence of tannins and alkaloids in the extract could possibly inhibit the growth of the microorganisms. It was demonstrated that *A. marmelos* leaf extract exhibited maximum activity against *E. coli* followed by *S. typhi*, *S. aureus*, *P. mirabilis*, *P. aeruginosa* at all concentrations tested. At a concentration of 200 ppm and above methanolic leaves extract significantly inhibited the growth of selected microbial strains. Similarly, methanolic extracts of *A. marmelos* flowers exhibited significant antimicrobial activity against *S. aureus* in all concentration followed by *P. mirabilis*, *E. coli*, *S. typhi* and *P. aeruginosa*. Likewise, Ulahannan *et al.*, demonstrated that minimum inhibitory concentration and minimum bactericidal concentration for *A. marmelos* methanolic leaf extract towards *Serratia marcescens*, exhibited MIC and MBC values of 200 mg/ml<sup>20</sup>. Recently, Gheisari *et al.*, evaluated antimicrobial activity of Bael fruit towards six food pathogenic strains of bacteria and fungi and implicated that Bael fruit has more antifungal activity than antibacterial properties<sup>21</sup>. On the other hand, the decoction of *A. marmelos* was shown to demonstrate significant anti-giardial and antirotaviral activity however, had no antibacterial activity<sup>22</sup>.

## Conclusion

In the present study antibacterial activity of *A. marmelos* extracts towards drug resistant/clinically significant microbes are reported and it was observed that the active constituents of plant material seep-out in organic solvents to display biological activity. The phytochemical extracts were less active against the probiotic organisms *Bacillus*, indicating that it is not going to affect the gut micro flora. Further, phytochemical studies for identification and elucidation of active constituents in the plant materials tested are expected to serve as lead in the development of novel bioactive antimicrobial compounds.

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