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# Antimicrobial activity of *Aegle marmelos* (Rutaceae) plant extracts

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**Abstract:** Antimicrobial assay was done for screening purpose for all the selected gram positive and gram negative microorganisms which showed zone of inhibition against test plant extract of *Aegle marmelos* (Rutaceae). The two plant extracts (fruit, leaf) showed antibacterial activity against all the used bacterial strains. Maximum zone of inhibition was observed against *Roultella plantikola* (11 mm). Minimum zone of inhibition was observed against *Pseudomonas aeruginosa* by using fruit extract (7 mm). Inhibition zones of 11 mm and 9 mm were observed by using leaf and fruit extract against *Roultella plantikola.* The plant extract showed maximum zone of inhibition (18mm)activity against fungal strains viz.*Penicillium chrysogenum* and minimum (7mm)against *Candida albicans.* The ability of the plant extracts of *Aegle marmelos* to inhibit growth of bacteria and fungi is an indication of its broad spectrum antimicrobial activity which could be a potential source for development of novel antimicrobial agents.

**Key words**: *Aegle marmelos* (Rutaceae), Antibacterial activity, *Pseudomonas aeruginosa, Roultella plantikola,* Antifungal activity, *Penicillium chrysogenum, Candida albicans.*

## Introduction

Human is using numerous plants and plant derived products to cure and for relief from various physical and mental illness. These plants are also used in traditional Chinese, Ayurveda, Siddha,Unani and Tibetan medicines. Ancient literature such as Rigveda, Yajurveda, Atharvaveda,Charak Samhita and Sushrut Samhita also describes the use of plants for the treatment of various health problems. In last five decades, these plants have been extensively studied by advanced scientific techniques and are reported for various medicinal properties viz, anticancer activity, antibacterial activity, antifungal activity, antidiabetic activity, antioxidant activity, hepatoprotective activity, haemolytic activity, larvicidal activity and anti-inflammatory activity etc. Plants have been utilized as a natural source of medicinal compounds since thousands of years. *A. marmelos* is a native plant of India. *A. marmelos* belongs to Rutaceae family and commonly known as wood apple .In India, *A. marmelos* is grown as a temple garden plant and the leaves are used to pray Lord Shiva. *A. marmelos* is an important medicinal plant with several ethnomedicinal applications in traditional and folk medicinal systems. Traditionally, *A. marmelos* is used in the treatment of diarrhea and dysentery. Leaves of this plant are used to cause infertility/abortion in women. Recently, the plant is screened for its medicinal properties by scientific techniques and reported for various medicinal properties. The present article aims to document the morphology, distribution, phytochemistry and medicinal properties of *A. marmelos* and its future prospects for the further scientific investigation for the development of effective therapeutic. (1) studied on leaf extract of *Aegle Marmelos* on Alloxane induced diabetes and reported that used extract was enough capable to reduce oxidative stress by scavenging lipid peroxidation and enhancing certain Anti oxidant levels which causes lowering of elevated blood glucose level. (2) worked on *Aegle marmelos* leaf extract on alcohol induced liver injury in albino rats and presented data of excellent hepatoprotective effects. Similar result was observed by (3,4) reported the antifungal activity of ethanolic extract of the *Aegle marmelos* leaves including anti-diarrhoeal, and

antimicrobial, activities.(5) evaluated anti fungal activity of essential oils isolated from the leaves of Bael using spore germination assay. (6) evaluated the anticancer potential of folk medicine used in Bangladeshi and used extracts of *Aegle marmelos* for cytotoxic action. Antimicrobial activity in plants is neither a generic character nor a family one but it is the feature of active principles present in the plant. The antibacterial study of the plant extracts of *A. marmelos* demonstrated that folk medicine could be as effective as modern medicine to combat pathogenic microorganisms.

## Material and Methods

### Collection of plant material:

Fresh plants or plant parts were collected from Botany Department University of Rajasthan Jaipur. Fresh plant material was washed under running tap water; air dried, homogenized to fine powder, and stored in tightened light-protected containers**.**

### Preparation of Extract:

Plant parts (leaf, fruit) were washed, air dried and grinded into powder form for preparation of extract. Aqueous plant extract was prepared by macerating powdered plant sample with 50 ml sterile distilled water. The macerate was filtered and filtrate was centrifuged at 8000 rpm for 15 minutes. Supernatant obtained after centrifugation was heat sterilized at 1200 C for 30 minutes. Extract obtained was preserved aseptically. Solvent extracts of plant parts were prepared in 70% methanol using Soxhlet extraction for 72 hours and extract was preserved at 40 C in air tight bottles. They were air dried and dissolved in Dimethyl sulfoxide (DMSO) in 1mg/1ml concentration and stored in refrigerator.

### The reference antibiotic discs:

Antimicrobial activity of the test samples was compared with antibiotics known to be effective against the test bacteria in their established doses. Amoxycilin was used for bacteria and Ketoconozole for fungi as reference for comparison.

### Fungal Media (Potato dextrose sugar):

200 gm of potato slices were boiled with distilled water. The potato infusion was used as water source of media preparation. 20 gm of dextrose was mixed with potato infusion. 20 gm of agar was added as a solidifying agent. These constituents were mixed and autoclaved. The solidified plates were bored with 6mm diameter cork borer. The plates with wells were used for antifungal studies.

### Test Fungal Strains:

The test fungal strains namely *Aspergillus niger* MTCC 282, *Penicillium chrysogenum* MTCC 171, *Candida albicans* MTCC 183, *Fusarium solani* MTCC 9667 were used to study antifungal potential. They were collected from, Durlabhji Hospital Jaipur.

### Antifungal activity assessment:

*Invitro* antimicrobial activity was screened by using Potato Dextrose agar (PDA) using agar well diffusion method [7]. Fungal strains were activated in Potato Dextrose broth (PDB) and incubated for 24 hours. 0.05ml of inoculum was uniformly spread on agar plates. Ethanolic, methanolic and aqueous extracts were introduced in agar wells in concentration of 25PPM, 50PPM, 75PPM and 100PPM. Control experiment was carried out with Glucanazole. Antifungal potential was then determined on the basis of diameter of zone of inhibition.

### Media preparation for nutrient agar media:

The bacterial cultures of gram positive and gram negative bacteria were maintained on nutrient agar medium (agar 15 gm, beef extract 3 gm, sodium chloride 5 gm and peptone 5 gm, in one liter distilled water). These micro-organisms were allowed to grow at 35°C-37°C temperature. A fresh inoculum of test microorganism in saline solution was prepared from a freshly grown agar slant before every antibacterial assay

by adjusting the concentration of micro-organism in the medium using spectronic-20 colorimeter (Bausch and Lomb) set at 630 nm, transmittance used bacteria was 40%.

**Preparation of inoculum:**

The antibacterial activity was tested by Whatman filter paper disc method. Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring loopful bacterial cells from the stock cultures to Erlenmeyer flask of nutrient broth that were incubated with agitation for 24 hrs at 37°C.

**Test microorganisms:**

The bacterial strains studied are *Roultella plantikola*, *Pseudomonas aeurioginosa, Agrobacterium tumifacian* and *Bacillus subtillis*. The fungal strains studied are *Aspergillus niger, Prenicillium chrysogenum, Candida albicans* and *Fusarium solani*. Microorganisms were maintained at 4 °C on nutrient agar slants. These test organisms were clinical isolates obtained from patients diagnosed for having bacterial infections and procured from the Durlabhji Hospital Jaipur.

**Antimicrobial screening**

The filter paper disc method was used for screening the extract for antibacterial activity. Standard size Whatman filter paper disc (6.0 mm diameter) were sterilized in an oven at 140°C for one hour, saturated with three plant extracts such as root, stem, leaf different streptomycin and air dried at room temperature to remove any residual solvent that might interfere with the determination of activity. The discs were then placed on the surface of sterilized nutrient agar medium that had been inoculated with test bacteria (using saline solution) and air dried to remove the surface moisture. The thickness of the agar medium was kept equal in all the pertriplates and the standard disc (streptomycin) was used as a control. Before incubation, the petriplates were placed for one hour in a cold room (5°C) to allow the diffusion of the compounds from the disc into the medium. Plates were incubated at 37°C for 20-24 hours after which the zone of inhibition or depressed growth could be easily measured. All the experiments were done in five replicates and the activity index was calculated for each of these.

$$\text{Activity index (A.I.)} = \frac{\text{Inhibition zone of the sample}}{\text{Inhibition zone of the standard}}$$

## Results and Discussion

The antibacterial assay done for screening purpose of selected gram positive and gram negative microorganisms showed zone of inhibition against test plant extract. Among these test micro organisms *Roultella plantikola* are the most susceptible to methanol extract of *Aegle marmelos.*The results of the antibacterial activity are presented in Table-1 and of antifungal activity in Table 2. All two Plants extracts (leaf,fruit) showed antibacterial activity against all used bacteria. Maximum zone of inhibition was observed against *Roultella plantikola* (11 mm). Minimum zone of inhibition was observed against *Pseudomonas aeruginosa* by using fruit extract (7 mm). Inhibition zones of 11 mm and 9 mm were observed by using leaf and fruit extract against *Roultella plantikola.* The plant extracts also showed activity against test fungal organism vix. Penicillium chrysogenum which was most susceptible to ethanolic fruit extract by forming inhibition zone of 18mm and lowest 7mm was shown by *Candida albicans*. This fungal strain was most susceptible to methanolic fruit extract by forming inhibition zone of 17mm and lowest zone of inhibition i.e 9mm for leaf extract was shown by penicillium species. The Candida albicans was most susceptible to aqueous fruit extract by forming inhibition zone of 14mm and lowest was shown by Aspergillus niger i.e 9mm in fruit extract.The antimicrobial principles and their distribution have been extensively reviewed by (8) followed by (9) who surveyed 174 plants belonging to 157 families of vascular plants. Antimicrobial activity of various plant parts has also been observed by several workers viz; *Begonia malabarica* (10) *Azadirachta indica*, (11and 12) Similarly antibacterial activity of different plant parts has also been observed by many workers viz.,Bauhinia purpurea (13),Eucalyptus gilli(14).

The vital role of flavonoids in defence against microorganism, due to antimicrobial activity has already been discussed by (8). Flavonoids such as quercetin, isorhamnetin and kaempferol have antimicrobial activity. Shyamala Gouri and K.Vasantha 2010 have carried out antimicrobial studies in *Syzygium cumini* leaf extracts and have proved that the methanolic extracts were more potent than the aqueous extracts. (15) have successfully carried out the phytochemical evaluation and antimicrobial activity for determination of bioactive compounds from the leaves of *Aegle marmelos*. Thus, it can be concluded that antimicrobial activity in plants is neither a generic character nor a family one but it is the feature of active principles present in the plant.

**Table 1: Antibacterial Activity of *Aegle Marmelos***

| **Bacterial Strain** | **Standard** | **Zone of inhibition** | | | | | |
|---|---|---|---|---|---|---|---|
| | | **Methanol** | | **Petrolium ether** | | **chloroform** | |
| | **Amoxycilin** | | | | | | |
| | | **Fruit** | **Leaf** | **Fruit** | **Leaf** | **Fruit** | **Leaf** |
| *Roultella plantikola* | 35 mm | 9 mm | 11mm | 9 mm | 10 mm | 12 mm | 13 mm |
| *Pseudomonas aeruginosa* | 14 mm | 7 mm | 8 mm | 9 mm | 10 mm | 10mm | 11 mm |
| *Bacillus subtillis* | 7 mm | 7 mm | 8 mm | 8mm | 9 mm | 10mm | 9 mm |
| *Agrobacterium tumifacian* | 11 mm | -- | -- | 6 mm | 7 mm | 7 mm | 7 mm |

**Table 2: Zone of Inhibition of Fruit, leaf and Stem of Methanol, Ethanol and Aqueous Extract with test fungal cultures and control drug**

| **Fungal strain** | **Zone of inhibition** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Methanolic** | | **Ethanolic** | | **Aqueous** | | **Control** |
| | Leaf | Fruit | Leaf | Fruit | Leaf | Fruit | Glucanazole |
| *Panicillium chrysogenum* | 9 | 15 | 12 | 18 | 9 | 9 | 9 |
| *Fusarium solani* | 11 | 12 | 12 | - | 13 | 10 | 7 |
| *Aspergillus nigar* | 9 | 13 | 13 | 15 | 12 | 9 | 17 |
| *Candida albicans* | 10 | 17 | 7 | 13 | 14 | 12 | 10 |

This research work states that the presence of phytoconstituents in the chloroform extract of *Aegle marmelos* were responsible for its antimicrobial activity. This study concludes that the crude extract of chloroform has potent antibacterial against clinical isolates of bacteria. Traditional herbal medicines must perforce be granted the benefits of modern science and technology to serve further global needs. The drugs derived from herbs may have the possibility of use in medicine because of their antimicrobial activity. It is therefore; from above findings recommended the further investigation on isolation and purification of bioactive compounds responsible for the antimicrobial activity should be carried out in order to unveil the various medicinal potentialities present in the plant.

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