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Comparative Antibacterial Activity of Fruit Extracts of *Emblica officinalis* Gaertn Against Gram-Positive versus Gram-Negative Bacteria

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ABSTRACT

Emblica officinalis Gaertn. is the most celebrated plant in the Indian traditional system of unani and ayurvedic medicine. The present study was carried out to assess quantitatively, the *in vitro* antibacterial activity of aqueous, ethanolic and acetone extracts of fruits of *Emblica officinalis* against Gram-positive versus Gram-negative bacteria employing *Staphylococcus aureus* and *E. coli*, respectively. All the extracts exhibited significant antibacterial activity, more against *S. aureus* than *E. coli*. Among the extracts, acetone extract maximally inhibited the growth of *S. aureus* and *E. coli* at minimum concentration of the extract (0.1 µg and 1.0 µg, respectively). MIC for ethanol and aqueous extracts were 0.3 and 1.0 µg and 1.5 and 3.75µg, respectively, for *S. aureus* and *E. coli*. It is concluded from the present study that *E. officinalis* is more inhibitory to gram-positive than the gram-negative bacteria.

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

INTRODUCTION

The use of medicinal plants as a therapeutic aid for alleviating human ailments can be traced back to over five millennia. Present day antibacterial therapy is mainly focussed on the administration of antibiotics and synthetic drugs. The indiscriminate use of antibacterials, particularly the antibiotics, has resulted in the emergence of resistant microbial strains and accumulation of metabolites in tissues and fluids, finally culminating in toxicity and adverse side effects[1]. The increased demand for effective, and more safe therapeutics has resulted in the renewed interest for use of natural products in improving health and fitness. Medicinal plants are rich sources of bioactive compounds such as alkaloids, flavonoids and phenolic compounds[2]. Herbs and spices have been an important constituent in human diet since time immemorial. Besides boosting up flavour, they are also known for their preservative and medicinal properties[3].

Emblica officinalis Gaertn. (Synonym *Phyllanthus emblica* Linn., commonly known as Nelli, Amla, Amalaki or Indian gooseberry) is a small and medium sized deciduous tree found throughout India, Sri Lanka and Malaca, the fruits of which are highly valued in traditional medicine[4,5]. Dried fruits of amla are used in the treatment of haemorrhage, diarrhoea and dysentery in Unani system of medicine[6]. The fruits of *E. officinalis* has diuretic, adaptogenic[7], hepatoprotective[8,9], antitumor[10], hypocholestrolemic[11], antioxidant[12,13], and antiulcerogenic[14] activities. *E. officinalis* is also reported to have antiviral, antibacterial[15,16,17,18,19,20], antifungal[21,22], antihelminthic[23] and anti-inflammatory properties[24]. Several of the bioactive compounds in *E. officinalis* such as flavonoids (quercetin),

ascorbic acid, gallic acid, alkaloids (phyllantine, phyllantidine), hydrolysable tannins (emblicanin A and B), punigluconin and pedunculagin[25,26] have been identified. The antioxidant activity of *E. officinalis* has been attributed to the presence of tannins such as emblicannin A and emblicannin B[13].

In view of its medicinal and antimicrobial properties the present study aimed at assaying quantitatively the antibacterial activity of extracts of fruits of *E. officinalis* against *Staphylococcus aureus* and *E. coli* and thereby compare differentially the antibacterial action on gram positive and gram negative bacteria.

MATERIALS AND METHODS

Collection of Sample

Fruits of small variety of *E. officinalis* were collected from the local markets of Ettumanoor, Amalagiri and Athirampuzha, Kottayam District, Kerala, India.

Bacterial Stains

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

The samples were surface sterilized following the modified procedure of Aneja[27]. The fruits of *E. officinalis* were washed in running tap water for 10 minutes followed by detergent wash in 10 % Extran (Merck, India) for 10 minutes. The fruits were then rinsed with distilled water. The fruits were made 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 diminished completely. These were spread out in clean trays for oven drying.

Preparation of Extracts

A comparative assay of fruit extracts of *Emblica* was carried out in this study. The cleaned and cut fruits of *E. officinalis* were oven dried at 60 °C, continuously, for 7 days. The dried samples were 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, ethanol and acetone, using the Sohxlet Apparatus at temperatures of 100, 70 and 60 °C, respectively. The 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 test bacteria 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, ethanolic and acetone extracts of fruits *E. officinalis*. *In vitro*, quantitative, antibacterial activity assay was carried out by well diffusion assay[28]. 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 extract as given in Table 1 and 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 and the minimum concentration of the respective extract inhibiting the growth of bacteria around the wells were taken as the minimum inhibitory concentration or MIC. The antibacterial activity of the extracts was quantitatively assayed by measuring the diameter of zone of inhibition around the wells bearing the minimum concentration of the extract, to the nearest mm.

RESULTS AND DISCUSSION

In this study the bioactive compounds in the fruit extracts of *E. officinalis* were evaluated for *in vitro* antibacterial activity. The antibacterial activities of fruit extracts were comparatively assayed against Gram-positive and Gram-negative pathogenic bacteria. *S. aureus*, to which the extracts produced maximal zone of inhibition in the qualitative assay (unpublished), was selected to represent the Gram-positive group of bacteria. *E. coli*, a commonly encountered foodborne pathogen and a coliform bacteria was also considerably affected by the extracts of *E. officinalis* in the qualitative assay (unpublished) and was selected to represent the Gram-negative group of bacteria. 5, 10, 15, 20, 25 µl of suitable dilutions of the aqueous, ethanol and acetone extracts of fruits of *E. officinalis* were assayed quantitatively by well diffusion method (Table 1).

In the present study the extracts of fruits in acetone, ethanol and water inhibited the growth of *S. aureus* to the maximum (Tables 1 and 2). This was in concordance with the results of Saeed and Tariq[20]. Patil *et al*[6], on the contrary, has observed earlier that acetone fruit extract has maximal antibacterial activity against *E. coli* whereas methanol and aqueous extracts were antibacterial to the maximum

Table 1: Dilutions prepared from Crude extracts of Fruit Extracts 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 Inhibitionin mm
Aqueous Extract of Fruit	25/100	15	9
	10/100	15	8
AcetoneExtract of Fruit	10/100	10	8
	2/100	5	10
EthanolExtract of Fruit	10/100	10	9
	2/100	15	10

Diameter of the Well = 6 mm

Table 2: Comparative Antibacterial Activity of Fruit 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 Fruit	*E. coli*	3.75
	S. aureus	1.5
AcetoneExtract of Fruit	*E. coli*	1.0
	S. aureus	0.1
EthanolExtract of Fruit	*E. coli*	1.0
	S. aureus	0.3

against *S. aureus* and *K. pneumonia*, respectively. The well diffusion assay in this study revealed 0.1, 0.3 and 1.5 µg, respectively, of acetone, ethanol and water extracts, to be the minimum concentration required for inhibiting the visual growth of *S. aureus* (Table 2). Hence this was determined as the MIC for these extracts. Acetone extract was the most effective against *S. aureus* when quantitatively analysed. For *E. coli* the MICs for the acetone, ethanol and water extracts were 1, 1 and 3.75 µg, respectively (Table 2). A similar study on the inhibitory action of extracts of *E. officinalis* in a quantitative level has been observed previously were the methanolic fractions of *Phyllanthus emblica* has shown lowest MIC values against *S. aureus* (0.08 ml/g) and *P. aeruginosa* (0.08 ml/g)[29]. It could be concluded from the present study also that the fruit extracts of *E. officinalis* were inhibiting the growth of *S. aureus* more than *E. coli*.

A previous study on the antibacterial activity of *P. emblica* L. against Gram-positive and Gram-negative bacteria employing *S. aureus* and *K. pneumonia* had revealed the extracts to be more inhibitory to the growth of 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)[30]. Similar results has been reported by Dhale and Mogle after studying the antibacterial activity of solvent extracts of leaves, fruits and bark of *Emblica officinalis* against Gram -positive and negative bacteria[31].

In the present study, it was evident that acetone, ethanol and water extracts of fruits of *E. officinalis* has maximal antibacterial activity, evidenced by the minimum inhibitory concentrations (MIC) against *S. aureus* as has also been reported earlier[20,29,30]. The extracts were inhibiting the growth of *E. coli* at higher concentrations than for *S. aureus*. It could be attributed from the present study that the bioactive compounds in fruits of *E. officinalis* are more inhibitory to the growth of Gram-positive than Gram-negative bacteria. This has to be further confirmed by extending the studies to more number of Gram positive and Gram negative bacteria. Further the bioactive compounds isolated and identified from *E. officinalis* such as quercetin, ascorbic acid, gallic acid, phyllantine, phyllantidine, emblicanin A, emblicanin B, punigluconin and pedunculagin have to be analyzed individually or in combinations for its differential antibacterial action on gram positive and gram negative bacteria for effective application in chemotherapy.

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