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Effect of herbal medicine on primary plaque colonizers: An *in vitro* study

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Abstract
Objective: The aim was to assess the antimicrobial efficacy of triphala gel at various concentrations against primary plaque colonizers.
Material & Methods: Triphala gel was prepared at various concentrations 6.25%, 12.5%, 25%, 50%.The antimicrobial efficacy testing of triphala gel against these bacteria was done by agar well disc diffusion method.
Results: All the concentrations inhibited the growth of primary plaque colonizers maximum zone of inhibition was seen at 50% for all the primary plaque colonizers.
Conclusion: Triphala gel has the potential to inhibit the growth of primary plaque colonizers and can be used as an antiplaque agent and used in prevention of dental and periodontal diseases.

Keywords: triphala, antimicrobial efficacy, antiplaque agent

Introduction

Dental plaque refers to the aggregates of bacterial cell embedded in a polysaccharide and protein matrix which adheres to the teeth gram positive streptococcus strains forms the major group of organisms called primary plaque colonizers. Streptococcus metabolizes sucrose in a peculiar way, producing an extracellular 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 [1].

Periodontal diseases appear to occur when pathogenic microbial plaque acts on susceptible host [2]. Bacterial plaque is the primary etiological agent in periodontal disease proper plaque removal can lower risk of initiation and progression of gingivitis, scaling and root planing forms the first phase of periodontal therapy, but factors such as inaccessibility or plaque retentive areas can compromise clinical and microbiological outcomes.

Chemical plaque control forms an effective adjunct to mechanical plaque control therapy in the treatment of gingivitis [3] various agents that are antimicrobial & prevent the bacterial proliferation phase of plaque development have been introduced into the market [4] 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.

This demonstrates a need for an antiplaque agent with minimal side effects which can be used as an effective adjunct to mechanical plaque control. Natural herbs are used as whole single herb or in combination have been scientifically proven to be safe and effective medicine against bleeding gums halitosis, mouth ulcers, tooth decay the major strength of these herbs is no side effects have been reported till date [5].

Triphala is a traditional ayurvedic herbal formulation consisting of the dried fruits of three medicinal plants Terminalia Chebula, Terminalia belerica and phyllanthus emblica also known as three myrobalan. Triphala means three fruits. Triphala is used in ayurvedic medicine in treating variety of conditions as it possesses anti-inflammatory, antibacterial, antioxidant, anti-aging, antitussive, analgesic, anti-arthritic, hypoglycemic.

The present study was undertaken to assess the antimicrobial efficacy of extracts of triphala at various concentrations against primary plaque colonizers.

Materials and Methods

Plaque sample was collected with minimum compliment of 20 teeth exhibiting mild to moderate supragingival plaque. Visible supragingival plaque was removed using hand scalers. This plaque sample was inoculated to the culture media one loopful of culture was made as a primary inoculum and was then distributed thinly over the plate by streaking it with the loop in a series of parallel lines in different segments of the plate and streak culture was obtained (shown in fig: 1a,b.) fig: 2 shows the microscopic colonies of streptococcus.

This cultured microorganisms were further subculture to isolate *Streptococcus mutans*, *Streptococcus sanguis*, *Streptococcus salivarius*, *Streptococcus mitis*.

Isolation of *Streptococcus mutans* and *Streptococcus sanguis*: The primary culture was subcultured on Mutans sanguis agar to isolate *Streptococcus mutans* and *Streptococcus sanguis* and incubated at 37 degree Celsius for 24 hrs the colonies of *Streptococcus mutans* appeared grayish yellow. The colonies of *Streptococcus sanguis* appeared whitish grey.

Isolation of *Streptococcus mitis* and *Streptococcus salivarius*: The primary culture was subcultured on mitis salivarius agar base to isolate *Streptococcus mitis* and *Streptococcus salivarius*. The culture was incubated at 37 degree Celsius for 24-48 hrs, with added 1% Potassium tellurite. The colonies of *Streptococcus salivarius* appeared "gum drop" colonies (shown in fig: 5) the colonies of *Streptococcus mitis* appeared small, flat, hard colonies, blue in color with a domed center.

Preparation of Triphala gel

Triphala gel was prepared at concentration of 6.25%, 12.5%, 25%, 50% (shown in fig: 3) in the lab of Department of Pharmacy, Y.B Chavan College of Pharmacy, Aurangabad.

Antimicrobial activity

Antimicrobial activity was checked by agar gel disk diffusion method to check the zone of inhibition for the concentrations of 6.25%, 12.5%, 25%, 50%.The zone of inhibition was measured using Metal scale after the completion of incubation period shown in fig: 4 & fig: 6)

Results

Comparison of the antibacterial activity of triphala at 6.25%, 12.5%, 25%, 50% on *Streptococcus mutans*, *Streptococcus sanguis*, *Streptococcus mitis*, *Streptococcus salivarius* by agar disk diffusion method. Maximum zone of inhibition was seen at 50% followed by 25%, 12.5%, 6.25% for *Streptococcus mutans*, *Streptococcus sanguis*, *Streptococcus mitis*, *Streptococcus salivarius*. The mean zone of inhibition for *Streptococcus mutans* at 6.25%, 12.5%, 25%, 50% was 19mm, 20mm, 25mm, 29mm & for *Streptococcus salivarius* was 20mm, 22mm, 25mm, 27mm.for *Streptococcus mitis* it was 15mm, 18mm, 20mm, 22mm For *Streptococcus sanguis* it was 12mm, 19mm, 22mm, 24mm.

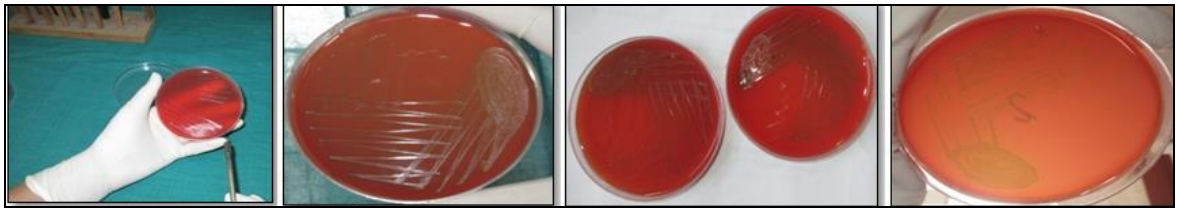

Fig 1a, b: Primary inoculum distributed evenly by streaking with loop, c & d showing primary

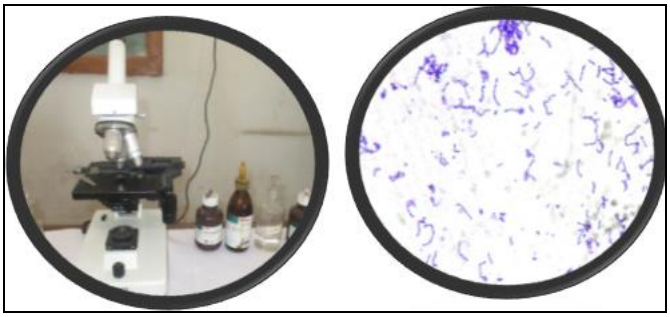

Fig 2: Showing armamentarium for staining and slide showing colonies of streptococcus in chains

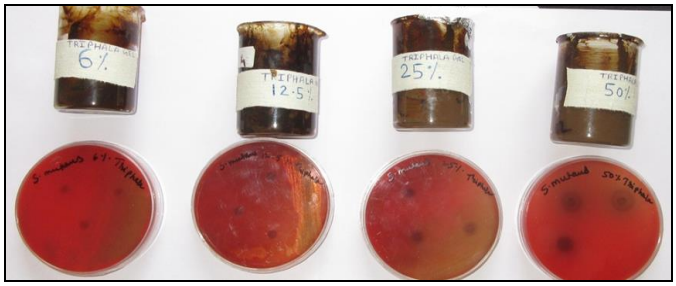

Fig 4: Showing concentrations of triphala gel and zone of inhibition for the particular concentration by agar well disc diffusion method for *Streptococcus mutans*.

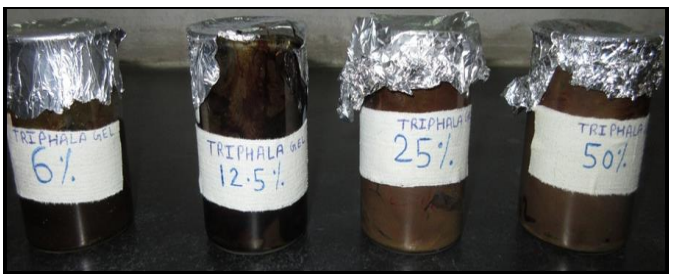

Fig 3: Showing triphala gel at concentrations of 6.25%, 12.5%, 25%, 50%

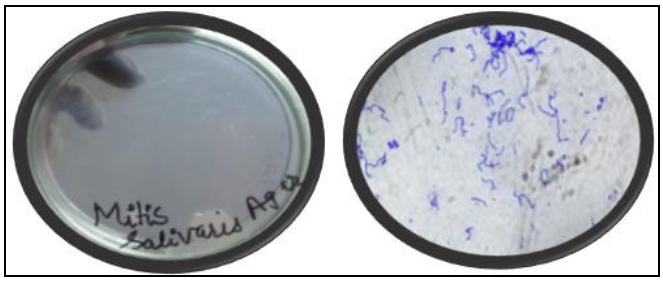

Fig 5: Showing growth of *Streptococcus salivarius* on Mitis salivarius agar agar and slide showing colonies of *Streptococcus salivarius*.

Fig 6: showing concentrations of triphala gel and zone of inhibition for the particular concentration by agar well disc diffusion method.

Discussion

In the present study antimicrobial activity of ayurvedic product triphala gel at concentrations 6.25%, 12.5%, 25%, 50%.Triphala is a well-known powdered preparation in the Indian system of medicine. Triphala consists of equal parts of Emblica officinalis, Terminalia Chebula and Terminalia belerica. Ingredient wise chemical constituents of triphala are Tannins, Quinones, Flavones, flavonoids, flavonols, Gallic acid, Vitamin C. Amalaki is known for its excellent healing properties. Terminalia chebula exhibited antibacterial activity against gram positive & gram negative human bacterial species. Terminalia belerica (Bibhitaki). T. belerica possesses antioxidant, antispasmodic, bronchodilatory, hypercholesterolemic, antibacterial, cardio protective, hepatoprotective, hypoglycemic and hypotensive properties. Triphala is known for anticaries activity, as a root canal irrigant, anticollagenase activity, antimicrobial, antioxidant effect, triphala as a mouthrinse [6]. In the present study antimicrobial activity of ayurvedic product triphala gel at concentrations 6.25%, 12.5%, 25%, 50%.

Jagadeesh *et al*, (2009) [7] conducted a study to determine effect of triphala on dental biofilm demonstrated the antioxidant and antimicrobial activity of triphala against *S. mutans* gram positive cocci, involved in plaque formation when it is adsorbed to the tooth surface.

Thomas *et al* (2011) [1] found the mean inhibition zone for the aqueous extract of triphala at 50%, 25% and 12.5% against clinical isolates of *S. mutans* was found to be 30mm, 28mm and 24mm using MTCC Strains.

Prajapathi and Raol (2014) [8] found the aqueous extract of triphala to inhibit *S. mutans*. The mean inhibition zone was 17mm against MTCC Strains and 19mm against clinical isolates.

Ruchika Gupta (2014) [9] conducted study to assess the antimicrobial efficacy of the aqueous and ethanolic extracts of triphala at various concentrations against primary plaque colonizers at 6.25%,12.5%,25%,50% and found maximum zone of inhibition at 50% and both aqueous and ethanolic extracts of triphala have the potential to be used antiplaque agents.

Triphala due to presence of Terminalia chebula is valuable in the prevention and treatment of several diseases of mouth such as dental caries, spongy and bleeding gums, gingivitis, and stomatitis. The antiplaque activity of triphala can be attributed to the presence of tannins, the antioxidant property of triphala can be attributed to presence of gallic acid, ellagic acid which prevents formation of free radicals. The ant collagenase effect of triphala is due to matrix metalloproteinase inhibiting property of triphala.

The literature comparing antimicrobial efficacy of triphala gel on oral bacteria is non-existent hence our results cannot be compared with any previous published literature. Further studies on secondary and tertiary colonizers is required for further beneficial uses of triphala.

Conclusion

Based on the results of our study the following conclusions can be drawn.

Triphala gel has significant antimicrobial activity and can be used effectively as an antiplaque agent.

It inhibits the growth of *Streptococcus sanguis*, *Streptococcus mitis*, *Streptococcus mutans*, *Streptococcus salivarius*, so can be effectively use to inhibit the primary plaque colonizers.

The mean diameter of inhibition zone increased with increasing concentrations with maximum inhibition at 50 % concentration.

Triphala gel seems to fulfill most of these requirements without any adverse effect on oral tissues and at very minimal cost as compared to commercially available products today.so further research on various therapeutic actions of triphala should be encouraged in dentistry.

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