



Preparation and evaluation of herbal mouthwash containing *Psidium guajava* leaf extract

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Abstract

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. 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 anti-inflammatory, anti-microbial 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 guava, 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 in mouthwash, *Psidium guajava* extract is useful against bad breath and for maintaining healthy gums.

Keywords: herbal mouthwash, oral hygiene, natural herbs, antibacterial activity

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 mouthwash available in the market today worldwide [1]. Mouthwash is an aqueous solution which is most often used for its deodorant, refreshing and antiseptic properties or for control of plaque. 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 chewing gums. Mouthwash (mouth rinses) are solutions or liquid intended to reduce the microbial load in the oral cavity [2]. 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 flossing [3]. Over 50% of the modern drugs are of natural products origin and as such natural products play an important role in drug development. 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 life. 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. But apart from this, mouthwashes also serve to refresh breath. Moreover, mouthwash also contains some ingredients that serve as digestive aids. Mouthwashes can be chemical or herbal in nature [4, 10]. 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, coloring agents and preservatives making our mouthwash economically more viable than commercial mouthwash [11, 16]. A herbal mouthwash preparation is developed using the extracts guava, neem, tulsi etc., in sterilized conditions having antibacterial, anti-cancer, anti-fungal activity. Guava leaves are known to reduce malodour and possess antibacterial property [17, 18].

Materials and Methods

Collection of Plant Materials

Guava (leaves), Spinach (leaves), tulsi (leaves), peppermint (leaves), were collected from the market and washed under running water to remove contaminants.

Extraction process

The collected plant materials were washed with distilled water, shadow dried, and stored in air-tight bottles separately. The aqueous extract of each plant material was prepared by soaking the powdered plant parts in distilled water and maintained in incubator at 37 °C for 72 hours. The herbal extract were filtered using filter paper; marc was washed with 10ml of distilled water and pressed.

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	Guava extract	<i>Psidium guajava</i>	Antibacterial	4 ml
2	Spinach	<i>Spinacia oleracea</i>	Antimicrobial	3 ml
3	Tulsi	<i>Ocimum sanctum</i>	Dental care	2 ml
4	Peppermint	<i>Mentha piperita</i>	Eliminate harmful bacteria	2 ml
5	Honey	-	Antibacterial	3ml
6	Methyl paraben	-	Preservative	1gm
7	Distilled water	-	Vehicle	q.s to make

Procedure: 4ml of *Psidium guajava* extract, 2ml of neem extract, 3ml of tulsi extract and honey were dissolved in distilled water and add peppermint then methyl paraben as preservative and add distilled water to make quantity sufficient for 20 ml.

Antimicrobial activity of extracts

Agar well diffusion method was used to determine the antimicrobial activity of Guava leaves extract in vitro. Agar was used to culture different micro-organisms examined in this study. Against the wall of the tube above the liquid to remove excess inoculum. The entire surface of agar plate was then swabbed 3 times with the cotton swab, transferring the inoculum, while the plates were rotated by approximately 60° between streaks to ensure even distribution. The overall procedure of inoculum preparation and inoculation of culture media remained the same for all three bacteria. Each bacterium was inoculated on one agar plates for three respective concentrations of the guava extract were inoculated to test all the two bacteria. Measure the recommended amount of agar and distilled water in to a clean, sterile flask or beaker. Heat resistant hand protection, hold the beaker/flask over a flame and stir the mixture gently using a sterile stir rod while heating. Continue boiling the mixture for about one minute, and then remove from heat. Place a sterile lab thermometer in the mixture and monitor until its temperature falls to about 47 degrees (45- 50 degrees). Pour melted agar in to the Petri dish to cover the bottom (about a quarter) and replace the lid immediately. Allow the agar plate to cool and set (the medium will set like gelatin at room temperature). It is ready for storage once it sets. During storage (in refrigerator, but not to freeze) the agar plates should be placed in an inverted position with the lid at the bottom. This prevents the condensation from dripping down on to the surface of the agar, which may allow for the movement of the organisms from one colony to another [8, 10]. Measure diameter of inhibition zone to nearest whole millimeter by holding the measuring device.

Results and Discussion

Evaluation of Herbal Mouthwash

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

pH: pH of mouthwash was measured by using pH paper.

Test for microbial growth in formulated mouthwash

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

Determination of Anti-bacterial Activity of the Extracts

Antibacterial potency against *S. aureus* and *E. coli* by the standard method using Ciprofloxacin as a standard drug was carried out.

Table 1: Antibacterial screening result of mouthwash by measuring the zone of inhibition

Sr. no	Concentration	<i>E. coli</i>	<i>S. aureus</i>
1	10 µg/ml	+++	++
2	25 µg/ml	++	+
3	50 µg/ml	+	++
4	100µg/ml (ciprofloxacin)	+++	++

Below 4mm shows (-) negative activity.

Between 4mm-6mm shows (+) slight activity.

Between 6mm-8mm shows (++) moderate activity.

Between 8mm – 10mm (+++) higher activity.

Conclusion

The pH of the formulation was found to be 6.1. As pH range of the formulation is suitable for oral disorders. 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 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 guava, neem, tulsi and other essential plant extracts have been found to reduce plaque and gingivitis when combined with daily brushing and flossing.

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