SCREENING OF ANTIBACTERIAL ACTIVITY OF EMBLICA OFFICINALIS L. FRUITS.

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Summary

In vitro 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*, Antibacterial activity, solvent extracts, Agar-well diffusion assay.

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* is a deciduous tree of the Euphorbiaceae family, widely growing in different parts of India. It is known for its edible fruit of the same name Indian gooseberry, with pale yellowish fleshy globose fruits. The fruits of *Emblica officinalis* are widely consumed raw, cooked or pickled, but they are also principle constituents of Ayurvedic preparations (1).

Amla as it is known in India is also used to treat hair disorders like premature falling and graying. It has been used to treat various diseases through Ayurvedic medicine therapy dating back to many centuries. It is also known to have the highest percentage of natural vitamin C present in any fruit (2). It is rich in quercetin, phyllambic compound, gallic acid, pectin and others (3). The effective compounds are flavonoids, tannins, vitamin C and are found in maximum concentration and are anti-oxidant in action (4). Amla is a component of Triphala and an important rasayana called Chyawanprash in ayurvedic medicine (5). *Emblica officinalis* famously known for its hepatoprotective and antioxidant activities (6, 7). Amla extract posses anticancer, antisclerotic, lipid lowering, hepatoprotective, anti-HIV activities (8, 9) and inhibits thioacetamide-induced oxidative stress and hyper proliferation in rat liver (10). The alcoholic and aqueous extract of amla has powerful retarding effect on Ochratoxin haemolysis on RBC (11). The wide use of *Emblica officinalis* fruits for various purposes prompted us to select for screening of antibacterial activity.

Materials and Methods

Collection of fruits

The fruits of *Emblica officinalis* were collected from Kambalakonda forest, Visakhapatnam. Fruits were dried under shade for one month and grounded with the help of an electrical grinder. Voucher specimen of the plant with fruit was dried and deposited at the herbarium of Department of Botany, Andhra University, Visakhapatnam.

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 (60-80^0 C) in Soxhlet apparatus. 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. 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 dimethyl-sulphoxide (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 organisms, *Escherichia coli* (ATCC 9637), *Klebsiella pneumoniae* (MTCC 2405), *Proteus vulgaris* (MTCC 0426), *Micrococcus luteus* (MTCC 1538), *Bacillus subtilis* (MTCC 2274), *Enterococcus faecalis* (MTCC 0439) and *Streprtococcus faecalis* (MTCC 0459) were procured from the Microbial type culture collection (MTCC), IMTECH, Chandigarah, India.

Nutrient broth was applied for growing and diluting the microorganism suspensions. Bacterial strains were grown in exponential phase in nutrient broth at 40^{0}C for 18 hours and adjusted to a final density of 10^8 CFU/ml by diluting fresh cultures and comparing with McFarland density. For susceptibility testing, the agar well diffusion assay was performed (13). 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 petridishes 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 HiAntibiotic ZoneScale-C (HiMedia 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.

Results 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/ Antibiotic	EC		KP		PV		ML		BS		EF		SF	
	*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. 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. 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 showed any ZOI indicated that it is not interfering in formation of zone of the inhibition.

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* posses 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.

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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