In vitro antibacterial and phytochemical potential of Aegle marmelos against multiple drug resistant (MDR) Escherichia coli

Srikala Ganapathy, Karpagam S

Abstract
Alarming increase of antibiotic resistant bacteria in recent years result in limited options for treatment of bacterial diseases. Escherichia coli is one of the most common Gram negative bacterial pathogen causing community and hospital acquired infections. Since the emergence of antibiotic resistant strains of Escherichia coli is at higher pace, there is an urgent need for new antibacterial compounds. Medicinal plants are alternative rich source of useful antibacterial agents. The present study assessed the phytochemical and in vitro antibacterial activity of ethanol, chloroform, hexane and water extracts of leaves and fruits of Aegle marmelos against MDR Escherichia coli. Zone of inhibition diameters were measured. The antibacterial activity was absent in hexane and water extracts. Chloroform extracts showed moderately good activity. However, highest antibacterial activity was found in ethanol extracts. The antibacterial compounds found in leaf and fruit extracts were tannins, flavonoids, saponins and alkaloids.

Keywords: Aegle marmelos extracts, multiple drug resistant (MDR) Escherichia coli, antibacterial activity.

1. Introduction
The emergence of antibiotic resistance is a global health concern. Gram negative bacterial resistance is of particular importance as there is dearth of novel antibiotics directed against these microorganisms [1]. The increase in antibiotic resistance is due to several factors but the major cause appears to be excessive use of antibiotics [2]. Increase in the incidence of multidrug resistant Escherichia coli has been reported in various studies [3]. Escherichia coli is the most common Gram negative bacterial pathogen causing community and hospital acquired urinary tract infections [4]. Enteric and diarrhoeal infections are major public health problems in developing countries and contribute to death of 3.3 to 6 million children annually. Enteric bacteria that includes Escherichia coli along with other bacterial pathogens are the major etiological agents of sporadic and epidemic diarrhea in both children and adults [5, 6]. An isolate is considered as multiple drug resistant if found to be resistant to three or more antimicrobials belonging to different classes or groups of antimicrobials. As multi drug resistant Escherichia coli are widely distributed in hospitals and increasingly being isolated in community, there is an urgent need to search for new antimicrobial agents [7, 8].

Aegle marmelos belongs to family Rutaceae, commonly known as Bael tree [9], is one of the most important tree species indigenous to India, China, Burma and Sri Lanka [10] and has been used by the inhabitants of Indian subcontinent for over 5000 years. It’s a slow growing, medium sized tree, up to 12-14 m tall with short trunk, thick bark, alternate leaves, borne singly or in group, composed of 3 to 5 oval, pointed leaflets [11]. Leaves, fruits, stem and roots of the tree have been used in ethno medicines for several medicinal properties like astringent, antidiarrhoeal, antidysenteric, demulcent, antipyretic, aphrodisiac and an antidote to snake venom [12]. Unripe fruit extract of Aegle marmelos has shown gastro protective and antidiarrhoeal properties [13]. The leaves are having astringent, laxative and expectorant effects and are also in the treatment of ophthalmia, deafness, inflammations, diabetes, diarrhea, dysentery and asthma [14]. Aegle marmelos has diverse pharmacological profile due to the presence of wide spectrum range of chemical entity [15]. Based on the background knowledge of therapeutic potential of the plant species and limited work on the antibacterial activity specifically against multiple drug resistant (MDR)
Escherichia coli and considering the urgent need to develop alternative therapeutic options, the present study was designed to assess the in vitro antibacterial and phytochemical screening of Aegle marmelos leaves and fruits against MDR Escherichia coli.

2. Materials and Methods

2.1. Plant collection and sample preparation

Aegle marmelos leaves and ripe fruits were collected from Thiruvallur District in Tamilnadu (voucher number qmc/2009/1325). The plant materials were washed thoroughly, shade dried for about 15 days and grounded into a powder. About 25g of plant powder was extracted with 100 ml solvents by using Soxhlet apparatus. Solvents used for extraction were ethanol, chloroform, hexane and water. The resultant extracts were filtered by using Whatman No 1 filter paper and then concentrated in a rotary evaporator and were stored in a refrigerator at 4 °C in small sterile glass bottles for further analysis.

2.2. Antibacterial activity

The dried plant extracts were dissolved in Dimethyl sulfoxide (DMSO) separately at the concentration of 1mg/ml for antibacterial assay. The bacterial culture used in the study was pure clinical isolates of multiple drug resistant (MDR) Escherichia coli obtained from Private Hospital, Chennai. Muller Hinton Agar (MHA) medium was used to study antibacterial activity. Prior to antibacterial screening, the bacterial culture was cultured in Muller Hinton Broth for about 4 hrs at 37°C. Antibacterial testing was carried out by Kirby Bauer disc diffusion method (Bauer et al., 1986) [16]. The bacterial culture was inoculated as lawn culture using sterile swab over the agar surface. The filter paper discs impregnated with 100 microl of plant extract (1mg/ml) were placed on the seeded agar plates. Dimethyl sulfoxide (DMSO) served as negative control and Streptomycin (10 microg) as reference. The plates were then labeled and incubated at 37 °C for 24 hours. After incubation, the plates were examined for clear inhibition zone and zone diameters were measured and recorded.

2.3. Phytochemical screening

The plant extracts were subjected to preliminary qualitative phytochemical screening method as described by Harbourne [17]. The plant extracts were investigated for tannins, alkaloids, flavonoids and saponins. The test of tannins was carried out by boiling 0.5g of sample in 20 ml distilled water followed by addition of 3 drops of 5% ferric chloride to the filtrate. Development of dark green colouration indicated positive by the presence of tannins. The flavonoids was determined using 0.2 g of plant extract was dissolved in dilute sodium hydroxide and adding drops of dilute hydrochloric acid. The development of yellow colouration was taken positive for flavonoids. The test for alkaloids was carried out by treating 1 g of plant extract with 5 ml methanol and 5ml of 2N HCl and then the filtrate was treated with Mayer’s reagent. Development of precipitate indicated the presence of alkaloids. Saponins were detected by boiling 1 g of the sample with 10 ml distilled water for 15 minutes and the cooled extract was shaken for froth formation.

3. Results and discussion

Our study highlights significant antibacterial activity of Aegle marmelos leaf and fruit extracts against MDR Escherichia coli (fig 1) it is interesting to observe that ethanol extracts of the fruits showed highest inhibitory activity against MDR E. coli with maximum zone of inhibition diameter when compared to standard antibiotic (20mm). Chloroform fruit extracts also showed moderately good inhibitory activity. Hexane and aqueous extracts showed no antibacterial activity. Interestingly, similar pattern of results was observed with Aegle marmelos leaves with ethanolic extracts showing the highest antibacterial activity in the study.

<table>
<thead>
<tr>
<th>Concentration of the Plant Extracts</th>
<th>Solvents</th>
<th>Positive Control (Streptomycin 10 Micro gram)</th>
<th>Aegle marmelos Fruits on MDR E.Coli ZOI (mm)</th>
<th>Aegle marmelos Leaves on MDR E.Coli ZOI (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mg/ml</td>
<td>Ethanol</td>
<td>20</td>
<td>44 44 44</td>
<td>41 41 40</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td></td>
<td>22 21 21</td>
<td>22 22 22</td>
</tr>
<tr>
<td></td>
<td>Hexane</td>
<td></td>
<td>--     --</td>
<td>-- --</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td></td>
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</tbody>
</table>

According to Tortora et al. the cell wall of Gram negative bacteria act as a barrier to a number of substances including antibiotics [18], but interestingly in the present study both Aegle marmelos fruit and leaves extracts showed significant antibacterial activity against MDR E. coli. Poovendran et al. [19] and Gangai amaran et al. [20] reported chloroform extracts responsible for significant antibacterial activity against E. coli. Other study has reported that organic solvents are better suited for consistent extraction of antibacterial substances in medicinal plants [21] while Chanda and Kaneria et al. have reported that the bioactive compounds concentration could vary in different plant parts [22], but in our present study leaf and fruit ethanolic extracts almost equivocally had accumulation of phytochemicals and activity.

![Fig 1: Zone of Inhibition results of antibacterial activity](image)
Preliminary phytochemical screening analysis showed the presence of tannins, flavonoids, saponins and alkaloids in both ethanolic leaf and fruit extracts of Aegle marmelos. The data is shown in Table 2.

Table 2: Qualitative Phytochemicals screening of Aegle marmelos ethanolic leaf and fruit extract.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Phytochemicals</th>
<th>Presence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Alkaloids</td>
<td>+</td>
</tr>
</tbody>
</table>

In our study better antibacterial activity was observed in ethanolic leaf and fruit extracts, this may be due to presence of more active phytochemicals in the extracts. The previous researchers have reported the presence of active phytochemicals responsible for antibacterial activity. Tannins are known to form irreversible complexes with protein and inhibit cell wall synthesis of the bacteria [23]. Previous reports have shown iron binding capacity of tannic acids responsible for bringing about inhibitory bacterial activity [25]. Saponins have ability to cause leakage of proteins and certain enzymes of the cell [25].

4. Conclusion
From the results obtained, it is concluded that ethanolic leaf and fruit extracts of Aegle marmelos showed significant antibacterial activity against Multi drug resistant (MDR) Escherichia coli. This study serves as a preliminary scientific validation of Aegle marmelos Ethanolic extracts and it is encouraging the plant shows promising potential for development of drugs for MDR E. coli infections. Phytochemicals isolation and characterization of ethanolic extracts of Aegle marmelos and their mechanism of action is our next focus of research.

5. Acknowledgements
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6. References

22. Chanda S, Kaneria M. Indian neutraectualc plant leaves as a potential source of natural antimicrobial agents.