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Antibacterial Activity of *Terminalia chebula* (Retz.), *Emblica officinalis* (Gaertn.) and *Terminalia bellirica* (Gaetn.) Roxb

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***Triphla*, 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 extracts of fruits of these plants were prepared and antibacterial activities were tested by agar well diffusion method against two enteric bacterial pathogens such as *Escherichia coli* and *Staphylococcus aureus*. The aqueous fruit extracts of all the three plants were found to be antibacterial to both the bacterial pathogens tested. The aqueous fruit extract of *T. chebula* showed maximum antibacterial activity followed by *T. bellirica* and *E. officinalis* .Thus it can be suggested that daily intake of Triphla may control enteric infections in human beings.**

Key words: *Triphla*, Antibacterial activities, *Terminalia chebula, Terminalia bellirica, Emblica officinalis*.

Medicinal plants are considerably useful and economically essential. They contain active constituents that are used in the treatment of many human diseases (Stary & Hans, 1998). Plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines. Most of the drugs today are obtained from natural sources or semi synthetic derivatives of natural products and used in the traditional system of medicine. Thus it is a logic approach in drug discovery to screen traditional natural products (Sukanya *et al.,* 2009).

Over the last 20 years, a large number of secondary metabolites from different plant species have been evaluated for their antimicrobial activity. The demand on plant-based therapeutics is increasing in both developing and developed countries due to growing recognition that they are natural products, non-narcotic, easily biodegradable, pose minimum environmental hazards, have no adverse side-effects and are easily available at affordable prices (Kannan *et al.*, 2009). Further, development of resistance to antibiotics by some pathogenic bacteria has lead to screening of several medicinal plants for their potential antimicrobial activity (Colombo & Bosisio, 1996).

'*Triphla*' is used in Ayurvedic medicine in treating a variety of conditions and also forms part of many other Ayurvedic formulations. Conditions for which Triphla is employed include headache, dyspepsia, constipation, liver conditions, ascites and leucorrhoea. It is also used as a blood purifier that can improve the mental faculties and it also possesses anti-inflammatory, analgesic, anti-arthritic, hypoglycaemic and anti-aging properties (Kaur *et al*., 2002).

The present study was focused to evaluate antibacterial activities of fruit extracts of

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T. chebula, T. bellirica and *E. officianlis* against two human pathogenic bacteria viz. *Escherichia coli* and *Staphylococcus aureus*.

MATERIALS AND METHODS

Procurement and preparation of crude extracts

Dried fruit powder of the test plants namely *Terminalia chebula, Terminalia bellirica* and *Emblica officinalis* were procured separately from Thakur Bhrata Shop, Shimla. The powders of test plants namely *E. officinalis, T. chebula* and *T. bellirica* were weighted 100 gm separately and mixed with 100 ml of distilled water. The resultant slurry was squeezed through whatman No. 1 filter paper to have clear solution. This served as 100% concentration (Shrivastava, 2008).

Procurement and maintenance of bacterial culture

The pure cultures of test bacteria namely *E. coli* and *S. aureus* were obtained from Department of Microbiology, I.G.M.C., Shimla. Pure cultures of *E. coli* and *S. aureus* were maintained in nutrient broth separately which were preserved in refrigerator. Subculturing was done at regular intervals in order to maintain cultures.

Methodology for determining antibacterial activity

Well diffusion technique

Agar well diffusion method was employed to determine antibacterial activity (Patil & Gaikwad, 2011).

Nutrient Agar medium (Yeast extract 2 gm, pepton 5 gm, Agar 20 gm, Beef extract 1 gm, sodium chloride 1 gm, distilled water, 1 lt) was used throughout the experiment. The medium was sterilized under 15 lb pressure for 30 minutes in an autoclave. The plates were left overnight at room temperature to check for any contamination. The pure cultures of test bacterial organisms were grown in nutrient broth for 24 hr. Bacterial lawns were prepared by using 100 µl of nutrient broth culture, of each test bacteria. 100 µl of bacterial suspension was spread on solidified nutrient agar plates. Agar wells of 8 mm diameter were prepared with the help of sterilized cork-borer. In one agar plate only one well is prepared. The well in each plate was loaded with 15%, 30%, 50% and 100% concentration prepared separately by dissolving extract in required amount of water. The plates containing extracts were incubated at 37^0C for 24 hrs. in incubation chamber. All the experiments were performed in triplicates. Diameter of bacterial colonies of treatment and control sets were measured in mutually perpendicular direction on second day.

Percentage inhibition of bacterial/fungal micro-organisms was calculated after subtracting the value of control from the value of extracts using control as standard (Hemashenpagam and Selvaraj, 2010).

$$\text{Percentage of growth inhibition} = \frac{\text{Control-test}}{\text{Control}} \times 100$$

(*Kannan et al*., 2009)

RESULTS AND DISCUSSION

The fruits of *Emblica officinalis* commonly known as Amla is highly valuable in traditional Indian medicine (Scarttezzini *et al*., 2006). In Unani medicine the dried fruits of Amla are used to treat haemorrhage, diarrhoea and

Table 1. Comparative analysis of Antibacterial screening of aqueous fruit extracts of *Terminalia chebula, Terminalia bellirica* and *Emblica officinalis* against two test bacteria namely *S. aureus* and *E. coli*.

Concentration (In %)	% inhibition of growth of test bacteria (mm ± S.E.)					
	Terminalia chebula		*Terminalia bellirica*		*Emblica officinalis*	
	S. aureus	*E. coli*	*S. aureus*	*E. coli*	*S. aureus*	*E. coli*
15%	4.66±0.06	3.33±0.57	3.33±0.57	0.00±0.00	0.00±0.00	0.00±0.00
30%	8.88±0.57	7.77±0.57	5.55±0.57	4.66±0.06	4.55±0.05	3.55±0.06
50%	11.88±0.05	11.66±0.06	7.88±0.06	7.44±0.05	7.00±0.06	5.55±0.57
100%	17.77±0.57	16.66±0.57	16.77±0.05	15.66±0.06	11.11±0.57	9.44±0.05

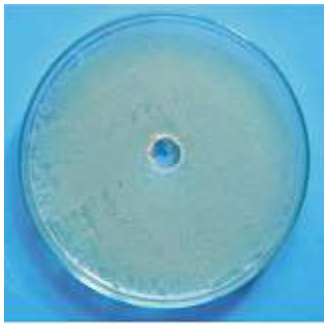

Plate 1. Pure culture of *Escherichia coli*

Plate 2. Pure culture of *Staphylococcus aureus*

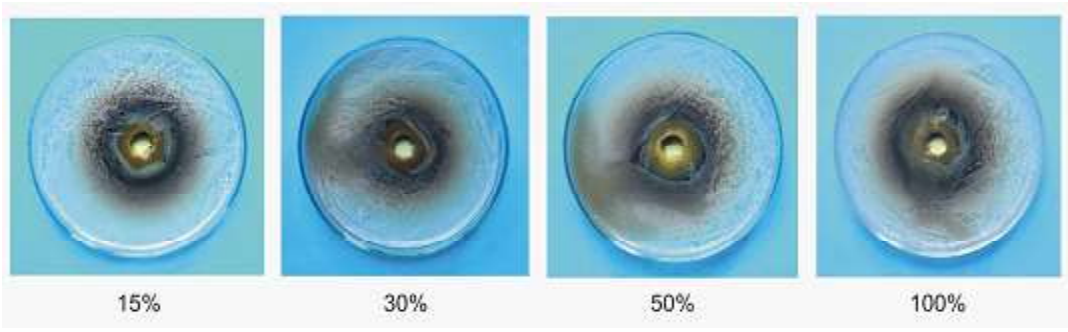

Plate 3. Percentage inhibition of growth of *E.coli* by *Terminalia chebule* fruit extract at different concentration

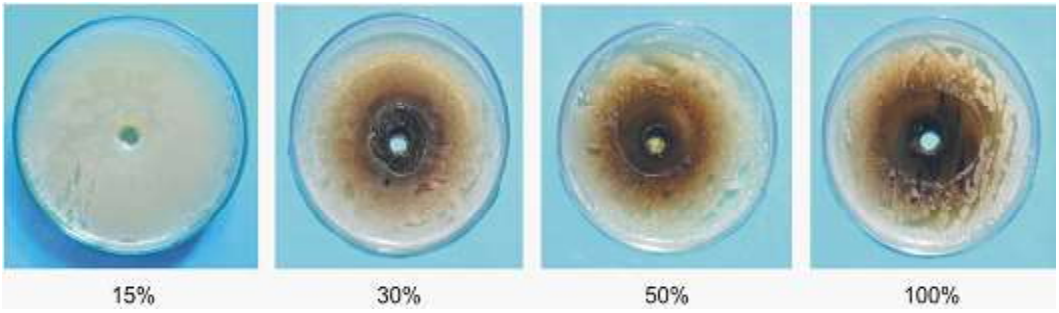

Plate 4. Percentage inhibition of growth of *E.coli* by *Terminalia bellirica* fruit extract at different concentrations

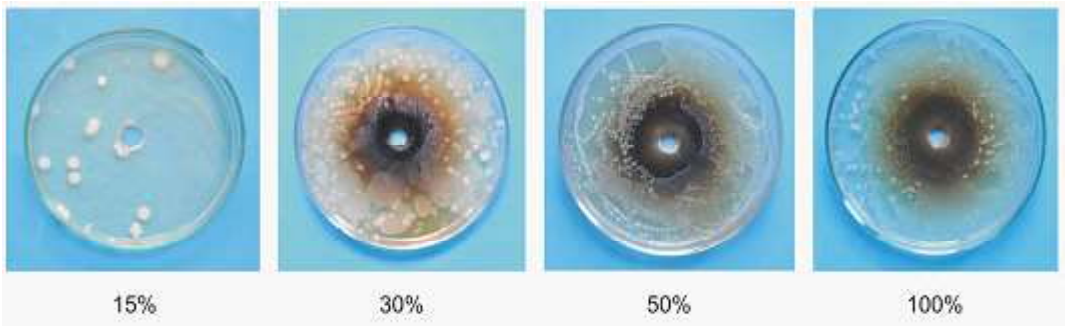

Plate 5. Percentage inhibition of growth of *E.coli* by *Emblica officinalis* fruit extract at different concentrations

dysentery (Parrota, 2001).

The cold infusion of fruits of *Terminalia chebula* is used as gargle in stomatitis and in chronic ulcers, carious teeth, asthma and urinary diseases. The ripe fruits of *Terminalia bellirica* are used as an astringent in combination with chebulic myrobalan and half ripe fruits are used as puragative (Farooq, 2005).

The antimicrobial sensitivity of plant extracts was observed using the well diffusion method by measuring the diameter of the growth inhibition zone. The *in vitro* anti-bacterial activity of aqueous fruit extracts of *T. chebula, T. bellirica* and *E. officinalis* are shown in Table-1 and Plate no. 3,4,5,6,7 and 8. The screening revealed that aqueous fruit extract of *T. chebula* was most effective in inhibition against *S. aureus* (17.77%, at 100% conc.) whereas in case of *E. coli* inhibition was (16.66% at 100% conc.). The aqueous fruit extract of *T. bellirica* was found to be most effective against *S. aureus* (16.77% at 100% conc.) whereas in case of *E. coli* the inhibition was

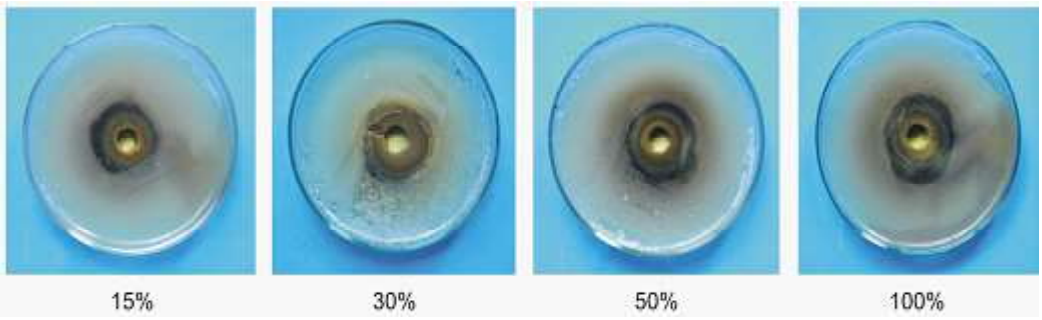

Plate 6. Percentage inhibition of growth of *Staphylococcus aureus* by *Terminalia chebula* fruit extract at different concentrations

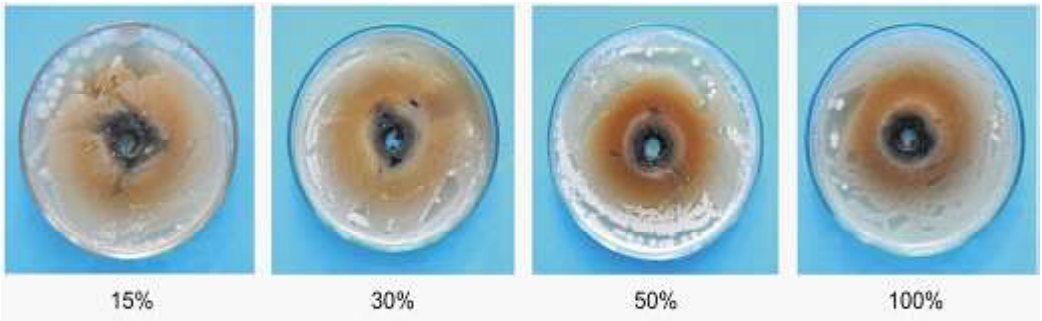

Plate 7. Percentage inhibition of growth of *Staphylococcus aureus* by *Terminalia bellirica* fruit extract at different concentrations

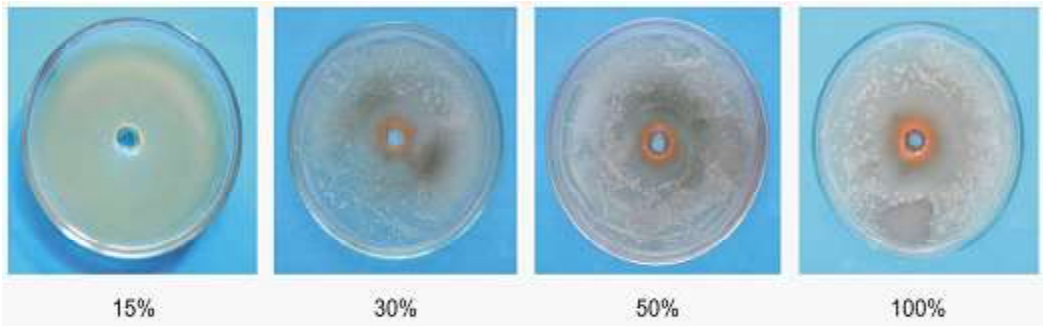

Plate 8. Percentage inhibition of growth of *Staphylococcus aureus* by *Emblica officinalis* fruit extract at different concentrations

(15.66% at 100% conc.). Similarly, the aqueous fruit extract of *E. officinalis* was found to be effective against *S. aureus* (11.11% at 100% conc.) whereas in case of *E. coli* inhibition was (9.44% at 100% conc.).

The aqueous fruit extracts of three medicinal plants namely *T. chebula, T. bellirica* and *E. officinalis* were found to be more effective against *S. aureus* as compared to *E. coli* at all concentrations.

Rani and Khullar (2004) screened some important plants in Ayurvedic system in India to treat enteric diseases and reported strong antibacterial activity of Triphla against multidrug resistant enteric *S. typhi*. Tambekar, Khante *et al.*, (2007) reported antibacterial properties of contens of Triphla against several enteric bacterial pathogens such as *E. coli, S. aureus, Pseudomonas aeruginosa, Proteus vulgaris* etc. Our study indicated that aqueous extract of *T. chebula* was strong antibacterial against *S. aureus* as compare to *E. coli*. Tambekar and Saratkar (2005) showed that *T. bellirica* and *T. chebula* were strong antimicrobial against enteric pathogens whereas *E. officinalis* was moderate.

Elizabeth (2005) reported strong antimicrobial activity of crude, and methanol extracts of *T. bellirica* against nine human microbial pathogens. Kannan *et al.* (2009) reported antibacterial activity of ethanolic extract of *T. chebula* fruit against *S. aureus* and *Salmonella typhi*. Kumar *et al*. (2009) studied antimicrobial activity of *T. chebula* against *S. aureus, S. epidermis, E. coli, S. flexineria* and *Pseudomonas aeruginosa*.

The effectiveness of medicinal plants were not due to one main active constituent, but to the combined action of other chemical compounds involved in it. Important subclasses in this group of compounds include phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins. These goups of compounds show antimicrobial effect and serve as plant defense mechanisms against pathogenic microorganism (Scalbert, 1991). The present study showed the efficacy of antimicrobial activity exclusively for bacterial pathogens which really shows the presence of biological principles.

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