Formulation and Characterization of polyherbal mouthwash containing *Achyranthes aspera* extract

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ABSTRACT:

The objective of this study is to formulate a mouthwash containing ethanolic extract of leaves of *Achyranthes aspera* and check its therapeutic efficacy. *A. aspera* is a plant with high medicinal values. Leaves of *A. aspera* were collected and ethanolic extract was obtained through maceration. Formulations named F1, F2, F3 and F4 with different concentration of extract were prepared and evaluated for physicochemical parameters (pH, colour, odour etc) as well as its stability. Mouthwash was also evaluated for antibacterial and antifungal activity against *Streptococcus mutans (ATCC 21571)* and *Candida albicans (MTCC 277)* using 0.2% chlorhexidine as control. The agar well diffusion method was used to test the in vitro antibacterial and antifungal activity for mouthwash, it was found that the mouthwash shows good antibacterial and antifungal property hence it can be used against oral microorganisms.

KEYWORDS: *Achyranthes aspera,Streptococcus mutans, Candida albicans,* Maceration, Agar well diffusion method, 0.2% chlorhexidine.

Introduction

A wide variety of microorganisms inhabit the mouth from birth, just as other body surfaces (all together known as the oral microbiota). Although bacteria are the most prevalent microorganisms in the oral cavity, it is also possible to find yeast, viruses, mycoplasmas, protozoa, and archaea there[1].

Dental caries remains one of the most prevalent global chronic diseases. One of the major contributors to dental caries is *S. mutans*. The lifestyle of *Streptococcus mutans* is not one of free living. The human mouth is *S. mutans*' natural home for growth; more particularly, dental plaque promotes its growth. In the human mouth, the bacteria lives in multispecies biofilms that form on the surfaces of teeth. [3]. A yeast called *Candida albicans* is typically found in the oral flora. Because it is the yeast that colonises the oral cavity with the greatest ease, it is the main cause of candidiasis[4].

No matter What your age is, taking care of your mouth is important for your general health and well-being. The ability to chew and nutritional intake can both be impacted by oral conditions such dental caries (tooth decay) and periodontal disease (gum disease). They can affect your social interaction and can impact on good quality of life. Hence, it is important to take care of your oral health, maintain good oral hygiene to protect your mouth[1]. The herb *Achyranthes aspera* is a member of the Amaranthaceae family; it is an annual plant that grows like a weed all throughout India. It is referred to as Apamarga traditionally. *A. aspera* has various therapeutic activities including antibacterial, immunomodulatory and antioxidant, anticancer, anti-inflammatory, thyroid stimulating, antiperoxidative etc. Ethanolic extract of leaves of *Achyranthes aspera* is proven to have antimicrobial activity against oral microorganisms[5]. Cloves possess antiseptic, analgesic properties and it is used in oral infections. *Glycyrrhiza glabra* belongs to family Leguminosae family. Saponin, one of the active components in *Glycyrrhiza glabra*, has a surfactant property and is used to increase the absorption of poorly absorbed drugs. The purpose of this study is to develop and assess a mouthwash containing herbal extract of leaves of *A. aspera* that has antimicrobial capabilities and can inhibit the growth of bacteria that cause cavities, stop bad breath, and maintain strong, healthy teeth and gums.

MATERIALS AND METHODS:

Materials:

Collection of leaves and roots: Leaves of *A. aspera* were collected from outskirts of Solapur, Maharashtra and roots of *Glycyrrhiza glabra* were obtained from Vasundhra agri mart Solapur, Maharashtra. They were authenticated by the Department of Botany, DBF Dayanand College of Arts & Science, Solapur as *Achyranthes aspera* and *Glycyrrhiza glabra.*

Test organisms: Strains of *Streptococcus mutans (ATCC 25175)* were obtained from National collection of industrial laboratories, Pune, Maharashtra, India and stains of *Candida albicans (MTCC 277)* was obtained from Department of Biotechnology V.G Shivdare college of arts and science, Solapur, Maharashtra.

Standard: Chlorhexidine mouthwash - 0.2% Chlorhexidine gluconate was used as standard antimicrobial agent

Method of extraction

The collected leaves of *A. aspera* plant and roots of *Glycyrrhiza glabra* were washed with sterile water, shadow dried, grinded into course powder using mixer grinder and then stored separately in air tight containers. The Ethanolic extract of leaves was prepared by soaking the powdered leaves(140gm) in ethanol for 7 days and extract of root was prepared by soaking

the powdered roots in ethanol: water (30;70) for 7 days. Using Whatman filter paper, the herbal extracts was filtered; the residue was then washed with 20 ml of ethanol and pressed. Obtained filtrates were transferred to petri plates and solvent was allowed to evaporate, 4 g and of *Achyranthes aspera* leaves extract and 6g of *Glycyrrhiza glabra* root extract was obtained. The extract was collected and stored at 4-5°c in air tight container.[7]

Method of preparation of mouthwash

All the ingredients were weighed accurately (Table 1). Extract of *Achyranthes aspera* leaves and

Glycyrrhiza glabra roots was dissolved with small quantity of water in one beaker (No.1). In a separate beaker (No.2) saccharine was dissolved properly in small quantity of water then clove oil was added drop by drop. Weighed quantity glycerol was added with proper stirring. Then the solution of extracts in the first beaker was added slowly to second beaker containing mixture of remaining ingredients and volume was adjusted with water, at last preservative was added, mixed well and the formulation was stored in a well closed container.

Table 1. Formulation of mouthwash

Ingredient	**Function**	**Formulation**			
		F1	**F2**	**F3**	**F4**
Achyranthes aspera L.	Active drug	250 mg	500 mg	1000 mg	1500 mg
Clove oil	Active drug	0.1 ml	0.15 ml	0.2 ml	0.25 ml
Saccharin	Sweetener	0.1 mg	0.1 mg	0.1 mg	0.1 mg
Liquorice (*Glycyrrhiza glabra* L.)	Surfactant	200 mg	200 mg	200 mg	200 mg
Glycerol	Cosurfactant	3.9 ml	3.9 ml	3.9 ml	3.9 ml
Alcohol	Preservative	1.2 ml	1.2 ml	1.2 ml	1.2 ml
Purified water	Up to 60 ml	60 ml	60 ml	60 ml	60 ml

Evaluation

Colour and Odour: Visual examination was used to assess physical characteristics like colour and odour.

pH: A digital pH metre was used to measure the pH. In order to calibrate the pH metre, standard

buffer solution was used.

The pH of the mouthwash was determined by dissolving 1 ml of mouthwash in 50 ml of distilled water.

Stability studies

Stability studies are conducted to assess the formulation's physical and chemical stability and, ultimately, the product's safety. The formulation is said to be stable when it is usable and maintains same characteristics for long term. Accelerated stability studies were carried out for short term for the prepared mouthwash. Physical stability of the mouthwash was determined through parameters like visual appearance, phase separation and homogeneity whereas pH was measured using digital pH meter. The samples were analysed at monthly intervals.

Test for microbial growth in formulated mouthwash

3 g of agar and 6.5 g of sabouraud dextrose agar was accurately weighed and transferred to two conical flasks with 100 ml of distilled water in each and heated on a water bath. Conical flask containing agar solutions and empty petri plates were sterilized in an autoclave (45 min, 121°C). After that solutions were cooled at room temperature it was transferred to the petri plates under sterile conditions and allowed to solidify. These petri plates were inoculated with formulated mouthwashes by streak plate method and control was prepared. The plates were incubated in an incubator at 37°C for 24 hours. After the incubation period was completed, the plates were removed and checked for microbial growth by comparing them to the control.

In vitro Antimicrobial activity

In vitro antimicrobial activity was carried out for the prepared mouthwash using *S. mutans* and *C. albicans* microorganisms. Appropriate quantity of agar and sabouraud dextrose agar medium was transferred to sterile petri plates and allowed to solidify under aseptic conditions. Samples of *S. mutans* and *C. albicans* were transferred to the petri plates with the help of micropipette and using a sterile spreader, it was evenly spread all over the petri plate. When the media got solidified, wells were made using a cup-borer of 5.5mm diameter. Then the Wells were filled with 100µl concentration of mouthwashes and a control was prepared using 0.2% chlorhexidine mouthwash.

The plates were incubated at 37° C for 48 hours. On completion of incubation period, plates were removed and zone of inhibition of growth was observed and measured in millimetres. These were compared with the zone of inhibition of growth of control.

Results

Stability studies

The prepared mouthwash was subjected to changed temperature and evaluated for parameters like visual appearance, phase separation, homogeneity and pH. It was found that there was no colour change, no phase separation was observed and it was homogenous. The pH of formulations was in the range 6.8 to 7.2 which is suitable for oral disorders. The results are depicted in (Table 2).

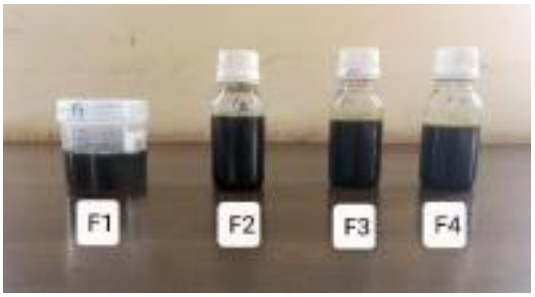


Figure 1. mouthwash formulation

Table 2. Results of stability study

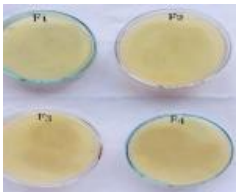
Temperature	**Evaluation parameters**	**Observation (months)**			
		0	1	2	3
3-5°C	Visual appearance	Dark Green	Dark green	Dark green	Dark green
	Phase separation	Nil	Nil	Nil	Nil
	Homogeneity	Good	Good	Good	Good
	pH	6.8	6.8	6.8	6.8

Room Temperature (25°C RH=60%)	Visual appearance	Dark Green	Dark green	Dark green	Dark green
	Phase separation	Nil	Nil	Nil	Nil
	Homogeneity	Good	Good	Good	Good
	pH	6.8	6.8	6.8	6.8
40°C±2°C RH=75%	Visual appearance	Dark green	Dark green	Dark green	Dark green
	Phase separation	Nil	Nil	Nil	Nil
	Homogeneity	Good	Good	Good	Good
	pH	6.8	6.9	6.9	6.8

Test for microbial growth in formulated mouthwash

Agar Petri plates that were inoculated only with mouthwash formulations were compared with the control. No microbial growth was observed on petri plates containing mouthwash formulations hence the formulations were found to be free from microorganisms.

A



B

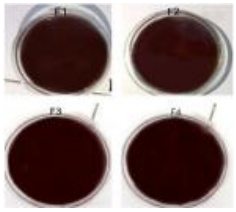


Figure 2. Microbial growth test (A) Only formulations (B) Control (*S. mutans)*

(C) Only formulations (D) Control *(C. albicans)*

In vitro Antimicrobial activity

The antibacterial and antifungal activity was performed using agar well diffusion method and zone of inhibition was measured. The zone of inhibition for *S. mutans* was found to be 22 mm, 24 mm, 20 mm and 20 mm for F1, F2, F3 and F4 respectively and for *C. albicans* it was 23 mm, 24 mm, 26 mm, 24 mm for F1, F2, F3 and F4 respectively. Zone of inhibition for 0.2 % chlorhexidine was 19 mm and 22 mm for *S. mutans* and *C. albicans* respectively. The results show that all mouthwash preparation shows antimicrobial and antifungal activity. F2 and F3 has highest zone of inhibition against *S. mutans* and *C. albicans* respectively.

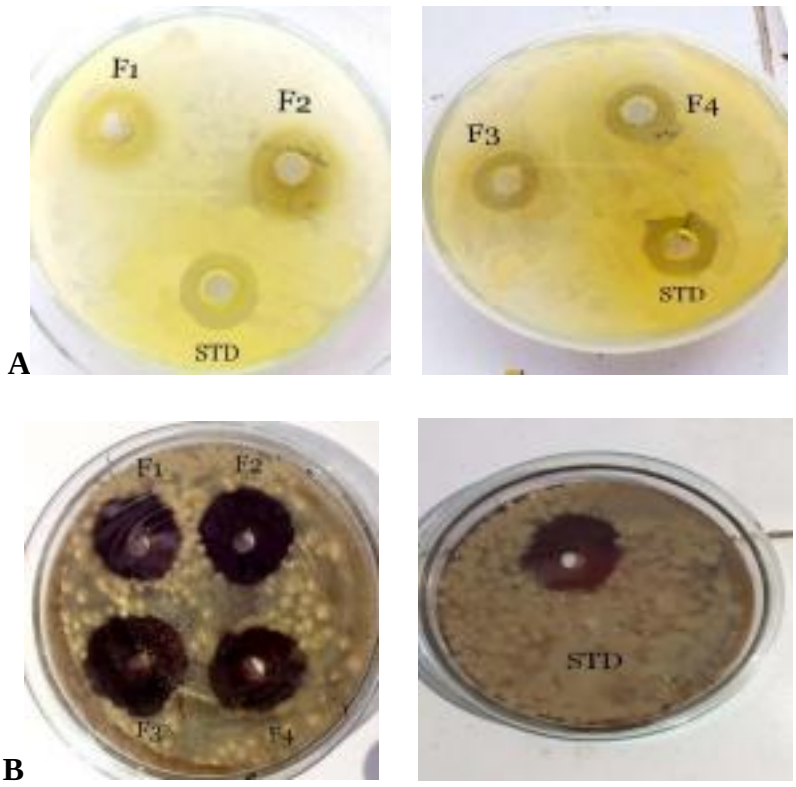


Figure 3. Agar well diffusion method (A) *S. mutans* (B) *C. albicans*

Table 3. Zone of inhibition (mm)at 100 µl concentration of respective formulations and standard against *Streptococcus mutans* and *Candida albicans*

Formulation	**Zone of inhibition (mm)**	
	Streptococcus mutans	***Candida albicans***
F1	22	23
F2	24	24
F3	20	26
F4	20	24
Standard (0.2% chlorhexidine)	19	22

Discussion

Ethanolic extract of *A. aspera* leaves has been proven to have antibacterial and antifungal activity specially against oral micrograms like *Streptococcus mutans* and *Candida albicans*. Mouthwash with herbal extracts is widely preferred due to its lesser side effects. *A. aspera*, clove oil used in mouthwash preparation is effective against oral micrograms that can cause dental caries and other oral issues. All the mouthwash formulations (F1-F4) were stable in variable conditions and free from micrograms because on inoculation in nutrient agar and sabouraud dextrose agarmedium the microbial growth was absent.

Antibacterial and antifungal activity was assessed using agar well diffusion method by measuring the zone of inhibition. The zone of inhibition for *S. mutans* was found to be 22 mm, 24 mm, 20 mm and 20 mm for F1, F2, F3 and F4 respectively and for *C. albicans* it was 23 mm, 24 mm, 26 mm, 24 mm for F1, F2, F3 and F4 respectively. Zone of inhibition for 0.2 % chlorhexidine was 19 mm and 22 mm for *S. mutans* and *C. albicans* respectively. According to the findings, all mouthwash preparations show antimicrobial and antifungal activity. F2 and F3 have the greatest inhibition zone against *S. mutans* and *C. albicans*, respectively.

Conclusion

Mouthwash is used temporarily to get rid of bad breath, to get a pleasant effect and to feel fresh. If a mouthwash contains herbal extract having therapeutic activity, such as antimicrobial properties then that is considered more effective as it can provide additional protection against oral issues. Antimicrobial drugs that are commonly used in mouthwashes can cause staining of teeth as well as alteration of taste. So, in order to avoid such artificial drugs and side effects related to it, herbal plants showing antimicrobial activity such as *Achyranthes aspera* can be used.

Achyranthes aspera plant has many therapeutic properties. Ethanolic extract of leaves of *Achyranthes aspera* has been proven to have antibacterial and antifungal activity against micrograms in oral cavity. The prepared mouthwash contains extract of leaves of *Achyranthes aspera* that shows good antimicrobial activity against oral microorganisms like *Streptococcus mutans* and *Candida albicans* that are mainly responsible for dental caries and fungal or yeast infections and helps to maintain good oral health.

Conflict Of Interest

The authors have no conflicts of interest regarding this investigation**.**

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