



Antimicrobial efficacy of aqueous and ethanolic extracts of Triphala on primary plaque colonizers: An *in vitro* study

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ABSTRACT

Objective: The aim was to assess the antimicrobial efficacy of the aqueous and ethanolic extracts of Triphala at various concentrations against primary plaque colonizers. **Materials and Methods:** Preparation of the herbal extracts for this *in vitro* study was done using cold infusion method. Ethanol and Millipore water were used as solvents for extraction. A stock solution was prepared by adding 1000 mg of dried extract in 1 ml of dimethyl sulfoxide (DMSO). The stock solution was further diluted to obtain 6.25%, 12.5%, 25% and 50% concentrations of the extract. The antimicrobial efficacy testing of Triphala extracts against these bacteria was done by agar well diffusion method. 0.2% chlorhexidine was used as a positive control, while DMSO acted as a negative control. One-way analysis of variance and independent sample *t*-test were used for comparing mean diameter of inhibition zones. **Results:** All the concentrations of ethanolic and aqueous extracts of Triphala inhibited the growth of *Streptococcus mutans*, *Streptococcus sanguis* and *Streptococcus salivarius*. In general, the efficacy increased with increasing concentration with maximum inhibition at 50% concentration. There was no statistically significant difference in the mean diameter of inhibition zone between the ethanolic and aqueous extracts of Triphala against *S. mutans*. **Conclusion:** Both aqueous and ethanolic extracts of Triphala have the potential to be used antiplaque agents.

Key words: Antimicrobial efficacy, dental caries, minimum inhibitory concentration, periodontal diseases, *Streptococcus mutans*, Triphala

INTRODUCTION

The prevalence of dental caries is steadily increasing, and periodontal diseases are amongst the most common

afflictions of mankind.¹⁻⁴ Dental plaque is a microbial ecosystem mainly consisting of densely packed microbial products and microbial byproducts.⁵ It is an important etiological factor for these two dental diseases.⁶

The current concept of disrupting the biofilm through professional and or home care practices are based on this association between dental plaque and periodontal diseases. The efficient plaque control demands a high degree of motivation and skill, which is beyond the scope of most individuals especially in rural settlements which are less educated, less aware about the importance of good oral hygiene.

Access this article online	
Journal Sponsor	Website: www.jyoungpharm.org
	DOI: 10.5530/jyp.2014.3.2

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Antimicrobial mouth rinses have been suggested as adjuncts for mechanical plaque control methods. The most commonly used antiplaque agent is chlorhexidine gluconate. The use of chlorhexidine has some potential drawbacks like altered taste sensation, staining of teeth and development of resistant microorganisms that incapacitate their long term use. This necessitates the development of innovative strategies. One such strategy would be to verify the enormous wealth of medicinal plants abundantly available in nature.

“Triphala” has been described as a classic Ayurveda remedy.⁷ It comprised of fruits of three medicinal trees, Amalaki - *Emblica officinalis*, Vibhitaki - *Terminalia bellerica*, Haritaki - *Terminalia chebula*. It has antibacterial, antiseptic, and anti-inflammatory properties along with many other properties.⁵

The studies assessing the beneficial effects of Triphala in preventing oral diseases is scanty. Hence, the present study was undertaken to assess the antimicrobial efficacy of the aqueous and ethanolic extracts of Triphala at various concentrations against primary plaque colonizers.

MATERIALS AND METHODS

This *in vitro* study was conducted at Center for Scientific Research and Development, People's University, Bhopal over a period of 2 months (December 2013 and January 2014).

Preparation of Triphala extracts

Ethanolic extract

The preparation of the herbal extract was done using cold infusion method. Two grams of commercially available Triphala Churna powder was dissolved in 10 ml of ethanol in a glass bottle. The mixture was then subjected to intermittent stirring for 48 h. The solution was filtered with Whatman filter paper. The filtrate was allowed to dry at room temperature. A stock solution was prepared by adding 1000 mg of dried extract in one ml of dimethyl sulfoxide (DMSO). The stock solution was further diluted to obtain 6.25%, 12.5%, 25% and 50% concentrations of the extract.

Aqueous extract

The procedure described above was employed to prepare 6.25%, 12.5%, 25% and 50% aqueous extract of Triphala using distilled water as a solvent for the extraction process.

Antimicrobial efficacy testing of ethanolic and aqueous extracts

Three primary plaque colonizers were used for antimicrobial efficacy testing in the present study. The pure laboratory

cultures of these bacteria were procured from American type culture collection (ATCC), USA. The antimicrobial efficacy testing of the ethanolic and aqueous extracts of Triphala against these bacteria was done by agar well diffusion method using Brain Heart Infusion agar. The zone of inhibition was measured using a metal scale at the end of 48 h of incubation. 0.2% chlorhexidine was used as a positive control, while DMSO acted as a negative control.

Minimal inhibitory concentration (MIC)

The MIC of ethanolic and aqueous extracts of Triphala on *Streptococcus mutans*, *Streptococcus sanguis* and *Streptococcus salivarius* was determined using the agar well diffusion method described earlier. The required number of serial dilutions was prepared by serially diluting a standard stock solution. The lowest concentration of the extract that inhibited the growth of *S. mutans*, *S. sanguis* and *S. salivarius* was considered the MIC of the extract against the particular bacteria.

Data analysis

The statistical analysis was performed using Statistical Package for Social Sciences version 20 (IBM, Chicago, USA). The mean inhibition zone between different concentrations of Triphala extract (ethanolic and aqueous) against each bacteria was compared using one-way analysis of variance and Tukey's *post-hoc* test. The mean inhibition zone between ethanolic and aqueous extracts of Triphala was compared using independent sample *t*-test. The statistical significance was fixed at 0.05.

RESULTS

The details of the plant ingredients in Triphala churna and their yield are presented in Table 1. The yield was more with aqueous extract than the ethanolic extract. The details of the bacteria used in the present study are denoted in Table 2.

Antimicrobial efficacy against *S. mutans*

The highest mean diameter of inhibition zone against *S. mutans* was observed with 50% concentrations of ethanolic (29.2 ± 0.7 mm) and aqueous extracts (29.5 ± 0.6 mm). The mean inhibition zone at all concentrations of ethanolic and aqueous extracts of Triphala against *S. mutans* was significantly higher compared with 0.2% chlorhexidine ($P = 0.001$, Table 3). DMSO failed to inhibit the growth of *S. mutans*. The multiple pair-wise comparisons revealed a significantly higher zone of inhibition with 50% concentration of the extract compared with other concentrations.

Table 1: Details of the plant extracts used in the present study

Extract	Botanical name	Common name	Family	Solvent used	Dry weight of extract (G)	Yield %
Triphala churna	<i>Terminalia chebula</i> Retz	Haritaki	Combretaceae	Ethanol	0.295	14.75
	<i>Terminalia bellirica</i>	Bahera (Bibhitaka)	Combretaceae			
	<i>Embolica officinalis</i>	Amla or Indian gooseberry	Euphorbiaceae	Millipore water	0.684	34.2

Table 2: Details of the bacteria used for antimicrobial efficacy testing in the present study

Bacteria	ATCC number	Selective media used for revival	Type of haemolysis on blood agar plate
<i>S. mutans</i>	25175	Brain heart infusion agar with 5% sheep blood	Gamma haemolysis
<i>S. sanguis</i>	10556	Brain heart infusion agar with 5% sheep blood	Alpha haemolysis
<i>S. salivarius</i>	13419	Brain heart infusion agar with 5% sheep blood	Gamma haemolysis

ATCC: American type culture collection, *S. mutans*: *Streptococcus mutans*, *S. sanguis*: *Streptococcus sanguis*, *S. salivarius*: *Streptococcus salivarius*

Table 3: Antimicrobial efficacy of ethanolic and aqueous extracts of triphala on *S. mutans*

Concentration	Mean diameter of inhibition zone in millimeters (SD)*		
	Ethanolic extract	Aqueous extract	Statistical inference
6.25%	24.17 (3.84)	20.50 (2.43)	t value: 1.976 P value: 0.076
12.5%	24.75 (2.04)	25.08 (1.07)	t value: -0.354 P value: 0.731
25%	25.58 (3.61)	26.33 (1.17)	t value: -0.484 P value: 0.639
50%	29.17 (0.68)	29.50 (0.63)	t value: -0.877 P value: 0.401
0.2% chlorhexidine	16.25 (0.27)	16.25 (0.27)	
Statistical inference	F value: 20.72	F value: 91.26	
	P value: 0.001	P value: 0.001	

*SD: Standard deviation

Antimicrobial efficacy against *S. sanguis*

The maximum inhibition was observed at 12.5% concentration (22.8 ± 3.1 mm) while using ethanolic extract and at 50% concentration (27.3 ± 4.8 mm) using aqueous extract. All the concentrations showed a significantly higher zone of inhibition compared to chlorhexidine, while using the ethanolic and aqueous extracts ($P = 0.001$, Table 4). However, there was no significant difference in the mean diameter of inhibition between 6.25% ethanolic extract

Table 4: Antimicrobial efficacy of ethanolic and aqueous extracts of triphala on *Streptococcus sanguis*

Concentration	Mean diameter of inhibition zone in millimeters (SD)*		
	Ethanolic extract	Aqueous extract	Statistical inference
6.25%	12.42 (0.74)	20.83 (3.86)	t value: -7.790 P value: 0.001
12.5%	22.83 (3.11)	23.75 (5.95)	t value: -0.496 P value: 0.625
25%	19.00 (3.66)	25.50 (4.41)	t value: -2.780 P value: 0.019
50%	22.58 (0.49)	27.33 (4.77)	t value: -2.426 P value: 0.036
0.2% chlorhexidine	12.42 (0.49)	12.42 (0.49)	
Statistical inference	F value: 33.50	F value: 11.09	
	P value: 0.001	P value: 0.001	

SD: Standard deviation

and chlorhexidine. The aqueous extract of Triphala yielded significantly higher mean diameter of inhibition zones compared with ethanolic extracts against *S. sanguis* at 6.25% ($P = 0.001$), 25% ($P = 0.019$) and 50% concentrations ($P = 0.036$, Table 4). However, the difference in the mean diameter of inhibition zone between the aqueous and ethanolic extracts of Triphala at 12.5% was not statistically significant ($P = 0.625$) though aqueous extract produced a marginally higher efficacy.

Antimicrobial efficacy against *S. salivarius*

The highest mean inhibition zone against *S. salivarius* was observed at 50% concentrations in both ethanolic (26.3 ± 2.9 mm) and aqueous extracts (29.3 ± 0.4 mm). There was a statistically significant difference in the mean inhibition zone between different categories ($P < 0.01$, Table 5). The *post-hoc* test revealed a significant difference between higher concentrations of the Triphala extracts (25% and 50% with ethanolic extract) and chlorhexidine while the lower concentrations (6.25% and 12.5%) showed no difference. The ethanolic extract of Triphala produced a significantly higher mean diameter of inhibition zone compared to aqueous extracts at 6.25% concentration ($P = 0.001$) while the aqueous extract produced a significantly

higher mean inhibition zone compared to ethanolic extract at 50% ($P = 0.027$). There was no significant difference in the mean diameter of inhibition zones between aqueous and ethanolic extracts at 12.5% ($P = 0.351$) and 25% concentrations ($P = 0.871$, Table 5). The inhibition zone produced by the ethanolic and aqueous extracts of Triphala on *S. mutans*, *S. sanguis* and *S. salivarius* is presented in Figure 1.

MIC

The ethanolic extract of Triphala inhibited *S. mutans*, *S. sanguis* and *S. salivarius* at 1 mg/ml, 0.5 mg/ml and 0.5 mg/ml, respectively. The MIC of aqueous extract of Triphala on *S. mutans*, *S. sanguis* and *S. salivarius* was 1 mg/ml, 0.5 mg/ml and 2 mg/ml, respectively (Figure 2, Table 6).

DISCUSSION

The aggregates of bacterial cell embedded in a polysaccharide and protein matrix adhering to the teeth is termed dental plaque.⁸ Several anti-plaque agents are available in the market. However, there is considerable interest in the development of other classes of antimicrobials for the control of infection owing to the rise in bacterial resistance

to antibiotics. Current advancement in drug discovery technology and search for novel chemical diversity has intensified the efforts of exploring herbal medicines. Triphala is one such herbal medicine that exhibits number of health benefits.⁸

The antimicrobial efficacy of Triphala at various concentrations against three dental plaque microorganisms considered to be the initial colonizers in the process of plaque formation was assessed.⁹

We found the ethanolic and aqueous extracts of Triphala at all concentrations to be effective against *S. mutans*, *S. sanguis* and *S. salivarius*.

T. chebula Retz contains polyphenols, terpenes, anthocyanins, flavonoids, alkaloids and glycosides.⁹ *Embllica officinalis* contains vitamin C and vitamin C complex.¹⁰ *T. bellirica* fruit contains chemical constituents such as tannins; viz gallic acid, ellagic acid, and Phylembilin.¹¹ Some components including a cardenolide, glycoside, oil containing palmitooleolinolein, 16-hentriacontanone, hexahydroxydiphenic acid ester, Friedelin, B-sitosterol, chebulagic acid are isolated from the fruit extract.¹²⁻¹⁵ The inhibitory effect of Triphala on *S. mutans* could be attributed to these phytochemical constituents.

Jagadeesh *et al.*, (2009)¹⁶ demonstrated the antioxidant and antimicrobial activity of Triphala against *S. mutans*. Jagtap and Karkera (1999)¹⁷ reported that extracts of *T. chebula* strongly inhibited the growth and adherence of *S. mutans*. Thomas *et al.*, (2011)⁸ found the mean inhibition zone for the aqueous extract of Triphala at 50%, 25% and 12.5% against *S. mutans* to be 30 mm, 28 mm, and 24 mm respectively using microbial type culture collection (MTCC) strains. 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 34 mm, 30 mm, and 28 mm, respectively. There was no significant difference in the efficacy with increasing concentrations. Although, the mean inhibition zones found in our study at various concentrations were comparable to the mean zones in this study, we found a significantly higher zone of inhibition at 50% concentration compared to other concentrations, contradictory to the findings of this study. A study by Prajapathi and Raol (2014)¹⁸ found the aqueous extract of Triphala to inhibit *S. mutans*. The mean inhibition zone was 17 mm against MTCC strains and 19 mm against clinical isolates. Here, 100 µl of 2% extract was used while we used 50 µl of 10% extract. They found variable results in terms of mean inhibition zone with maximum inhibition observed with acetone extract. The minor variations in the mean zone between our findings and these studies

Table 5: Antimicrobial efficacy of ethanolic and aqueous extracts of triphala on *Streptococcus salivarius*

Concentration	Mean diameter of inhibition zone in millimeters (SD)*		
	Ethanolic extract	Aqueous extract	Statistical inference
6.25%	21.92 (4.13)	17.33 (1.33)	t value: 4.773 P value: 0.001
12.5%	23.08 (4.51)	21.75 (4.49)	t value: 0.946 P value: 0.351
25%	25.17 (4.06)	25.50 (2.78)	t value: -0.166 P value: 0.871
50%	26.25 (2.88)	29.33 (0.41)	t value: -2.599 P value: 0.027
0.2% chlorhexidine	17.92 (0.58)	17.92 (0.58)	
Statistical inference	F value: 5.065 P value: 0.004	F value: 25.84 P value: 0.001	

SD: Standard deviation

Table 6: Minimum inhibitory concentration of ethanolic and aqueous extracts of triphala on *S. mutans*, *S. sanguis* and *S. salivarius*

Bacteria	Milligram/ml (%)	
	Ethanolic extract	Aqueous extract
<i>S. mutans</i>	1 mg/ml (0.1)	1 mg/ml (0.1)
<i>S. sanguis</i>	0.5 mg/ml (0.05)	0.5 mg/ml (0.05)
<i>S. salivarius</i>	0.5 mg/ml (0.05)	2 mg/ml (0.2)

S. mutans: *Streptococcus mutans*, *S. sanguis*: *Streptococcus sanguis*, *S. salivarius*: *Streptococcus salivarius*



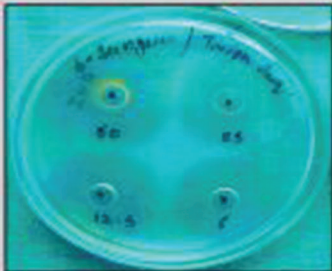

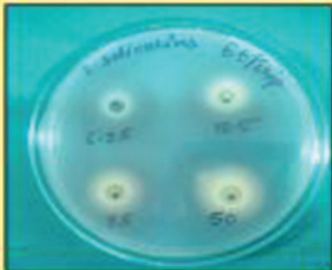

Bacteria	Ethanollic extract	Aqueous extract
<i>S. mutans</i>		
<i>S. sanguis</i>		
<i>S. salivarius</i>		

Figure 1: Mean diameter of inhibition zone by ethanolic and aqueous extracts of Triphala on *Streptococcus mutans*, *Streptococcus sanguis* and *Streptococcus salivarius*

could be attributed to methodological differences involved in the extraction process, antimicrobial efficacy testing, differences in the bacterial strains, concentration and volume of the extract used. The inhibition zone in our study is computed after subtracting the diameter of the well (7 mm) from the actual diameter of inhibition zone. Thomas *et al.*, (2011)⁸ found the MIC of ethanolic extract of Triphala on MTCC strains and clinical isolates of *S. mutans* to be 6.25% and 3.12%, respectively. These MIC values against *S. mutans* are high compared to MIC values in our study. The difference in the bacterial strains between our study and this study may explain these differences. The literature comparing the antimicrobial efficacy of aqueous and ethanolic extracts of Triphala on oral bacteria is non-

existent. Hence, our results could not be compared with any previous published literature. The present *in vitro* study was conducted using ethanol and aqueous extracts of Triphala on ATCC strains of *S. mutans*, *S. sanguis* and *S. salivarius*. The antimicrobial efficacy testing using other solvent systems and clinical isolates of these bacteria could offer different results. Moreover, further studies on secondary and tertiary plaque colonizers could add to beneficial effects of Triphala before considering this for clinical use.

CONCLUSION

Based on the results of the present study, the following conclusions are drawn:

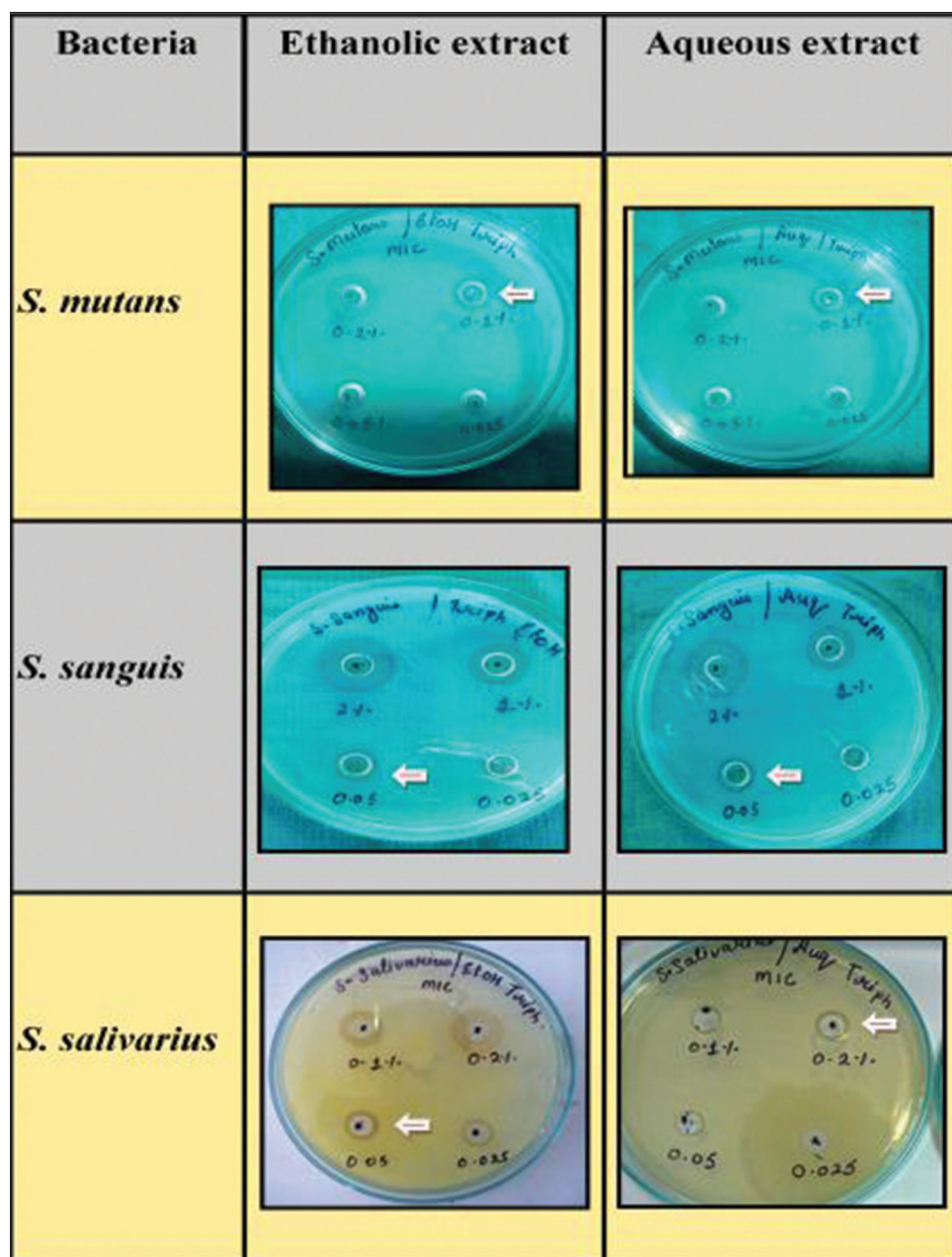


Figure 2: Minimum inhibitory concentration of the ethanollic and aqueous extracts of Triphala on *Streptococcus mutans*, *Streptococcus sanguis* and *Streptococcus salivarius*

- Both aqueous and ethanollic extracts of Triphala inhibited the growth of *S. mutans*, *S. sanguis* and *S. salivarius*. Hence, they could be considered as alternates to chlorhexidine.
- The mean diameter of inhibition zone in general increased with increasing concentration with maximum efficacy observed at 50% concentration and least at 6.25%.
- There was no statistically significant difference in the mean diameter of inhibition zone between the ethanollic and aqueous extracts of Triphala against *S. mutans*.
- The aqueous extract of Triphala showed a higher mean diameter of inhibition zone against *S. sanguis* compared with ethanollic extracts.
- The ethanollic extract of Triphala produced a significantly higher mean diameter of inhibition zone against *S. salivarius* compared to aqueous extracts at 6.25% concentration, while aqueous extract produced a significantly higher mean inhibition zone compared to ethanollic extract at 50% concentration.

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