



ETHANOLIC EXTRACTS OF BHRINGRAJ, HARITAKI AND SHANKAPUSHPAM VS CHLORHEXIDINE GLUCONATE AGAINST *S. MUTANS*: AN IN VITRO ANALYSIS

Dental Science

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ABSTRACT

Aim: To evaluate the antimicrobial efficacy of ethanolic extracts of Bhringraj, Haritaki and Shankapushpam against *S. mutans* and compare with commercially available 0.2% Chlorhexidine gluconate (CHX).

Materials And Methods: Ethanolic extracts of Bhringraj, Haritaki and Shankapushpam were obtained by subjecting them to hot continuous extraction process and test extracts of 25%, 12.5%, 6.25% and 3.12% concentrations were prepared. Their antimicrobial efficacy was evaluated and compared with 0.2% CHX against *S. mutans* using the agar dilution method. The results were statistically analyzed using 1-way ANOVA and LSD Post Hoc tests.

Results: Haritaki seemed to have superior and statistically significant antimicrobial activity when compared to the test extracts at all the concentrations at which they were tested. It was followed by 0.2% CHX, which also showed considerable antimicrobial activity even at a low concentration.

Conclusion: Ethanolic extract of Haritaki has better antimicrobial efficacy compared to 0.2% CHX at the tested concentrations and can be considered as a safe herbal alternative for it.

KEYWORDS

Bhringraj, Haritaki, Shankapushpam, *S. mutans*

INTRODUCTION:

Human oral cavity serves as an excellent reservoir for several microorganisms, therefore, maintenance of good oral hygiene is the key to prevention of dental diseases. Dental caries is one of the most common diseases of the oral cavity affecting almost 50-60% of the Indian population^{1,2}. Early childhood caries (ECC), an aggressive form of caries, shows a heavy infection of *Mutans Streptococci* (MS), which may exceed the total plaque-biofilm flora by 30% at times³. In humans usually two species of MS are found: *Streptococcus mutans* (*S. mutans*) and *Streptococcus sobrinus* (*S. sobrinus*)⁴. Therefore, maintenance of good oral hygiene is the key to prevention of dental diseases.

It is believed that 0.2% chlorhexidine gluconate (CHX) reduces caries by preventing plaque formation that houses *S. mutans* and *Lactobacilli spp*⁵, but few studies have shown that in adolescents and children, it does not concurrently reduce caries⁶. Moreover, various adverse effects have been reported with the regular use of oral products containing this chemical⁷, thus raising concerns about it. Also, the emergence of multidrug resistant microorganisms⁸ led to renewed interest in Ayurveda as a substitute.

Evidence of the use of plants for medicinal purposes dates some 60,000 years back in both Western and Eastern cultures⁹. Herbal remedies have a long history of use for gum and tooth problems. It is well known that our ancestors have used unrefined sea salt, neem seed oil, twigs of mango or neem tree to clean their teeth. Even today, in many parts of rural India, people practice these ancient methods of tooth cleaning¹⁰. Although most of the plants used for medicinal and dental purposes have been identified and their applications are well documented and described by different authors, the antimicrobial efficacy of many plants is yet to be verified¹¹.

Earlier studies established that the ripe fruit of *Terminalia chebula* (*T. chebula*) or *Haritaki* is valuable in the prevention and treatment of oral diseases like gingivitis, stomatitis and subsequently the extract of *T. chebula* was reported to be potential anticariogenic mouthwash¹⁰. In view of its medicinal properties and applications in dentistry this plant was selected in the present work to examine the antimicrobial efficacy against dental pathogens.

It has been proven by several authors that *Clitoria ternatea* (*C. ternatea*) or *Shankapushpam* is a not only a long known effective

natural remedy for variety of ailments, but has also shown powerful antimicrobial activity against *Escherichia coli*, *Vibrio cholerae*, *Staphylococcus aureus*, etc.¹². Previous studies indicated that *Wedelia chinensis* (*W. chinensis*) or *Bhringraj* has anti-inflammatory action and is a potential analgesic and its efficacy can be comparable to standard drugs like aspirin, morphine, etc.¹³. However, there is paucity of documented literature concerning the antimicrobial activity of *C. ternatea* and *W. chinensis* against dental pathogens. Therefore, the present study was undertaken to evaluate the antimicrobial efficacy of *Haritaki*, *Shankapushpam* and *Bhringraj* against *S. mutans*, the proven initiator of dental caries.

MATERIALS AND METHODS:

Preparation of ethanolic extracts¹⁴:

The powders of *Bhringraj*, *Haritaki* and *Shankapushpam* were procured from the local market along with ethanol that was to be used as the organic solvent for extraction. The active extracts were obtained by hot continuous Soxhlet extraction method. In three separate Soxhlet apparatuses, 300 grams of the test powders were placed. In the round bottom flask, 300 ml of ethanol was added and 400 ml was used to wet the powder in the thimble of the apparatus.

Ethanol in the round bottom flask was heated using the isomantle at around 50-60°C and was allowed to evaporate, moving through the apparatus to the condenser. The condensate then dripped into the reservoir containing the thimble. Once the level of solvent reached the siphon, it poured back and a new cycle initiated. This process was terminated once the extract pouring into the siphon either became totally colourless or light in colour.

The collected extract was allowed to cool and the ethanol was left to evaporate. Once all of ethanol evaporated, the extracts were collected in vials and stored in a dessicator till the time of use.

Preparations of the dilutions of the test extracts:

Dimethyl sulfoxide (DMSO) was used as the solvent for preparing the dilutions of the test extracts. To get the desired dilution, the extracts were dissolved in 1 ml of DMSO in the quantity mentioned below:

25% = 250 mg of test extract in 1000 µl DMSO

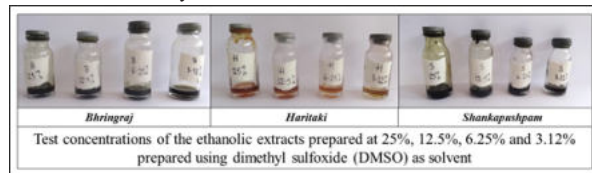
12.5% = 125 mg of test extract in 1000 µl DMSO

6.25% = 62.5 mg of test extract in 1000 µl DMSO

3.12% = 31.2 mg of test extract in 1000 µl DMSO

Agar well diffusion method¹⁴:

S. mutans was isolated using standardized microbiological techniques from the saliva collected from a patient with Early Childhood Caries (ECC) after securing informed consent from the patient's guardian and acquiring ethical clearance from the Institutional Ethical Committee. Further on, it was cultured in Brain-Heart Infusion (BHI) broth and subsequently the inoculum size was adjusted so as to deliver a final inoculum of approximately 10^8 CFU/ml using 0.5 McFarland's standard. For the agar well diffusion method, twelve culture plates of BHI-A were prepared onto which six wells of 8 mm diameter each were punched using a sterile cork borer. The plates were labelled with the name of the test extract and the concentrations at which they were being tested. 0.2% CHX was considered as the positive control and DMSO as the negative control and also to rule out if it had any antimicrobial activity of its own.



The diluted extracts were poured into the wells using a micropipette that was adjusted to deliver 50 μ l of the test solutions. Each extract, 0.2% CHX and DMSO were tested in triplicate. The plates were then kept for incubation at a temperature of 35-37°C for 24 hours. After the incubation period, the zones of inhibition formed around the wells were measured using a millimeter scale with a handle.



The data obtained was tabulated, computerized and analyzed using the Statistical Package for Social Sciences software version (SPSS) 17.0. The mean and standard deviation were calculated. One way analysis of variance (1 way-ANOVA) was used for multiple group comparison and LSD Post Hoc test was used for pairwise comparison. The value $p < 0.05$ was considered statistically significant.

RESULTS:

The ethanolic extracts were tested at 25%, 12.5%, 6.25% and 3.12% and were compared with CHX against *S. mutans*.

DMSO was used as an inert solvent for preparing the extracts. To rule out the possibility of any antimicrobial activity of DMSO, we used it as a negative control.

At 25% concentration, *Haritaki* showed maximum antimicrobial activity (27.66 ± 0.47), followed by CHX (15.13 ± 0.94), *Bhiringraj* (10.33 ± 0.47) and *Shankapushpam* (9.33 ± 0.94) respectively. The differences were highly significant ($p < 0.001$) except for the differences in activity between *Bhiringraj* and *Shankapushpam* ($p > 0.05$). The efficacy was observed in the increasing order – *Haritaki* > CHX > *Bhiringraj*, *Shankapushpam* > DMSO.

At 12.5% concentration, the antimicrobial activity was observed in the following order - *Haritaki* > CHX > *Bhiringraj*, *Shankapushpam* > DMSO. *Haritaki* showed maximum activity against *S. mutans* (24.66 ± 1.24). The differences were highly significant ($p < 0.001$) against all the test solutions except for the differences between *Bhiringraj* and *Shankapushpam* ($p > 0.05$).

Table 1: Antimicrobial Activity Of Test Extracts And 0.2% Chlorhexidine Gluconate Against *S. Mutans*

Legend: *HS: Highly Significant; [†]: numbers in this row correspond to the respective serial number of the test extract

Sr. No.	Extract	Zone of inhibition (mm) $\pm$ SD			
		25%	12.5%	6.25%	3.12%
1	<i>Bhiringraj</i>	10.33 ± 0.47	8.33 ± 0.47	0	0

2	<i>Haritaki</i>	27.66 ± 0.47	24.66 ± 1.24	20.33 ± 3.29	17.33 ± 3.29
3	<i>Shankapushpam</i>	9.33 ± 0.94	8.33 ± 0.94	0	0
4	CHX	15.13 ± 0.94	15.13 ± 0.94	15.13 ± 0.94	15.13 ± 0.94
5	DMSO	0	0	0	0
ANOVA		F = 596.78 $p < 0.001$ (HS*)	F = 278.29 $p < 0.001$ (HS*)	F = 86.725 $p < 0.001$ (HS*)	F = 70.719 $p < 0.001$ (HS*)
[†] LSD Post Hoc		2 > 4 > 1, 3 > 5	2 > 4 > 1, 3 > 5	2, 4 > 1, 3, 5	2 > 4 > 1, 3, 5

At 6.25%, the antimicrobial efficacy of *Bhiringraj* and *Shankapushpam* seemed to be negligible. *Haritaki* and CHX showed considerable antimicrobial activity (20.33 ± 3.29 and 15.13 ± 0.94 respectively) in comparison to the other test solutions and the differences between them were highly significant ($p < 0.001$). The order of activity observed at this concentration was *Haritaki* > CHX > *Bhiringraj*, *Shankapushpam*, DMSO.

At 3.12%, it was observed that *Haritaki* seemed to have greater antimicrobial activity in comparison to the other test solutions (17.33 ± 3.29). *Bhiringraj* and *Shankapushpam* had negligible activity at this concentration too. The antimicrobial activity was observed in the order *Haritaki* > CHX > *Bhiringraj*, *Shankapushpam*, DMSO at this concentration too.

DISCUSSION:

The pathology of ECC is highly complex. Majority of studies point to the *Streptococcus* group as being the main etiological agent of dental caries and that this microorganism can also lead to non-oral infections, principally bacterial endocarditis¹⁵ which is a serious problem.

Though oral cavity contains a wide variety of oral bacteria, only a few specific species of bacteria are believed to cause dental caries. Some studies^{16,17} indicate that the initiation and progression of dental caries is closely associated with *S. mutans*. It can therefore be inferred that *S. mutans* play an important role in the aetiology of dental caries.

If left untreated, dental caries can lead to pain, tooth loss and infection. Destroyed tooth structure does not fully regenerate, although remineralization of very small carious lesions may occur if dental hygiene is kept at optimal level. Therefore, it is desirable to prevent caries in order to preserve tooth structure.

Chlorhexidine is widely used as an ideal mouth wash for several years in western countries. Our study also shows that even at a concentration as low as 0.2%, CHX is a highly effective anticariogenic agent. But despite its effectiveness in combating dental caries, 0.2% CHX is not free from controversy surrounding its usefulness⁷. Most of the reviews conducted in recent years have concluded that although some formulations of 0.2% CHX might appear to be effective in inhibiting caries in children and adolescents, the evidence for an overall caries-preventive effect of Chlorhexidine was lacking and inconclusive¹⁸ and regular use of oral care products containing this chemical are often associated with various adverse effects⁷.

The emergence of multi drug-resistant bacterial and fungal strains has increased substantially in the recent years and is posing a serious therapeutic problem worldwide. One of the methods to reduce the resistance to antibiotics is by utilizing antibiotic resistant inhibitors produced from medicinal plants. Researchers stated that plant extracts show target sites other than those used by antibiotics, which will be active against drug-resistant pathogens⁹. Plants contain phytochemicals such as alkaloids, tannins, essential oils, flavanoids, etc., which have pronounced antimicrobial activity and other properties. The phytoconstituents present in the plants exhibit anti-cariogenic effects through various modes of action, including bactericidal effects on oral bacteria, prevention of adherence of bacteria to the tooth surfaces, inhibition of glucan production, and inhibition of amylases²⁰.

It is a proven fact in pharmacognosy and pharmaceuticals that when alcohol, especially ethanol, when used as a solvent for extraction has a potential to dissolve comparatively more phytochemical constituents compared to distilled water. The available literature had tested the

efficacy of aqueous extracts of *Bhringraj*, *Haritaki* and *Shankapushpam*²¹. Therefore, we undertook this study to evaluate the antimicrobial efficacy of the ethanolic extracts of these herbs.

Our study showed that the ethanolic extracts of *Haritaki* had a considerable antimicrobial activity against *S. mutans* even at a concentration of 3.12%. A study²² showed that 10% mouthwash of its aqueous extract had a potential to inhibit the salivary bacterial count and the *S. mutans* count for upto three hours compared to placebo. The study at Gowd et al²¹ showed that *S. mutans* was mildly susceptible to the aqueous extracts of *Haritaki* at 25% concentration. In our study, it was seen that *S. mutans* was highly susceptible to the ethanolic extract of *Haritaki* at the same concentration (Table 1). However more in vivo interventions would be needed to introduce it commercially. The antimicrobial effect of this herb apparently could be attributed to the property of its phytoconstituents to block their ability to utilize sugars²³.

Bhringraj has been used as a component in herbal dentrifice formulations, but less literature is available in particular related to their anticariogenic activity. Thus, this study was taken up to evaluate its antimicrobial activity against *S. mutans*. Our study showed that this herb has mild antimicrobial activity against *S. mutans*, which is in contrast to the study performed by Rehana Banu and Nagarajan N.²⁴ that showed the *Streptococcus spp.* to be more susceptible to this herbal extract. When compared to the study done to test the antimicrobial efficacy of its aqueous extracts at 25% against *S. mutans*²¹, we found that ethanolic extracts of *Bhringraj* at the same concentration showed mild antimicrobial activity against *S. mutans* (Table 1). This could be due to the chemical property of ethyl alcohol to dissolve a large number of chemical constituents such as alkaloids, alkaloidal salts, glycosides, tannins, anthraquinone derivatives, volatile oils and resins²³. Another possible reason for its mild anticariogenic activity could be because of the presence of small amounts of fluoride in the herb²⁶.

Shankapushpam is a drug that has been used for the treatment of multiple ailments, but its efficacy against dental caries microbes had not been studied extensively. Gowd et al.²¹ had tested the efficacy of the aqueous extract of this herb on *S. mutans*, *Staphylococcus aureus* (*S. aureus*) and *Lactobacillus casei* (*L. casei*) and found that it was mildly susceptible against these three microorganisms. In our study, we tested the ethanolic extract of *Shankapushpam* at 25%, 12.5%, 6.25% and 3.12%. We found that at there was mild activity at 25% and 12.5% concentrations (Table 1). Our study as well as the study by Gowd et al²¹ show that this herb had minimal or no activity against *S. mutans*.

It is known that CHX used in high concentrations has an immediate bactericidal effect during rinsing; it penetrates to the bacterial cell wall and leads to precipitation of the cytoplasm²⁷. Lower concentrations have a bacteriostatic effect²⁸. But these benefits of CHX are often associated with side effects. This emphasizes the need to find a suitable alternative for it. The results of our study concur with several studies^{22, 29} that propose herbal extracts as an alternative to CHX.

Though we see that *Haritaki* has better antimicrobial activity than the solutions tested in this study, we need more human interventions to establish it as a better anticariogenic agent. Also, long term studies will have to be carried out to evaluate the possible side-effects and toxicity issues of this herb, if any, before actually introducing it as an apt substitute for 0.2% CHX.

CONCLUSION:

Haritaki showed superior antimicrobial activity against *S. mutans* at 25%, 12.5%, 6.25% and 3.12% concentration when compared with *Bhringraj* and *Shankapushpam* at the same concentrations and 0.2% chlorhexidine gluconate.

Bhringraj and *Shankapushpam* did not have significant antimicrobial activity against *S. mutans* at the tested concentrations.

At 3.12%, *Haritaki* and 0.2% CHX showed differences in their antimicrobial activity but were not statistically significant.

Dimethyl sulfoxide did not influence the antimicrobial activity of *Haritaki*, *Bhringraj* or *Shankapushpam*.

Chlorhexidine gluconate showed considerable antimicrobial activity

even at a concentration as low as 0.2%.

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