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ANTIBACTERIAL PROPERTIES OF CONTENTS OF TRIPHALA: A TRADITIONAL INDIAN HERBAL PREPARATION

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ABSTRACT

Triphala, a combination of three tropical fruits preparation comprised of equal parts of *Terminalia chebula, Emblica officinalis, and Terminalia bellirica*, which gently promotes internal detoxification of all conditions of stagnation and improving digestion and assimilation. The aqueous, acetone, ethanol and methanol extracts of these plant's fruits were prepared and antibacterial activities were tested by disc and well diffusion method against enteric bacterial pathogens such as *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus vulgaris, Staphylococcus epidermidis, Salmonella typhi, Salmonella typhimurium, Enterobacter aerogenes.* The extracts of *E. officinalis, T. bellirica and T. chebula* were found to antibacterial to all bacterial pathogen tested. Thus it can be suggested that daily intake of triphala may control enteric infections in human being.

KEY WORDS: Triphala, antibacterial properties, *Terminalia chebula, Emblica officinalis, Terminalia bellirica*)

INTRODUCTION

Triphala, a combination of three tropical fruits preparation comprised of equal parts of *Terminalia chebula, Emblica officinalis, and Terminalia bellirica*, which gently promotes internal detoxification of all conditions of stagnation and improving digestion and assimilation. In India these medicinal plants have an important therapeutic and antimicrobial aid in various ailments. Today, there is widespread interest in drugs derived from plants, which leads to the screening of several medicinal plants for their potential antimicrobial activity (Hussain et al, 2007). Efforts are thus directed to identify plant products used in the treatment of various diseases, which have broad–spectrum antimicrobial properties and no ill effects (Tambekar and Saratkar, 2005, Chattopadhyay and Bhattacharyya, 2007).

Multiple microbial resistances among Gram-negative microorganisms have been a long term and well-recognized problem with enteric infections (Saeed and Tariq, 2007) The fruit of E. officinalis, is potent antimicrobial (Ahmed *et al*., 1998), antifungal (Dutta et al., 1998) and used in hemorrhage, diarrhea and dysentery (Parrotta, 2001). T. chebula exhibited antibacterial activity against a number of bacterial species (Ahmed *et al*., 1998), inhibit Helicobacter pylori (Malckzadeh, *et al*, 2001), methicillin-resistant Staphylococcus aureus (Sato, *et al*, 1997), Salmonella typhi (Rani and Khullar, 2004), intestinal bacteria (Kim, *et al*., 2006) and antifungal against a number of dermatophytes and yeasts (Vonshak, *et al*, 2003). T. bellerica fruit has digestive, laxative, anti allergic, antihelminthic antimalerial, antifungal (Valsaraj *et al*, 1997) and antibacterial (Aqil *et al*, 2005) properties. The antimicrobial activities of all these three plants components of Triphala were scanty, particularly on enteric bacterial pathogens. Therefore, an attempt has been made to study the antimicrobial activity of the crude and organic extracts of its dry fruit on certain enteric bacterial pathogens. In this study, these three selected plants of Melghat forest, Maharashtra State, India, were screened for their potential antibacterial activities.

MATERIALS AND METHODS

Preparation of crude extracts

Table 1: Antibacterial activity of extracts of *Emblica officinalis, Terminalia bellerica and Terminalia chebula* against bacterial pathogens (Zone of growth inhibition in mm)

Bacterial Pathogens	Ampicillin	*Negative control*				*Plant Fruits*	Aqueous extract					Acetone extract					Ethanol extract					Methanol extract				
		Aqueous	Acetone	Ethanol	Methanol		100%	50%	20%	10%	5%	100%	50%	20%	10%	5%	100%	50%	20%	10%	5%	100%	50%	20%	10%	5%
Escherchia coli (MTCC 452)	*18*	-	-	*11*	*11*	*Eo*	16	-	-	-	-	20	16	13	-	-	16	14	-	-	-	20	17	-	-	-
						Tb	13	-	-	-	-	23	20	18	-	-	19	15	-	-	-	24	22	19	17	14
						Tc	14	14	-	-	-	19	16	14	12		17	16	13	-	-	18	16	-	-	-
Staphylococcus aureus (MTCC 87)	16	-	12	-	12	*Eo*	14	-	-	-	-	15	-	-	-	-	16	-	-	-	-	15	-	-	-	-
						Tb	15	-	-	-	-	24	23	21	19	15	13	-	-	-	-	25	23	21	19	16
						Tc	16	-	-	-	-	19	18	16	14	11	17	14	-	-	-	16	14	-	-	-
Enterobacter aerogenes (MTCC 111)	16	-	11	11	14	*Eo*	16	-	-	-	-	20	15	13	-	-	17	14	-	-	-	21	18	16	14	13
						Tb	16	-	-	-	-	26	23	20	17	13	19	16	-	-	-	21	19	17	14	12
						Tc	15	-	-	-	-	15	13	-	-	-	23	21	19	17		22	19	15	-	-
Pseudomonas aeruginosa (MTCC 424)	10	-	12	14	-	*Eo*	18	16	12	-	-	23	18	15	-	-	16	12	-	-	-	19	14	-	-	-
						Tb	17	-	-	-	-	16	12	-	-	-	17	13	-	-	-	24	22	19	17	13
						Tc	18	16	13	-	-	16	13	-	-	-	11		-	-	-	18	14	-	-	-
Salmonella typhi (MTCC 733)	18	-	13	-	13	*Eo*	18	15	-	-	-	29	21	16	-	-	23	17	13	-	-	24	21	16	14	-
						Tb	13	-	-	-	-	17	13	-	-	-	16	12	-	-	-	13	-	-	-	-
						Tc	21	19	17	15	11	14	11	-	-	-	15	13	-	-	-	20	17	-	-	-
Staphylococcus epidermidis (MTCC 435)	18	-	-	-	15	*Eo*	19	14	-	-	-	22	17	14	-	-	16	13	-	-	-	23	19	16	14	-
						Tb	15	-	-	-	-	16	15	-	-	-	17	-	-	-	-	20	18	16	13	-
						Tc	23	21	19	14	-	21	19	18	15	13	22	19	17	-	-	25	18	-	-	-
Salmonella. Typhimurium (MTCC 98)	17	11	11	11	12	*Eo*	16	-	-	-	-	27	20	16	-	-	19	16	12	-	-	26	16	-	-	-
						Tb	14	-	-	-	-	27	25	23	18	15	16	-	-	-	-	20	17	13	-	-
						Tc	20	19	15	-	-	16	13	-	-	-	17	15	13	-	-	18	17	-	-	-
Proteus vulgaris (MTCC 426)	16	11	11	-	12	*Eo*	16	13	-	-	-	17	15	13	-	-	17	14	-	-	-	19	16	-	-	-
						Tb	13	-	-	-	-	18	15	11	-	-	17	-	-	-	-	13	13	-	-	-
						Tc	20	18	14	-	-	18	11	-	-	-	19	14	11	-	-	16	14	-	-	-

Where*: Eo- Emblica officinalis; Tb - Terminalia bellerica; Tc - Terminalia chebula*

Fruits of E. officinalis, T. chebula and T. bellerica were collected from Melghat Forest (Amravati district, India) and washed with distilled water. Fruits were cut into small pieces and dried at 60^0C overnight. They were crushed to powder in a mechanical mortar. Extracts of the powders were prepared in aqueous, acetone, ethanol, and methanol solvents. Ten gm-dried powder was dissolved in 100ml water/ organic solvents and soaked for 24h. The mixture was then refluxed in soxlet apparatus and filtered. Filtrate obtained was evaporated to dry in a controlled temperature conditions. The percentage yield for each extract was determined. Aqueous extracts were dissolved in water and organic solvent extracts were dissolved in dimethylsulfonide (DMSO) before use.

Bacterial cultures: The standard pathogenic bacterial cultures were procured from IMTECH, Chandigarh, India and used in the present study. The bacteria rejuvenated in Mueller-Hinton broth (Hi-media laboratories, Mumbai, India) at 37^0C for 18 hours and then stocked at 4^0C in Mueller-Hinton Agar. Subcultures were prepared from the stock for bioassay. A loopful of culture was inoculated in 10 mL of sterile nutrient broth and incubated at 37^0 C for 3 hours. Turbidity of the culture was standardized to 10^5 CFU with the help of SPC and Nephloturbidometer.

Agar gel diffusion antibacterial activities: For antibacterial properties, 0.1 ml bacterial suspension of 10^5 CFU ml^{-1} was uniformly spread on Mueller-Hinton Agar plate to form lawn cultures. The various organic

extracts of acetone, ethanol and methanol were prepared in dimethyl sulfoxide (DMSO) at the concentration of 20 mg ml^{-1}.

Well diffusion technique:
Screening of antibacterial activity was performed by well diffusion technique (Saeed and Tariq, 2007), the MHA plates were seeded with 0.1 ml of the standardized inoculum of each test organism. The inoculum was spread evenly over plate with loop or sterile glass spreader. A standard cork borer of 10 mm diameter was used to cut uniform wells on the surface of the MHA and 100 µl of each crude and organic extracts of E. officinalis, T. chebula and T. bellerica was introduced in the well.

Paper disc agar diffusion method:

Sterile Whatman filter paper discs (10 mm) were soaked with each crude and organic extracts of E. officinalis, T. chebula and T. bellerica diluted to 250 mg/ml by DMSO, so that each disc was impregnated with 2.5 mg of residue. Both wet and dry discs (dried at 37^0C overnight) were applied to the surface of MHA plates seeded with 3-h broth culture of the tested bacteria. The plates were then incubated for 18 h at 37^0C. Antibiotic susceptibility discs ampicillin 10µg were used as positive control while disc soaked in various organic solvents and dried were placed on lawns as negative control. The antibacterial activity was evaluated by measuring the diameter of inhibition zone. The experiment was performed in duplicate and the mean of the diameter of the inhibition zones was calculated.

RESULTS
Present study indicated great variation in antimicrobial activities of medicinal plants. The results showed that the aqueous extracts of *E. officinalis* exhibited maximum activity against *S. typhi* and *P. aeruginosa* with 16±1 mm mean zone of inhibition respectively and also exhibited potent antibacterial activities against all bacterial isolates tested except *S. aureus* with 14±1 mm mean zone of inhibition. Findings of present study are similar to those reported by Khanna and Nag (1973) against a range of enteric pathogenic bacteria including *S. aureus, E. coli, Candida albicans and S. typhosa.*

The aqueous extracts of *T. chebula* were insensitive to all tested pathogens except concentrate form, which showed zone of inhibition in the range of 15±2 mm. Out of all these extracts, acetone extracts of *E. officinalis and T. bellerica* was highly antibacterial where as methanol extract of *T. chebula* produced maximum antibacterial effect followed by aqueous, ethanol, and acetone extracts. Dilution up to 50 % also produced remarkable antibacterial activity in these extracts. In case of acetone extract of *E. officinalis, S. typhi* was most sensitive test pathogen followed by *S. typhimurium, P. aeruginosa, S. epidermidis, E. aerogenes, E. coli, P. vulgaris and S. aureus*. The maximum antibacterial activity was also recorded against *S. typhi* in other three extracts. The acetone extracts of *T. bellirica* were highly potent against *S. typhimurium, E. aerogenes, S. aureus, and E. coli*. The methanol extract of T. *bellirica* (up to 50%) was also antibacterial to all test pathogens except for *P.vulgaris*. In comparison, the methanol extracts of *T. chebula* showed maximum antibacterial activities followed by aqueous, ethanol and acetone extracts (Table 1).

DISCUSSION
Rani and Khullar (2004) screened some important plants in Ayurvedic system in India to treat enteric diseases and reported strong antibacterial activity of triphala against multidrug resistant enteric *S. typhi*. Elizabeth (2005) showed strong antimicrobial activity of crude and methanol extract of *T. bellerica* against human microbial pathogens. Our study indicated that methanol extracts of *T. bellerica* was strong antibacterial than crude extract against most of the microbes tested except *E. coli and P. aeruginosa.* Tambekar and Saratkar (2005) showed that *T. bellirica and T. chebula* were strong antimicrobial agents against enteric pathogens where as *E. officinalis* was moderate. Panthi and Chaudhary (2006) examined extracts of T. chebula and recorded potent antibacterial against *S. aureus, Shigella boydii, P. aeruginosa and E. coli.* Jagtap and Karkera (1999) reported that the extract of *T. chebula* inhibited the salivary bacteria and potential an anti-caries agent. All the antibacterial data obtained in the present study are in concord with the finding of these scientists.

Thus present study revealed the importance of natural products to control enteric bacterial pathogens, which are being a threat to human health and suggested that daily intake of triphala, may control enteric infections in human being.

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