Abstract

The antibacterial activity of the methanol, chloroform and aqueous extracts from the leaves, bark and fruit of A. marmelos was studied using disc diffusion method against Bacillus subtilis, Staphylococcus aureus (Gram Positive), Klebsiella pneumoniae, Proteus mirabilis, Escherichia coli, Salmonella paratyphi A and Salmonella paratyphi B (Gram Negative). Results suggest that the methanolic 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.

Key Words: Antibacterial activity, A.marmelos, Inhibition zones, Infectious diseases

Introduction

Failure of modern medicine, side effects of chemical drugs, alarmingly rising cost of allopathy treatment make people look for an alternative system of medicine. This includes medicinal plant therapy, Ayurveda, Unani, Siddha. Acupuncture and Acupressure and the like. (Sheth 1998). It is estimated that in modern age 80% of the medical care is provided by home care and traditional native systems of medicine and major part of these therapies involve the use of plant extracts or their active principles (Ramesh et al., 1985). Angiosperm plants are store houses of effective chemotherapeutants and results of biological screening of these plants for a wide range of activities proved that these can be used for treating diseases like diarrhoea, dysentery, dyspepsia,
stomach pain (Elsamma Thomas et al., 1999). Over the last 20 years a large number of plant species have been evaluated for the antimicrobial activity. Therefore it is necessary to establish the scientific basis for the therapeutic action of these plants. The present study is an attempt to evaluate the antibacterial activity of leaves, bark and fruits of *A. marmelos*.

*Aegle marmelos*, corr. (Fam.Rutaceae) is commonly known as “Bael” in Bengal and Vilvam in Tamil is distributed throughout India in dry forests, and also cultivated. The leaves are trifoliate, flowers are greenish white, bark is yellowish brown in colour and fruits are green in colour with orange brown sweet gummy pulps. The extracts of the various parts of the plant is used for controlling diarrhoea, dysentery, dyspepsia, stomach pain, seminal weakness, uropathy, vomiting, diabetes, snake bite, fish poisoning and some antiviral activities (Krithikar and Basu, 1999).

Considering the uses of *A. marmelos* in traditional system of medicine, an attempt is made to assess the antibacterial activity of leaf, bark and fruit extracts.

**Materials And Methods**

**Collection of plant material and extraction:**

The leaves, barks and fruits of *A. marmelos* were collected in and around Namakkal district. The plant materials collected were shade dried, crushed and broken using a pestle and mortar, and stored in a cool dry place. The powders (5g each) were subjected to extraction using Soxhlet apparatus with chloroform, methanol and water separately. The three extracts were concentrated into paste consistency. From each extract 100 mg was dissolved in 10 ml of Dimethyl Sulphoxide (DMSO) and stored in airtight containers.

**Microorganisms**

The following strains of bacteria were used *Bacillus subtilis* (ATCC 1813), *Klebsiella pneumoniae* (ATCC 6071), *Proteus mirabilis* (ATCC 14153), *Staphylococcus aureus* (ATCC 2112), *Escherichia coli* (ATCC 11229), *Salmonella paratyphi* A and *Salmonella paratyphi* B (Isolated from patients suffering from typhoid).

**Antibacterial activity**

The above mentioned bacterial isolates were grown in nutrient agar at 37°C and reactivated them for further use in nutrient broth. The different extracts of *A. marmelos* were tested for antimicrobial activity against the test organism using the agar diffusion method of Kirby et al., (1966).

Muller Hinton agar media was prepared and the plates were swabbed with 24 hrs cultures of respective bacteria grown in nutrient broth overnight. Sterile discs of 6mm diameter were impregnated with 20µl of each extract separately. Blank disc impregnated with DMSO was used as negative control and discs of tetracycline (30µg) as positive control.
The plates were then incubated at 37°C for 24 hrs. Each experiment was repeated in triplicates.

**Result And Discussion**

The inhibitory effect of phytoextracts of leaf, bark and fruits of *A. marmelos* in methanolic, chloroform and aqueous extracts of leaves, bark and fruits of *A. marmelos* were found to be effective against the bacteria tested. The zone of inhibition of methanolic extracts of the leaf, bark and fruit (6mm, 2mm and 4mm) were found to be less effective when compared to commercial antibiotic. Where as only the fruit extract (4mm) showed inhibition zone against chloroform extract and the aqueous extract did not show any activity against *Klebsiella pneumoniae*.

The methanolic extracts (13mm, 10mm, 8mm), chloroform extract (10mm, 8mm, 4mm) and aqueous extract (4mm, 2mm, 0) of *A. marmelos* were found to have inhibitory activity against *Proteus mirabilis* and the zone of inhibition coincides with tetracycline. The other bacteria exhibited minimum zone of inhibition against the different extracts as shown in Table 1.

The results indicate that greater activity resides in methanolic leaf extracts compared to chloroform and aqueous extract. This may be due to the antibacterial principles/chemical constituents which are either polar or non polar and can be effectively extracted only through the organic solvent medium (Essawi and Srours, 2000). Mohan et al., (2005) reported that the aqueous, acetone and petroleum ether extract of *A.marmelos* were found to be effective against *B. coagulans, B. subtilis, B. thuringiensis, P.aeruginosa and S.aureus*.

The present findings support the applicability of *A.marmelos* in traditional system for its claimed uses and can be recommended by the scientific community as an accessible alternative to synthetic antibiotics. This study is a preliminary evaluation of antibacterial activity of *A. marmelos* and isolation of the compounds responsible for antibacterial activity would be taken up later.
Table 1: Antibacterial activity of *A. marmelos* against leaf, bark and fruit extracts.

| Bacteria                  | Zone of inhibition (mm) | Tetracycline (mm) |
|---------------------------|-------------------------|-------------------|
|                           | DMSO        | Methanol | Chloroform | Aqueous | Methanol | Chloroform | Aqueous | Methanol | Chloroform | Aqueous | Methanol | Chloroform | Aqueous |
| Klebsiella sps            | -           | 6        | 2          | 4       | -        | -          | 4       | -        | -          | -       | 6        | 2          | 4       | 15       |
| Proteus mirabilis         | -           | 13       | 10         | 8       | 10       | 8          | 4       | 4        | 2          | 2       | 14       |
| Staphylococcus aureus     | -           | 14       | 7          | 11      | 6        | 2          | 5       | 2        | -          | 10      | 22       |
| Salmonella paratyphi A    | -           | 24       | 8          | 10      | 16       | 4          | 7       | -        | -          | -       | 20       |
| Salmonella paratyphi B    | -           | 22       | 7          | 18      | 19       | 5          | 14      | 6        | -          | -       | 20       |
| Bacillus sps              | -           | 6        | 2          | 4       | 2        | 2          | 4       | -        | -          | -       | 11       |
| E. coli                  | -           | 10       | 4          | 8       | 8        | 4          | 2       | 4        | -          | -       | 12       |

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