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Research Article

Formulation And Evaluation of Herbal Mouthwash

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ABSTRACT

This study aimed to develop and evaluate an herbal mouthwash and its effectiveness against microbial growth in the oral cavity. Plant materials were collected and extracted to obtain water-soluble ingredients. The mouthwash that was prepared was subsequently assessed for its physicochemical characteristics and its antimicrobial effectiveness. This mouthwash possesses good antibacterial properties. The findings from the stability study further validate the efficacy of the formulation. This mouthwash is a liquid preparation that normally contains antibacterial and antiseptic agents. These solutions can reduce microbial growth in the oral cavity and may also be used for other reasons, such as their analgesic action, and anti-inflammatory properties. Herbal mouthwash is increasingly sought after over chemical options due to its effectiveness against oral pathogens and microbes, rapid pain relief, and absence of harmful side effects. The prepared formulation was further evaluated for physical properties such as pH, color, and stability. This mouthwash possesses good antibacterial properties. This preparation is stable at different temperatures. This mouthwash is a liquid preparation that normally contains antibacterial agents. These solutions can reduce microbial growth and infection in the oral cavity.

INTRODUCTION

Mouthwash can be suggested to address infections, decrease inflammation, alleviate pain, and combat bad breath, or to administer fluoride directly for the prevention of cavities. The purpose of using mouthwash is mainly to control dental caries, and its therapeutic use is to inhibit or reduce plaque-associated bacteria. The International Standards Organization (ISO) recommends and considers general aspects of mouth rinse safety, such as toothpaste. Possible risks and side effects associated with mouth rinses arise from three factors: the physicochemical characteristics of the rinse, the active components in the products, and additional ingredients. The mouth harbors

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different microorganisms that come through food intake, warm temperatures, and moisture, which provide a suitable environment for these microorganisms to grow easily and produce dental plaque[1,2]. A significant portion of the population is afflicted by plaque-associated oral illness, which is one of the main causes of tooth loss. Herbal products are highly valued compared with chemical products. Because of their antibacterial and antifungal capabilities against human infections, medicinal plants have been used to cure illnesses for a long time[3,4]. Throughout history, plants have served to prevent or address a variety of illnesses globally, starting from the emergence of contemporary pharmaceutical medications. Plants contain substances that can be used for therapeutic purposes or as precursors for synthesizing useful drugs. Thus, over 50% of these modern drugs are directly or indirectly derived from natural products. Consequently, these natural substances are essential in the drug development process within the pharmaceutical sector.[5,6]. It is easier to prevent oral diseases than to treat them. The widespread use of mouthwashes as an aid to oral hygiene is a relatively recent phenomenon in developing countries. Herbal mouthwashes are in high demand because they act on oral pathogens, relieve pain instantly, and are also less side-effective[7,8,9]. The mouthwashes are concentrated aqueous antibacterial solutions that are used against oral microbes to counter oral infection, cleansing to remove bad breath refreshing, and antiseptic. Mouthwash plays a prominent role in oral hygiene, helping to relieve the symptoms of inflamed gum gingivitis[10]. Teeth's supporting tissues are impacted by periodontal disorders. Gingivitis, which is the least severe type of periodontal disease, is typically a result of inadequate oral hygiene. Gum swelling and bleeding are signs of gingivitis. The primary factor contributing to gingivitis is the plaque that develops on the teeth and gum surfaces. As a mainstay for maintaining oral hygiene, mechanical plaque control measures are used. Methods for controlling plaque mechanically are often time-intensive and necessitate a certain level of motivation and skill to execute effectively; therefore, antimicrobial agents have been widely used as supplements to mechanical cleaning[11,12]. Several herbal mouthwashes and herbal extracts have been tested in vitro and in vivo in search of a suitable adjunct to mechanical therapy for long-term use. In this study, one such herbal mouthwash was selected, which has proven to be a promising antimicrobial agent in past studies and has shown anti-plaque properties[13].Jamun leaves have antibacterial properties and are believed to decrease bad odor. Using natural mouthwash alongside brushing and flossing is an excellent approach for minimizing oral bacteria and maintaining optimal oral health and hygiene. Clove extracts are used to treat. periodontitis and stop Staphylococcus aureus from growing. It also has anti-inflammatory, antioxidant, and antimicrobial properties[14,15]. An herbal mouthwash preparation with antibacterial properties was created using extracts of jamun leaves, mint leaves, clove buds, and licorice root under sterilized conditions.

1. MATERIALS AND METHODS

2.1 Collection of plants

Collection of jamun leaves (*Syzygium cumini)*, mint leaves (*Lamiaceae)*, clove buds (*Eugenia caryophyllus*), and licorice root (*Glycyrrhiza glabra*) from mature plants.

2.2 Extraction Process[16,17]

The leaves of mature plants were collected and washed 2-3 times with sterile water to remove dust and dirt. The leaves underwent a shadow drying technique in which the leaves were spread in

container trays and maintained at room temperature for 6-7 days. After 7 days, dried leaves were taken and powdered using a sterile mixer under aseptic conditions. Pulverized leaves were transferred to airtight sterile container jars. A total of 100 ml of sterile distilled water was gathered from four 250 ml conical flasks, and the powdered leaves were measured and placed in distilled water while maintaining sterile conditions. The preparation was heat sterilized at 40°C for 5-10 min and incubated at 37°C for 72 h. After incubation, the extracts were filtered using a sterile Whattmann filter paper no. 1 and a funnel under laboratory conditions. The filtered extracts are boiled vigorously again to kill the bacterial spores, which prevents contamination. After heating, the extract is ready to be used in the formulation of mouthwash.

Fig. 1: Collection of A) jamun leaves, B) mint leaves, C) clove, and D) licorice

Fig. 2: Shadow drying in a container.

Fig. 3: Powder of all dried items.

Fig. 4: The drug was stored airtight under sterile conditions.

Fig. 5: Powdered drug mixed with 100 ml of water.

Fig. 6: Preparation in an incubator for incubation.

Fig. 7: Extract filtered using Whattmann filter paper.

Fig. 8: Boiled filtered extract.

Table. 1: Formulation Of the Herbal Mouthwash.

Sr.no	Ingredients	Role	Formulation1	Formulation2	Formulation3
1.	Jamun	Active drug	7ml	5ml	5ml
2.	Mint	Active drug	3ml	4ml	5ml

3.	Clove	Active drug	5ml	7ml	6ml
4.	Licorice	Sweetener	2ml	3ml	4ml
5.	Salt solution	Preservative	5ml	6ml	6ml
6.	Distilled water	Vehicle	28ml	25ml	24ml
7.	Total volume	-	50ml	50ml	50ml

2.3 Procedure for the preparation of mouthwash containing the extract[18,19]

A weighed quantity of each ingredient is taken. Extracts were taken and mixed thoroughly in a mortar and pestle with a small quantity of water. Mix all ingredients properly, taking care to avoid lump formation. Finally, water is added to increase the volume and preservatives are added, and the product is packed in an attractive, well-closed container.

3. Evaluation of mouthwash[19,20]

3.1 Colour and Odour

Physical parameters, such as odour and color, were examined by visual examination.

3.2 Measurement of pH

The pH of the prepared herbal mouthwash was measured using a digital pH meter (kncp-ELICOLI- 120). The pH meter was calibrated using standard buffer solutions of pH 7, 9.2; approximately 1 ml of mouthwash was weighed and dissolved in 50 ml of distilled water, and its pH was measured.

3.3 Homogeneity

All mouthwash formulations were placed on a platform and tested for homogeneity by visual inspection. They were tested for their appearance and the presence of any lump flocculates, or aggregates.

3.4 Test for microbial growth in developed mouthwash.

The developed mouthwash was inoculated into plates of agar media using the streak-the-plate method, and a control was prepared. The plates were placed in an incubator and incubated at 37°C for 24 h. After the incubation period, the plates were removed, and microbial growth was checked by comparing them with the control.

3.5 Stability studies

The formulation and preparation of any pharmaceutical product is incomplete without proper stability studies of the prepared product. A stability study is done 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, in which the product is subjected to elevated temperatures as per the ICH guidelines. A short-term accelerated stability study was conducted for 3 months for the prepared formulation. The samples were stored under the following conditions at 35°C. Finally, the samples kept under the accelerated study were withdrawn at monthly intervals and analyzed.

3.6 In vitro antibacterial activity

In vitro, antibacterial activity was assessed in isolated colonies of *Streptococcus aureus*. The agar well diffusion technique was used to determine the zone of inhibition and minimum inhibitory concentrations (MIC). The strains of *S. Aureus* were inoculated into a prefabricated agar

plate. Plates were dried, and four wells were made with the help of a 6-mm agar well cutter. 1 ml, 2 ml, and 3 ml of prepared mouthwash were loaded into the wells. The agar plates were kept undisturbed to allow the passive diffusion of herbal mouthwash into the agar culture medium. The plates were then incubated at 37°C for 24 h. The commercial antibiotic ampicillin (50mg/ml) was used as a positive control for S. aureus. The zone of inhibition (mm) was recorded for each plate and compared with the control.

4. RESULTS AND DISCUSSION

Table 2: Evaluation parameters of the herbal mouthwash

Formulation	Colour	Homogeneity	pH
F-1	Light brown	Good	5.94±0.04
F-2	Light brown	Good	6.3±0.02
F-3	Light brown	Good	6.2±0.01

Table 3: Results of the agar well diffusion antibacterial assay

Organism	Zone of inhibition(mm)		
	1ml	2ml	3ml
Staphylococcus Aureus	10	12	13
Standard ampicillin	12	13	15

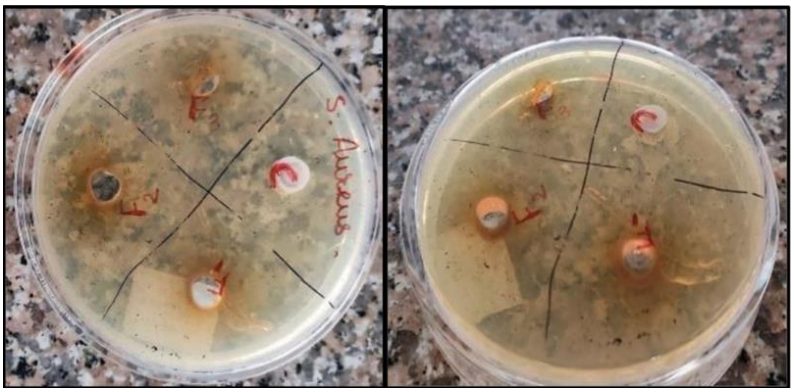

Fig. 9: Agar diffusion method for assessing antibacterial activity.

Table 4: Stability Study

Temperature	Evaluation parameter	Observation (Month)			
35°C		0	1	2	3
	Visual Appearance	Light brown	Light brown	Light brown	Light brown
	Phase Separation	Nil	Nil	Nil	Nil
	Homogeneity	Good	Good	Good	Good
	Odor	No change	No change	No change	No change
	pH	6.3	5.94	6.3	6.2

5. CONCLUSION

Natural remedies are more acceptable in the belief that they are safer and have fewer side effects than synthetic ones. Herbal formulations have a growing demand in the world market. This is a super attempt to establish an herbal mouthwash containing jamun leaves extract, mint leaves

extract, clove extract, and licorice powder at various concentrations. The studies revealed that the developed single herbal formulation F2 consisting of a proportion of jamun leaves extract, mint leaves extract, clove extract, and licorice extract was comparatively better than other formulations. Still, all formulations were non-irritant and did not show any toxicity when used. An extensive amount of work has been put into combining the active components of various extracts to produce an efficient, alcohol-free poly-herbal mouthwash. Because of their alleged antibacterial capabilities, this study included extracts of jamun, mint, clove, and licorice. The results of the zone of inhibition test demonstrated the effectiveness of this herbal mouthwash as a plaque inhibitor and the test duration in the mouth following washing. To treat plaque-induced gingivitis, they can be used in conjunction with mechanical therapy. The creation of an efficient and affordable herbal oral health intervention for underdeveloped areas will be significantly impacted by the results of the current investigation. Longer-term research with larger samples is needed, nevertheless, as this was a short-term study. Clinical studies have demonstrated that the natural herbs included in this formula can aid with bad breath and oral hygiene. Numerous study findings support the long-standing belief that certain herbs have miraculous healing properties. A person can simply rinse their mouth with this herbal mouthwash to prevent a variety of oral health issues.

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