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Formulation and Evaluation of Clove Oil-Based Mouthwash

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Abstract

Mouthwash is a therapeutic liquid retained in the mouth and swished by the perioral muscle to remove oral pathogens. The current study aims to develop, test, and assess Mouthwash's efficacy against the oral cavity's microbial burden. For making Mouthwash, clove oil, thymol, and menthol were chosen, and the finished product was then tested for antibacterial activity against a staphylococcus aureus culture. Physical characteristics, including pH, color, odor, and stability, are further assessed. The current Mouthwash has strong antibacterial properties. In a variety of temperature conditions, this preparation remains stable. Current Mouthwash is a liquid formulation that often has antiseptic and antibacterial properties. This solution can lessen halitosis and bacteria development in the oral cavity.

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Keywords: Antimicrobial, Clove Oil, Halitosis, Mouthwash, Staphylococcus Aureus.

1. Introduction

According to the 2003 World Oral Health Report(WOHR), oral health is a crucial aspect of overall health. Most chemical products contain an antiseptic, essential for avoiding plaque accumulation (Vijapur et al., 2022). Since the beginning of civilization till the 21st century, People have been aware of how important it is to have a clean mouth and teeth (Namdeo, Singh, & Sharma, 2021). Numerous bacteria in the mouth are stored in the oral cavity. Mouth bacteria collect to produce biofilms that stick to the mouth cavity's soft and hard surfaces.

While some oral bacterial species are harmless, others can cause mouth problems, dental plaque, and bad smell. Diabetes, osteoporosis, and cardiovascular disorders have all been connected to oral microorganisms. For example, Porphyromonas gingivalis, lactobacillus acidophilus, and Streptococcus mutans are thought to be the primary culprits of dental caries and other oral disorders. An aqueous gargling solution known as a mouthwash is frequently used due to its cooling, antibacterial, and effective tooth plaque control qualities (Nafea et al., 2020).

1.1. *Types of Mouthwashes*

1) **Flouride Mouthwash**: Sodium fluoride helps shield teeth from decay and cavities.
2) **Antiseptic Mouthwash**: People with mouth infections commonly use this Mouthwash, which typically contains alcohol, to prevent the growth of bacteria. It frequently helps those who have Halitosis as well.
3) **Cosmetic Mouthwash**: A mouthwash that helps to mask bad breath and provides freshness to the mouth.
4) **Natural Mouthwash**: A natural mouthwash might perform the same functions as other types but with natural components ("Mouthwash and What the Different Types Can Do for You," 2017).

1.2. *Halitosis*

An unpleasant breath odor that is continuously released is the hallmark of the oral health disorder halitosisAnaerobic bacteria that reside in the mouth create volatile sulfur compounds (VSCs), which are the leading cause of Halitosis (Usra, Alimuddin, & Aisyah, 2023). Figure 1 depicts an example of Halitosis.

Fig. 1. Halitosis an Example.

Unpleasant odors from mouth cavities are known as foul breath, Halitosis, and oral malodor. Malodour is a prevalent issue among many individuals, with up to 50% reporting frequent episodes. Today's media features adverts highlighting the American culture's fixation on having clean breath. The etiology of Halitosis may have an intra-oral or extra-oral cause. Food particles and biofilm accumulation on the teeth and tongue are common causes of Halitosis. Malodourous volatile sulfur compounds are created when oral waste is putrefied by microorganisms, giving rise to the stench that comes from inside the mouth. Halitosis may also be exacerbated by extra-oral or systemic diseases that generate volatile substances expelled through the air (Armstrong, Sensat, & Stoltenberg, 2010).

1.2.1. *Classification of Halitosis*

Oral malodor can be categorized based on its cause as:

- **Genuine Halitosis**: evident malodor with a degree of severity that is higher than what is considered socially acceptable.
- **Physiological Halitosis**: Malodour that comes from the mouth cavity, and a particular illness or pathological condition does not bring it on. The tongue dorsum is most likely the primary source of origin.
- **Pathologic Intra-Oral Halitosis**: caused by pathogenic processes or diseases affecting the oral cavity.
- **Pathologic Extra-Oral Halitosis**: caused by disease or pathologic processes related to nasal, paranasal, or laryngeal regions.
- **Halitophobia**: The patients think they have oral malodor even if there isn't any malodor that other people can detect. Patients with halitophobia continue to believe they have Halitosis despite assurances, therapy, and counseling.

1.2.2. *Epidemiology*

According to epidemiology research, approximately 2.4% of adults have unpleasant Halitosis. The National Institute of Dental Research estimates that over 65 million people worldwide experience Halitosis at some point. Convenience samples of persons from over the globe have been used in descriptive studies to examine the prevalence of Halitosis. Several evaluations and cut-off points are available (Aminu, Muhammad, & Yabo, 2021). The cavitation of inorganic components in the enamel and dentin results from interactions between the host, bacteria, food, and time. Dental cavities are complicated, preventable, and localized illnesses.

Mouth bacteria cause the infectious disease known as caries. Lactobacillus development and streptococcus mutans are the main initiators of caries (Mulyanti et al., 2020). Together with periodontal infections, dental caries is one of the most prevalent infectious disorders that many individuals experience. Periodontitis is a dangerous dental condition that can damage your teeth and gums. Dental caries were

widespread in children and adolescents due to inadequate oral hygiene practices. An inflammation and bleeding of the gums is known as gingivitis.

Plaque accumulation on the gums and surfaces of the teeth is the primary cause of gingivitis (Kad et al., 2024). Cloves are aromatic. It is used to treat oral infections, a group of illnesses that impact the oral cavity, called mouth infections. It contains antiseptic, stimulant, flavoring, and local anesthetic agents (Nigam, Verma, & Chhajed, 2020).

An essential oil called clove oil is extracted from the leaves of clove trees. Eugenol, carvacrol, thymol, and cinnamon aldehyde are examples of phenylpropanoids that are the main components of clove oil. It has several uses: treat gum disease, relieve dental discomfort and bad breath, and be found in flavoring and aroma products.

Clove oil also has the natural capacity to suppress bacterial development, which can benefit the management of oral and throat ailments (Singh et al., 2022). Menthol contains the primary component of peppermint oil. It is utilized as a flavoring agent. Because it can prevent bad breath and offer overall freshness in the breath, it is also used in the production of oral dentifrices.

Thymol, sometimes called 2-isopropyl-5-methylphenol, is a white crystalline compound with potent antibacterial qualities derived from thymus vulgaris, common thyme (Rathi & Rathi, 2017). Benzoic acid is used as a preservative. It has been demonstrated that common food preservatives like sorbates and benzoates have antibacterial plaque-inhibiting properties (Singgih et al., 2014).

1.2.3. *Advantages of Mouthwashes*

a) Mouthwashes provide fresh breath.
b) They use sodium chloride to lessen tooth decay.
c) Decrease gum irritation by eliminating germs.
d) Brightens tooth.
e) It is used as an antiseptic or antiplaque.
f) Mouthwash helps to prevent gingivitis and gum disease.
g) It can prevent dental decay by strengthening enamel, preventing plaque buildup, and promoting the demineralization of teeth (Shahidulla et al., 2024).

2. Materials

A list of materials such as Clove oil, thymol, menthol, sucrose, and apple green (coloring agent) was acquired from Republic Grandigi Shop, Chitradurga, Karnataka. Tween 80, Benzoic acid, and alcohol were sourced from Vasa Scientific Suppliers, Bengaluru, Karnataka.

List of types of equipment such as measuring cylinder, beaker, conical flask, stirrer, burner, Petric dish, digital pH meter, and incubator. Collected from the college laboratory of Sri Raghavendra College of Pharmacy, Chitradurga, Karnataka. These instruments were purchased from J.K. Agencies, Bengaluru, Karnataka.

2.1. *Phytochemical Investigation of Clove Oil*

2.1.1. *Test for Eugenol*

Thin layer chromatography: The technique known as thin layer chromatography is used to find non-volatile combinations. The studies were carried out on a sheet of glass, plastic, or aluminum foil covered with a thin layer of adsorbent material, commonly silica gel, cellulose, or aluminum oxide.

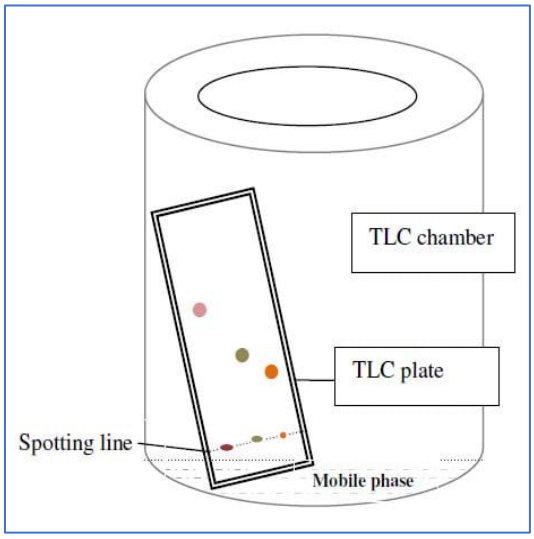


Fig. 2. Test for Eugenol.

The plates are then stood in a suitable solvent system (hexane: acetone,9:1) and left to develop. Each component shows up as vertical spots after the separation is complete. The retention factor (Rf) for each spot is calculated as follows: Rf = distance traveled by sample/distance traveled by solvent.

2.1.2. *$Fecl_3$ Test*

Treat a solution of clove oil with ferric chloride. The mixture will turn blue due to the presence of eugenol.

2.1.3. *Bromine water Test*

It is a qualitative test determining whether an organic molecule has unsaturation (double or triple bond). Eugenol contains phenolic and alkene groups, so that it can be tested with bromine water. Add a few drops of bromine water to a small amount of clove oil (which contains eugenol) in a test tube. The color changes if eugenol is present. The reddish-brown color of bromine water will decolorize (turn colorless).

3. Method

3.1. *Preparation of Mouthwash (50 ML)*

Table 1. Mouthwash Preparation – Ingredients.

SI.No.	Ingredients	Functions	F1(mg)	F2(mg)	F3(mg)
1.	Clove oil	Antimicrobial, Analgesic	1 ml	0.10 ml	0.5 ml
2.	Thymol	Antiseptic, Antibacterial	0.032gm	0.032gm	0.032gm
3.	Menthol	Flavoring agent, Freshener	0.21gm	0.21 gm	0.21 gm
4.	Tween 80	Surfactant	2.5ml	2.5 ml	2.5 ml
5.	Benzoic acid	Preservative	0. 5 gm	0. 5 gm	0. 5 gm
6.	Alcohol	Solvent, Preservative	10 ml	10 ml	10 ml
7.	Sucrose	Sweetening agent	0. 5 gm	0. 5 gm	0. 5 gm
8.	Food Color	Coloring agent	0. 01 gm	0. 01 gm	0. 01 gm
9.	Water	Quantity sufficient	QS	QS	QS

3.2. *Procedure*

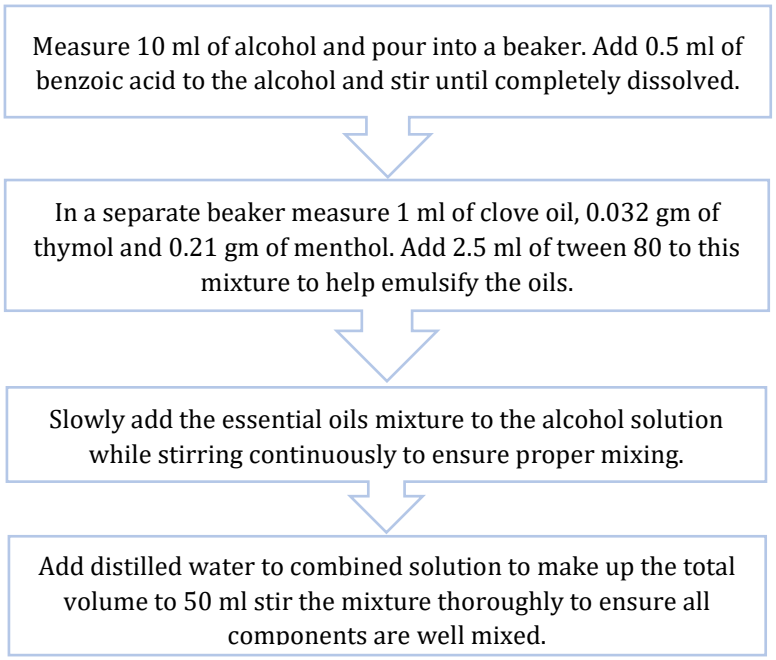


Fig. 3. Procedure of Preparation of Mouthwash.

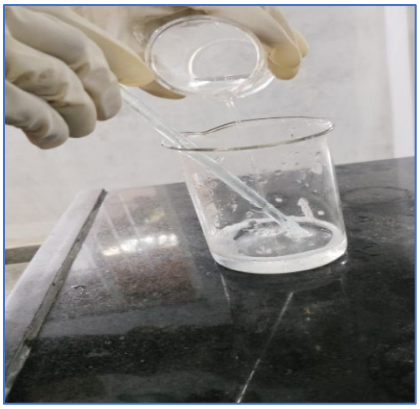


Fig. 4. Mixing and Stirring.

4. Evaluation of Prepared Mouthwash

4.1. *Physical Evaluation*

Physical factors, including taste, color, and odor, were investigated.

4.2. *pH determination*

Here, the pH value is determined using a pH meter. The glass electrode was placed in 30ml Mouthwash, and the pH value was measured.

4.3. *Antimicrobial Study*

In vitro, antimicrobial activity was assessed in isolated Streptococcus aureus colonies. The strains of S. Aureus were inoculated in premade agar plates, and the zone of inhibition and minimum inhibitory concentrations (MIC) were determined using the agar well diffusion technique. Wells were created once the plates were dried. 20 µl of produced Mouthwash was added to each well. The agar plates were undisturbed to diffuse Mouthwash passively into the agar growth media. Plates were then incubated for 24 hours at 37 °C. In millimeters, the zone of inhibition was computed.

4.4. *Stability Studies*

Without adequate stability assessments of the final product, no product's formulation and preparation are complete. Accelerated stability studies are a common way to anticipate a product's stability. Where the product is heated following ICH regulations. For the prepared formulation, a three-week short-term stability study was conducted. The samples were kept at a temperature of 37 degrees Celsius.

Ultimately, the sample maintained for the stability research was taken out and examined once a week.

5. Results and Discussion

Collection of ingredients: Clove oil, Menthol, and Thymol were obtained from Republic Grandigi Shop, Chitradurga, on September 04, 2024.

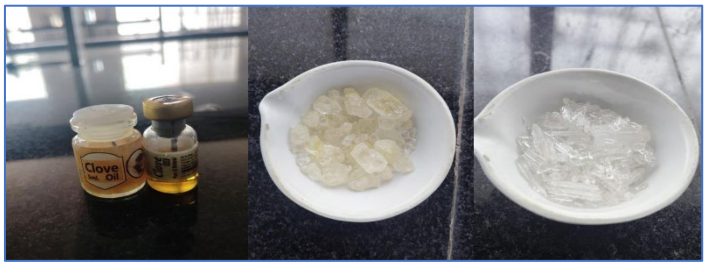


Fig. 5. Image of clove oil, Thymol, Menthol.

Preparation of clove oil-based mouthwash- Mouthwash was prepared using a mixing method.

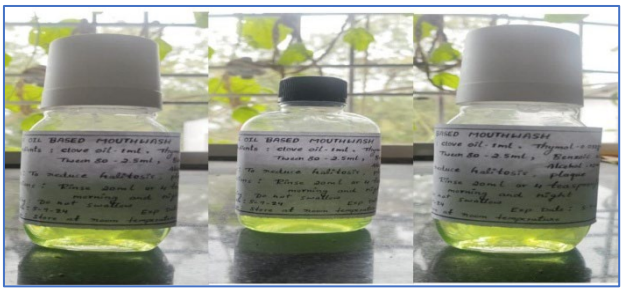


Fig. 6. Prepared Mouthwash.

5.1. *Confirmatory Test for Eugenol*

5.1.1. *Thin layer chromatography:*

Results: The R_f value of eugenol in clove oil was 0.43,0.62.

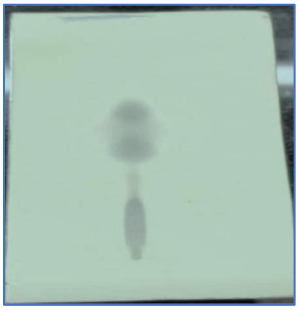


Fig. 7. Thin layer chromatography.

5.1.2. *$Fecl_3$ Test*

The mixture turned blue due to the presence of eugenol.

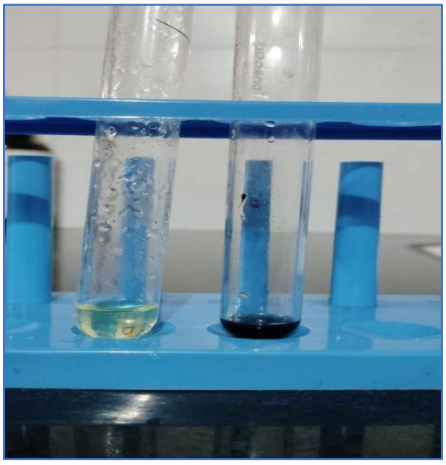


Fig. 8. $Fecl_3$ Test.

5.1.3. *Bromine Water Test*

The reddish-brown color of bromine water turned colorless(decolorized). That confirmed eugenol is present.

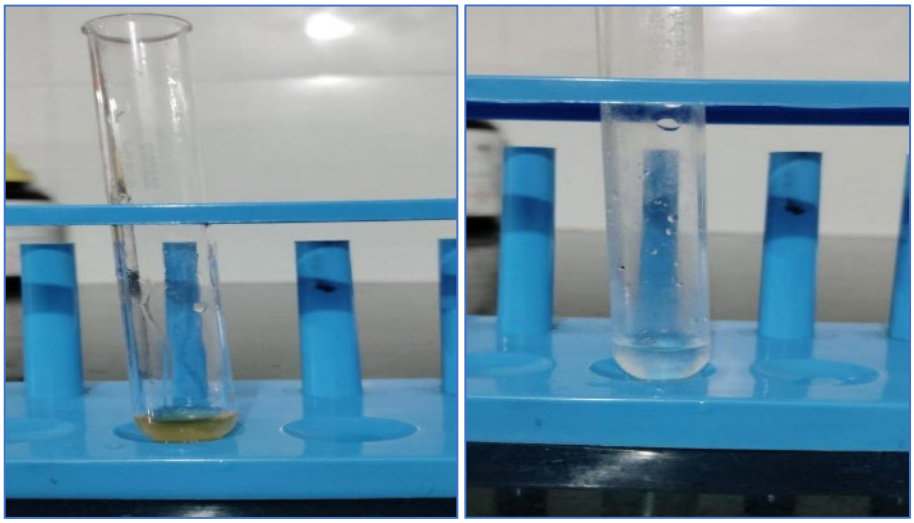


Fig. 8. Bromine Water Test.

6. Physical Characteristics of Mouthwash

Organoleptic characteristics of Mouthwash are given in Table 2.

Table 2. Organoleptic characteristics of Mouthwash.

Sl.No.	Formulations	Parameters	Observation
1.	F1	Color	Light Green
		Odor	Cool Mint
		Taste	Slightly Pungent
2.	F2	Color	Light Green
		Odor	Pleasant Odour
		Taste	highly Pungent
3.	F3	Color	Light Green
		Odor	Pleasant Odour
		Taste	Highly Pungent

Physical characteristics will be assessed, including color, taste, and odor.

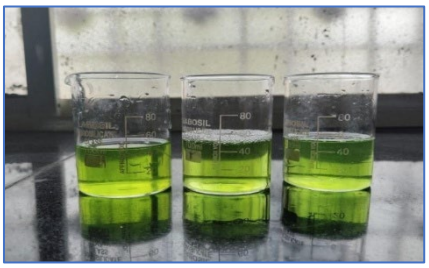


Fig. 9. Organoleptic Characters of Mouthwash: F-1, F-2, F-3.

6.1. *pH Analysis*

The pH of all three formulations was measured by a Digital pH meter. The results are shown in Table 3.

Table 3. Measurement of pH Formulation.

SI.NO	Formulation	pH
1	F-1	6.06
2	F-2	5.81
3	F-3	5.83

F-1 **F-2** **F-3**

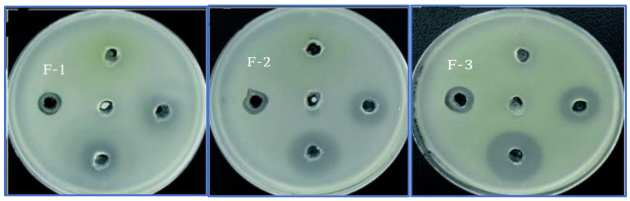
Fig. 10: The Mouthwash Formulations pH.

6.2. *Anti –Microbial Activity*

In this work, the formulated Mouthwash was investigated for antimicrobial activity against staphylococcus aureus (fig No:16), and the result showed that the formulated Mouthwash exhibited antimicrobial activity. At the same time, F-1 formulation shows high antimicrobial activity compared to F-2 and F-3. The mouthwash formulation demonstrated significant antibacterial activity, and the current product can stop germs from growing in the oral cavity.

Table 4. Measurement of pH Formulation.

Formulation Batch			Zone of Inhibition (mm)		
Sl.No.	**Ingredient**	**Organism**	**F1**	**F2**	**F3**
1	Prepared formulations	S aureus	15	18	20



F-1(15mm) F-2(18mm) F-3(20mm)

Fig. 11. Antimicrobial Assay

6.3. *Stability Analysis of Mouthwash*

Table 5. Agar Well Diffusion Antimicrobial Assay.

Temperature		Evaluationparameters	Observation (Weeks)			
			1	**2**	**3**	**4**
F-1	**37° c**	**Visual Appearance**	Light Green	Light Green	Light Green	Light Green
		Odor	No Change	No Change	No Change	No Change
		pH	6.06	6.06	6.06	6.06
		Taste	No Change	No Change	No Change	No Change
F-2	**37° c**	**Visual Appearance**	Light Green	Light Green	Light Green	Light Green
		Odor	No Change	No Change	No Change	No Change
		pH	5.81	5.81	5.81	5.81
		Taste	No Change	No Change	No Change	No Change
F-3	**37° c**	**Visual Appearance**	Light Green	Light Green	Light Green	Light Green
		Odor	No Change	No Change	No Change	No Change
		pH	5.83	5.83	5.83	5.83
		Taste	No Change	No Change	No Change	No Change

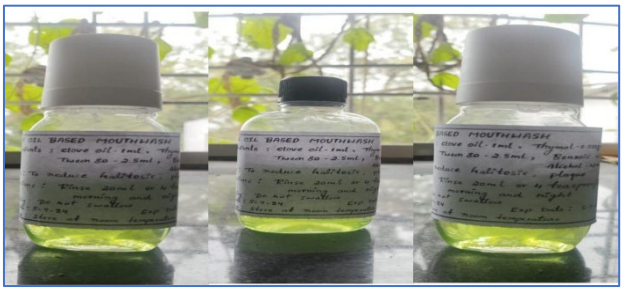


Fig. 12. Formulated Mouthwash Stored at 37° c (F-1, F2, F3).

7. Conclusion

Based on the findings reported in this study, it was determined that the Mouthwash made using clove oil has considerable antibacterial activity, is therapeutically effective, and prevents Halitosis in the oral cavity. The current generation of liquid Mouthwash can significantly reduce bad breath and other oral health issues. We chose F1 because it has an optimized formulation that exhibits good organoleptic, antimicrobial, pH, and stability properties. The physicochemical evaluation results confirm that the current formulation's color and odor are acceptable, with a pleasant odor and better effects afterward. The results of the zone of inhibition also confirmed that this Mouthwash was found to be a potent plaque inhibitor.

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