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Research Article

ANTIBACTERIAL ACTIVITIES AGAINST DIFFERENT SELECTIVE MIC EXTRACTION OF AMLA

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Abstract

Present investigation focused on antimicrobial potential of aqueous extracts and solvent extracts (ethanolic, methanolic, ethyl acetate and chloroform) of Emblica officinalis (amla) against selected bacterial strains. The agar well diffusion technique and disc diffusion methods were employed. Amla fruit protein extracts exhibited antibacterial activity against Staphylococcus aureus, Bacillus cereus, Bacillus subtilis and Escherichia coli.

Keywords: Salinity, Growth, Activity, Antioxidant enzymes, Chili *(Capsicum annuum* L.).

Introduction

Herbs and spices are the most important part of human diet. In addition to boosting flavor, herbs and spices are also known for their preservative and medicinal value (DeSouza *et al.*, 2005; Saeed & Tariq, 2006), which forms one of the oldest sciences. It is only in recent years that modern science has started paying attention to the properties of spices (Chaudhry & Tariq, 2006). Because of the concern about the side effects of conventional medicine, the use of natural products as an alternative to conventional treatment in healing and treatment of various diseases has been on the rise in the last few decades (Ansari *et al.*, 2006).

The fruit of *Emblica officinalis* commonly known as amla is highly valued in traditional Indian medicine (Scartezzini *et al.*, 2006). In Unani medicine the dried fruits of amla are used to treat haemorrhage, diarrhoea and dysentery (Parrotta, 2001). In addition, the fruit of *E. officinalis* is diuretic (Anon., 2006), adaptogenic (Rege *et al*., 1999), hepatoprotective (Jeena *et al*., 1999; Jose & Kuttan, 2000), antitumor (Jose *et al*., 2001), hypocholestrolemic (Kim *et al*., 2005), antioxidant (Bhattacharya *et al*., 1999) and antiulcerogenic (Sairam *et al*., 2002). The fruits are also reported to be anti-inflammatory (Sharma *et al*., 2003), analgestic and antipyretic. Several constituents of *E. officinalis* fruit has been identified, mainly the hydrolysable tannins, emblicanin A, emblicanin B, punigluconin and pedunculagin (Perianayagam *et al*., 2005). Emblicanin A and B have been proposed to be the active constituents with significant *In vitro* antioxidant activity (Ghosal *et al*., 1996).

Earlier studies have demonstrated potent antimicrobial properties of *E. officinalis* (Ahmed *et al*., 1998) and it is used as antiviral for cold and flu. In the respiratory infections, it has an antibiotic activity against a wide range of bacteria, used traditionally in the treatment of lungs (Chopra & Simon, 2000). It also has shown antifungal activity *In vitro* (Dutta *et al*., 1998).

The present study was therefore conducted to evaluate the antibacterial potential of aqueous extracts and solvent extracts of *Emblica officinalis* against selected bacteria strains viz., *Escherichia. coli* ATCC 632, *Klebsiella aerogenes* ATCC 9621, *Klebsiella pneumoniae* ATCC 31488, *Salmonella typhi* ATCC 13311, *Pseudomonas aeruginosa* ATCC 13525, *Bacillus cereus* ATCC 128263, *Bacillus subtilis* ATCC 128263, *Staphylococus aureus* ATCC 12600, *Streptococcus faecium* ATCC 8043, *Listeria seeligeri* ATCC 35967.

Materials and Methods

Maintenance of isolates: Bacterial strains were maintained on tryptone soy agar (TSA) (Oxoid).

Preparation of aqueous extracts: Aqueous extracts of *E. officinalis was* prepared by macerating 25 gm plant powder in 50 ml sterile distilled water with the help of pestle and mortar. Macerate was filtered through four layers of muslin cloth and then filtrate was centrifuged at 8,000 rpm for 15 minutes at room temperature. Supernatant was filtered through Whatman No. 1 filter paper and heat sterilized at 120ºc for 30 minutes. The extract was preserved aseptically in a brown bottle at 4ºc until further use.

Preparation of solvent extracts: 25 gm of shade dried powder of plant materials were filled separately in the thimble and extracted successively with 150 ml each of methanol, ethanol, ethyl acetate and chloroform using a Soxhlet extractor for 48 h. All the extracts were concentrated using rotary flash evaporator. After complete solvent evaporation, each of these solvent extract was weighed and preserved at 4ºc in airtight bottles until further use. 1 gm of each solvent residue was dissolved in 10 ml of respective solvents were used as the test extracts for antimicrobial activity assay.

Screening of antibacterial activity

Media: The molten Mueller-Hinton agar (MHA) (Merck) was inoculated with 100 µl of the inoculam (1 × 108 cfu/ml) and poured in to the petriplate (Hi- media).

Antimicrobial assay: The antimicrobial assay was performed by two methods viz. agar disc diffusion method for aqueous extract and agar well diffusion method for solvent extract. For agar disc diffusion method the disc (0.7 cm) (Hi- Media) was saturated with 100 µl of the test compound, allowed to dry and was introduced on the upper layer of the seeded agar plate. For agar well diffusion method, a well was prepared in the plates with the help of a cork- borer (0.85 cm). 100 µl of the test compound was introduced in to the well.

Incubation: The inoculated plates were incubated at 35-37ºc for 24 hours and zone

of inhibition was measured to the nearest millimeter (mm).

Statistical analysis: The observations of each parameter were mathematically averaged and then statistically analysed.

Results and Discussion

Among the *Phyllanthus emblica* fruit extracts, maximum zone of inhibition of bacterial growth was recorded (Ghosal *et. al.*, 1996) at 100 mg/ml concentration at pH 8.0 followed by *Klebsiella Pneumoniae* ATCC 31488 (ZOI 42 mm) and *Streptococcus* faecium ATCC 8043 (ZOI 42 mm) in ethanol solution extract.

Minimum zone of inhibition of bacterial growth was recorded at 20 mg/ml concentration all the treatments at pH 7.0 in aqueous solution by *Pseudomonas aeruginosa* ATCC 13525. There were found a regular increase in zone of inhibitions with the advancement of concentrations

Table: 1. Antibacterial activity of *Phyllanthus* ***emblica*** **fruit proteins** extract at pH 8.

S.No.	Bacterial Strains	Ethanol Solution		Methanol Solution		Chloroform Solution		Aqueous Solution	
		ZOI (mm)	MBC (mg/ml)	ZOI (mm)	MBC (mg/ml)	ZOI (mm)	MBC (mg/ml)	ZOI (mm)	MBC (mg/ml)
1.	*E. coli* ATCC 632	11	20	14	20	15	20	9.5	20
2.	*Klebsiella aerogenes* ATCC 9621	17	20	14.5	20	15	20	12	20
3.	*Klebsiella Pneumoniae* ATCC 31488	15	20	12	20	14	20	09	20
4.	*Salmonella typhi* ATCC 13311	12	20	11.5	20	12	20	11	20
5.	*Pseudomonas aeruginosa* ATCC 13525	11	20	10	20	08	20	07	20
6.	*Bacillus Cereus* ATCC 128263	10	20	10.5	20	11	20	09	20
7.	*Bacillus subtilis* ATCC 128263	11	20	11	20	12	20	10	20
8.	*Staphylococus aureus* ATCC 12600	12	20	11	20	11	20	9.5	20
9.	*Streptococcus* faecium ATCC 8043	16	20	15	20	16	20	11	20
10.	*Listeria seeligeri*	13	20	14	20	12	20	10	20

in all bacterial strains (Ahmed *et.al*., 1998). Zone of inhibition of test microorganisms *E. coli* ATCC 632, *Klebsiella aerogenes* ATCC 9621, *Klebsiella pneumoniae* ATCC 31488, *Salmonella typhi* ATCC 13311, *Pseudomonas aeruginosa* ATCC 13525, *Bacillus cereus* ATCC 128263, *Bacillus subtilis* ATCC 128263, *Staphylococus aureus ATCC* 12600, *Streptococcus faecium* ATCC 8043 and *Listeria seeligeri* ATCC 35967, were recorded 8.0, 12.5, 12, 7.5, 8.0, 07, 9.5, 12.5 and 9.0 mm at 20 mg/ml concentration all the treatments at pH 6.0, respectively in ethanol solution. The corresponding values for methanol solution at pH 6.0 zone of inhibition were 10, 11, 12, 08, 04, 7.5, 7.0, 7.5, 11 and 08 mm for test organisms test microorganisms *E. coli* ATCC 632, *Klebsiella aerogenes* ATCC9621, *Klebsiella pneumoniae* ATCC 31488, *Salmonella typhi* ATCC 13311, *Pseudomonas aeruginosa* ATCC 13525, *Bacillus Cereus* ATCC 128263, *Bacillus subtilis* ATCC 128263, *Staphylococus aureus ATCC* 12600, *Streptococcus faecium* ATCC 8043 and *Listeria seeligeri* ATCC 35967, respectively. The zone of inhibition were observed in chloroform and aqueous solutions 11, 12, 11, 07, 5.5, 07, 09, 08, 12, 09 mm and 07, 10, 10.5, 07, 03, 06, 06, 07, 8.5, 07 mm at pH 6 in 20mg/ml concentration using by test microorganisms *E. coli* ATCC 632, *Klebsiella aerogenes* ATCC 9621, *Klebsiella pneumoniae* ATCC 31488, *Salmonella typhi* ATCC 13311, *Pseudomonas aeruginosa* ATCC 13525, *Bacillus Cereus* ATCC 128263, *Bacillus subtilis* ATCC 128263, *Staphylococus aureus ATCC* 12600, *Streptococcus faecium* ATCC 8043 and *Listeria seeligeri* ATCC 35967, respectively.

The present study has revealed the importance of natural products to control antibiotic resistant bacteria which are a threat to human health and can serve as an important platform for the development of inexpensive, safe and effective medicines.

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