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Research Article

Title of the Manuscript: Formulation and Evaluation of Herbal Mouthwash

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ABSTRACT

Oral health is as crucial as overall health. Nowadays, individuals may encounter more oral issues such as periodontal disease, sore throats, gingivitis, plaque, and others. To support good oral health, various products and formulations are developed. It is advised to use a mouthwash to manage bacteria, bad breath, plaque, and toothaches. Because herbal mouthwash has fewer adverse effects, is non-irritating, less poisonous, and does not contain alcohol, it is favoured over chemical mouthwash. Medicinal herbs play a significant part in healing and sickness prevention due to their antiviral and antibacterial properties against human pathogens. Compared to manufactured mouthwash, herbal mouthwash has fewer or no negative effects because it contains extracts from crude medications. Several plant extracts are used to make herbal mouthwashes. Tulsi , guava, and peppermint oil are among the herbs discussed in this article that have dental applications. The general dental health of the populace could be improved by mouthwashes that anyone could easily make and use safely at home using natural materials. The main objective of this review is to use natural mouthwash to maintain oral health.

INTRODUCTION

Oral infections are eliminated by holding mouthwash, an aqueous solution typically used to dissolve plaque, in the mouth and swishing it around with the perioral muscle. Herbal medicine

takes an active approach. The primary advantage of this natural herb is that, aside from the fact that no herbal mouthwash contains alcohol or sugar, there have been no known adverse effects from its

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use. These goods are problematic because the bacteria that cause halitosis and foul breath prefer to eat them and produce the halitosis-causing metabolites. Thus, using herbal mouthwash to stay away from dangerous substances is a good place to start. In dental practice, plaque-induced gingivitis a very prevalent periodontal disease—is frequently observed. Plaque buildup can be avoided and managed by employing several strategies that improve oral hygiene standards.

Goal of herbal mouthwash:

- To improve oral hygiene.
- It helps control dental plaque.
- It can be applied to gum disease.
- Used to get rid of oral bacteria.
- It both freshens and covers up foul breath.
- Using mouthwash is essential to preventing gum disease.
- Septic tanks are cleaned with it.
- It reduces pain and inflammation.

Advantages of Herbal Mouthwash:

- The use of herbal mouthwash has grown advantage over chemical mouthwashes due to their non-irritant and non-staining properties and it does not contain alcohol.
- They have very minimal or no side effect and they are less harmful.
- All herbal mouthwashes do not contain alcohol and/or sugar.
- Herbal mouthwashes are gentle for even the most sensitive mouth.

- Herbal mouthwashes have naturally antibacterial property.
- It contains no harsh additives.
- Herbal mouthwash doesn't cause dry mouth.
- It is highly in demand.
- It keeps your mouth healthy [1]

Disadvantages of the mouthwash:

- Infants under the age of six should avoid using mouthwash.
- Since mouthwash sometimes contains an excessive amount of alcohol, it might cause cancer [4].
- Mouthwash has the potential to harm some oral tissues as well as stain and darken teeth.[2]

Types of mouthwash:

- 1. Fluoride mouthwash**
- 2. Antiseptic mouthwash**
- 3. Cosmetic mouthwash**
- 4. Natural mouthwash**

1. Fluoride mouthwash:

Fluoride in mouthwashes contains salt which help protect the teeth from cavities. Fluoride is also included in toothpaste and water, therefore it's best to use caution while using this kind of mouthwash because consuming too much fluoride is bad for your general health .

2. Antiseptic mouthwash:

This is the most widely used mouthwash. People who have mouth infections commonly use this



mouthwash, which contains alcohol, to prevent bacterial growth. People with halitosis, or bad breath, can also benefit from this. This is frequently used in conjunction with regular tooth brushing and flossing to help prevent bacteria that lead to mouth infections and foul breath. For instance, Equate Antiseptic Mouthwash.

3. Cosmetic mouthwash:

A mouthwash that just works to freshen or cover up foul breath and has no effect on your overall oral health.

Ex: Himalaya Herbals Complete Care Mouthwash, for instance.

4. Natural mouthwash:

It is also a well-liked choice for mouthwash without alcohol. Compared to other types of mouthwash, their chemicals are safer to use.

LITERATURE REVIEW:

Sr. No	Title of research article	Name of herbal plant used	Use of mouthwash	Name of Author	Name of Journal & Year of publication
1	Formulation and Evaluation of Antibacterial herbal mouthwash against oral disorders [5]	Stem and bark of neem buds clovebud of cinnamon and root of liquorice	Antimicrobial, anti-inflammatory	Shafi Ahmad, Saloni Sinha, Smriti Ojha et al.	Indo global journal of pharmaceutical science,2018;8(2):37-40
2	Effect Of Mouth Wash Extracted From <i>Salvadora Persica</i> (Miswak) On Dental Plaque Formation: A Clinical Trial [6]	Miswak	Antibacterial	Foud hussein al- bayaty, aiman hamad al- koubaisi, et al.	Journal of medicinal plants research,4(14),pp.1446-1454,18 july 2010
3	Preparation of antibacterial herbal mouthwash against oral pathogens [7]	Neem, tulsi, mint, pomegrante, turmeric	Antibacterial	J Nasreen Banu, V. Gayathri	International journal of current microbiology and applied science,2016,5(11):205-221
4	Design, development and evaluation of herbal mouthwash for antibacterial potency against oral bacteria.[8]	Neem, spinach,tulsi,peper mint	Antibacterial	Shweta S. Patil, Akshay R Yadav, Dr. Atul R Chopade, et al.	Journal of university of shanghai for science and technology, vol 22, issue 11 nov 2020
5	Evaluation of holy basil mouthwash as an adjunctive plaque control agent in a four day Plaque regrowth model [9]	Holy basil	Antioxidant activity, anti cancer, anti-inflammatory , Antipyretic.	Mansa Hosamane, Anirudh B. Acharya, Chhavi Vij, et al.	Journal of clinical experimental dentistry,2014;6(5)



6	Formulation of Antibacterial Mouthwash from Local Herbs: A Mini Review [10]	Clove, oak gall, turmeric, neem, rosemary, ginger, peppermint, tea leaves	Anti-inflammatory , antibacterial	Juman Nafea, Harisun Yaaub, Mohamed Faraj Edbeib, et al.	Journal Of Biochemistry, Microbiology and Biotechnology, 2020(8), No 2,7-12
7	Antimicrobial Activity of Medicinal Plant Leaf Extracts Against Pathogenic Bacteria [11]	Green Tea, Guava, Neem, Marigold Leaves	Antimicrobial Activity or Anti-Bacterial	Atikya Farjana, Nagma Zerine, Md. Shahidul Kabir	Asian Pacific Journal Of Tropical Disease,2014,4(Suppl 2):S920-S923

NEED OF HERBAL MOUTHWASH:

- Preserves oral hygiene by lowering the amount of microorganisms in the mouth and aiding in the removal of food particles.
- Breath freshening: Offers a rapid and simple solution for halitosis, or bad breath.
- Decreases tartar and plaque: helps avoid accumulation that causes cavities and gum disease.
- Prevents and treats gum disease: can lessen gingivitis risk, bleeding, and irritation.
- Supports dental care in general: enhances brushing and flossing to provide a more thorough dental hygiene regimen.

AIM: Formulation and Evaluation of herbal mouthwash

OBJECTIVE:

1. To develop formulation of herbal mouthwash:

The present results therefore offer a greater use for traditional use of herbal mouth wash.

2. Safety:

Herbal mouthwash was safe and there was neither report of adverse reactions.

3. Effectiveness:

The objective of present work is to formulate and evaluate herbal mouthwash and to evaluate its effectiveness against microbial load of oral cavity.

4. To maintain oral hygiene:

Oral health is important as overall health. Now-a-days people may face more oral problems like periodontal disease, sore throat, gingivitis, plaque and so on. For maintaining good oral health various formulations are formulated.

5. To Prevention, control and reduction of oral infection:

It can reduce the plaque growth in your mouth, decrease your chances of developing gum disease, and prevent tooth decay.

6. To reduce side effects by promoting herbal use:

The use of herbs in dentistry should be based on evidence of effectiveness and safety. Herbal medicines, derived from botanical sources, have been applied in dentistry for a long history to



inhibit microorganisms, reduce inflammation, soothe irritation, and relieve pain.

7. Herbal medicines:

Ayurvedic medicines give a holistic approach toward entire human beings. It can maintain the balance between general and oral health as well as an environment which is in this era necessary for the well-being of humans.

- Literature Survey .
- Collection of Plant Material .
- Selection of Excipients for Formulation.
- Extraction of Herbal Drugs .
- Development of Formulation .
- Evaluation of Prepared Formulation.[12]

PLAN OF WORK:

DRUG PROFILE:

Table No 1: Drug profile

SR.NO	PLANT/HERB	CATEGORY/ USES
1	Tulsi Leave	Antimicrobial, Antioxidant, Anti-inflammatory.
2	Guava Leave	Antibacterial, Antioxidant, Anti-inflammatory.
3	Peppermint Leave	Flavouring-agent, Reducing-agent, Fresh- Breath.

Chemicals, Glasswares And Instruments:

These are obtained from Research-lab fine chemicals industries, Mumbai 400002 (India) Research- lab. , salunke@gmail.com A GMP Certified company chemicals and Glassware used

in the experimental work were calibrated in the laboratory the glassware was washed until it was used for experiment .

LIST OF CHEMICAL:

Table No 2: List of chemical

Sr. No	Chemical	Grade	Manufactured By
1	Propylene glycol	Lab Reagent	Research-lab fine chemicals industries, Mumbai 400002 (India) Research-lab. , salunke@gmail.com
2	Sodium benzoate	Lab Reagent	Research-lab fine chemicals industries, Mumbai 400002 (India) Research-lab. , salunke@gmail.com
3	Tween-80	Lab Reagent	Research-lab fine chemicals industries, Mumbai 400002 (India) Research-lab. , salunke@gmail.com
4	Glycerol	Lab Reagent	Research-lab fine chemicals industries, Mumbai 400002 (India) Research-lab. , salunke@gmail.com
5	Ethanol	Lab Reagent	Research-lab fine chemicals industries, Mumbai 400002 (India) Research-lab. , salunke@gmail.com
6	Water	Lab Reagent	Research-lab fine chemicals industries, Mumbai 400002 (India) Research-lab. , salunke@gmail.com





Fig No 1: Chemical Reagent

Instruments:

- Beakers
- Measuring cylinders
- Electronic weighing balance
- Water bath
- Whatman filter paper
- Steam distillation apparatus
- pH meter
- Ostwald viscometer
- Petri dishes

Materials And Method Of Extraction

1. Tulsi (*Ocimum sanctum*)

The herb (Tulsi) is useful in teeth disorders. You can use its sun-dried and powdered leaves to wash your teeth. Additionally, it can be combined with mustered oil to create a paste that can be used as toothpaste.

- **Biological source:** It is the fresh and dried leaves of plants from the *Ocimum* genus, primarily *Ocimum sanctum* L. (also known as *Ocimum tenuiflorum*)
- **Kingdom:** plantae.
- **Order:** lamiales .
- **Family:** lamiaceae.
- **Genus:** *Ocimum* Species: *sanctum*.
- **Geographical Source:** North-central India and surrounding South Asian regions.
- **Parts used:** Leaves, Seeds and Roots.[13]

1.1 Chemical constituents:

- Eugenol, nerol, eugenol methyl ether.
- Caryophyllene, terpinene-4-ol-decylaldehyde.
- Camphor and carvacrol.
- Essential oils, ascorbic acid, carotene, calcium, phosphorus and insoluble oxalates.
- It also contains terpenes, mucilage, fixed oil and fatty acids.
- Volatile Oil-0.8% .[14]

1.2 Uses of Tulsi plant:

- In reduction of oral malodour.
- Anticancer activity of *O. sanctum*: Tulsi has been shown to possess an excellent anticancer activity.
- As an antioxidant, it also has the ability to scavenge highly reactive-free radicals.
- Modulates immunity.
- As analgesic, antipyretic, and anti-inflammatory Activity.[15]



Fig No 2: Tulsi leave [16]

1.1 Preparation of *Ocimum sanctum* Leaf Extract. (Tulsi)

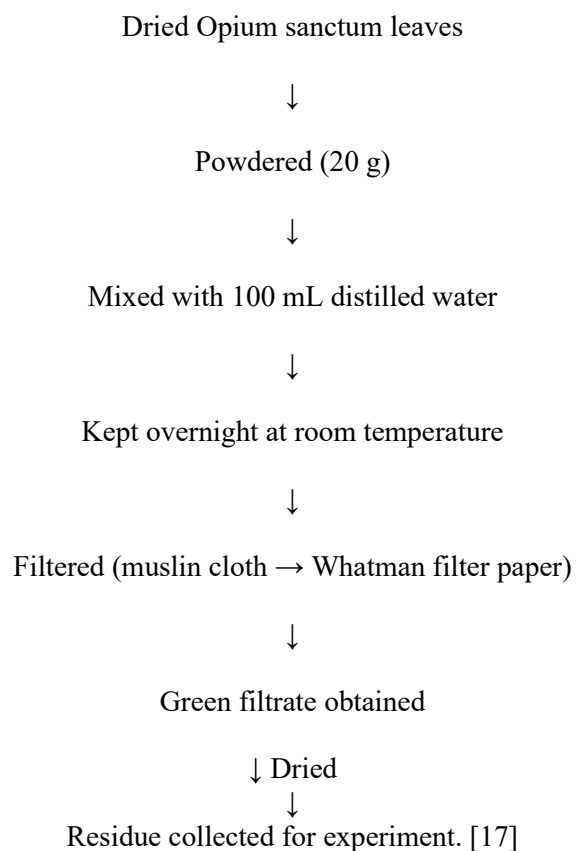


Fig No 3: Tulsi Extract

2. Guava (*Psidium guajava*)

Psidium Guajava is the plant that gave rise to guava. It is a member of the Myrtaceae family. Shape: Depending on the species, guava fruits can be round or oval in shape and range in length from 4 to 12 centimeters (1.6 to 4.7 inches) .[18]

- **Biological source:** guava is the evergreen tree *Psidium guajava* L.

- **Kingdom:** Plantae.
 - **Order:** Myrtales.
 - **Family:** Myrtaceae Genus: Psidium.
 - **Geographical Source:** America, specifically a region extending from Mexico and Central America to northern South America. Historically, it is believed to have originated in the savannas and semi-deciduous forests of South America.
 - **Species:** *Psidium guajava*. [19]
- **Tannins:** Known for their antimicrobial properties.
 - **Terpenoids:** Which can produce relaxation effects.
 - **Saponins:** Compounds with potential health benefits.
 - **Vitamins:** Including ascorbic acid (vitamin C).

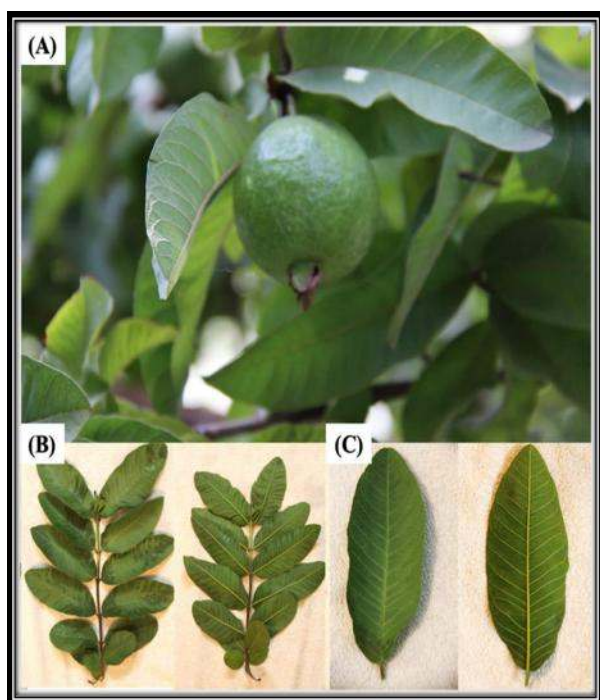


Fig No 4: Guava with leaf [21]

2.1 Chemical constituents:

- **Phenolic compounds:** These are powerful antioxidants that have health-promoting properties.
- **Flavonoids:** Such as quercetin, avicularin, apigenin, guaijaverin, kaempferol, and myricetin, which have been studied for their

anticancer, antidiabetic, and antioxidant activities.

2.2 Uses of guava leaves:

- Antibacterial properties.
- Anti-inflammatory effects, Reduces dental plaque, Relief from toothache.

2.3 Extraction process of guava leaves:

Take 25 gm of leaf powder → into a beaker
↓
Add 200 ml of sterile double distilled water → to the beaker
↓
Heat the mixture → in a water bath
↓
Reduce the volume → till it's less than 50 ml
↓
Filter the liquid
↓
By using Whatman's filter paper

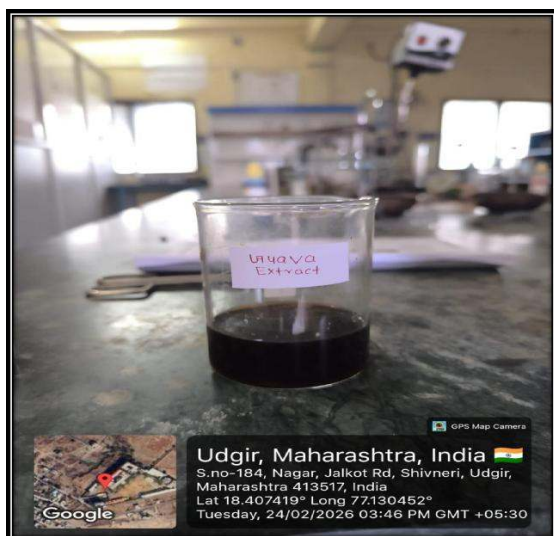


Fig No 5: Guava Extract

3. Peppermint oil:

Peppermint oil appears to be safe when taken orally (by mouth) or applied topically in the doses commonly used. Peppermint oil has been safely used in many clinical trials. Possible side effects of peppermint oil taken orally include heartburn, nausea, abdominal pain, and dry mouth. [23]

- **Biological Source:** Peppermint consists of the leaves and flowering tops of the plant *Mentha piperita* L.
- **Kingdom:** Plantae (Plants).
- **Order:** Lamiales.
- **Family:** Lamiaceae (also known as the mint family).
- **Geographical Source:** it originated in Europe and the Middle East, but the majority of the world's peppermint leaves are now harvested in India (Uttar Pradesh), the United States (Pacific Northwest), and Morocco.
- **Species:** *Mentha piperita* L.

3.1 Chemical constituents

- Essential oil including menthol, menthone, menthofuran.
- 1,8-cineole, and menthyl acetate. [24]
- 1,8-cineole, limonene.
- Beta-pinene and beta- caryophyllene.[25]

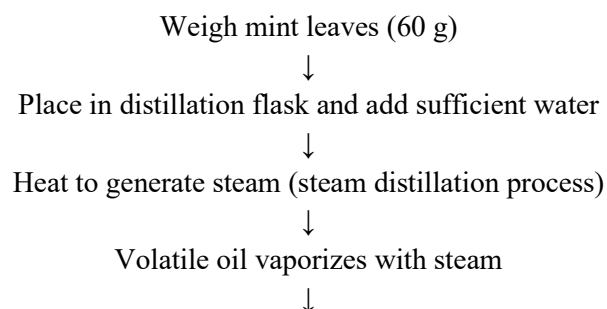
3.2 Uses of Peppermint Oil:

- Antioxidant.
- Anti-inflammatory.
- Anti-bacterial properties.[26]



Fig No 6: Peppermint leaves [27]

1.1 Extraction process of peppermint leave:



Condense vapours (oil + water) using condenser

↓
Collect distillate

↓
Separate oil using separating funnel

↓
Collect and weigh peppermint oil [28]



Fig No 7: Peppermint Extract

Methodology:

1. Maceration method / Aqueous extraction (hot)

Used for Tulsi leaves (boiling leaf powder in water and filtering).

2. Hot Aqueous Extraction

Used for Guava leaves (boiling leaf powder in water and filtering).

3. Steam distillation method

Used for Peppermint oil extraction.

4. Simple mixing / Solution Method

Used for final formulation of herbal mouthwash (mixing extracts, excipients, preservative, & vehicle).

Formulation Of Herbal Mouthwash:

Table No 3: Formulation of herbal mouthwash

Sr.no.	Ingredients	Formulation (50 ml)	Uses
1.	Guava leaves extract	25 ml	Antibacterial, antioxidants, anti- inflammatory Agents.
2.	Tulsi leaves extract	5ml	Antimicrobial, anti- oxidant, anti- Inflammatory.
3	Peppermint extract (oil)	1 ml	Flavoring agent Reducing pain, fresh breath.
4.	Propylene glycol	7.5 ml	Thickener, Sweetener.
5.	Sodium benzoate	0.1 gm	Preservative.
6.	Tween -80	0.5 ml	Surfactant.
7.	Glycerol	5.96 ml	Humectant.
8.	Ethanol	1 ml	Co-solvent.
9.	Water	q.s	Vehicle.

METHODS OF PREPARATION:

Wash all laboratory apparatus and accurately weigh all ingredients



Collect fresh guava leaves



Dry guava leaves for 10 days at room temperature



Grind dried leaves to obtain coarse powder



Store powder in airtight container

(cool, dry place; away from sunlight)



Boil guava leaf powder at 90 °C for 15–20 minutes & Filter extract using filter paper



Beaker I: Add ethanol and peppermint oil and mix



Beaker II: Add glycerol, propylene glycol, Tulsi extract, tween-80, warm water, and guava extract; dilute



Add sodium benzoate & Add FDA-approved green colorant



Make up final volume with purified water



Transfer prepared mouthwash into tightly closed container. [29]

Evaluation Test Of Herbal Mouthwash:

- **Physical parameters:** such as color and odor, were assessed visually.
- **PH:** A digital pH meter was used to measure the prepared herbal mouthwash's . A standard buffer solution was used to calibrate the pH meter. One milliliter of mouthwash was weighed, dissolved in fifty milliliters of purified water, and its pH was then measured. [30]
- **Determination of Antibacterial Property of Mouthwash:**
 1. Agar media was prepared then the formulated mouthwash was inoculated on the plates agar media by steak plate method and controlled is prepared by mouthwash. The plates were placed in the incubator and are incubated 37°C for 24 hours. After the incubation period the plates were taken out and the Microbial growth were checked and compared with the control.
 2. The antibacterial activities were evaluated by measuring the zones of inhibition (in mm).[31]
- **Viscosity:** A Ostwald viscometer was used to measure the mouthwash's viscosity .The table provides a description of the viscosity results for each created formulation. [32]



- **Foam test:** The product's ability to foam was assessed by adding a small amount of water to a measuring cylinder, noting the starting volume, then shaking the cylinder 10 times. The final foam volume was recorded. [33]

Table No 5: pH of Herbal Mouthwash

Day of measurements	pH range of sample	Ph range of standard
0 to 15day	5-41	5-6
15 to 30day	5-41	5-6

Result And Discussion:

1. Physical Parameter:

Table No 4: Physical parameter of Herbal Mouthwash

Parameters	Formulation
Color	Dark brown
Taste	Slightly bitter
Odour	Minty



Fig No 8: pH Analysis of Mouthwash

2. pH Stability analysis:.

3. Antibacterial activity:

Table No 6: Antibacterial Activity of Mouthwash

Storage of sample	temperature	Storage life (log cfu/ml) – 0 th
Ambient (normal room temp.)		No growth
Refrigerated	No growth	No growth



(A) Before keeping in incubator

(B) After incubator

Fig no 9: Microbial Activity

1. Viscosity:

Table No 7: Viscosity of Herbal Mouthwash

Formulations	Viscosity (in Centipoise)
Herbal mouthwash	1.690
Standard	2.352



Fig No 10: Viscosity of Herbal Mouthwash by Ostwald viscometer

6. Foam test: Final volume of foam of formulation was noted to around 1 ml.

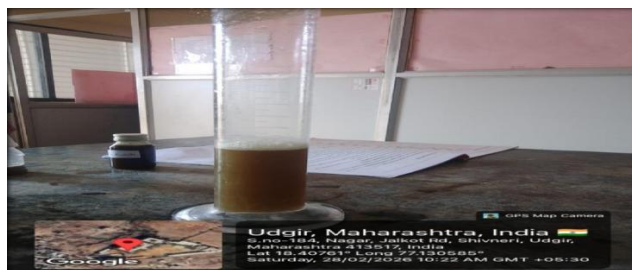


Fig No 11: Foam test of herbal Mouthwash

CONCLUSION:

Many dental diseases, including foul breath, can be effectively treated with the current liquid herbal mouthwash. Furthermore, the absence of any hazardous substances in this preparation gives us peace of mind. The findings of the physicochemical examination verify that the current herbal formulation's color and smell are acceptable, with a pleasant scent and improved

aftereffects. The patients favored this herbal mouthwash due to its taste, ease of use, and test length in their mouths after rinsing, and the zone of inhibition results also revealed that it was a powerful plaque inhibitor. As a result, they can be utilized to treat plaque-induced gingivitis in addition to mechanical therapy. The current study has a significant impact on the development of an affordable and successful herbal oral health intervention for low socioeconomic communities. Herbal mouthwashes are an excellent natural option for long-term oral health management since they provide substantial advantages for preventing common dental issues with fewer side effects. They are a useful, scientifically supported supplement to everyday oral care.

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