RESEARCH ARTICLE

Comparative evaluation of Antimicrobial Activity of Triphala and commercially available Toothpastes: An in-vitro study

Biju Thomas, Sunaina Shetty Y, Agrima Vasudeva, Veena Shetty

Abstract

Background: Triphala is an ayurvedic herbal formulation consisting of the dried fruits of three medicinal plants Terminalia chebula, Terminalia belerica and Phyllanthus embelica, which is used for conditions like headache, constipation, liver conditions and it possess anti-inflammatory, analgesic, anti-aging properties. Aims: The present study was undertaken to assess the antimicrobial properties of Triphala in comparison with commercially available toothpastes. Materials and Methods. The standard stock culture of Streptococcus mutans strain from Microbial type culture collection (MTCC), Chandigarh and clinical culture of Streptococcus mutans isolated from the plaque samples of the patients were used. The antimicrobial activity of ethanol extracts of Triphala and commercially available toothpastes (Product 1 and Product 2) against MTCC strain and clinical isolate of Streptococcus mutans was evaluated using agar gel diffusion method. Further, Minimum Inhibitory Concentration (MIC)/Minimum Bactericidal Concentration (MBC) values were obtained using broth dilution method. Statistical analysis: The collected data were statistically analyzed by one-way analysis of variance (ANOVA) to evaluate the differences. The threshold for the statistical significance was set at P<0.05. Results: The present study showed that Triphala has significant antimicrobial activity when compared to commercially available Product 1 and Product 2 toothpaste (P<0.05). Conclusions: Triphala has significant antimicrobial activity and thus can be employed as an effective anti-plaque agent and can be used in the prevention of dental caries. Since Triphala is of herbal nature, it can be easily extracted and is cost effective.

Key Words: Antimicrobial, Antiplaque, S. Mutans, Triphala.

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Introduction

Human dental plaque was one of the ecosystems 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 (1). Gram positive streptococcus strains form the major group of organisms during first few hours of plaque formation. Streptococcus mutans 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 (2).

Several anti-plaque agents are being available in the market. However with the rise in bacterial resistance to antibiotics, there is considerable interest in the development of other classes of antimicrobials for the control of infection (3).

Current advancement in drug discovery technology and search for novel chemical diversity have intensified the efforts of exploring products from ayurveda the traditional system of medicine in India(4).

While screening a number of traditional Vedic formulas scientists discovered one of the most revered of all ayurvedic compounds – Triphala, which exhibits a number of health benefits (5).

Triphala is a traditional ayurvedic herbal formulation consisting of the dried fruits of three medicinal plants Terminalia chebula, Terminalia belerica and Phyllanthus embelica, also known as ‘three myrobalan’. Triphala means ‘three’ [tri] ‘fruits’ [phala] (6).

Triphala is used in Ayurvedic medicine in treating a variety of conditions and also forms part of many other Ayurvedic formulations. Conditions for which Triphala 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 possesses anti-inflammatory, analgesic, anti-arthritic, hypoglycemic and anti-aging properties (7,8). T. chebula which acts as anticaries agents strongly inhibits the sucrose or glucan induced
aggregations of S. mutans (9) and strengthens the gums, prevents and treats several diseases of mouth such as dental caries, spongy and bleeding gums gingivitis and stomatitis (10).

Prabhakar et al (11) conducted a study to evaluate antimicrobial efficacy of herbal alternatives (Triphala and green tea polyphenols), MTAD and 5% sodium hypochlorite against Enterococcus faecalis biofilm formed on tooth substrate and concluded that Triphala showed statistically significant antibacterial activity.

Srikumar et al (12) concluded that both individual and combined aqueous and ethanol extracts of Triphala have antibacterial activity against the bacterial isolates of HIV infected patients. Hence, in this present study an attempt has been made to study the antimicrobial properties of Triphala in comparison with commercially available toothpastes.

Materials and Methods

Stock Culture Strain: Standard Streptococcus mutans strain (code 890) from Microbial Type Culture Collection and gene bank (MTCC), Institute of microbial Technology, Chandigarh was used as a standard stock culture.

Isolated Strain: Streptococcus mutans were isolated from the plaque samples of the patients.

Collection of Plaque Sample: Patients with minimum compliment of 20 teeth, exhibiting mild to moderate supragingival plaque and no evidence of gingival inflammation and stains were selected. Visible supragingival plaque was removed using hand scalers. It was immediately transferrred to thioglycollate broth. This was further incubated for 4 hours and then subcultured.

Isolation of Streptococcus mutans from clinical samples: Plaque samples which were inoculated onto thioglycollate medium were further subcultured after 4-6 hours on Mutans sanguis agar and incubated at 370 C for 48 hrs. The colonies formed were identified further by gram staining methods and biochemical tests. Gram positive cocci in chains were observed by gram staining methods and biochemical tests included catalase test negative, esculin test positive, starch hydrolysis positive, raffinose and mannitol fermentation test to confirm the colonies identified.

Preparation of the extracts: Triphala churna which was used in the experiment was commercially available in the market. The aqueous product triphala churna was prepared by suspending 100 grams in 100ml of double distilled water and boiling it for 45 minutes. It was further cooled and filtered. The filtrate of Triphala churna thus obtained was used for antimicrobial activity study. The commercial toothpastes Product 1 (containing 0.24% sodium fluoride, 0.3% triclosan, calcium carbonate and silica) and Product 2 (containing 0.32% sodium fluoride sorbitol, water, hydrated silica, sodium lauryl sulphate and cellulose gum) were purchased from the local market and their aqueous product was prepared by suspending 1 gram each in double distilled water.

Antimicrobial activity: Antimicrobial activity was checked by agar gel diffusion method. The cultures, both MTCC stock culture and clinical isolate were grown in Mueller-Hinton broth and incubated at 370C for 24 hours. After incubation period was finished the optical density of the culture was adjusted to 0.1 with sterile Mueller-Hinton broth. The 0.1 ml of the culture was seeded in 25 ml molten Mueller-Hinton agar butts, mixed and poured into sterile petri plate and allowed to solidify. The wells were bored with 8mm borer in seeded agar. Then the particular concentrations (50%, 25%, 12.5 %,) of Triphala and product 1 and product 2 extracts were added in each well. After it normalized to room temperature, plates were incubated at 370C for 24 hours. The zone of inhibition was measured and recorded after the completion of incubation period.

Determination of Minimum Inhibitory Concentration (MIC)/Minimum Bactericidal Concentration (MBC) : To measure the MIC values, micro-broth dilution method was used. The reconstituted extracts was serially diluted 2-fold in Mueller-Hinton broth medium to obtain various concentrations of the stock 50, 25, 12.5, 6.25, 3.125 % and were assayed against S.mutans. Equal volume of the various concentration of each extract and Mueller Hinton broth were mixed in micro-tubes to make up 0.5ml of solution. 0.5ml of McFarland standard of the organism suspension was added to each tube.

The tubes were incubated aerobically at 370C for 24 hours. Two control tubes were maintained for each test batch. These included tube-containing extract without inoculum and the tube containing the growth medium and inoculum. The MBC was determined by sub culturing the test dilution on Mueller Hinton Agar and further incubated for 24 hours. The highest dilution that yielded no single bacterial colony
was taken as the Minimum Inhibitory Concentration and Minimum Bactericidal Concentration.

Statistical analysis: The collected data were statistically analyzed by one-way analysis of variance (ANOVA) to evaluate the differences. The threshold for the statistical significance was set at p<0.05.

Results

Comparison of The antibacterial activity of Triphala and Commercially available toothpastes (Product 1 and Product 2) on Streptococcus mutans MTCC strain and a clinical isolate by Agar diffusion method (Table 1) was done.

<table>
<thead>
<tr>
<th>% of Triphala</th>
<th>Zone of inhibition</th>
<th>% of Product 1</th>
<th>Zone of inhibition</th>
<th>% of Product 2</th>
<th>Zone of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>30mm</td>
<td>25mm</td>
<td>20mm</td>
<td>15mm</td>
<td></td>
</tr>
<tr>
<td>25%</td>
<td>28mm</td>
<td>24mm</td>
<td>18mm</td>
<td>12mm</td>
<td></td>
</tr>
<tr>
<td>12.5%</td>
<td>24mm</td>
<td>20mm</td>
<td>18mm</td>
<td>12mm</td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td>0.146 (NS)</td>
<td></td>
<td>0.064 (NS)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1: The Antibacterial Activity of Triphala and Commercially Available Toothpastes on Streptococcus Mutans MTCC Strain and a Clinical Isolate by Agar Diffusion Method (one-way ANOVA)

Around 50%, 25% and 12.5% of Triphala showed 30mm, 28mm and 24mm zone of inhibition respectively on S.mutans MTCC strain and 34mm, 30mm, 28mm respectively for S.mutans clinical isolate. At concentration of 50%, 25% and 12.5%, Product 1 showed zone of inhibition of 25, 20 and 15 mm respectively for S.mutans MTCC strain and 28, 24 and 20mm respectively for S.mutans clinical isolate.

At the same concentrations, Product 2 showed zone of inhibition of 25, 18, 12mm respectively for S.mutans MTCC strain and 26, 22 and 18mm for S.mutans clinical isolate. This result showed that the zone of inhibition was more for Triphala as compared to Product 1 and Product 2. However there was no statistical significance.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Triphala</th>
<th>Product 1</th>
<th>Product 2</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus mutans MTCC strain</td>
<td>6.25%</td>
<td>12.5%</td>
<td>12.5%</td>
<td></td>
</tr>
<tr>
<td>Streptococcus mutans Clinical Isolate</td>
<td>3.12%</td>
<td>6.25%</td>
<td>6.25%</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

Table 2: Table showing the Minimum inhibition concentrations (MIC) / Minimum Bactericidal Concentration (MBC) Of Triphala, Product 1 and Product 2. (One way ANOVA).

Comparison of Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of Triphala, Product 1 and Product 2 on S.mutans strains was done. (Table 2).

The MIC/MBC values of ethanol extracts of Triphala and commercially available toothpastes (Product 1 and Product 2) were obtained by Broth dilution method. Triphala showed Minimal Inhibitory Concentration / Minimal Bactericidal Concentration at 6.25% for MTCC strain of S.mutans and 3.12% for clinical isolate of S.mutans whereas Product 1 and Product 2 had Minimal Inhibitory/ Minimal Bactericidal Concentration at 12.5% for MTCC strain of S.mutans and 6.25% on clinical isolate of S.mutans. The statistically significant results were obtained with P value of 0.001(P<0.05).

Discussion

In the present study the antimicrobial activity of an ayurvedic product, Triphala was assessed. Ethanol extracts of Triphala was used to evaluate antimicrobial activity on both clinical and MTCC strain of S.mutans. Antimicrobial activity was found by using agar gel diffusion method and broth dilution method. The antimicrobial activity of Triphala was also compared with commercially available toothpastes, Product 1 and Product 2.

Jagadish et al (13) conducted a study to determine the effect of Triphala on dental biofilm and concluded that Triphala 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.

A study was conducted to explore the antimicrobial activity of Triphala and Triphala Mashi, and concluded that Triphala and Triphala Mashi exhibited a broad-spectrum antimicrobial
activity against all the microorganisms from human secretions and from pathology labs with prior diagnosis. It inhibited the growth of all Gram-positive and Gram-negative bacteria (14). Jagtap and Karkera (9) reported that extracts of Terminalia chebula strongly inhibit the growth and adherence of Streptococcus (S. mutans), a virulent cavity-inducing organism. Oral rinsing with an extract of Terminalia chebula significantly reduced both total bacterial counts and streptococcal counts in saliva samples. The protective effect lasted for up to 3 hours after rinsing, demonstrating a potential role for Terminalia chebula in the prevention of dental caries.

Our results show that ethanol extracts of Triphala has more antimicrobial activity on clinical isolate than MTCC strains of S. mutans. Also, when compared to Product 1 and Product 2, it demonstrated more efficacy on these strains. This is measured by gel diffusion and broth diffusion method. Our report hence co-relates with the studies done by other authors exhibiting antimicrobial activity in relation to Triphala (13, 14).

Since Ayurvedic System of Medicine has a long history of therapeutic potential, it can be used as a logical approach to drug discovery, to screen the traditional natural products such as Triphala which shows a scientific proof of its superior antimicrobial potential.

The results obtained by other authors show that the free radical scavenging property and anti-plaque activity of Triphala may be important as an effective agent to treat patients with dental caries and to prevent formation of dental plaque in the near future (13, 14).

Conclusions

Triphala has significant antimicrobial activity and thus can be employed as an effective anti-plaque agent and can be used in the prevention of dental caries. Since Triphala is of herbal nature, it can be easily extracted and is cost effective. In future it can be explored further for its other parameters such as toxicity study, inhibition of biofilm formation before recommending it for usage in the clinical practice.

Further studies are required to highlight the antimicrobial activity of many such herbal products available in nature and to promote the discovery of new natural bioactive compounds.

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Affiliations of Authors: 1. Dr. Biju Thomas, MDS, Professor and Head, 2. Dr. Sunaina Shetty, Y. Post Graduate Student, Department of Periodontics, 3. Dr. Agrima Vasudeva, Under Graduate Student, 4. Dr. Veena Shetty, Reader, Department of Microbiology, A.B. Shetty Memorial Institute of Dental Sciences, Mangalore, 575 018, Karnataka, India.

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Corresponding Author
Dr. Sunaina Shetty Y. BDS, (MDS). Post Graduate Student, Department of Periodontics A.B. Shetty Memorial Institute of Dental Sciences, Mangalore, 575 018, Karnataka (India). Contact No.: +91 9739249396 E-Mail: raghavendra77@yahoo.com