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Study of Antibacterial Activity of Emblica Officinalis (Amla) Fruit Extract against Bacterial Pathogens

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Abstract
The present study was carried out to antibacterial activity of hexane, chloroform and methanol crude extracts of Emblica officinalis fruits were tested against seven bacterial strains using agar-well diffusion method. Hexane, chloroform and methanol extracts were obtained using the Soxhlet apparatus. To measure the zone of the inhibition values, two concentrations (50 mg/ml and 100 mg/ml) of the extracts used, Enterococcus faecalis was the most susceptible bacteria. Hexane extract was less potent when compared to chloroform and hexane extracts. This study demonstrates that Emblica officinalis fruits have a potent anti-bacterial activity. The results supported the ethnomedicinal use of fruits of Emblica officinalis for the treatment of various bacterial related diseases.

Keywords
Emblica officinalis, Hexane, chloroform and methanol extracts, Enterococcus faecalis and medicinal plant

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INTRODUCTION
Plants have provided mankind with herbal remedies for many diseases for many centuries and even today. They continue to play a major role in primary healthcare as therapeutic remedies in developing countries. In India, herbal medicines have been the basis of treatment and cure for various diseases in traditional methods practiced such as Ayurveda, Unani and Sidha. *Emblica officinalis* (EO) enjoys a hallowed position in Ayurveda- an Indian indigenous system of medicine. According to believe in ancient Indian mythology, it is the first tree to be created in the universe. It belongs to family Euphorbiaceae. It is also named as Amla, *Phyllanthus Emblica or* Indian gooseberry and 'Nelli' in tamil. The species is native to India and also grows in tropical and subtropical regions including Pakistan, Uzbekistan, Srilanka, South East Asia, China and Malaysia (1).

It is widely grown in all over India. Their fruits are edible and are pale yellowish and fleshy in nature. It is the highest source of natural vitamin C [3]. The fruits of EO are widely used in the Ayurveda and are believed to increase defence against diseases. It has its beneficial role in cancer, diabetics, liver treatment, heart trouble, ulcer, anaemia and various other diseases (2). Similarly, it has application as antioxidant, immunomodulatory, antipyretic, analgesic, cytoprotective, antitussive and gastroprotective. Additionally, it is useful in memory enhancing, ophthalmic disorders and lowering

cholesterol level. It is also helpful in neutralizing snake venom and as an antimicrobial (3).

The wide use of *Emblica officinalis* for various purposes prompted us to select for screening of antibacterial activity. The present study aimed at identification of antibacterial ability of plant. *In vitro* studies on this plant strongly help in the identification of the plant constituents with antimicrobial activity capable of exerting protective effects against human bacterial pathogens (4). This study involves the *in vitro* antimicrobial activity of this plant extracts against some gram positive and gram-negative pathogenic microorganisms and to ascertain the chemical constituents that may be present in plant extracts. The wide use of *Emblica officinalis* fruits for various purposes prompted us to select for screening of antibacterial activity.

MATERIALS AND METHODS

Selection of fruit

In the present work, *Emblica officinalis* (Fig 1) fruit were screened for its potent antibacterial activity.

Fig 1: *Emblica officinalis (*Amla)

Collection of fruits

The fruits of *Emblica officinalis* were collected from Siddha Medical College, Munchirai. Fruits were dried under shade for one month and grounded with the help of an electrical grinder

Preparation of the extracts

Powdered fruit material was extracted using Soxhlet extractor each for 6 to 8 hours with three different solvents viz. hexane, chloroform and methanol (12). Hundred grams of powdered material was exhaustively extracted with hexane (60-800 C) in Soxhlet apparatus (5). The hexane extract was filtered and evaporated under reduced pressure. The extracted fruit material was then air dried, repacked in the Soxhlet apparatus and exhaustively extracted with chloroform and methanol successively (6). Chloroform and methanol extracts were filtered and evaporated under reduced pressure using Rota-vapor (Heidolph, Heizbad, Laborota 4001, Germany 2002). The extracts were dissolved in dimethylsulphoxide (DMSO) to reach a final concentrations 50 mg/ml and 100 mg/ml, which kept in refrigerator till used.

Screening of antibacterial activity

The test bacterial strains such a, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Micrococcus luteus*, *Bacillus subtilis*, *Enterococcus faecalis* and *Streprtococcus faecalis* were obtained from stock culture in the Department of Microbiology, Sri Paramakalyani College, Alwarkurichi, Tirunelveli, India (7). The test bacterial strains were prepared on Nutrient agar medium and incubated for 24 hrs at 37°C. Then the cultures were stored at 4°C till test as stock cultures. Active cultures for experiments were prepared by transferring a loop full of cells from the stock cultures to test tubes of Nutrient broth that were incubated in shaker at 37°C for 24 hrs that were used as the inoculums (8).

Agar well diffusion assay

For susceptibility testing, the agar well diffusion assay was performed (9). About 100 µl of inoculated nutrient broth was inoculated into 100ml of nutrient agar and care was taken in ensure proper homogenization and poured into petri dishes and allowed them to cool strict aseptic conditions. After medium was solidified a well was made with the help of sterile metal borer (6mm). 50µl of each extract was filled in well by adjustable digital finn pipette. After proper incubation, antibacterial assay was determined by measuring the diameter of the zone of the inhibition (ZOI) around the well by using Hi Antibiotic Zone Scale-C (Hi Media Laboratories Pvt.

Limited) and the activity was compared with Ciprofloxacine (10 µg). Simultaneously, control DMSO was also maintained without extract. Triplicates were carried out for each extract against each of the test organism (10).

Result and discussion

The results of antibacterial activity of fruits of *Emblica officinalis* are presented in the Table 1. The values of ZOI of hexane, chloroform and methanol extracts were expressed in millimeters. The three extracts were exhibited inhibition zones against all tested bacteria, except *Bacillus subtilis*, it did not show any inhibition zone to the hexane extract. Fruits of *Emblica officinalis* expressed ZOI values were dose dependant, the values were increased when the concentration of extract was increased.

Table 1. Inhibition zones expressed by solvent extracts of *Emblica officinalis.*

Extracts/	EC		KP		PV		ML		BS		EF		SF	
Antibiotic	***50**	**100**	**50**	**100**	**50**	**100**	**50**	**100**	**50**	**100**	**50**	**100**	**50**	**100**
Hexane	16	18	15	17	10	11	14	16	--	--	18	20	15	19
Chloroform	28	30	29	30	20	22	23	25	22	24	32	34	24	26
Methanol	30	32	33	36	22	24	25	27	28	31	34	36	24	26
Ciprofloxacin (10µg)	15		20		20		22		17		11		16	
DMSO	--		--		--		--		--		--		--	

***: all concentrations are mg/ml and values expressed in mm**

--: no activity

EC: *Escherichia coli*

KP: *Klebsiella pneumoniae*

PV: *Proteus vulgaris*

ML: *Micrococcus luteus*

BS: *Bacillus subtilis*

EF: *Enterococcus faecalis*

SF: *Streprtococcus faecalis*

Hexane extract of *Emblica officinalis* fruits were found the most susceptible to *Enterococcus faecalis* followed by *Streprtococcus faecalis*, *Escherichia coli, Klebsiella pneumoniae, Micrococcus luteus* and less activity against *Proteus vulgaris*. Chloroform extract of *Emblica officinalis* fruits were found sensitive against all tested bacteria with the maximum ZOI against *Enterococcus faecalis*.*Proteus vulgaris* was showed intermediate antibacterial activity and *Streprtococcus faecalis* was susceptible only at high concentration of chloroform extract(11). Methanol extract of *Emblica officinalis* fruits showed promising results of antibacterial activity against all tested bacteria with ZOI range from 22-36 mm and the highest antibacterial activity was found against *Klebsiella pneumoniae* and *Enterococcus faecalis*. Comparatively other tested bacteria Gram-negative bacteria, *Escherichia coli,Klebsiella pneumoniae* and Gram-positive bacteria, *Enterococcus faecalis* were the most susceptible bacteria to the both methanol and chloroform extracts(12). Ciprofloxacin (10 µg) was used as positive control and showed ZOI values against all tested bacteria and these values were lower than above three solvent extracts of *Emblica officinalis* fruits against *Escherichia coli*, *Enterococcus faecalis* and *Streprtococcus faecalis*. Whereas methanol extract of *Emblica officinalis* found ZOI values were more than 10 µg of Ciprofloxacine. Hence it indicated that fruits of *Emblica officinalis* had broad spectrum of antibacterial activity against all tested bacteria. DMSO, a negative control, it did not show any ZOI indicated that it is not interfering information of zone of the inhibition (13). The excellent activity of *Emblica officinalis* against *Escherichia coli*, *Klebsiella pneumoniae*,*Proteus vulgaris*, *Micrococcus luteus*, *Bacillus subtilis*, *Enterococcus faecalis* and *Streprtococcus faecalis* shows a very good potential to treat infectious diseases caused by bacteria. The possible reason for the antibacterial activity of *Emblica officinalis* might be due to the tannins present in its fruits.

The fruits have 28% of the total tannins distributed in the fruits. The fruits contain tannins Emblicanin A and B, which have antimicrobial activities (14). The results of the present study are similar to Saeed and Tariq (15) those reported that *Emblica officinalis* possess potent antibacterial activity against *Escherichia coli*, *K. ozaenae*, *K. pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa, S. paratyphi A, S. paratyphi B* and *Serratia marcescens*. Therefore, these results clearly support the usefulness of *Emblica officinalis* fruits as a broad-spectrum anti-

microbial agent against a wide range of microbes (16).

CONCLUSION

The present results therefore offer a scientific basis for traditional use of solvent extracts of *Emblica officinalis* fruits could be a possible source to obtain new and effective herbal medicine to treat infectious diseases caused by multi-drug resistant strains of bacteria. In fact, its promising influence on *Enterococcus faecalis* clearly suggests the *Emblica officinalis* fruits as a potent antimicrobial agent. However, it is necessary to determine the toxicity of the active constituents, their side effects and pharmaco-kinetic properties.

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