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Effect of Triphala on dental bio-film

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Abstract: The free radical scavenging property and antimicrobial activity of Triphala- the herbal product made of equal proportion of *Terminalia chebula, Terminalia belerica* and *Emblica officinalis,* were evaluated. Ethanol extracts of the formulation were tested for its total antioxidant activity using improved ABTS radical cation decolorizing assay and antibiotic assay against *Streptococcus mutans* (predominantly involved in bio-film formation on human teeth). An effort was also made to correlate its antiplaque activity using an *in-vitro* assay (conditions were kept similar to oral cavity) with Triphala and two commercial toothpastes (Product 1 and product 2). The herbal extract effectively inhibited the bio-film formation and the better antioxidant activity exhibited by the extract might protect the gum cells effectively from free radicals than the commercial toothpastes. Thus Triphala can be used as an effective antiplaque agent.

Keywords: ABTS, antiplaque, *Terminalia*, triphala.

Introduction

Human dental plaque was one of the eco-systems in which microorganism was first observed. Dental plaque refers to the aggregates of bacterial cell embedded in a polysaccharide and protein matrix which adheres to the teeth by a characteristic bacterium, *Streptococcus mutans* (Scherp, 1971). This organism metabolizes sucrose in a peculiar way, producing an extra-cellular adhesive polysaccharide (dextran), a sticky insoluble glucan which promotes the firm adherence of the organisms to the tooth surface contribute the formation of dental plaque, subsequently leads to localized decalcification of the enamel surface (Ooshima *et al.,* 1994).

Several anti-plaque agents are being available in the market. However, due to several undesirable side effects associated with these agents stimulated the search for alternate agents (Schee, 1989). In recent years, there has been focus on plants or plant products used in folk dental practice or presumed in Unani, homeopathic or Ayurvedic remedies (Memory, 1986). Natural compounds contained in the herbal cocktail can act in a synergetic manner within the human body, and can provide unique therapeutic properties with minimum or no undesirable side effects.

One such herbal cocktail is Triphala, the herbal product of equal proportion of dry powder of *Terminalia chebula*, *Terminialia belerica* and *Emblica officinalis* being used extensively in Indian system of medicine having anti-diabetic and antioxidant activity (Sabu & Kuttan, 2002). It has also been used as laxative in Siddha and Ayurveda medical systems. *T. chebula* which acts as anticaries agents strongly inhibits the sucrose or glucan induced aggregations of *S. mutans* (Jagtap & Karkera, 1999) and strengthens the gums, prevents and treats several diseases of mouth such as dental caries, spongy and bleeding gums gingivitis and stomatids (Date & Kulkarni, 1995). Hence, an attempt was made to study antioxidant and antimicrobial properties of Triphala in comparison with commercially available tooth pastes, Product 1 and Product 2.

Materials and methods

Preparation of the extract

Triphala churna, an Ayurvedic medicine used in the experiment is manufactured in India by Dabur India Limited. The commercial tooth pastes, Product 1and Product 2 were purchased from the local market. The samples were suspended in 100 ml ethanol and kept in shaker for 48 hours; extracts were separated using rotary vaccum evaporator.

Total Antioxidant Activity

The Trolox equivalent antioxidant capacity (TEAC) of Triphala compared with commercially available tooth paste Product 1 and Product 2 was estimated using the Feryl Myoglobulin/ ABTS method for total antioxidant activity (Pellegrini *et al.*, 1999). This technique measures the relative ability of antioxidant substance to scavenge the ABTS radical cation ($ABTS^{\cdot+}$) generated in aqueous phase, compared with standard synthetic antioxidant Trolox (6-hydroxy-2-5-8 tetra methyl chroman-2-carboxilic acid) a vitamin-E analogue. Test samples prepared in different concentrations *i.e.,* 50, 100, 150, 200 and 250 µg/ml were added to the tube containing 7 mM ABTS and 140 mM potassium per-sulphate diluted with ethanol to O.D. 0.7 at 734 nm. The antioxidant property was determined by reduction in the O.D. compared with the standard Trolox.

Antimicrobial activity

Agar well diffusion method: The effect of ethanol extract of Triphala and commercial tooth pastes against *S. mutans* was determined by agar well diffusion method. The 18 hours old culture of *S. mutans* was incorporated in nutrient agar and poured into the Petri-plates, allowed to set. Wells

were made using cork-borer (8 mm diameter) and were loaded with extracts along with appropriate controls. The zone of inhibition obtained with various concentrations of the extracts was observed after 24 hours incubation at 37ºC.

Minimum inhibitory concentration (MIC): Analysis of minimum inhibitory and minimum bactericidal concentration (MIC & MBC) was determined for *S. mutans*. The test was carried out for the ethanol extract of Triphala, Product 1 and Product 2. A two fold dilutions of the extracts were prepared and the MIC & MBC were determined.

In vitro assay reflecting oral condition

An *in-vitro* assay (Evans *et al.,* 1977) using saliva treated human teeth for determining the potential of chemotherapeutic agent's ability to adsorb, to the tooth surface and act against plaque forming bacteria was carried out. The tooth was coated by shaking in sterile saliva on a rotating platform at 150 rpm at room temperature for one hour. The coated tooth was then rinsed twice in sterile phosphate buffer saline. The rinsed tooth was placed in small plastic Petri-dishes containing various concentrations of the test drugs (Triphala, Product 1 & Product 2) and left for two minutes with gentle agitation, after that they were rinsed in 5 ml phosphate buffer saline for two minutes for two times, and immersed in sterile distilled water for two minutes with a control. The rinsed tooth was placed in cotton stopper glass tube containing 1 ml of medium. Each tube was inoculated with 50 µl of 24 hours culture of test organism (*S. mutans*) and incubated at 37ºC for 24 hours under aerobic conditions.

After incubation, non adherent cells were removed carefully by decanting the tube, cells adhered to the tooth surface were washed twice with 0.5 ml phosphate buffer saline and the washing was combined with previously removed non adherent cells. The tooth was then transferred to 1 ml of 0.1N sodium hydroxide and suspended for sonification at 20 watts for 20 seconds. The optical density of the fractions, adherent and non-adherent was taken at 540 nm. The cells adhere to the tooth will referred as plaque forming unit's responsible for plaque formation on tooth.

Results

Antioxidant property of Triphala compared with commercial tooth paste

Ethanolic extracts of Triphala, Product 1 and Product 2 were found to scavenge the free radicals generated by $ABTS^{\cdot+}$, as measured from the reduction in radical cation absorbance at 734 nm.

Table1. In vitro antioxidant activity of Triphala. Product 1 and Product 2

Ethanolic extracts	Trolox equivalent antioxidant activity mmol/kg				
	50µg[a]	100 µg	150 µg	200 µg	250 µg
Triphala	2.38	2.58	2.9	3.0	3.16
Product 1	0.06	0.18	0.62	0.72	1.8
Product 2	0.16	0.78	1.48	2.1	2.28

[a] *Means ± SD from triplicate determination*

The results demonstrated that extracts reacted with $ABTS^{\cdot+}$ at different concentrations (50,100, 150, 200 and 250µg) and Triphala extract (250 µg) reacting for 2.5 mins showed a maximum decolorisation up to 99.71%, whereas the commercial tooth paste Product 1 decolorized up to 57.85% and Product 2 up to 72% (Fig. 1). In control group, no decolorisation was observed.

The extent of reduction in the absorbance of the $ABTS^{\cdot+}$ is plotted as a function of concentrate in order to determine the Trolox equivalent antioxidant activity (TEAC). The unit of antioxidant activity is defined as the concentration (mmol/litre) of trolox having the equivalent antioxidant activity to a 1 mM or 1 mg/ml solution of the substance under investigation or expressed as mmol/kg of food/compound (Table 1).

Antimicrobial activity against S. mutans

The antibacterial activity of ethanolic extract of Triphala and the commercial pastes (Product 1 & Product 2) was assessed. Triphala showed minimal inhibitory and minimal bactericidal concentration at 50µg/ml itself whereas the other two had the minimum inhibitory concentration at

Fig. 1. In-vitro antioxidant activity of ethanolic extract of Triphala, Product 1 and Product 2, trolox equivalent (Pelligrini et al., 1999).

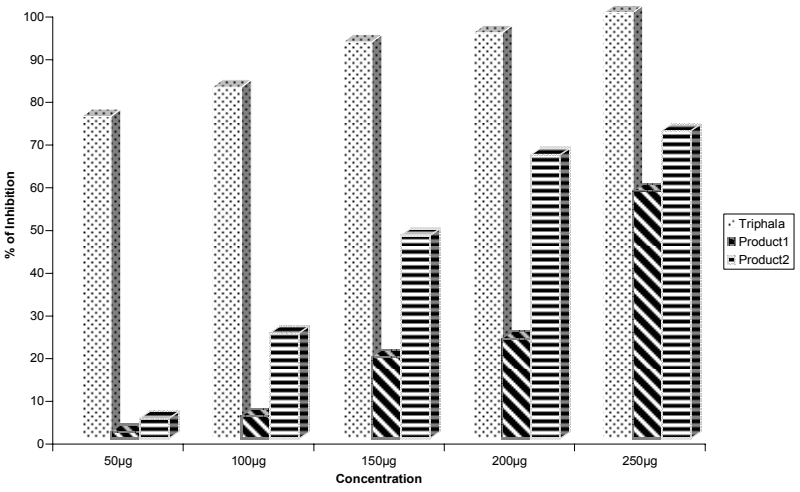


Table 2. Anti-Streptococcus activity of Triphala, Product 1 and Product 2.

Organism	Ethanol Extract	Concentration µg/ml				
		800	400	200	100	50
S. mutans	Triphala	NT[a]	NT	NT	NT	NT
S. mutans	Product 1	NT	NT	NT	NT	T[b]
S.mutans	Product 2	NT	NT	NT	NT	T

[a]No turbidity (No growth); [b]Turbid (Presence of growth)

100µg/ml against *S. mutans* (Table 2).

In-vitro assay reflecting oral condition

The assay was employed to test the effect of Triphala and commercial tooth pastes like Product 1 & Product 2 against growth and plaque formation by *S. mutans* with concentrations of 5% and 10%. Triphala effectively controlled the plaque formation by inhibiting 83.72% growth of *S. mutans* in 5% solution and 86.34% in 10%; whereas the activity range for Product 1 and Product 2 was 75% in 10% solution and 35% in 5% of the solution (Fig. 2), respectively.

Discussion and conclusion

The unique aspect of the work is to confirm the importance of herbal product for its medicinal, antioxidant and antimicrobial relation. The results obtained indicate that Triphala (equal proportions of *Terminalia chebula*, *Terminalia belerica* and *Emblica offfficinalis*) had potent antioxidant and antimicrobial activity and inhibited the growth of *S. mutans,* gram positive cocci, involved in plaque formation when it adsorbed to the tooth surface.

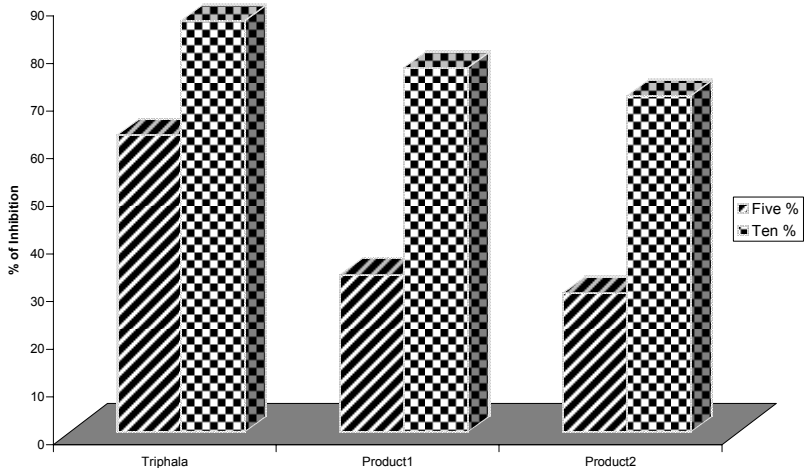
The strong antioxidant activity of Triphala may be partially responsible for many of the biological properties (Sabu & Kuttan, 2002). *T. belerica* was the most active antioxidant followed by *E. officinalis* and *T. chebula.* The major ingredients of *T.bellerica* are ellagic and gallic-acid; *E. officinalis* has several gallic acid derivatives including epigallocatechin gallate and in *T. chebula* gallic acid is the major ingredient (Sabu & Kuttan, 2002; Lee *et al.,* 2005). The presence of these active ingredients of phenolic nature may be responsible to scavenge the free radicals generated by $ABTS^{\cdot+}$ in a solution.

Tannic acid represents the major constituent of the ripe fruit of *T .chebula*, *T.belerica* and *E. officinalis* (Chopra & Handa, 1958). Earlier studies reported that tannic acid is bacteriostatic or bactericidal to some gram positive and gram negative pathogens (Kau, 1980). Similarly the ethanol extract of herbal powder showed antibacterial activity in minimum concentration of 50µg/ml against *S. mutans* in the present study.

S. mutans adheres to the tooth surface by virtue of its glucan binding protein that leads to the formation of dental plaque. An *in-vitro* assay on the formation of a pellicle over the human tooth surface was performed before and after exposure to the drug. Adherent and non-adherent cells could be measured (Turesky *et al*., 1972) for the relative effectiveness of the drug. The results obtained by us showed the effectiveness of Triphala as a strong antiplaque agent. Probably the tannic acid (in Triphala), can be adsorbed well to the hydroxyapatite of the tooth or to the salivary mucins, alternately it can be bound to anionic groups on the surface of the bacterial cells, which resulted in protein denature and ultimately to the bacterial cell death (Bonesvoll *et al.,* 1974). The increased oxidative stress has been postulated in the diabetic state (Boynes, 1991). Hence, the effective antioxidant property present in the extract may be useful in the treatment of diabetic patients having dental carries; whereas the sweetener present in the commercial pastes can delay the healing process or can harm the tooth.

The extract of Triphala can be employed as an effective agent to treat dental carries and to prevent the formation of dental plaques. Since the formulation is of herbal nature, it is renewable and can be made cheaper.

Fig. 2. Effect of Triphala, Product 1, Product 2 on the biofilm formation on human tooth under in-vitro condition (Evans et al., 1977).



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