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EVALUATION OF *IN VITRO* ANTIBACTERIAL ACTIVITY WHOLE PLANT (FRUITS, SEEDS, STEM, LEAVES AND ROOTS) OF *EMBLICA OFFICINALIS* GAERTN.

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Abstract

In this study the antibacterial activity of *Emblica officinalis* Gaertn. was evaluated against *E. coli, Salmonella typhi, Salmonella paratyphi, Staphylococcus aureus, Bacillus* sp., *Proteus* sp., *Pseudomonas* sp. and *Klebsiella* sp. The antibacterial activity was determined in aqueous extracts of fruits, seed, stem, leaves and root of *Emblica officinalis*. Aqueous extracts of all parts effectively inhibited the growth of the test bacteria except for the root extract. All the eight strains of bacteria were resistant to the aqueous root extract. *Staphylococcus aureus* exhibited the maximal antibacterial activity against the fruit extract of *Emblica* followed by leaf, seed and stem extracts. Minimum activity of the extracts was observed against *Salmonella paratyphi*. The bactericidal activities of extracts of Emblica officinalis were comparatively assayed against major antibiotics acting in different modes against bacteria.

Keywords: Antibacterial, aqueous extract, disc diffusion, *Emblica officinalis*, whole plant

1. Introduction

Herbs and spices constitute the most important part of human diet. In addition to flavor, they are famous for their nutritive, medicinal and preservative value[1]. In spite of the slow recovery process the therapeutic aid of *Emblica officinalis* is more popular now-a-days because of their low resistance in microorganisms and lesser side effects[2]. *Emblica officinalis* Gaertn., a medicinal plant native of India, is extensively used in Ayurvedic and Unani systems of medicine[3].

Emblica officinalis Gaertn. (synonym *Phyllanthus emblica* Linn.) enjoys a hallowed position in Ayurveda, an indigenous system of medicine in India. *E. officinalis* or Indian gooseberry, commonly known as, Amla, Nelli and Amalaki, belongs to the family Euphorbiaceae. It is a medium sized tree, native to India, but also grows in tropical and sub-tropical regions of Pakistan, Uzbekistan, Sri Lanka, South East Asia, China and Malaysia. *E. officinalis* is one of the three constituents of 'Tribhala', a rejuvenating medicinal formula, popular for its effectiveness in the treatment of intestinal disorders, fever, cough and eye diseases[4]. Two varieties of *E. officinalis* are reported worldwide, the wild ones with smaller fruits and the cultivated ones, also known as 'Banarasi', with larger fruits[5].

The fruits of *Emblica*, naturally rich in vitamin C, are used in many of the Ayurvedic medicinal preparations. Fruits are globose, fleshy, pale yellow in colour and is proved to be effective against diabetes, cough, asthma, bronchitis, dyspepsia, colitis, flatulence, hyper acidity, peptic ulcer, skin diseases, leprosy, inflammations, anaemia, hepatopathy, jaundice, diarrhoea, dysentery, haemorrhage, leucorrhoea, cardiac disorders, intermittent fevers and greying of hair and is given to cancer[6]. The plant is known for digestion power, improving liver functions and is liver protective. It is also a very good blood purifier 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[6]. Fruits are reported as anti-inflammatory, analgesic, antidiarrhoeal and anti-pyretic[7,8].

Several of the chemical constituents of *E. officinalis* have been identified. The major principles in *Emblica officinalis*, active against microbes, include flavonoids (quercetin), ascorbic acid, gallic acid, alkaloids (phyllantine, phyllantidine) and hydrolysable tannins (emblicanin A and B)[9]. Fruits of *E. officinalis* are the richest source of Vitamin C, tannin and flavonoids, most of which are antioxidant in action. Extracts of various plant parts such as leaves, stem, root, seeds and fruits have been widely used in treatment of

various diseases. In this study attempts were made to assay the *in vitro* antibacterial activity of fruits, seed, stem, leaf and root extracts of *E. officinalis* against eight species of pathogenic bacteria.

2. Materials and methods

2.1. Collection of Sample

Fruits of small variety of *Emblica* were collected from the local markets of Ettumanoor, Amalagiri and Athirampuzha, Kottayam District, Kerala, India. Leaves, stem, and roots of the small variety of *Emblica* were collected from Amalagiri (Kottayam District) and Konni (Pathanamthitta District), Kerala, India.

2.2. 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 *E. coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Salmonella paratyphi*, *Bacillus* sp., *Proteus* sp., *Klebsiella* sp. and *Pseudomonas* sp. were included in this study. The bacterial strains were maintained on Nutrient Agar plates or slants and were stored at 4 °C before use.

2.3. Surface Cleaning and Sterilization of the Samples

In this study the various samples were surface sterilized following the modified procedure of Aneja[10]. The samples namely *Emblica* fruits, seeds, stem, roots and leaves were washed in running tap water for 10 minutes followed by detergent wash in 10 % Extran (Merck) for 10 minutes. The samples were rinsed with distilled water. After this the samples were made into small pieces using a sterile scalpel. The cut samples were rinsed in 70 % ethanol for 30 seconds and washed again in distilled water till the ethanol smell was completely diminished. The samples were spread out in clean trays for oven drying.

2.4. Preparation of Extracts

A comparative assay of whole plant of *Emblica* was carried out using the fruit, seed, stem, root and leaves of the small variety. Fresh samples 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. 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.

2.5. Determination of Antimicrobial Activity

2.5.1. Antibiotic Discs

Antibiotic discs were purchased from HiMedia, Mumbai, India. The antibiotics used in the present study include Penicillin, Erythromycin, Polymyxin B, Tetracycline, Rifampicin, and Bacitracin. The discs were stored at 4 °C before use.

2.5.2. Sensitivity Discs

Sterile sensitivity discs of 6 mm diameter were purchased from HiMedia, Mumbai, India. The discs were stored at 4 °C before use. The discs were soaked in the extracts for 10 minutes and allowed to dry. The dried discs were used for disc diffusion assay.

2.5.3. Preparation of Bacterial Suspension

Pure isolated colonies of the bacteria were inoculated into 1 % peptone water and incubated at 37 °C for 48 h and were used as inoculum for lawn culture on MHA.

2.5.4. Antimicrobial Assay by Disc diffusion Method

Mueller-Hinton Agar (MHA) was used as base medium for screening of antibacterial activity. About 15 to 20 ml of MHA medium (HiMedia, Mumbai, India) was poured in sterile Petri dishes and allowed to solidify. Using sterile cotton swab, 0.2 ml of 24 hr old culture was inoculated evenly on to the surface of MHA to make a lawn culture. The antibiotic discs were used as such in the assay. For analysing the antibacterial activity of *Emblica* extracts, the discs carrying the respective extract were impregnated on the seeded agar plate (2 discs per plate). Discs carrying sterile distilled water were used as controls. The experiments were performed in duplicates. The plates were incubated at 37 °C for 24 hrs and observed for zone of inhibition of growth around the discs. The antibacterial activity of the extracts against each bacterial species was assayed by measuring the diameter of zone of inhibition to the nearest mm.

3. Results and Discussion

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. In this study the *in vitro* antibacterial activity of extracts of fruit, seed, stem, leaves and root of *Emblica* were assayed alone, and also in comparison with each other and with commonly used the antibiotics. Pathogenic bacterial strains belonging to eight different species of bacteria namely

Bacillus, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Salmonella paratyphi*, *Pseudomonas* and *Klebsiella* were used as the test organisms.

The plant, *Emblica officinalis*, has potent antibacterial activity against *E. coli*, *K. pneumonia*, *K. ozaenae*, *Proteus mirabilis*, *P. aeruginosa*, *S. paratyphi* A and *S. paratyphi* B and *Serratia marcescens*[11]. In the present study aqueous extracts of the entire plant - parts such as fruit, seed, stem, leaves and root - were evaluated for antibacterial activity. Roots extracts were ineffective against all the bacterial species investigated producing no zone of growth inhibition around the sensitivity discs. Aqueous extracts of all other plant parts considerably inhibited the growth of bacteria in varied levels except for *S. typhi* which was resistant to the stem extract (Table 1). Maximal antibacterial activity was obtained for *S. aureus* for the fruit extract with zone of growth inhibition of diameter 19 mm, followed by leaf, seed and stem extracts in the descending order (Table 1). Minimum response was recorded against *S. paratyphi* for all the extracts.

The results of former studies on the antibacterial activity of extracts of bark, leaves and fruits of *E. officinalis* were in concordance with the results of the present study where antibacterial activity against *S. aureus* was observed for extracts of bark, leaves and fruits[12]. Concordant results were also observed by others where they 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)[13]. Studies on the antibacterial activity of *P. emblica* L. against gram-positive and gram-negative bacteria employing *S. aureus* and *K. pneumonia* as the representative organisms has revealed that the *P. emblica* extracts were more inhibiting the growth of *S.* aureus[14]. A parallel study by the authors employing ethanolic, aqueous and acetone extracts of fruits of *Emblica officinalis* against *Staphylococcus aureus* and *E. coli* revealed that the extracts were antibacterial against gram positive than gram negative group of bacteria[15].

Table 1. Antibacterial Assay of Aqueous Extract of Whole Plant of *Emblica officinalis* Against Pathogenic Bacteria by Disc Diffusion Method

Bacterial Strains Used in the Study	**Aqueous Extract of *Emblica officinalis* (Average Diameter of Zone of Inhibition of Growth in mm)**				
	Root	**Leaves**	**Stem**	**Fruits**	**Seeds**
E. coli	0	8	7	14	10
S. typhi	0	10	0	13	8
S. aureus	0	16	10	19	14
Bacillus sp.	0	11	7	18	12
S. paratyphi	0	8	7	13	8
Proteus sp.	0	12	7	14	11
Klebsiella sp.	0	10	8	15	11
Pseudomonas sp.	0	12	7	16	11

Diameter of the disc = 6 mm

Saeed and Tariq[16] also observed effective activity of *Emblica* against *S. aureus.* Patil *et* al[9] has observed maximal antibacterial activity for methanol extract of fruits against *S. aureus* whereas the acetone and aqueous extracts inhibited the growth of *E. coli* and *K. Pneumonia* maximally. Synergistic antibacterial activity of seeds of *E. officinalis* against *S. aureus* in combination with *Nymphaea odorata* stamens have been reported earlier[17]. Earlier *S. typhosa, Candida albicans S. aureus, E. coli* and *M. tuberculosis* have been reported to be sensitive to *E. officinalis*[18].

The bactericidal activity of the fruit, seed, stem and leaf extracts of *Emblica officinalis* were compared with that of the commonly used antibiotics having varied mode of action. None of the antibiotics were superior to the *Emblica* extracts against *Pseudomonas* (Table 2). Other than the stem extract, the bioactive compounds in fruit, seed and leaves were more inhibitory to the growth of *Proteus* than the test antibiotics except for penicillin (Tables 1 and 2). The aqueous extract of fruit, though inferior to erythromycin, proved to be a potent antibacterial agent against most of the bacterial strains of the study, particularly *E. coli, S. typhi, S. paratyphi, Klebsiella, Proteus* and *Pseudomonas*. Growth of *S. aureus* was inhibited by tetracycline, erythromycin and rifampicin more effectively than the fruit extracts of *Emblica* (Tables 1 and 2). *Bacillus* also exhibited concordant results, where the seed, stem and leaf extracts, with the exception of fruits, inferiorly inhibited the growth of the bacteria. *Klebsiella* is a common opportunistic pathogen in patients suffering from tuberculosis. In this study the aqueous extracts of *Emblica* exhibited fairly good antibacterial activity against *Klebsiella* which could be effectively exploited in the treatment of pneumonia in tuberculosis patients.

Table 2. *In vitro* Antibacterial Activity of Antibiotics Against the Test Bacteria

Bacterial Strains Used in the Study	**Antibiotics Used for *In vitro* Antibacterial Assay (Diameter of Zone of Inhibition of Growth in mm)**					
	Penicillin	**Tetracycline**	**Polymyxin B**	**Erythromycin**	**Rifampicin**	**Bacitracin**
E. coli	0	13.5	11	21	14	0
S. aureus	15	24	9.5	21	26	11
S. typhi	0	20	10	13	12.5	11
Bacillus sp.	28	27	10	13.6	19.5	11.5
S. paratyphi	12	21	11	10.5	8	0
Klebsiella sp.	0	19	10.5	0	0	0
Proteus sp.	16	0	0	0	10.5	0
Pseudomonas sp.	0	0	13	0	0	0

Diameter of the disc = 6 mm

Emblica, especially fruits, has been extensively used in majority of the ayurvedic medicinal preparations like kashayam, arishtam and lehyam where water the decoctions, extracts or processed concentrates of fruits are employed. The present study has undoubtedly proved the potential of the water

soluble bioactive compounds in fruit, seed, stem and leaves of *Emblica officinalis* in their bactericidal action against pathogenic bacteria. Besides, the study has observed the superiority of extracts of *Emblica* against major antibacterial antibiotics. The bactericidal activity of *E. officinalis* could be attributed to the bioactive compounds present in *E. officinalis* namely flavonoids, phenols, saponins, and tannins such as emblicanin A and B which could be effectively employed as effective chemotherapeutic agents in antibacterial treatment and therapy[19,20]. Besides, these compounds could be a good alternative for antibiotics especially in cases of hypersensitivity reactions against penicillin.

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