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# Antibacterial Activity of Extracts from *Aegle marmelos* against Standard Pathogenic Bacterial Strains

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**Abstract:** The principal objective of the present research work was to determine the antibacterial potential of *Aegle marmelos* against seven standard pathogenic bacterial strains. To evaluate antibacterial activity the agar-well diffusion assay was used. All the three extracts showed the highest and significant antibacterial activity against both Gram-negative and Gram-positive bacteria. It is the strain *Bacillus subtilis* that is almost resistant to the three extracts of *Aegle marmelos.*

**Key Words:** Antibacterial activity, Standard pathogenic bacteria, *Aegle marmelos.*

## Introduction

Use of traditional medicine is one of the common practices in India due to their wide pharmacological activities[1]. Developed and developing countries use traditional medicine at the primary health care level. Many currently used drugs are expensive or not readily available and a major set back to their continued usage is the development of resistance. This situation urgently forced scientists for searching new, inexpensive drugs that will be able to act for longer periods before resistance sets in[2].

The main aim of the present research work was to determine the antibacterial potential of *Aegle marmelos* against seven standard pathogenic bacterial strains. This selection was guided by lack or incompleteness of information in literature on antibacterial activity of their extracts. Three extracts of *Aegle marmelos* were tested against both Gram-negative (*Escherichia coli* ATCC 9637, *Klebsiella pneumoniae* MTCC 2405, *Proteus vulgaris* MTCC 0426 and *Micrococcus luteus* MTCC 0439) and Gram-positive (Bacillus *subtilis* MTCC 2274, *Enterococcus faecalis* MTCC 0439 and *Streptococcus faecalis* MTCC 0459) bacteria.

## Experimental[3-5]

### Plant Material:

The leaves of *Aegle marmelos* were collected from A.P. Forest of Endada. The plant was identified in the Department of Botany, Andhra University by comparison with herbarium specimen of the Department. The leaves were dried under shade for 3 weeks and pulverized with a mechanical grinder.

### Preparation of Extracts:

Different extracts were prepared by a modification of the method according to Robinson[3]. 140g of plant powder was macerated with ethanol in a conical flask for 24hrs. Mother liquor (crude ethanol extract) was filtered out and the dried residual plant material was again macerated with hexane (60-80$^0$ C)for 24hrs, filtered and was evaporated to dryness at room temperature (hexane extract). The residual plant material after macerated with hexane was again macerated with methanol (cold) for 48hrs, followed and was evaporated to obtain cold methanol extract. The residual plant material after cold maceration with methanol was again subjected to soxhlet apparatus and exhaustively extracted with methanol (hot) until solvent turned pure and colorless. The methanol extract was filtered and evaporated under reduced pressure using rotavapor (Heidolph, Heizbad, Laborota 4001, Germany, 2000). All extracts were stored in sterilized containers at room temperature until used for antibacterial testing. At the time of testing, the extracts were reconstituted to a concentration of 100mg/ml in Dimethyl Sulphoxide (DMSO).

## Screening of Antibacterial Assay

The antibacterial activity was tested by agar-well diffusion method[4]. Mueller-Hinton broth was applied for growing and diluting the bacterial suspensions. Bacterial strains were grown to exponential phase in Mueller-Hinton at $37^0$ C for 18hrs and adjusted to a final density of $10^8$ CFU/ml by diluting fresh cultures and comparison with Mc Farland density.

For susceptibility testing, 500µl of Mueller-Hinton broth was inoculated into 250 ml of nutrient agar and allowed them to cool under strict aseptic conditions. After the medium was solidified a well was made in petriplates with the help of a sterile metal borer (6mm). 50 µl of each extracts were filled in each well by using adjustable volume digital finn pipette. After that the plates were incubated at $37^0$ C for 24hrs. After proper incubation, antibacterial activity was determined by measuring the diameter of the zone of the inhibition around the well by using Hiantibiotic zone scale – C and the activity was compared with Ciprofloxacine (5 µg/ml) (Multidisk, Axiom, Axiom Laboratories, New Delhi). Simultaneously, control (DMSO) was also maintained with extract. Three replicates were carried out for each extract against each of the test organism.

## Results and Discussion

The antibacterial activity of crude extracts (hexane, cold methanol and hot methanol extracts at a concentration of 100mg/ml) showing positive results. The results of diameters of the zones of inhibition of extracts and antibiotic are presented in Table-1, and were interpreted as sensitive (18 mm), intermediate (14-17 mm) and resistant[5] (<14 mm).

Hexane extract of *Aegle marmelos* was found effective against *Klebsiella pneumoniae* (17 mm), *Micrococcus luteus* (14 mm), *Entercococcus faecalis* (18 mm) and *Streptococcus faecalis* (19 mm). Bacterial species *Escherichia coli* and *Proteus vulgaris* were found to be resistant (IZ = 11-13 mm). Cold methanol extract showed promising result against all tested bacteria (IZ = range between 18-27 mm) except *Klebsiella pneumoniae* and *Micrococcus luteus* were found to be resistant (IZ = 12mm). Hot methanol extract showed high antibacterial activity against all tested Gram-negative and Gram-positive bacteria (IZ= range between 14-29 mm). Where as three extracts of *Aegle marmelos* were did not showed antibacterial activity against *Bacillus subtilis*. Most of the bacterial species showed fairly high degree of sensitivity to the methanol (both cold and hot) extracts of *Aegle marmelos* (IZ = ranging between 17-29 mm). It indicates *Aegle marmelos* may serve as source for compounds with therapeutic potency.

Although this study investigated the preliminary screening of antibacterial activity, the results showed that the extracts from *Aegle marmelos* posses good antibacterial activity, conferring the great potentials of this plant used in folk-lore medicine for the production of bioactive compounds and are useful for rationalizing the use of this medicinal plant in preliminary health care. It is therefore, the above findings recommended the further investigation on isolation and purification of bioactive compounds responsible for the antibacterial activity.

**Table-1. Antibacterial Activity of Extracts from *Aegle marmelos* against Standard Bacterial Strains.**

| Extracts | Test organisms* | | | | | | |
|---|---|---|---|---|---|---|---|
| | E.c | K.n | P.v | M.l | B.s | E.f | S.f |
| Hexane | 13 | 17 | 11 | 14 | -- | 18 | 19 |
| Cold methanol | 18 | 12 | 18 | 12 | -- | 20 | 27 |
| Hot methanol | 19 | 14 | 20 | 16 | -- | 21 | 29 |
| Ciprofloxacine | 15 | 20 | 20 | 22 | 21 | 14 | 22 |

*E.c: *Escherichia coli*, K.n: *Klebsiella pneumoniae*, P.v: *Proteus vulgaris*, M.l: *Micrococcus luteus*, B.s: *Bacillus subtilis*, E.f: *Enterococcus faecalis*, S.f: *Streptococcus faecalis*. Values are the mean of three replicates; --: no inhibition.

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