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Research Article

Formulation Evaluation and Comparative Study of Herbal Mouthwash with Marketed Preparation

Aman Mohata*, Seema Wakodkar, Jagdish Baheti

Kamla Nehru College of Pharmacy Butibori, Nagpur, Maharashtra (India)-441122

A B S T R A C T

The current study aims to create and assess a herbal mouthwash and assess its efficacy in combating the oral cavity's microbial load. To extract the hydroalcoholic and water-soluble components, plant materials were gathered. Piper nigrum seeds and hibiscus flowers were used to prepare herbal mouthwash for extraction. The prepared mouthwash's pH, antibacterial activity, and physicochemical characteristics were further assessed. The mouthwash that is currently available has good antibacterial qualities. Currently, available mouthwash is a liquid formulation that often includes antiseptic and antibacterial ingredients. The major benefits of utilizing mouthwashes are that they can be used regularly at home to maintain good oral hygiene, that they have antibacterial activity, that they can be used as prophylaxis before and after oral surgery procedures like tooth extractions, and that they clean teeth after brushing. These solutions may be used for various purposes, such as their analgesic, anti-inflammatory, or anti-fungal properties, in addition to their ability to lessen microbial development, gingivitis, and halitosis in the oral cavity.

Keywords: - Herbal Mouthwash, Hibiscus Rosa sinensis, Piper nigrum, Chlorhexidine.

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*Address for Correspondence:

Aman Mohata, Kamla Nehru College of Pharmacy Butibori, Nagpur, Maharashtra (India)-441122

INTRODUCTION

From the dawn of civilization to the twenty-first century, people have understood the significance of keeping their mouth and teeth clean. Many mouthwash products are available to patients and oral health professionals, each with a unique combination of active and inactive components. Many herbal products, including extracts from guava, pomegranate, neem, liquorice, tulsi, green tea, cranberries, and grapefruit, have demonstrated notable benefits over synthetic alternatives. There could be a lot of benefits to using natural mouthwash instead of conventional ones.

Mouthwash is an aqueous solution that is primarily used for controlling plaque or for its deodorizing, refreshing, and antibacterial qualities. Alcohol, glycerin, artificial sweeteners, flavorings, colorings, surface active agents, and so forth may be present. To use mouthwashes effectively, the oral condition must be correctly diagnosed, and the product must be thoroughly understood.[1]Mouthwash is useful for several diseases like Gingivitis, Halitosis, etc.

Gingivitis is an inflammatory reaction to a dental plaque that is reversible, affecting the gingival tissue. It is the most common human disease of the oral cavity.[2] Periodontitis is a destructive inflammatory disease of the supporting tissues of the teeth, resulting in the progressive destruction of the periodontal ligament, alveolar bone with pocket formation and gingival recession caused by specific or group of specific microorganisms.[3]

Bad breath is referred to as halitosis, which comes from the Latin halitus (breath) and the Greek suffix osis (pathological process).[4] Halitosis is an irritating or disagreeable mouth odour that causes discomfort and psychological shame. Oral problems, specifically oral malodor, are the source of halitosis in about 80% of cases.[5]Herbal mouthwashes target oral pathogens, provide immediate pain relief, have fewer side effects, and act on oral pathogens, they are in high demand. Chemical mouthwashes contain cetylpyridinium chloride, hydrogen peroxide, and chlorine dioxide as an instant teeth whitener, sterilizer, and pain reliever. However, they tend to cause tooth discolouration and may have adverse effects, even though they are reasonably priced. Dental

infections and periodontal diseases are among the most prevalent infectious diseases that many people experience. As a serious mouth illness, gingivitis can impact the Dental caries including the development of cavities, the eruption of enamel, gum enlargement, bleeding gums, and the appearance of a hollow, black eruption on the tooth's surface. Because they do not practice good oral hygiene, children and adolescents have a high rate of dental cavities in the beginning.[6]Hibiscus rosa sinensis belongs to thefamily "Malvaceae" which gives the anti-inflammatory activity. It is a glabrous shrub with many forms and a range of flower hues that is commonly grown as anattractive plant in the tropics. However, the red-flower type is recommended in medicine.[7]The evergreen shrub Hibiscus rosa-sinensis reaches a height of 4 meters. Small trees with ovate branches and stalks that are 10 cm wide and 15 cm long are referred to as "shrubs" in this species.[8-9]The genus Piper L. (family Piperaceae) has more than a thousand species, the majority of which are found in tropical regions of the world.[10]The most famous species in this genus is Piper nigrum L., also known as black pepper; it is referred to as the "king of spices" due to its strong piperine flavor and widely utilized as a food spice around the world.[11]It shows the antimicrobial activity, antibacterial activity, etc

Advantages of mouthwash:–

- It can help fight cavities. Mouthwash can help rinse away bacteria that might otherwise cling to your teeth and contribute to decay.
- It can help keep your gums healthy. Because mouthwash helps rinse away bacteria, this means bacteria is less likely to stick around the gums and provoke inflammation, infection, or gum disease.

Disadvantages of mouthwash: -

- It may causeirritation. Mouthwashes with a high alcohol content may have strong antibacterial properties, but they can also irritate the tissues.
- It may not be safe for (accidental) consumption. Some mouthwashes contain ingredients that are harmful if accidentally swallowed. This is a big reason why mouthwash is generally not recommended for use by children under the age of six.

MATERIALS AND METHOD

- Plant and seed collection of Hibiscus rosa sinensis and Piper nigrum.
- The Flower of Hibiscus rosa sinensiswas collected from the medicinal garden area of Kamla Nehru College of Pharmacy, Butibori, Nagpur. And local area
- The seeds of Piper nigrum were purchased from the local shop.

Authentication of Hibiscus rosa sinesis and Piper nigrum

The plant was collected around Kamla Nehru College of Pharmacy, Butibori, and near local areas and Piper nigrum seeds were collected from local shop and packed into zip lock packet it was identified and authenticated by Associate Professor, Department of Botany, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, Maharashtra, India. The voucher herbarium specimen number of Piper nigrum is 313 and Hibiscus rosa sinensis is 314.

Preparation of Extract

Hibiscus

A total of 30 g of dried flower powder was soaked into the 150 ml of hot water which was then boiled for 30 min and kept for 24 hours undisturbed and filtered through sterile filter paper, evaporated by using solvent distillation apparatus. The extract was centrifuged at 10,000 rpm for 5min and the supernatant was stored at 4^{o} C for further use.[12]

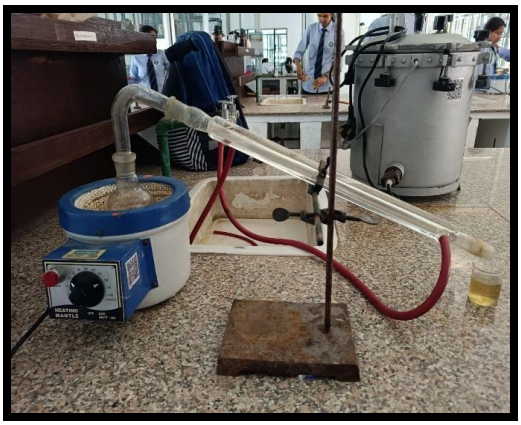

Figure 1: Solvent evaporation method

Piper nigrum

30 gm of black pepper powder extracted with 250ml 95% ethanol in Soxhlet extractor for 2 hours. The solution was filtered and concentrated on the water bath at 60oC. 10 ml 10% of alcoholic potassium hydroxide was added to the filtrate with continuous stirring. The insoluble residue was filtered and alcoholic solution was left overnight and filtered through a filter paper.[13]

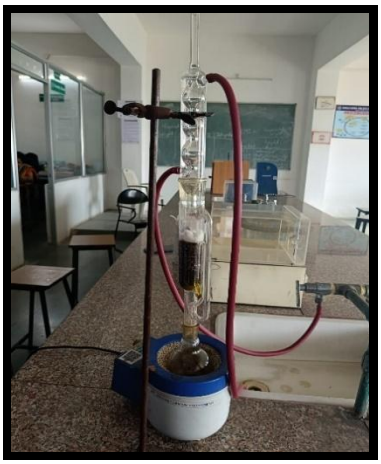

Figure 2: Extraction of Piper nigrum by Soxhlet apparatus

Phytochemical analysis of plant extracts for active components of Hibiscus rosa sinesis and piper nigrum:

Hibiscus

The phytochemical tests for Hibiscus rosa sinensis compounds contain tannins, flavonoids, alkaloids, and glycosides in accordance with the methods with little modifications.

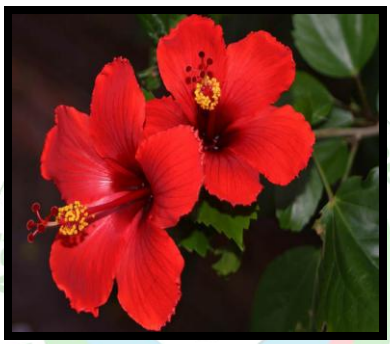

Figure 3: Hibiscus rosa sinensis

Table 1: Phytochemical testing of Hibiscus rosa sinesis

Phytochemical	**Test**	**Reagent**	**Result**	**Pass or fail**
Alkaloids	Dragendroff test	Dragendroff reagent	Yellow ppt	Pass
Tannins	Fecl3 test	5% Fecl3	Brown or Black color	Pas
Flavonoids	NaOH Test Lead acetate test	20% NaOH, HCL 10% lead acetate	Yellow color on addition of HCL turns to colorless White ppt	Pass Pass
Glycosides	Killer killani test	1mL filtrate + 1.5mL glacial acetic acid +1 drop of 5% ferric chloride + conc. H2SO4	Brownish green ppt	Pass

Piper nigrum: -

Figure 4: Black pepper seeds

Table 2: Phytochemical testing of Piper nigrum

Phytochemical	Test	Reagent	Result	Pass or Fail
Alkaloids	Dragendroff test	Dragendroff reagent	Orange red ppt	Pass
	Mayers test	Mayers reagent	Yellow or white ppt	Pass
Saponin	Foam test	2ml water shake with extract	Formation ofFoam	Pass
	Forth test	20 ml water with extract shake 15 min	Formationof1cm forth	Pass
Protein	Millon test	Millon's reagent	White precipitate	Pass
Carbohydrate	Molisch test	2 drops of Molisch reagent with 2ml of conc.H_2SO_4	Reddish violet or purple colour	Pass
	Benedict test	5ml reagent boil over flame with extract for 2min let it cool down	Red ppt	Pass

Procedure for Mouthwash

Weighted quantities of each ingredient will be taken. The extractwas mixed thoroughly in mortar and pestle properly with a small quantity of water. All other remaining ingredients will be gradually added with good mixing. Drop by drop clove oil and mint oil will be added and mixed properly taking care to avoid lump formation. PEG 40 and Glycerol will then be added drop by drop and mixed well. Finally, water added to make volume and preservative will be added and the product will be packed in an attractive, well closed container.[14]

Formulation table of Mouthwash:

Table: Formulations of Mouthwash:

Ingredient	Formulation 1	Formulation 2	Functions
Hibiscus	1ml	2ml	Active drug
Black pepper	1ml	2ml	Active drug
Clove oil	0.1ml	0.15ml	Flavor / Active drug
Mint oil	0.1ml	0.1ml	Flavour
Saccharin	0.001g	0.001g	Sweeteners
PEG-40	6g	6g	Surfactant
Glycerol	6.5ml	6.5ml	Cosurfactant
Alcohol	2ml	2ml	Preservatives
Purified water	Up to 100ml	Up to 100ml	Solvent

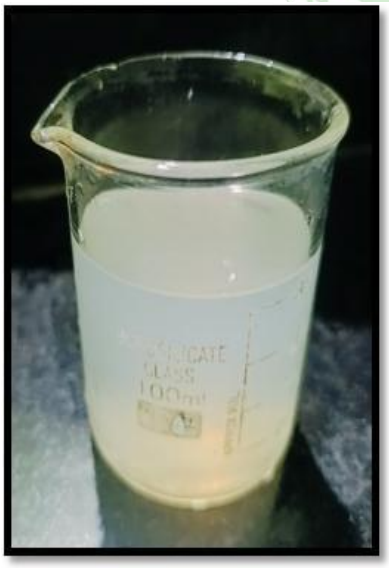

Figure 5: Formulation 1

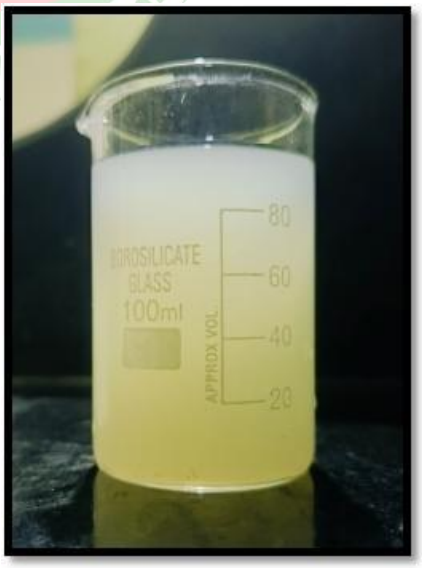

Figure 6: Formulation 2

Marketed Formulation For Comparison Study: Chlorhexidine

- One of the most widely used antiseptics for disinfecting skin and mucous membranes is chlorhexidine (CHX). is employed in dentistry as gradual release devices, mouth rinses . Dental caries treated with chlorhexidine, oropharyngeal cleansing, and endodontic therapy.[15–16]
- CHX is effective against viruses, yeasts, and bacteria, both Gram-positive and Gram-negative. One of the most popular antiseptics, particularly in dentistry, is

chlorhexidine (CHX). CHX functions as a bacteriostatic at low doses and as a bactericidal at high quantities, cytolyzing cells to death.[17]

Mode of Action

- Chlorhexidine's antibacterial activity varies with dose low concentrations of chlorhexidine (0.02%–0.06%) have bacteriostatic properties, while higher amounts (> 0.12%) have bactericidalproperties.[18]
- CHX is a cationic molecule that attaches itself to the negatively charged phospholipids in bacterial membranes in a nonspecific manner. CHX influences the bacterial cell's osmotic balance shift at low concentrations. Potassium, phosphorus, and other low-weight molecules are released as a result. [19]

Application of chlorhexidine

- CHX is offered as dental varnishes (1%, 10%, 40%), gels (0.12-1%), aerosols and spray formulations (0.12-0.2%), and oral rinses. Dental floss, toothpaste, and tooth cleaning gels all contain CHX. When CHX is used in mouthwashes at concentrations of 0.12% to 0.2%, gingival inflammationand plaque indexes are significantly reduced.[20]
- Chlorhexidine has favorable effects in lowering bleeding levels and plaque buildup.

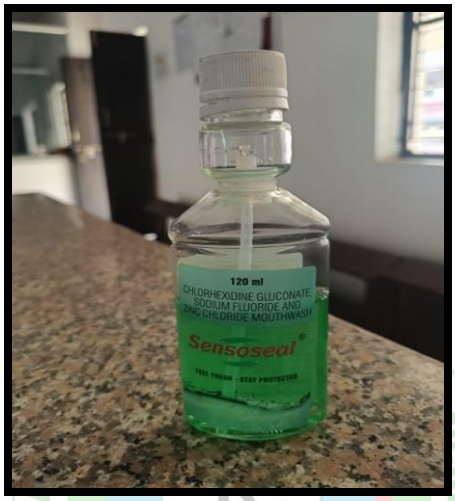

Figure 7: Chlorhexidine as marketed formulation

Evaluation test

Evaluation of formulated herbal mouthwash and marketed mouth wash -

Organoleptic evaluation: -

Physical parameters like odor and color were tested by visual examination. [21]

pH: -

A digital pH meter was used to measure the pH of the produced herbal mouthwash. A pH meter was used to weigh and dissolve 1 ml of mouthwash in 50 ml of distilled water.[21]

Test for microbial growth in formulated mouthwash: -

Using the streak plate method, the mouthwash formulation was inoculated into the agar medium plates, with chlorhexidine serving as the standard. After being put in the incubator, the plates are incubated for one hour at 37°C. Following the incubation period, the plates were removed and examined for highly active cultures of Staphylococcus aureus and Escherichia coli for microbial growth. These cultures were then incubated for a further 24 hours, after which the zone of inhibition was determined. [21]

RESULT AND DISCUSSION

Sr. No	Evaluation parameters	Formulation 1	Formulation 2	Marketed Formulation (Chlorhexidine)
1.	Color	Whitish	Lime	Greenish
2.	Odor	Aromatic	Aromatic	Aromatic
3.	pH	3.58	3.80	4.84
4.	Microbial activity (zone of inhibition)	10µl -15mm 20µl – 37mm 30µl – 61mm	10 µl -61mm 20µl- No 30µl- 53mm	**Standard** F1- 45mm F2- 53mm

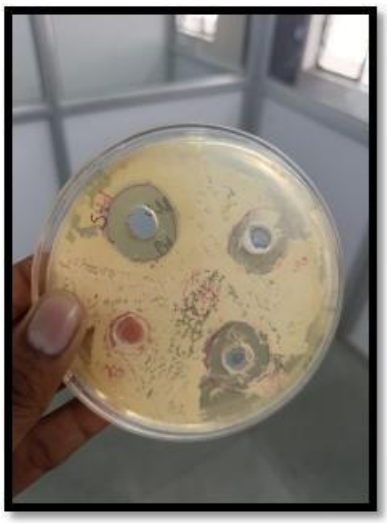

Figure 8: Microbial activity of F1

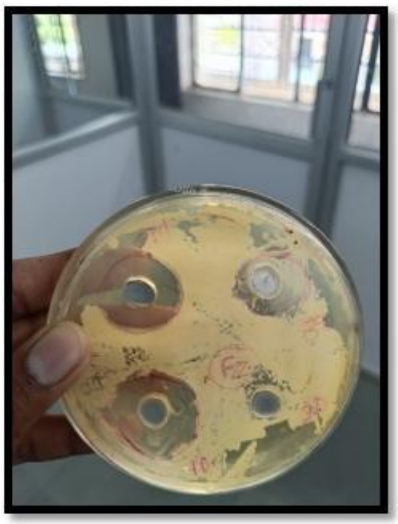

Figure 9: Microbial activity of F2

CONCLUSION

It is concluded from the present study that the developed herbal mouthwash containing Hibiscus rosa sinensis and Piper nigrum as significant, therapeutically efficacious and are with high potential. Both of the herbs contain antibacterial properties and anti-inflammatory activity. The use of herbs in dentistry should be based on evidence of effectiveness and safety. The present results therefore offer a greater use for traditional use of herbal mouth wash. The results also showed anti-microbial activity in both mouthwash formulations. Therefore, these two preparations can be used for further studies to establish their efficacy and safety as antibacterial mouthwashes. Herbal mouthwash formulations can be prepared by using different plant sources with varying concentrations. The organoleptic evaluation and pH was determined and was found to be F1 and F2 were 3.5 and 3.80 which was compared with chlorhexidine and its Ph was found to be 4.8. In this study, the mouthwash formulations F1 and F2, which contained equal amounts of alcohol, were the best formulations compared with the chlorhexidine. The herbal mouthwash which is formulated generally made up of herbal ingredients gives the best result as compared to chlorhexidine mouthwash. Herbal mouthwash preparation has potent action and minimal side effects when compared with that of the other marketed mouthwashes, hence there is need for increased usage of herbal preparation to avoid the adverse effects.

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