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# Evaluation of Growth Inhibitory Activities of Extracts of Whole Plant of *Emblica officinalis* Gaertn. on Gram-Positive and Gram-Negative Bacteria

**Research article**

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**Abstract**

The present study focussed on the quantitative estimation of *in vitro* antibacterial activity of aqueous extracts of fruits, stem, root, leaves and seeds of *Emblica officinalis* against Gram-positive versus Gram-negative bacteria. The study employed *Staphylococcus aureus* and *Escherichia coli*, respectively, as the test organisms and the assay was carried out by using agar well diffusion method. *S. aureus* was more susceptible to the extracts of fruits, stem, leaves and seeds of *Emblica*. The aqueous extract of leaves maximally inhibited the growth of *S. aureus* at its minimum concentration (0.1 µg) when compared to *E. coli* (1.0 µg). MIC for fruit and seed extracts were 1.5 and 3.75 µg and 1.5 and 5.0 µg, respectively, for *S. aureus* and *E. coli*. The aqueous extract of stem had the least activity where more quantities of the extract were required to inhibit the growth of both *S. aureus* (15.0 µg) and *E. coli* (20.0 µg). Both the test organisms were resistant to the aqueous extracts of root of *Emblica officinalis*. It could be attributed from the present study that the inhibitory effect of *Emblica officinalis* is more against Gram-positive than Gram-negative bacteria. The leaf of *Emblica* is more inhibitory to the growth of bacteria than fruits, seeds or stem.

**Key words:** *Emblica officinalis*, antibacterial assay, well diffusion, aqueous extract, *S. aureus*, *E. coli*

## Introduction:

Medicinal plants contain wide array of bioactive compounds or phytochemicals that are extensively used as antimicrobial agents and chemotherapeutic agents. The bioactive compounds in these plants are effective therapeutics, devoid of any toxic or other adverse side effects (1). 'Green medicine', obtained from medicinal plants, has high curative potential and is being advocated now a day as dependable therapeutic aid than the costly synthetic drugs (2). *Emblica officinalis* Gaertn. is an Indian medicinal plant, extensively used in Ayurvedic and Unani systems of medicine (3). In spite of the slow recovery process, *Emblica officinalis* is more popular because of their low resistance in microorganisms and lesser side effects (4).

*Emblica officinals* or Indian gooseberry, (Synonym *Phyllanthus emblica* Linn.; commonly known as Amla, Nelli and Amalaki) belongs to the family Euphorbiaceae (5). The fruits of *E. officinalis* are widely used in many of the Ayurvedic medicinal preparations such as kashayam, lehyam and arishtam. The species is native to India, though it grows well in tropical and sub-tropical regions of

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Pakistan, Uzbekistan, Srilanka, South East Asia, China and Malaysia (6). *E. officinalis* is one among the three constituents of tribhala, a rejuvenating medicinal formula, popular for its effectiveness in the treatment of intestinal disorders, fever, cough and eye diseases (5). Two varieties of *Emblica* have been reported worldwide, the wild ones with smaller fruits and the cultivated ones or the 'Banarasi' variety, with larger fruits (7). The plant *E. officinalis* is known for its digestion power, improving liver functions and is liver protective (2). It is a very good purifier of blood which in turn improves the health of liver by keeping the toxins and infections away. It has anti-viral, anti-bacterial, anti-cancer, anti-allergic, and anti-mutagenic properties (2). Fruits are reported as anti-inflammatory, analgesic and anti-pyretic (8, 9). Amla is the prime ingredient in most of the herbal medicines for skin ailments.

*E. officinalis* is a small to medium sized deciduous tree, 8-15 meters tall, having thin light grey bark that exfoliates in small thin irregular flakes. The leaves are simple, sub-sessile, closely set along the branchlets and light green in colour, having the appearance of pinnate leaves. Flowers are greenish yellow, in axillary fascicles, unisexual, actinomorphic and trimerous. Male flowers are numerous on short slender pedicel. The female flowers are few, sub-sessile, pistillate, hypogynous and the ovary three celled (2). Fruits are globose, fleshy, pale yellow in colour, with six obscure vertical furrows enclosing six trigonous seeds.

Extracts of various parts of *E. officinalis* such as leaves, stem, root, seeds and fruits have been widely used in treatment of various diseases. Several of the bioactive compounds in *E. officinalis* have been identified which includes the flavonoids (quercetin), ascorbic acid, gallic acid, alkaloids (phyllantine, phyllantidine), hydrolysable tannins (emblicanin A and B), punigluconin and pedunculagin (10, 1, 7). The fruits of *E. officinalis* are rich in anti oxidants, contributed by the tannins and flavonoids contained in them (1). They are also the natural source of vitamin C. In this study attempts were made to assay the *in vitro* antibacterial activity of extracts of whole plant parts (fruits, seed, stem, root and leaves) of *E. officinalis* against *E coli* and *S. aureus*, thereby trying to compare their bactericidal activity against Gram-positive and Gram-negative bacteria.

## Materials and Methods

### Collection of Sample

Fruits, seeds, stem, roots and leaves of small variety of *Emblica* were collected from Kottayam and Pathanamthitta districts, Kerala, India.

### Bacterial Stains Used

Bacterial cultures used in this study were obtained from the culture collections of School of Biosciences, Mahatma Gandhi University, Kottayam, Kerala, India. Bacterial cultures namely *Staphylococcus aureus* and *E. coli* were included in this study as representatives of Gram-positive and Gram-negative group of bacteria. The bacterial strains were maintained on Nutrient Agar (HiMedia, India) plates or slants and were stored at 4 °C before use.

### Surface Cleaning and Sterilization of the Samples

In this study the various samples were surface sterilized following the modified procedure of Aneja (11). The samples namely fruits, stem, root, seeds and leaves were washed in running tap water for 10 minutes followed by detergent wash in 10 % Extran (Merck, India) for 10 minutes. The samples were then rinsed with distilled water and cut into small pieces using a sterile scalpel. The cut pieces were rinsed in 70 % ethanol for 30 seconds and washed again in distilled water till the ethanol smell completely

diminished. These were spread out in clean trays for oven drying.

**Preparation of Extracts**

A comparative assay of extracts of fruits, stem, root, seeds and leaves of *Emblica* was carried out in this study. The cleaned and cut samples of *E. officinalis* were oven dried at 60 °C, continuously, for 7 days. The dried samples were dried and powdered using a clean grinder. The powder was stored in air sealed containers at room temperature before extraction. A fixed weight of 30 gm of each powdered material was weighed out in aseptic condition and was extracted with distilled water using the Soxhlet Apparatus at a temperature of 100 °C, respectively. The Soxhlet extraction was carried out continuously for 8 hrs. Each extract was concentrated by evaporation and made up to a final volume of 20 ml. The extracts were stored at room temperature, in sterile screw capped containers, till use.

**Determination of Antimicrobial Activity**
**Preparation of Bacterial Suspension**

Pure isolated colonies of the *E. coli* and *S. aureus* were inoculated into 1 % peptone water and incubated at 37 °C for 48 h and were used as inoculum for lawn culture on Mueller Hinton Agar (HiMedia, India).

**Antimicrobial Assay by Well Diffusion Assay**

A comparative, rather, quantitative assay was carried out using the suitable dilutions of aqueous extracts of fruits, seeds, stem, root and leaves of *E. officinalis*. *In vitro*, quantitative, antibacterial activity assay was carried out by well diffusion assay (12). Mueller-Hinton Agar (MHA) was used as base medium for screening of antibacterial activity. About 15 to 20 ml of MHA medium was poured in sterile Petri dishes and allowed to solidify. In this method, wells of diameter 6 mm were dug out using a sterile cork borer in solidified MHA medium. Using sterile cotton swab, 0.2 ml of 24 hr old cultures of *S. aureus* and *E. coli* were inoculated evenly on to the surface of the MHA plate to make a lawn culture. Dilutions were made from the crude extracts that were used for the well diffusion assay. From the dilutions 5, 10, 15, 20, 25 and 30 µl each of the various extracts were added to the respectively labelled wells. The plates were incubated at 37 °C for 24 hrs and observed for zone of inhibition of growth around the wells.

**Zone Analysis and Determination of MIC**

The wells were checked for zone of clearance around it. The antibacterial activity of the extracts was assayed by measuring the diameter of zone of inhibition around the wells. The minimum concentration of the respective extract inhibiting the growth of *E. coli* and *S. aureus*, around the wells, were taken as the minimum inhibitory concentration or MIC.

**Study, Observations and Results**

*Emblica officinalis* Gaertn. is a plant of great medicinal importance and has been extensively used as antipyretic, analgesic and immunomodulatory agent. It has antimicrobial activity; potentially against various bacterial, viral and fungal pathogens. Early studies have reported the identification of bioactive compounds in *E. officinalis* (10,1). The potent antibacterial activity *E. officinalis* against *E. coli*, *K. pneumonia*, *K. ozaenae*, *Proteus mirabilis*, *P. aeruginosa*, *S. paratyphi* A and *S. paratyphi* B and *Serratia marcescens* has been reported previously (13). The bioactive compounds in the entire plant of *E. officinalis*, from aqueous extracts of stem, leaf, fruit and seed were evaluated for antibacterial activity against pathogenic bacterial strains belonging to Gram-positive and Gram-negative bacteria, represented by *S. aureus* and *E. coli*,

respectively. Here the *in vitro* antibacterial activity of extracts of *E. officinalis* were assayed alone, and also in comparison with the extracts from various plant parts.

**Table 1. Dilutions Prepared from Crude Extracts of Fruits, Seeds, Leaves and Stem of *Emblica officinalis* for MIC assay**

| Extract Used | Dilutions Prepared From the Crude Extracts | | |
|---|---|---|---|
| | Dilution Used in µl (Extract/Solvent) | Quantity at which Zone was obtained (µl) | Average Diameter of Zone of Inhibition in mm |
| Aqueous Extract of Fruits | 25/100 | 15 | 9 |
| | 10/100 | 15 | 8 |
| Aqueous Extract of Stem | Crude extract (no dilution) | 20 | 10 |
| | Crude extract (No dilution) | 15 | 9 |
| Aqueous Extract of Leaves | 20/100 | 10 | 12 |
| | 5/100 | 5 | 8 |
| Aqueous Extract of Seeds | 20/100 | 25 | 9 |
| | 10/100 | 15 | 9 |

Diameter of the Well = 6 mm

In this study the bioactive compounds present in the fruits, seeds, stem, roots and leaves of *E. officinalis* were extracted out in water from which dilutions were prepared and aliquots of 5, 10, 15, 20, 25 and 30 µl of the dilutions were assayed quantitatively by well diffusion method (Table 1). Both the test bacteria viz. *E. coli* and *S. aureus* were sensitive to the aqueous extracts of the plant parts such as fruit, seed, stem and leaves (Table 2). The root extracts, on the contrary, produced no zone of inhibition of growth (0 mm) of bacteria around the wells (unpublished). The root extract was not used in the study further since it was not inhibitory to the growth of any of the bacterial species used in the assay.

**Table 2. Comparative Antibacterial Activity of Fruits, Seeds, Leaves and Stem Extracts of *Emblica officinalis* Against Gram-Positive and Gram-Negative Bacteria**

| Extract Used | Quantitative Assay and MIC | |
|---|---|---|
| | Organism Used | MIC (µg of Original Crude Extract) |
| Aqueous Extract of Fruits | *E. coli* | 3.75 |
| | *S. aureus* | 1.5 |
| Aqueous Extract of Stem | *E. coli* | 20.0 |
| | *S. aureus* | 15.0 |
| Aqueous Extract of Leaves | *E. coli* | 1.0 |
| | *S. aureus* | 0.1 |
| Aqueous Extract of Seeds | *E. coli* | 5.0 |
| | *S. aureus* | 1.5 |

Diameter of the Well = 6 mm

In this study, the lowest MIC was recorded for the leaf extracts for both *E. coli* and *S. aureus*, having inhibition zones around wells containing 1.0 µg and 0.1 µg of the extracts, respectively (Table 2). Stem had the least activity as indicated by the MIC of 20 and 15 µg, respectively, for *E. coli* and *S. aureus*. Fruit and seed extracts were similar in their activity against *E. coli* and *S. aureus*. The MIC for fruit and seed extracts against *E. coli* were 3.75 and 5 µg, respectively. For the fruit and seed extract the MIC values against *E. coli* and *S. aureus* were 1.5 µg each (Table 2).

**Discussion**

The present study evaluated the bioactive compounds in the entire plant of *Emblica officinalis*, from aqueous extracts of stem, leaves, fruits and seeds for antibacterial activity against *E. coli* (Gram-negative bacteria) and *S. aureus* (Gram-positive bacteria). The antibacterial activity of extracts of *E. officinalis* bark, leaves and fruits has been assayed previously by Dhale and Mogle (14), where they observed significant bactericidal activity of alcohol extracts of *Emblica* leaves against *S. aureus* at 20 mg/ml concentrations, followed by *B. subtilis*, *E. coli* and *P. aeruginosa*. The least activity of bark has been reportedly against *E. coli* whereas the maximum was recorded against *S. aureus* (10 mg/ml) for petroleum ether extracts. In their study the fruit extracts also exhibited superior activity against *S. aureus*, at a concentration of 20 mg/ml. The current study also revealed similar results where the entire extracts of *Emblica*, except of root, inhibited the growth of *S. aureus* more, than that of *E. coli*. The bioactive compounds in leaves had maximal activity against *S. aureus* (Table 2) because the leaf extract inhibited the bacterial growth at the most minimum concentration of 0.1 µg. The decoctions extracted out of stem of *E. officinalis* had the least activity against both Gram-positive *S. aureus* and Gram-negative *E. coli* though *S. aureus* was more sensitive than *E. coli* (Table 2).

Dhale and Mogle (14), in a similar study, has also reported the superior activity of solvent extracts of leaves, fruits and bark of *E. officinalis* against the Gram-positive *S. aureus*, than the Gram-negative *P. aeruginosa* and *E. coli*. Patil *et al* (1) has observed that aqueous extract of fruits of *E. officinalis* were antibacterial to the maximum against *S. aureus* whereas the acetone and methanol extracts of fruits had maximal antibacterial activity against *E. coli* and *K. pneumonia*, respectively. The current study revealed that the aqueous extracts of fruits and seeds were equally inhibitory to the growth of *S. aureus*, an inhibitory concentration of 1.5 µg each, even though the fruit extract inhibited the growth of *E. coli* more effectively than the seed extract (Table 2).

Venkanna and Estari (15) have reported lowest MIC values for methanolic fractions of *Phyllanthus emblica* against *S. aureus* (0.08 ml/g) and for *P. aeruginosa* (0.08 ml/g). The antibacterial activity of *Emblica* against Gram-positive and Gram-negative bacteria by Reghu and Ravindra (16) has revealed it to be inhibitorier to *S. aureus* (MIC of 0.261 for methanol extract, 0.432 for chloroform extract and 0.512 for diethyl ether extract) than to *K. pneumonia* (0.342 for methanol extract, 0.542 for chloroform extract and 0.612 for diethyl extract). It could also be concluded from the present study that irrespective of the plant part used, the extracts were superiorly inhibiting the growth of *S. aureus* than to *E. coli*

Saeed and Tariq (17) has observed effective activity of *E. officinalis* against a range of bacteria including *S. aureus*, *S. hemolyticus*, *S. saprophyticus*, *Micrococcus varians*, *Bacillus subtilis* and also against the yeast *Candida albicans*, the maximum of which was exhibited against *S. typhi*. Khanna and Nag (18) have also observed that *E. officinalis* is

active against *S. aureus*, *E. coli*, *M. tuberculosis*, *S. typhosa* and *Candida albicans*. Synergistic antibacterial activity of *E. officinalis* seeds against *S. aureus* in combination with *Nymphaea odorata* stamens have been reported earlier (19). It is apparent from the quantitative assay in this study that the aqueous extracts of *E. officinalis* had maximal antibacterial activity, as evidenced by the minimum inhibitory concentrations (MIC) against *S. aureus, i.e.,* against Gram-positive bacteria than Gram-negative *E. coli*. Similar observation has also been reported by Saeed and Tariq (17), Venkanna and Estari (15) and Reghu and Ravindra (16). However the superiority of *E. officinalis* against Gram-positive bacteria has hitherto not been reported.

## Conclusion

The bactericidal activity of *E. officinalis* has been attributed to the bioactive compounds present in *E. officinalis* namely flavonoids, phenols, saponins, and tannins such as emblicanin A and B. The present study is a preliminary level investigation on the comparative analysis of the antibacterial activity of the fruits fruits, seeds, stem, root and leaves of *E. officinalis* Gaertn. against Gram-positive and Gram-negative bacteria. The experimental evidences revealed that the aqueous extracts of all the samples except root of *E. officinalis* had more antibacterial activity against Gram-positive bacteria than Gram-negative bacteria, producing wider zones of inhibition of growth around *S. aureus* than *E. coli*. The root extract neither inhibited the growth of *S. aureus* nor *E. coli*. These results have to be further confirmed by extending the studies to more number of Gram-positive and Gram-negative bacterial strains. The bioactive compounds have to be assayed individually, at the same time, selectively, for their activity against Gram-positive and Gram-negative bacteria.

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