

Design, Development and Evaluation of Herbal Mouthwash for Antibacterial Potency against Oral Bacteria

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ABSTRACT

Several herbal mouthwash and herbal extracts have been tested in vitro and in vivo in search of suitable adjunct to mechanical therapy for long term use. In this study, we aimed to look at the antimicrobial effect of herbal mouthwash on selected microorganisms. The main purposes of using mouthwashes are it can be used at home as routine to maintain good oral hygiene, mouthwash provides antimicrobial activity, it is used prior to and after oral surgery procedures such as tooth extraction as prophylaxis, the purpose of mouthwash after brushing is to clean. Natural herbs such as spinach, neem and tulsi and many others are used as single or in combination have been scientifically proven to be safe and effective medicine against oral health problems such as bleeding gums, halitosis, mouth ulcers, and preventing tooth decay without side. The anti-inflammatory and anti-infectious properties of tulsi make it a powerful treatment for gum disease. Chewing of tulsi leaves helps clear ulcers and infections of the mouth. As a mouthwash, it is useful against bad breath and for maintaining healthy gums.

Keywords: *Herbal mouthwash, Natural herbs, Oral hygiene, Antibacterial activity.*

1. INTRODUCTION

Ideally, it is required that any antimicrobial agent used should be able to modify the oral environment by being specifically effective against pathogens without altering the normal flora. There are several types of mouth available in the market today worldwide¹. Mouthwash is an aqueous solution which is most often used for its deodorant, refreshing and antiseptic properties or for control of plaque². Maintenance of oral hygiene is imperative in preventing the buildup of plaque, a sticky film of bacteria and food that accumulates on teeth. Oral

hygiene measures include mechanical aids such as toothbrushes, interdental cleansers and chemotherapeutic agents such as mouthwashes, dentrifices and chewing³. Mouthwash (mouth rinses) are solutions or liquid intended to reduce the microbial load in the oral cavity. It may contain alcohol, glycerin, synthetic sweetness, surface active agents, flavoring agents, colouring agents, etc. Many popular herbal products have helped to control dental plaque and gingivitis and they have so far been used as adjunct to other oral hygiene measures such as brushing and flossing⁴. Over 50% of the modern drugs are of natural products origin and as such natural products play an important role in drug development⁵. A common problem among immune compromised, elderly, and chronically ill patients is oral candidiasis. Precipitation of oral candidiasis causes burning sensation and altered taste which further lowers the quality of life⁶. To overcome such problems, the WHO has advised researchers to investigate the possible use of natural products in the management of infections. Various kinds of mouthwashes have evolved following oral hygienical problems. Moreover, mouthwash also contains some ingredients that serve as digestive aids. Mouthwashes can be chemical or herbal in nature. Mouthwash is a liquid accessory to clean and maintain the health of our teeth for oral hygiene. Nowadays, we use commercial mouthwash which contains many chemical compounds like sodium lauryl sulfate, thymol, methyl salicylate, benzalkonium chloride, hydrogen peroxide, alcohol which are harmful to our buccal cavity. We have developed a mouthwash with some common food materials and herbs and which can replace costly chemicals like alcohol, colouring agents and preservatives making our mouthwash economically more viable than commercial mouthwash. A herbal mouthwash preparation is developed using the extracts spinach, neem, tulsi etc., in sterilized conditions having antibacterial, anticancer, antifungal activity. Basil leaves are known to reduce malodor and possess antibacterial property. Herbal products and their extracts such as spinach, Neem, Tulsi, etc, have shown significant advantages over the chemical ones. Herbal Mouthwash are in high demand, because they act on oral pathogens and relieve the pain instantly and are also less side effective⁷⁻¹⁰. Microwave extraction has proved to be more effective and efficient than its conventional counterpart, the soxhlet extraction method. The Soxhlet extraction, which is a standard technique, is a continuous solvent extraction method¹¹⁻¹⁷. Extraction systems are used to conduct routine solvent extractions of soils, sediments, sludge, polymers and plastics, pulp and paper, biological tissues, textiles and food samples. Experiments have proved that microwaves, in comparison with the soxhlet extraction, use a lesser volume of solvent and sample and perform extraction at a much faster rate¹⁸⁻²⁹. In the discovery of effective medicines for prevention and treatment, an outbreak of coronavirus disease

(COVID-19) caused by the novel extreme acute respiratory syndrome coronavirus-2 (SARS-CoV-2) poses an unprecedented obstacle. The proximity to the patient during dental care, high generation of aerosols, and the identification of SARS-CoV-2 in saliva have suggested the oral cavity as a potential reservoir for COVID-19 transmission. Mouthwashes are widely-used solutions due to their ability to reduce the number of microorganisms in the oral cavity. Given the rapid pace of scientific research and clinical data provided by the large number of people who are rapidly infected with SARS-CoV-2, clinicians need reliable evidence of good medical care for this infection, as it is simple to do in-silico analysis in the initial stage with the aid of molecular docking software with help of chemical structure of compound. It is necessary to enhance both enzymatic stability and membrane permeation in the formulating drug delivery system for protein and peptide drugs. Soon, someday, you might be making your own drugs at home. That is because researchers have adapted a 3D printer from basic, readily available medicinal active agents fed into a drug delivery system³⁰⁻⁴³. Due to the high impact of multidrug resistant and extensively drug-resistant treatment, there is an urgent need for new drugs to treat this disease efficiently. Hence, there is emerging demand for the development of new mouthwash. Using the natural mouthwash in conjunction with the brushing and flossing is a great way to reduce oral bacteria and even maintain the optimal oral health and hygiene. "Smile till you have teeth."- An ancient and famous saying. Extracts from neem inhibit the growth of *S. mutans* and used in the treatment of periodontitis. It contains anti-microbial, anti-inflammatory, and anti-oxidant property. Tulsi (*Ocimum sanctum*) as a mouthwash is quite effective for the ulcer and infections in the mouth⁴⁴⁻⁴⁶.

2. MATERIALS AND METHODS

Collection of plants

Leaves of *Azadirachta indica* (neem), spinach, tulsi and Peppermint were purchased from the local market and these specimens were authenticated by Y. C College, Karad.

Extraction process

Shade drying was done for almost a month as to avoid chemical degradation due to sunlight. Grinding of the dried material was done, with the aid of a grinder and converted into coarse powder. Extraction was done by microwave extraction. The crude powder was defatted subjected to extraction with ethyl acetate using a microwave extraction. The excess solvent present was evaporated.

Formulation of herbal mouthwash

The herbal mouthwash was prepared by the formula given in table 1. The extracted ingredients are mixed in a fixed ratio.

Table 1. Formulation Table for Herbal Mouthwash

Sr. no	Ingredients	Botanical name	Role	Quantity
1	Spinach	Spinacia oleracea	Antibacterial	4 ml
2	Neem	Azadirachta indica	antimicrobial	2 ml
3	Tulsi	Ocimum sanctum	Dental care	2 ml
4	Peppermint	Mentha piperita	Eliminate harmful bacteria	0.2 ml
5	Honey	-	Antibacterial	0.2ml
6	Methyl paraben	-	preservative	0.2 gm
7	Distilled water	-	Vehicle	q.s to make

Procedure

4ml of spinach, 2ml of neem extract and 2ml of tulsi extract were dissolved in distilled water and add peppermint then methyl paraben as preservative and add distilled water to make quantity sufficient for 10 ml.

Evaluation of Herbal Mouthwash:***Colour and odour***

Physical parameters like odour and colour were examined by visual examinations.

pH

pH of prepared herbal mouthwash was measured by using digital pH meter. The pH meter was calibrated using standard buffer solution about 1 ml of mouthwash was weighed and dissolved in 50ml of distilled water and its pH was measured.

Test for microbial growth in formulated mouthwash

The formulated mouthwash was inoculated in the plates of agar media by streak plate method and a control was prepared. The plates were placed in the incubator and are incubated at 37°C for 24 hours. After the incubation period plates were taken out and checked for microbial growth by comparing it with the control.

Stability Studies

The formulation and preparation of any pharmaceutical product is incomplete without proper stability studies of the prepared product. This is done in order to determine the physical and chemical stability of the prepared product and thus determine the safety of the product. A general method for predicting the stability of any product is accelerated stability studies, where the product is subjected to elevated temperatures as per the ICH guidelines. A short term accelerated stability study was carried out for the period of 3 months for the prepared formulation. The samples were stored at under the following conditions of temperature as 3-5°C, 25°C RH=60%, 40°C \pm 2% RH= 75%. Finally the samples kept under accelerated study were withdrawn on monthly intervals and were analyzed.

In-vitro Antibacterial Activity

In vitro antibacterial activity was performed on isolated colonies of Streptococcus mutans. The Agar well diffusion technique was used for determining the zone of inhibition and minimum inhibitory concentrations (MIC). The strains of S. mutans were inoculated in prefabricated blood agar plate. Plates were dried and 4 wells were made with the help of 6 mm agar well cutter. 20 μ l, 40 μ l, 60 μ l, 80 μ l of prepared mouthwash was loaded in all the respective wells. The agar plates were kept undisturbed to allow the passive diffusion of herbal mouth wash into the agar culture medium. Then the plates were incubated at 37°C for 24 hours. The zone of inhibition was calculated in mm⁴⁷⁻⁵⁶.

3. RESULT AND DISCUSSION

The pH of the formulation was found to be 6.1. As the skin is having an acidic pH around 5.5 this pH range of the formulation is suitable for oral disorders. The formulation was found to be free from heavy metals. The formulation was free from microbes as they have not produced any microbial growth when they got inoculated in the agar medium. This

mouthwash is a purely herbal prepared without the addition of any kind of alcohol and any other additives as other products found in the market. The formulation was undertaken stability studies for physical and chemical change. No considerable variations in properties of the formulation were observed. The results of stability stability studies are shown in the given table 2. Alcohol consumption as well as alcohol and tobacco use are known risk factors for head and neck cancers. It has always been the question of whether use of alcohol containing mouthwash increases the risk of cancer. When used in mouthwashes antimicrobial ingredient like neem, clove and other essential plant extracts have been found to reduce plaque and gingivitis when combined with daily brushing and flossing. Volatile sulfur compounds are the major contributing factor to bad oral odour. They arise from a variety of sources that is breakdown of food, dental plaque and bacteria associated with oral disease. The antibacterial activity was evaluated by agar diffusion method for different concentrations of mouthwash. The result of zone of inhibition for *S. mutans* was found to be 18 mm for 80 μ l, 15 mm for 60 μ l, 12 mm for 40 μ l and 7 mm for 20 μ l respectively and 20 mm for 80 μ l, 22 mm for 60 μ l, 17 mm for 40 μ l and 14 mm for 20 μ l respectively for *S. salivarius*. These results showed that the herbal mouthwash has significant antibacterial activity and the present preparation is able to inhibit bacterial growth in oral cavity. The association of oral microbial load on oral diseases is well established.

Table 2. Results of Stability study of Herbal Mouthwash

Temperature	Evaluation parameters	Observation (months)			
		1	2	3	4
3-5°C	Visual Appearance	Light brown	Light brown	Light brown	Light brown
	Phase Separation	Nil	Nil	Nil	Nil
	Homogeneity	Good	Good	Good	Good
Room Temperature (25°C RH=60%)	Visual Appearance	Light brown	Light brown	Light brown	Light brown
	Phase Separation	Nil	Nil	Nil	Nil
	Homogeneity	Good	Good	Good	Good
40°C±2°C RH=75%	Visual Appearance	Light brown	Light brown	Light brown	Light brown
	Phase Separation	Nil	Nil	Nil	Nil
	Homogeneity	Good	Good	Good	Good

Table 3. Result of agar well diffusion antibacterial assay

Organism	Zone of inhibition (mm)			
	20 µl	40 µl	60 µl	80 µl
<i>S. mutans</i>	7	12	15	18
<i>S. salivarius</i>	14	17	22	20

4. CONCLUSION

Mouthwash is a liquid accessory to clean and maintain the health of our teeth for oral hygiene. Several herbal mouthwash and herbal extracts have been tested in-vitro and in-vivo in search of suitable adjunct to mechanical therapy for long term use. The pH of the formulation was found to be 6.1. As pH range of the formulation is suitable for oral disorders.

This mouthwash is purely herbal without adding any kind of alcohol and any other additives as other products found in the the market. When used in mouthwashes antimicrobial ingredients like spinach, neem and tulsi plant extracts have been found to reduce plaque and gingivitis when combined with daily brushing and flossing. The results of zone of inhibition also confirmed that this herbal mouth rinses was found to be a potent plaque inhibitor, and were preferred by the patients for its taste, convenience of use and test duration in their mouth after rinsing. Thus, these can be used as an adjunct to mechanical therapy for treating plaque induced gingivitis. Present study has an important impact in order to create an effective and inexpensive herbal oral health intervention for low social economic communities. However this study was short-term study so long term studies are required with larger. The natural herbs used in present formulation have been medicinally proven to prevent the problem of oral hygiene and bad breath. Since years and decades, these herbs have been known for working wonders as reflected in many research findings. Person can easily rinse his mouth using this herbal mouthwash and stay clear of wide variety of oral health issues.

5. CONFLICT OF INTEREST

The authors declare that there is no conflict of interest to reveal.

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