



ISSN (E): 2277-7695

ISSN (P): 2349-8242

NAAS Rating: 5.23

TPI 2023; 12(6): 78-84

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[www.thepharmajournal.com](http://www.thepharmajournal.com)

Received: 25-04-2023

Accepted: 29-05-2023

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## Formulation and *in vitro* evaluation of polyherbal antibacterial mouthwash

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### Abstract

Many plants derived medicines used in medicinal system have been reported to treat infection against dental carries and periodontal diseases. The main aim of this research was to formulate and evaluate the anti-bacterial activity of herbal mouthwash formulation containing the extract of clove, neem, tulsi, liquorice, which has been previously reported to have anti-bacterial activity. Thus, the clove, neem, tulsi, liquorice was extracted with ethanol and three base formulation were prepared using propylene glycol, tween 80, menthol, and sodium benzoate. The resulting formulation exhibit a smell of mint and had a refreshing minty taste.

**Result:** The pH of the herbal formulations were determined and ranged from 4.0-6.0 and F3 formulation exhibit more stability and larger zone of inhibition against *Streptococcus mutans* when compare to F1 and F3 formulation.

**Conclusion:** The formulation features the ideal physical and stability characteristics of mouthwash and possesses antibacterial properties that can potentially combat *streptococcus mutans*.

**Keywords:** Polyherbal mouthwash, plague, antibacterial activity, oral health, *streptococcus mutans*

### Introduction

Mouthwash is a term that refers to a liquid preparation, typically antiseptic, used to clean teeth and mouths or to refresh breath. Dentistry frequently recommends mouthwash for the prevention and management of a number of oral disorders. Recently, there has been a significant increase in the usage of naturally occurring items, also known as "grandmother's remedy." This has prompted the development of more modern mouthwashes, but this study examines whether they compare favourably to the gold standard or are even superior to it.

Due to their antioxidant and antibacterial properties, spices like clove, basil, mint, neem, and cardamom have been used as food preservatives and medicinal plants for ages. The antibacterial, antifungal, antiviral, and anticarcinogenic effects of spice plants are now well acknowledged in reports. Mouthwashes are liquids with analgesic, anti-microbial, and anti-inflammatory properties. Mouthwashes come in two varieties: chemical and herbal. Natural components found in herbal mouthwash, known as phytochemicals, have the desired anti-microbial and anti-inflammatory benefits. Herbal mouthwash is becoming more and more popular because it functions without alcohol or artificial flavours, colours, or preservatives. Because it contains natural herbs, which naturally cleanse and repair gums and teeth. Many herbal mouthwashes contain herbs with antimicrobial property such as neem, some of the herbs that are used in mouthwashes are clove, which is traditionally used for oral health because of their antiseptic, antibacterial and antiviral property, menthol which gives cooling effect to the mouth. Natural Herbs such as basil, Neem, Clove oil, mint and many others are used as single or in combination have been Scientifically Proven to be Safe and Effective Medicine against Oral Health Problems such as Bleeding Gums, Mouth Ulcers, and Preventing Tooth Decay without side effects.

Since ancient times, many dental disorders have been treated and their onset prevented by using neem bark and leaf. The analgesic and anti-inflammatory properties of clove. It is employed as a short-term toothache remedy. It has been shown to lessen oral cavity localised irritation. Cardamom contains anti-inflammatory and antibacterial properties. Additionally, it gives many oral formulations a tasty flavour. It is said to safeguard oral health and naturally refresh breath. Glycyrrhiza is a demulcent, analgesic, and anti-inflammatory. This plant is said to improve issues related to dental health.

One such well-known plant in Ayurvedic medicine is *Ocimum sanctum* (Tulsi), which is frequently utilised in the treatment of numerous systemic disorders due to its antibacterial property. Non-ionic surfactant Tween 80, also known as polysorbate 80 or polyoxyethylene sorbitan monooleate, is frequently used as an emulsifier in cosmetic, pharmaceutical, and culinary applications. Propylene glycol serves as a thickening, a sweetener, and prevents items from drying out.

The local geological conditions have a significant impact on the composition of water. The fact that water contains trace amounts of gases, minerals, and naturally occurring organic matter means that neither groundwater nor surface water has ever been chemically pure. Deionized or reverse osmosis-treated water, distilled water, and artificially manufactured demineralized waters have all been utilised primarily in industrial and scientific settings. In order to clean the mouth, this is also a component in mouthwash formulations. In order to explore short its antibacterial characteristics and shelf life duration, our current project effort is based on the development of a new type of herbal mouthwash from neem leaves, clove, tulsi leaves, and menthol.

### Types of mouthwash

#### Cosmetic mouthwash

As the name suggests, cosmetic mouthwashes do not kill germs like a mouthwash containing germ-killing components can, but instead work to temporarily regulate breath odour and leave a pleasing taste in the mouth. As a result, they do not have a long-lasting refreshing effect.

#### Fluoride mouthwash

Fluoride mouthwash aids in remineralizing damaged tooth enamel, strengthening teeth's resistance to decay and erosion. By using fluoride mouthwash, you can prevent tooth decay and its early symptoms while also maintaining the health of your teeth.

Fluoride can be found in many naturally occurring sources, including some foods and the majority of water sources including rivers, lakes, and wells; however, the concentration of fluoride in these sources is below the level required to give your teeth the necessary protection. Using a fluoride mouthwash like Listerine® Fluoride defence guarantees that you receive the required dose of fluoride to aid in cavity prevention. It includes more fluoride, essential oils, and green tea extract to help prevent tooth decay by fortifying the teeth's enamel.

#### Antiseptic mouthwash

Since antiseptic mouthwash contains germ-killing agents, it aids in getting rid of the bacteria that produce bad breath. Some Antiseptic mouthwash preparations contain the powerful antibacterial ingredient eucalyptus oil to help battle plaque and destroy bacteria. It is derived from the eucalyptus tree (*Eucalyptus globulus*), which has been used for centuries by traditional Aboriginal Australians as an antiseptic to destroy bacteria.<sup>6</sup> Consider using Listerine® Cool Mint Mouthwash, an antibacterial mouthwash that works to efficiently combat plaque and guard against bad breath.

#### Natural mouthwash

A natural mouthwash is the best option for those who, for whatever reason, prefer to use non-alcoholic goods! Natural mouthwashes are gentler, have a milder flavour, and don't

include alcohol, but they nevertheless provide the same advantages as other mouthwashes.

Listerine® Cool Mint Milder Taste Mouthwash and Listerine® Total Care Teeth Protect Milder Taste Mouthwash are two options for Listerine mouthwashes that contain no alcohol.

### Whitening mouthwash

Dental treatment now goes beyond just looking after your teeth and gums; it now includes maintaining a smile that makes you look good. The desire for oral care products with whitening capabilities has increased significantly. In order to assist eliminate stains and brighten stained teeth, a whitening mouthwash can be the ideal addition to your at-home teeth whitening regimen.

You must continuously take care of your teeth to maintain them safe, strong, and cavity-free. Your efforts are more successful and your mouth stays fresher for longer when you use a mouthwash that is helpful in eliminating germs and microorganisms that endure after brushing.

### Advantages of mouthwash

1. A fresh breath.
2. Using sodium fluoride to prevent tooth decay.
3. Decreasing gum irritation by eliminating bacteria.
4. Whitening teeth with a bleaching agent
5. Using an antiseptic or anti-plaque component to prevent gum disease.
6. By eliminating the bacteria that might otherwise infect the gums and dental sockets, mouthwash prevents gingivitis and gum disease.
7. It can demineralize your teeth, reinforce the enamel, and stop plaque formation, all of which help you prevent dental decay.

### Materials and Method

#### Ingredients

##### Clove



Fig 1: Clove

Biological Source- *Eugenia caryophyllus*, a member of the *Myrtaceae* family of plants, produces the dried flower buds that make up cloves.

Chemical constituents- Eugenol, caryophyllene, methyl amyl ketone.

#### Uses

1. Clove works as a dental analgesic
2. Treats bad breath
3. Prevents cavities

4. Increases circulation
5. Provides temporary relief from toothache The eugenol ingredient present in the clove has anti-inflammatory properties that can help the ailing tooth
6. Clove has natural ingredients that can numb the part of the skin that are in close contact with the teeth.
7. Due to its antibacterial and antifungal properties it is widely used in preparations of toothpaste.

### Neem



**Fig 2:** Neem

**Biological Source-** The plant *Azadirachta indica*, a member of the *Meliaceae* family, is utilised for its leaves.

**Chemical constituent-** Azadirachtin, Nimbin, Nimbdin, Nimbinin.

It prevents the development of plaque and bacterial growth. Neem seeds, twigs, and leaves have all been employed to clean teeth and treat bacterial infections. Because it prevents the development of plaque and the growth of germs, neem extract is effective in the treatment of gingivitis and oral infectious diseases. Neem leaves, twigs, and seeds have been used to clean teeth and treat bacterial and fungal infections in India and South Asia for thousands of years. Because it prevents plaque formation and bacterial growth, neem extract is suitable for treating gingivitis and oral infections. Gram-positive and gram-negative bacteria, as well as other bacteria like *E. coli* and streptococcus that cause a wide range of illnesses in humans and animals, have all been demonstrated to be significantly affected by neem.

### Tulsi



**Fig 3:** Tulsi

**Biological Source:** Tulsi is made up of fresh and dried leaves from the *Lamiaceae* family of plant *Ocimum sanctum* Linn. (Syn. *Ocimum tenuiflorum*). It must have a minimum of 0.40% dry-weight eugenol content.

**Active Constituents:** It contains approximately eugenol (70%), carvacrol (3%), and eugenol-methyl-ether (20%).

**Uses:**

1. Powder made from dried leaves is used to wash teeth.
2. Tulsi leaf preparation is used in toothpaste for oral health to relieve a cough and a cold.
3. It exhibit bacteriostatic, anti-oxidant, and immune-modulating qualities, as well as effective against different bacterial strains.
4. Additionally, it is employed as a treatment for periodontal and gingival illnesses.

### Liquorice



**Fig 4:** Liquorice

**Biological Source-** It is an extract from the plant *Glycyrrhiza glabra*, which is a member of the *Fabaceae* family.

**Chemical constituent-** Glycyrrhizin.

**Uses:**

1. It serves as a natural sweetener
2. Flavor addition
3. Common oral and dental illnesses include oral candidiasis, restricted plaque buildup, gingival inflammation, caries, and periodontitis can all be successfully treated with the bioactive ingredients.

### Tween 80

Tween 80, also known as polysorbate 80 or polyoxyethylene sorbitan monooleate, is a non-ionic surfactant that's frequently used in food, medicine, and cosmetic items as an emulsifier.

### Propylene Glycol

Propylene glycol (propanediol, IUPAC propane-1,2-diol) is a common constituent in numerous foods, topical and systemic medications, and mouthwashes. Propylene glycol has largely taken the place of ethyl alcohol in mouthwashes as a result of concerns about its safety or potential adverse effects, such as mucosal irritation. It is a viscous alcohol with two hydroxyl functional groups that is colourless and only a little bit sweet. It has been employed as a more effective emollient and solvent than glycerine, dissolving substances including phenols, vitamins A and D, and a variety of local anaesthetics. Additionally, it acts as a preservative. It functions as an

antiseptic similarly to ethanol. The safety of propylene glycol is universally acknowledged. To our knowledge, there hasn't been any research done on how frequent usage affects the salivary glands and oral tissues. The liquid used in electronic

cigarettes contains propylene glycol as well, although no toxicologically significant consequences have been found.

### Ingredient Table

**Table 1:** Composition of polyherbal mouthwash

Ingredients	Scientific Name	Chemical Constituents	Activity
Clove	<i>Eugenia caryophyllus</i>	Eugenol	Dental analgesic, Anti-microbial agent.
Neem	<i>Azadirachta indica</i>	Nimbin, Nimdin, Azadirachtin	Antiseptic, inhibit plague formation.
Tulsi	<i>Ocimum sanctum</i>	Eugenol, carvacrol, and eugenol-methyl-ether.	Anti-microbial activity against various bacterial strains.
Menthol	<i>Mentha piperita</i>	Menthol	Local anesthetic, cooling agent
Liquorice	<i>Glycyrrhiza glabra</i>	Glycyrrhizin	Sweetening agent
Sodium benzoate	–		Preservative
Tween 80	–		Non-ionic emulsifier
Propylene glycol	–		Humectant
Water	–		Vehicle

### List of ingredients

1. Neem
2. Clove
3. Tulsi
4. Liquorice
5. Menthol
6. Sodium Benzoate
7. Tween 80
8. Propylene Glycol
9. Water.

### List of equipment

1. Measuring cylinder
2. Beaker
3. Magnetic stirrer
4. Conical flask
5. Funnel
6. Whatman filter paper
7. Tripod stand
8. Petri dish
9. pH meter
10. Incubator
11. Weighing balance
12. Laminar air flow
13. Hot air oven
14. Incubator

### Method

#### Extraction Process

Neem (*Azadirachta indica*), clove (*Syzygium aromaticum*), tulsi (*Ocimum sanctum*), liquorice (*Glycyrrhiza glabra*) were

collected from local market and authenticated. The samples were washed, dried in shade and were powdered. These samples were separately suspended in 300 ml of Ethanol (Et OH) in a soxhlet apparatus for 6 hours. Then the extract was filtered and evaporated on water bath at 60-80 °C. The above extracts were tested individually for antimicrobial activity by zone of inhibition.



**Fig 5:** Extract of herbs

#### Preparation of polyherbal extract

For each formulation, mouthwash containing active ingredients such as clove, neem, tulsi, and licorice was created in quantities of up to 100 ml. Table 2 lists the polyherbal mouthwash formulations (designated as F1, F2, and F3).

**Table 2:** Composition of different formulations

S. No	Ingredients	F1	F2	F3
1	Clove	0.3 gm	0.6 gm	0.9 gm
2	Neem	0.2 gm	0.4 gm	0.6 gm
3	Tulsi	0.2 gm	0.4 gm	0.6 gm
4	Liquorice	0.3 gm	0.6 gm	0.9 gm
5	Menthol	1 gm	1 gm	1 gm
6	Propylene glycol	10 ml	10 ml	10 ml
7	Tween 80	5 ml	5 ml	5 ml
8	Sodium benzoate	1 gm	1 gm	1 gm
9	Distilled water	q.s	q.s	q.s

The extract indicated above was mixed with propylene glycol and put in a glass beaker. The temperature was then increased to 60 °C, and Tween 80 and menthol were added while stirring at 300 rpm with a magnetic stirrer.

Sodium benzoate was dissolved in distilled water before being added to the mixture and homogenising it with a magnetic stirrer. The solution was then mixed with 100 ml of the distilled water until it turned transparent.



**Fig 6:** F1, F2, F3 Formulations

**Evaluation Parameters**

**Morphological evaluation**

Response was recorded by testing three polyherbal mouthwash formulations. The evaluation of preparation procedure was done to capture observations on taste, color, odour and form.

**pH testing**

During the seven weeks of storage, the pH was measured using a Hanna pH metre that had been calibrated with a buffer solution (pH 4 and pH 7). After being cleansed with distilled water, the electrodes were dried. A beaker was used to make each polyherbal mouthwash, and measurements were obtained.

**Stability**

Three cycles of the freeze-thaw test were used to conduct stability testing on the polyherbal mouthwash formulations. After 48 hours of storage at 4 °C and then again after 48 hours at 40 °C, each cycle was examined. This procedure lasted for

6 days.

**Anti-bacterial activity**



**Fig 7:** Nutrient agar media

The nonselective medium on an agar plate is injected with a suspension of streptococcus mutans to produce a confluent lawn of growth in the disk-diffusion susceptibility test. Discs bearing known concentrations of an antimicrobial agent are put on the surface of the plate. The antimicrobial agent diffuses into the medium, resulting in a zone of growth inhibition of the strain surrounding the disc that corresponds to the strain's sensitivity to the agent. In order to classify an isolate as susceptible, intermediate (or demonstrating diminished sensitivity), or resistant to an antimicrobial agent, interpretative inhibitory zone sizes for susceptibility test findings have been created.

**Result**

**Morphological evaluation**

The morphological examination was carried out over the course of one week, with the findings displayed in the table. The F1 solution was moss green, had a strong minty aroma, and tasted minty. The F2 solution was olive green, smelled strongly like mint, and tasted similarly. The F3 solution was army green, had a strong mint flavour, and smelled like mint.

**Table 3:** Morphological evaluation of different formulation

Observations	F1	F2	F3
Color	Moss green	Olive green	Army green
Odour	Minty smell	Minty smell	Minty smell
Taste	Refreshing mint	Refreshing mint	Refreshing mint
Form	Liquid	Liquid	Liquid

**pH testing**

The results of the pH testing are listed in a table. Over the course of seven weeks of storage, the pH values of each

formulation decreased. F1 had a pH range of 4.12 to 5, F2 between 4.15 and 5.50, while F3 ranged from 4.56 to 6.

**Table 4:** Determination of pH of different formulations

pH testing	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
F1	5	4.9	4.7	4.5	4.4	4.2	4.12
F2	5.5	5.3	5.1	4.85	4.62	4.31	4.15
F3	6	5.75	5.64	5.42	5.18	4.8	4.56

### Stability

The freeze-thaw test was conducted on each of the three polyherbal mouthwash formulations at 4 °C and 40 °C for

three cycles and showed no separation.

### Anti-bacterial activity



**Fig 8:** Antibacterial assay on streptococcus mutans and *E. coli*

Anti-bacterial effects of polyherbal mouthwash against *Streptococcus mutans* showed a larger inhibition zone in F3 ( $15 \pm 2$ ), compared with F2 ( $13 \pm 2$ ) and F1 ( $10 \pm 2$ ) as shown in table.

**Table 5:** Determination of antibacterial activity by zone of inhibition

	Zone of inhibition
F1	$10 \pm 2$
F2	$13 \pm 2$
F3	$15 \pm 2$

### Discussion

Due to growing public awareness of the benefits of complementary and alternative medicine, the usage of herbal mouthwashes has increased recently. The far stronger perception that alternative therapies have fewer adverse effects is another factor. Herbal substances or components vary in terms of chemical structure and makeup, according to research. The designated items lacked labels that provided a full history of the chemicals' composition, and it was also established that they were polluted with heavy metals and other natural pollutants, raising concerns about their suitability for further use. According to the studies presented above, clove has greater antibacterial activity than other active substances and also has the ability to reduce bad breath. The fact that F3 contains more cloves than the other two formulations is thought to increase its antibacterial action.

### Conclusion

The current liquid herbal mouthwash may be really effective in assisting folks to get rid of foul breath and numerous oral health issues. Additionally, the absence of any harmful additives in this recipe gives us peace of mind and comfort. The findings of the physicochemical examination show that the current herbal formulation's color and scent are acceptable, with a pleasant aroma and improved after effects. The zone of inhibition results further supported the findings that this herbal mouthwash is a powerful plaque inhibitor. The current study has a significant influence on efforts to develop a herbal oral health intervention for poor socio economic populations that is both efficient and affordable. However, since this research was brief, more investigations with bigger sample sizes are necessary. The natural herbs utilised in this

composition have been shown to have medical benefits for treating oral hygiene issues and foul breath. Numerous studies have shown that these plants have a long history of being used successfully. With the help of this herbal mouthwash, a person may effortlessly rinse their mouth and avoid a range of oral health problems.

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