Formulation and evaluation of mouthwash containing herbal ingredients to prevent the mouth problems

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Abstract

The main objective of present study is to formulate and evaluate the herbal mouthwash containing antibacterial and antiseptic agents like neem, tulsi, and clove. In current study the material from plants were collected and extraction process were performed after collection from the plants. Further from the all extracted material the three formulations were formulated and further evaluation process were performed on the basis of following parameters likephysical examination, pH, microbial test, microscopic examination, phase separation, temperature. From the evaluation study it was concluded that all the three formulations showed significant antimicrobial activity, prevent bad breath and prepared formulations were able to inhibit the microbial growth.

Keyword: -*Herbal Mouthwash, Neem, Oral disorders, Tulsi, Anti-microbial*

1. INTRODUCTION

A topical treatment with a water basis called mouthwash works by rinsing and gargling in the mouth. The multifactorial treatment of complicated oral disorders such halitosis, gingivitis, periodontitis, oral mucositis, and even dry mouth can be supported by the use of over-the-counter and prescription mouthwashes that include a variety of active components. Many disorders, such as halitosis, gum disease, periodontitis, oral malodor, and plaque, are treated with mouthwash. It has been noted that bacterial activity in an unclean mouth is the primary cause of bad breath. Specifically anaerobic bacteria that accumulate on food detritus high in protein and stick to the tongue, bacteria that break down protein and cause bad breath with methyl mercaptan and hydrogen sulphide [1][2]

Plaque is an accumulation of microorganisms on the teeth, such as bacterial cells, salivary polymers, and bacterial extracellular products. Human illnesses are traditionally treated with herbal plants. In order to kill gramme positive (Streptococcus mutans) and gramme negative (Escherichia coli) germs and to freshen the mouth, Mentha piperita (Mint) includes ketones and metyhyl acetate [1]. As the "Queen of herbs," Ocimun sanctum (Tulsi) is known for its phenolic chemical Eugenol, which has antibacterial properties and is used to treat periodontal illnesses as well as Streptococcus pyogenes and Staphylococcus aureus. Quercetin, polyphenolic, and beta-sitosterol components found in Azadirachta indica (Neem) have antibacterial activity against S. mutans and eliminate dental caries. The natural anaesthetic eugenol, which is contained in Syzgiumaromaticum (Clove), is used to cure toothaches [2] [4]

Types of Mouthwashes [1]

1. **Fluoride mouthwash** includes salts that help prevent teeth from cavities and other dental problems. When using this kind of mouthwash, caution should be taken because consuming chemicals is bad for your overall health.
2. **Antiseptic Mouthwash**: The most popular type of mouthwash is antiseptic. People with mouth infections typically use this alcohol-containing mouthwash to prevent bacterial development. Also, it frequently aids

those who have bad breath. To avoid mouth infections and bacteria that cause bad breath, it is frequently used in conjunction with good brushing and flossing.

3. **Cosmetic Mouthwashes**: These mouthwashes just work to hide or refresh foul breath; they don't contribute to your entire dental care regimen.

Need of Herbal mouthwash [3] [4]

Because herbal mouthwash has fewer adverse effects than chemical mouthwash, the latter is less popular. Phytochemicals from herbal plants, which are natural compounds, have inherent antibacterial properties. Given the state of the economy, herbal plants are widely accessible.

Sulfates and triclosan, which can have severe effects on the oral mucosa, are added to chemical mouthwash. These unpleasant side effects can be prevented using herbal mouthwash. Natural herbal mouthwash does not have these issues, however high alcohol content mouthwash decreases salivation, which leads to dry mouth and gum disease. Oral sensitivity is brought on by overbrushing and medical problems. The delicate tissue of the oral cavity is moisturized by herbal mouthwash. Chemical components in synthetic mouthwash are unsafe.

The following ingredient commonly used in herbal mouthwash and their mechanism and uses is given below:

Table 1: Ingredients, their mechanism and uses in herbal mouthwash [5] [6]

Sr.No.	Ingredients	Mechanism and uses
1.	**Clove oil**	The main active constituent of clove is *Eugenol*. Inhibit prostaglandins synthesis. Reduce painful symptoms.
2.	**Neem**	The main active constituent of neem is *Azadirachtin*. Disrupting the microorganism (bacteria) cell membrane Provide relief from odontogenic pain.
3.	**Peppermint Oil**	Peppermint (*Mentha piperita*) oil is one of the foremost prevalent and widely used EOs. In the EO from *M. piperita*, menthol is distinguished as the major compound; take after by methyl acetate and menthofuran. Peppermint oil appears as inhibitory impact on the expansion of staphylococci.
4.	**Tulsi**	The main active constituents of tulsi are *Eugenol*. Inhibit cox-2 enzyme and inhibit the conversion of Arachidonic acid to prostaglandin H2 Provide relief from toothache,throat pain and inflammation

Ideal properties of mouthwash [1]

- It should be not irritate
- It should be capable of cleaning and freshening the mouth cavity
- It should have a pleasing flavor and aroma.
- It should be inexpensive and possess analgesic, anti-inflammatory, anti-microbial, and anti-malodor properties.

Advantages [1] [2]

- Herbal mouthwash works as bactericidal
- It is easy to apply.
- It prevents bad breath, Halitosis, Gingivitis, Periodontitis and dryness of mouth.
- It maintains Ph of the oral cavity.

- This type of mouthwash shows the very less harmful effects.

Disadvantages

- Herbal drugs are not easily available.
- Pungent in smell.

2. METHODS AND MATERIALS

2.1 Materials

Leaves of *Mentha piperitas* (Mint), *Azadirachtaindica*(Neem), *Sazygiumaromaticum* (Clove) , *Ocimum sanctum* (Tulsi), were collected from plants. Polyethyleneglycol (PEG) , Glycerol purchased from Central Drug House Pvt. Ltd.

2.2 Preparation of Herbal Mouth Wash [1] [6] [7][8]

1. Extraction preparation

All the leaves which were collected from plants were washed with sterile water.

Dry the leaves in oven at 60-70℃ for 48 hours.

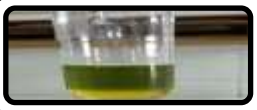
Triturate the leaves individually

Then powder passed through sieve

After this the extract was prepared by soaking the powdered form of Neem and Tulsi in distilled water

Incubated at 37 degree Celsius for 72 hours

After incubation the herbal extracts were filtered by using the Whatmann filter paper.



After filteration stored it in sample container

2. Methods of Mouthwash Preparation [1]

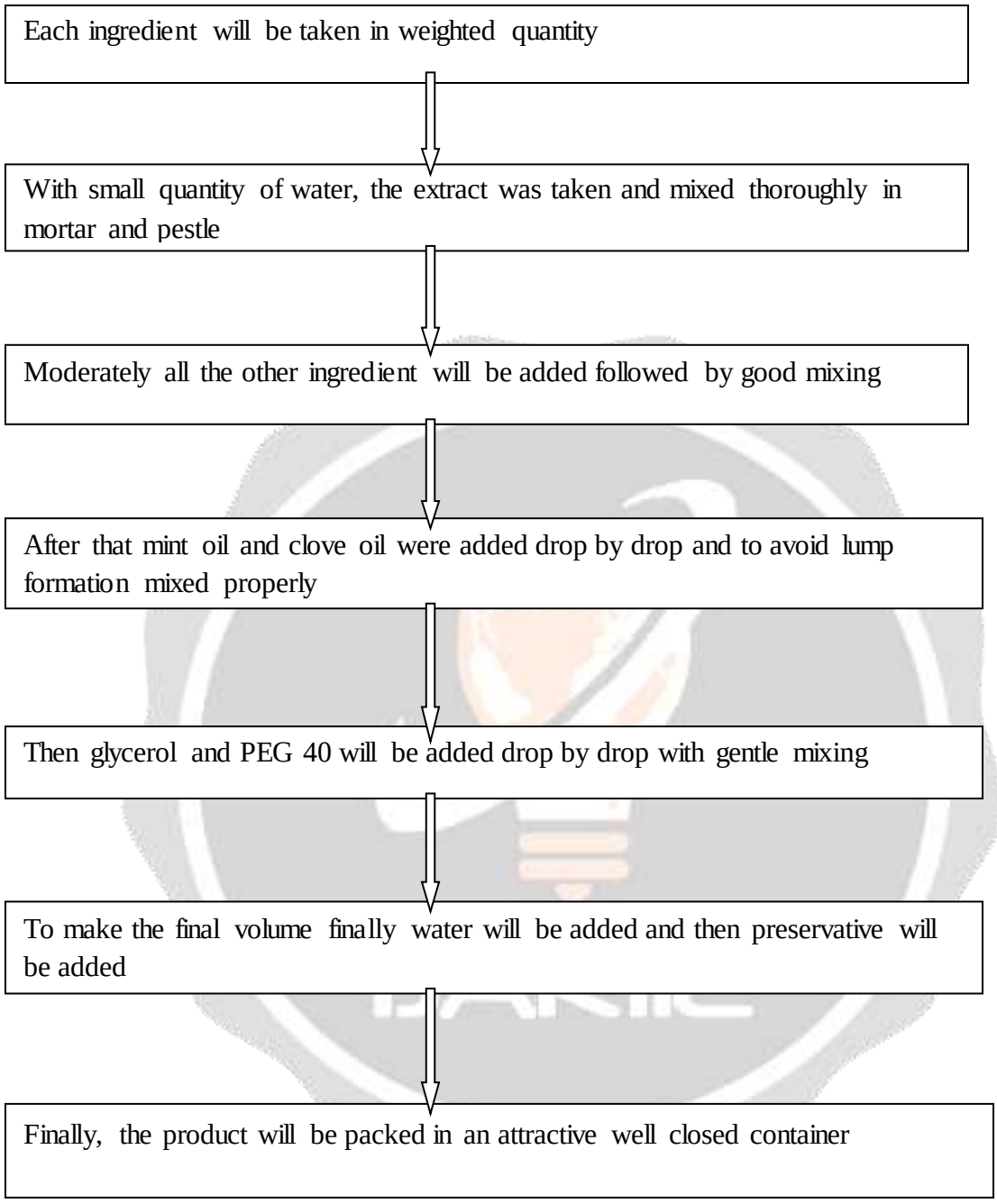


Table 2: Composition of Mouthwash[1] [2][9]

Ingredients	**Botanical name**	**Plant parts**	**Function**	**Formulations**		
				F1 (mg)	**F2 (mg)**	**F3 (mg)**
Neem	Azadirachta indica	Leaves	Analgesic, anti-inflammatory anti-microbial, anti gingivitis.	200	400	800

Tulsi	Ocimum sanctum	Leaves	Remove oral malador, anti-ulcer.	200	400	800
Clove	Syzygiumaromaticum	Leaves/seeds	Anaesthetic, antiseptic, analgesic, Antifungal.	0.15ml	0.20ml	0.25ml
Mint oil	Mentha piperita	Leaves	Mouthfreshener, cooling, cleaning agent.	0.5ml	0.10ml	0.15ml
Saccharin			Sweetener	5mg	10mg	15mg

2.3. EVALUATION PARAMETERS

2.3.1. Physical Examination: Through visible examination, physical characteristics including odour and colouring have been checked [1] [10].

2.3.2. pH: A virtual pH meter was used to measure the pH of a prepared natural mouthwash. The Ph meter was calibrated with the aid of a modern buffer A pH meter was used to assess the mouthwash's pH after it had been weighed, dissolved in 50 cc of pure water, and weighted again [1].

2.3.3. Temperature: During this test, mouthwash is stored either at room temperature, 25°C, or in the refrigerator, 8 degrees Celsius [13] [14].

2.3.4. Viscosity: With an Ostwald viscometer, the mouthwash's viscosity was determined.

2.3.5. Microbial test: Using the streak plate technique, the produced mouthwash was inoculated into the agar medium plates, and a sample was then formed. The plates have been placed inside the incubator, where they are being incubated for twenty-four hours at 37°C. When the incubation length plates have been removed and compared to the control, they have been examined for microbial growth [11] [12]

2.3.6. Centrifugation test: Using test tubes, the prepared mouthwash was centrifuged in the centrifugation machine to examine the phase separation [15][16].

2.3.7. Microscopic test: A compound microscope with magnification powers of 10 and 40 was used to assess the mouthwash formulation's transparency [1]

3. Result and Discussion:

3.1 Physical Examination: visually examining the physical characterization of the medicament was performed, and the results are given in the table for various physical characterizations

Table 3: Physical examination of prepared mouthwash

Formulation	Color	Odor	Taste	Appearance
F1	Light green	Fragrant	Astringent sensation	Clear

F2	Light green	Fragrant	Astringent sensation	Clear
F3	Light green	Fragrant	Astringent sensation	Clear

(F1) (F2) (F3)

Figure 1: Photograph of mouthwash containing herbal ingredients

3.2. pH observation: To measure pH, we used pH paper. A piece of pH paper was dipped into 5 ml of mouthwash. It supported a shadeation that identified the pH range between 6 to 7 by comparing it to a recognised pH shadeation range. Thus, the pH that was found between 6 to 7 [1]

Table 4: Results of pH examination of prepared mouthwash

Formulation	**Ph**
F1	6.35
F2	6.35
F3	5-6

3.3 Microbial Test: The mouthwash was inoculated on agar medium plates using the streak plate method, and a control was created. The plates were placed in the incubator and let to incubate for 24 hours at 37°C. After the incubation period, the plates were removed and compared to the control to see if any microbial growth had occurred. When they were inoculated in the agar medium, they did not produce any microbial growth, indicating that the formulation was free of microorganisms.

Table 5: Result of microbial test of mouthwash formulations

Formulation	**Incubator**	**Temperature**	**Time**	**Microbes**
F1	Biological incubator	37°C	72 hr	No microbial growth
F2	Biological incubator	37°C	72 hr	No microbial growth
F3	Biological incubator	37°C	72 hr	No microbial growth

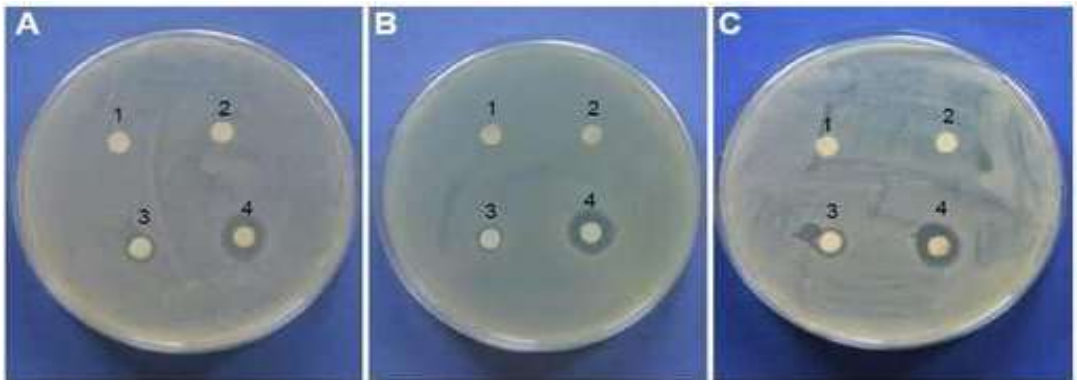


Figure 2: Agar diffusion method for assessment of anti-microbial study

3.4 Temperature: Following exposure to various storage temperatures, the physical features of various mouthwash formulations.

Table 6: Mouthwash formulation exposure to different temperature

Formulation	Room temperature	Refrigerator temperature	Time period	Changes
F1	25° C	2-8 °C	1 week	No change
F2	25 °C	2-8 °C	1 week	No change
F3	25 °C	2-8° C	1 week	No change

3.5Microscopic test:-by using compound microscope with magnification powers of 10 and 40, it was observed that the mouthwash formulations are transparent was used to assess the mouthwash formulation's transparency.

Table 7: Result of microscopic test of mouthwashformulations

Formulation	Microscope	Magnification power	Observations
F1	Compound microscope	10&40	Transparent
F2	Compound microscope	10&40	Transparent
F3	Compound microscope	10&40	Transparent

3.6 Centrifugationtest:-Using cetrifuge tubes, the prepared mouthwash was centrifuged in the centrifugation machine to examine the phase separation and it was found that there is no phase separation.

Table 8: Result of centrifugation test of mouthwash formulations

Formulation	Time in	Time out	Sedimentation rate
F1	2:20 2:25	2:25 2:40	No phase separation
F2	2:30 2:35	2:35 2:50	No phase separation
F3	2:40 2:45	2:45 3:00	No phase separation

4. Conclusion:-

This research is about the herbal mouthwash a liquid dosages form might be useful as compared with the synthetic mouthwash. In this research three formulations of herbal mouthwash were prepared and evaluated on the basis of their physiochemical properties like colour, odour, taste, pH, temperature, microscopic test, microbial test, phase separation.

The formulation were contained herbal ingredients like Neem (as a antibacterial, analgesics), Tulsi (for bad odor), Clove (As a anesthetics, antiseptic) and Mint (as a mouth freshener). From the evaluation studies it was concluded that all the three formulationsconfirms the acceptable colour, odour ad gave a better aftertaste. Microbial studiesshowed that the all the formulations did not produce any microbial growth and indicating that the formulations was free of microorganisms.

Therefore from the evaluation results of all the formulations was found to be in favorable Consideration and in this herbal mouthwash all the herbal ingredients used, have been medicinally proven to prohibit the oral hygiene problems and bad breath.

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