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FORMULATION AND EVALUATION OF AN HERBAL MOUTHWASH FOR FUTURE DENTAL APPLICATIONS

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Abstract:
Mouthwash is typically an antiseptic solution used for cleaning the mouth and teeth or for freshening the breath. It is frequently recommended in dentistry to prevent and treat various oral conditions. Recently, the use of natural products, commonly referred to as grandmother remedies, has become widespread. Herbal mouthwashes are in high demand because they effectively target oral pathogens, provide immediate pain relief, and have fewer side effects. Liquid herbal mouthwash can significantly help alleviate bad breath and various oral disorders. These liquids contain anti-inflammatory, antimicrobial, and analgesic properties. In this study, three formulations of herbal mouthwash were developed using spirulina, clove oil, peppermint oil, liquorice, sodium benzoate, salt, and water. Herbal mouthwash includes natural phytochemicals known for their antimicrobial and anti-inflammatory effects. The prepared formulations were further evaluated for color, odor, flavor, pH, and microbial activity using the agar diffusion method.

Key Words: Herbal mouthwash, Formulation, Natural Phytochemicals

Introduction:

Oral health is becoming a significant concern worldwide. Mouthwashes, which possess anti-inflammatory, antimicrobial, and analgesic properties, are commonly used for maintaining oral hygiene. There are two main types of mouthwashes: chemical and herbal. Chlorhexidine digluconate is widely regarded as the gold standard antiplaque agent, but its side effects can limit patient acceptance, particularly for prolonged use[1].

Herbal mouthwashes, on the other hand, contain natural phytochemicals with antimicrobial and anti-inflammatory effects. These mouthwashes utilize natural herbs known for their cleaning and healing properties for teeth and gums. Common herbs used in herbal mouthwashes include neem, Yovani sattva, raga Vali, Thriphala, Tulsi, Clove oil, and Pudina, either individually or in combination[2].

The purpose of this study is to prepare an herbal mouthwash and evaluate its antimicrobial efficacy to determine its effectiveness.

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Classification of Mouthwashes:

- **Cosmetic Mouthwash:** Designed to temporarily control breath odor and leave a pleasant taste in the mouth, cosmetic mouthwashes do not eliminate germs like those containing germ-killing ingredients.
- **Fluoride Mouthwash:** This type of mouthwash aids in the remineralization of weakened tooth enamel, making teeth more resistant to decay and erosion.
- **Antiseptic Mouthwash:** Containing bacteria-killing substances, antiseptic mouthwashes help eliminate the bacteria that cause bad breath.
- **Natural Mouthwash:** Ideal for those who prefer non-alcoholic products, natural mouthwashes provide similar benefits to other mouthwashes but are gentler, have a milder taste, and are alcohol-free.
- **Whitening Mouthwash:** Beyond dental and oral health, whitening mouthwashes help achieve bright white teeth and an attractive smile, reflecting the growing importance of aesthetics in dental care.

MATERIALS AND INSTRUMENTS:

Liquorice: Glycyrrhiza glabra, commonly known as liquorice, is one of the most popular medicinal plants in the Fabaceae family (Leguminosae). The genus name Glycyrrhiza is derived from the Greek words "glykos" (sweet) and "rhiza" (root). Liquorice is also referred to as sweet wood and Liquorice radix in English, süssholz and lakritzenwurzel in German, reglisse and bois doux in French, shirin bayan and make in Persian, and liquirizia and regaliz in Italian and Spanish, respectively. Native to Mediterranean regions, this species is now also found in India, Russia, and China[3]. Liquorice extracts are widely used in the pharmaceutical and food industries, as well as in the production of functional foods and dietary supplements.

Fig-1 Liquorice

Clove Oil: Eugenol, commonly known as clove oil, is an aromatic oil extracted from cloves. It is widely used as a flavoring agent in foods and teas, and topically as an herbal remedy for toothaches. Occasionally, it is taken orally to treat gastrointestinal and respiratory issues. Eugenol constitutes 70% to 90% of the aromatic oil from cloves, which are commonly used to flavor meats, stews, cakes, and teas. It is also present in lower concentrations in cinnamon and other aromatic spices[4]. Eugenol, the primary component of clove oil, is believed to be responsible for its aromatic qualities and both its beneficial and harmful effects. In vitro

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studies have demonstrated that eugenol possesses antibacterial, antifungal, antioxidant, and antineoplastic properties.

Fig-2 Clove oil

Mint Oil: Peppermint oil is commonly used topically to address issues such as headaches, muscle aches, joint pain, and itching. In aromatherapy, it is used to treat coughs and colds, alleviate pain, enhance mental function, and reduce stress. Both peppermint leaves and peppermint essential oil have been utilized for health benefits. The essential oil is extracted from the flowering parts and leaves of the peppermint plant, containing the concentrated substances responsible for the plant's characteristic odor and flavor. Peppermint is widely used as a flavoring agent in foods and beverages, while peppermint oil is also used as a fragrance in soaps and cosmetics.

Fig-3 Mint oil

Sodium Benzoate: Sodium benzoate, also known as benzoate of soda, is the sodium salt of benzoic acid. It is widely used as a food preservative (designated E211) and as a pickling agent. It appears as a white crystalline substance with the chemical formula C6H5COONa. Benzoic acid, its salts, and esters occur naturally in many foods, particularly in fruits and vegetables such as cranberries and bilberries. Other sources include seafood like prawns and various dairy products. Sodium benzoate is used to treat urea cycle disorders because it binds to amino acids, facilitating their excretion and reducing ammonia levels. Recent studies suggest that sodium benzoate may be beneficial as an adjunct therapy for schizophrenia, with a dosage of 1 gram per day leading to a 21% reduction in Total Positive and Negative Syndrome Scale scores compared to a placebo.

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Fig-4 Sodium benzoate

Spirulina: Spirulina is a type of blue-green algae known for its high content of protein, vitamins, minerals, carotenoids, and antioxidants, which help protect cells from damage. It contains a range of nutrients, including B complex vitamins, beta-carotene, vitamin E, manganese, zinc, copper, iron, selenium, and gamma-linolenic acid (an essential fatty acid). However, like other blue-green algae, spirulina can be contaminated with toxic substances called microcystins and can absorb heavy metals from its growing environment. Despite the potentially low amount of spirulina in some supplements, it may offer health benefits such as lowering blood pressure, slowing blood clotting, boosting immune system activity, and reducing blood sugar levels.

Fig-5 Spirulina

6.Salt: Sodium is an essential nutrient for human health, playing a crucial role as an electrolyte and osmotic solute. However, excessive salt intake can increase the risk of cardiovascular diseases, such as hypertension, in both children and adults.

Fig-6 Salt

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7.Charcoal: Charcoal has been used since ancient times for various purposes, including art and medicine, but its most significant use has been as a metallurgical fuel. It is the traditional fuel for blacksmith's forges and other applications requiring intense heat. Historically, charcoal was also ground into a black pigment, which was important to early chemists and used in formulations like black powder.

Fig-7 Charcoal

Methyl Blue: Methyl blue, with the molecular formula C37H27N3Na2O9S3, is a chemical substance used primarily as a stain in histology. It stains collagen blue in tissue sections and can mediate electron transfer in microbial fuel cells. It is also used in differential staining methods such as Mallory's connective tissue stain and Gömöri trichrome stain, and it stains fungal cell walls. In solutions containing phenol, glycerol, and lactic acid, it is known as Lactophenol cotton blue (LPCB), which is used to observe fungi under a microscope. Methyl blue, along with water blue, is marketed under various names including Aniline Blue WS, Aniline blue, China blue, and Soluble blue.

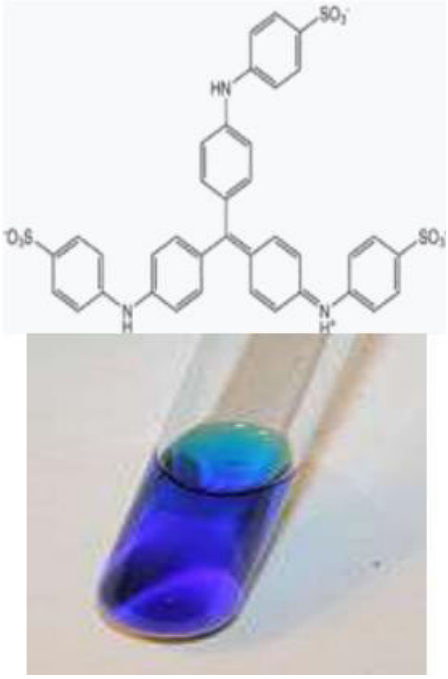

Fig-8 Methyl Blue

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Distilled Water: In chemical, biological laboratories, and industrial settings, deionized water or reverse osmosis water are sometimes used as more economical alternatives to distilled water. However, for applications requiring exceptionally high-purity water, double distilled water is preferred.

Method:

Procedure for Mouthwash Preparation:

1. Weigh the following ingredients:
    - Spirulina (200 mg)
    - Clove oil (1 ml)
    - Peppermint oil (1 ml)
    - Liquorice (5 mg)
    - Sodium benzoate (0.5 mg)
    - Methyl paraben (0.5 mg)
    - Salt (0.5 mg)
    - Methylene blue (1 drop)
2. Add distilled water to make up to 100 ml.
3. Dissolve spirulina in distilled water.
4. Add a pinch of charcoal to the solution and filter using filter paper.
5. After filtration, add the remaining ingredients (clove oil, peppermint oil, sodium benzoate, liquorice) to the distilled water and make up the volume to 100 ml.
6. Add methylene blue and transfer the solution into a clean beaker.

This procedure outlines the preparation steps for the mouthwash formulation containing spirulina, clove oil, peppermint oil, liquorice, sodium benzoate, methyl paraben, salt, charcoal, and methylene blue dissolved in distilled water.

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Evaluation of Mouthwash:

1. **Physical Evaluation:** Color, odor, and consistency of the mouthwash were assessed visually.
2. **pH Measurement:** The pH of the prepared herbal mouthwash was measured using a digital pH meter. Prior to measurement, the pH meter was calibrated using standard buffer solutions.
3. **Microbial Assay:** Antibacterial activity was evaluated using the Agar diffusion method to measure zones of inhibition (in mm). Agar media plates were prepared and inoculated with the formulated mouthwash using the streak plate method. A control plate without mouthwash was also prepared. The plates were then incubated at 37°C for 24 hours in an incubator. After incubation, microbial growth was observed and compared between the test and control plates.
4. **Stability Studies:** Physical parameters such as color, odor, consistency, and pH were assessed at room temperature to evaluate stability. The results of the stability studies are summarized in a table.

RESULTS AND DISCUSSION:

The prepared mouthwash was subjected to the following evaluation parameters to ensure its quality:

1. **Physical Evaluation:** The morphological parameters of the mouthwash were assessed and are detailed in the table. The formulation appeared yellow in color with a sweet, spicy, clove-like woody odor.
2. **pH Measurement:** The pH meter was calibrated using standard buffer solutions, and the pH of the herbal mouthwash was determined to be 6.0.
3. **Microbial Evaluation:** Agar plates were prepared, and the formulated mouthwash was inoculated onto the agar using the streak plate method. A control plate without mouthwash was also prepared. The plates were then incubated at 37°C for 24 hours in the incubator.

These evaluations provide insights into the physical characteristics, pH level, and antimicrobial activity of the prepared herbal mouthwash.

S.NO	PARAMETERS	OBSERVATION
1	Colour	Blue
2	Odour	Sweet spicy wood clove
3	Texture	Liquid

Table 2

AFTER INCUBATION:

S.NO	CONTENT	F1	F2	F3
1	Formulations	Zone of Inhibition		
2	Herbal formulation	12mm	12mm	12mm
3	Marketed formulation	12mm	12mm	12mm

Table 3

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Stability Studies: The results of stability testing are presented in the table. No changes in color, odor, or texture were observed. However, slight variations in the pH of the formulation were noted at room temperature.

S.NO	PARAMETER	F1	F2	F3
1	Colour	No changes	No changes	No changes
2	Odour	No changes	No changes	No changes
3	Texture	Liquid	Liquid	Liquid
4	pH	6	6	6

F2Formulation

F3 Formulation

CONCLUSION:

The herbal mouthwash prepared in this study effectively addresses bad breath and oral disorders. Physiochemical evaluations affirm that the color and odor of the current herbal formulation are satisfactory, characterized by a pleasant aroma. The antimicrobial evaluation demonstrated that the F3 formulation exhibited the highest zone of inhibition. The formulation's pH was measured at 6.1.

This study underscores the significant potential of herbal mouthwash as a cost-effective and efficient intervention for improving oral health, particularly beneficial for low socioeconomic

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communities. The inclusion of natural ingredients such as spirulina, clove oil, and peppermint oil has proven effective in preventing oral hygiene issues.

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