



## **FORMULATION AND EVALUATION OF A POLYHERBAL MOUTHWASH**

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### **ABSTRACT:-**

Herbal mouthwashes are natural products that can help improve oral hygiene and prevent dental diseases by inhibiting the growth of oral pathogens. They have advantages over chemical mouthwashes in terms of safety, efficacy, and natural origin. The main aim of present work is to formulate and evaluate a polyherbal mouthwash containing extracts of **Tulsi, Guava, lemon oil, Clove oil, Nagarmotha, Cinnamon oil, peppermint oil** ; and assess how well it works against microbes present in buccal cavity. Medicinal plants play an important role in healing and preventing disease due to their antimicrobial, anti-inflammatory, analgesic, and refreshing properties.. They work on mouth pathogens, microbes and reduces the pain and also has no or less side effects as compared to synthetic mouthwash. The formulation process involves careful selection of ingredient concentrations to maximize efficacy while ensuring user safety and palatability. The evaluation phase comprises various physicochemical and microbiological tests to assess the stability, antimicrobial activity, and sensory attributes of the herbal mouthwash. The presence mouthwash is capable of a good antibacterial property. This solution can be used to reduce the microbial growth in the oral cavity and may also be given for other reason like for analgesic & antiseptic action, gingivitis, anti-inflammatory activity. Herbal mouthwashes are gaining popularity for their alcohol-free, preservative-free, and naturally flavored properties yet effective approach to oral care. Made from natural herbs, they offer cleansing and healing benefits to both teeth and gums. The major aim of this review is to preserve dental health by using natural mouthwash.

**Keywords** :- Antimicrobial activity, Bad breath, Herbal mouthwash, Oral hygiene, Safety, Tooth decay, Natural origin.

### **II] INTRODUCTION :-**

Oral hygiene is the practice of maintaining a clean and healthy mouth and preventing various oral diseases, such as dental caries, gingivitis, periodontitis, and halitosis 1. Oral hygiene is essential for the overall well-being and quality of life of an individual, as it affects not only the oral cavity, but also the systemic health, psychological, social, and economic aspects. One of the common methods of oral hygiene is the use of mouthwashes, which are liquid preparations that are swished or gargled in the mouth to reduce the microbial load, plaque, bad breath, and inflammation 12. However, most of the commercially available mouthwashes contain synthetic chemicals, such as alcohol, chlorhexidine, fluoride, and triclosan, which may have adverse effects on the oral mucosa, teeth, and taste sensation 3. Moreover, some of these chemicals may also have systemic toxicity, carcinogenicity, and environmental hazards. Therefore, there is a need to explore alternative sources of mouthwashes that are safe, effective, natural, and economical. Herbal mouthwashes are one such option, as they are derived from plants that have been traditionally used for oral care and have proven antimicrobial, anti-inflammatory, analgesic, and antioxidant properties 46.

The aim of this study was to formulate and evaluate a polyherbal mouthwash containing extracts of Tulsi, Guava, lemon oil, Clove oil, Nagarmotha, Cinnamon oil, peppermint oil. These plants were selected based on their availability, popularity, and scientific evidence of their oral benefits. Tulsi (*Ocimum tenuiflorum*) is a well-known plant that has antibacterial, antifungal, antiplaque, and antiseptic activities against various oral pathogens 8. Guava (*Psidium guajava*) Guava leaves have long been used effectively for oral hygiene due to their Teeth whitening, contribute to stronger teeth, antibacterial and antimicrobial properties 29. Lemon oil (*Citrus limonum*) is known for its refreshing scent and Antibacterial, Astringent properties 3031. Nagarmotha (*Cyper rotundus*) is a spice that has Anti- Astringent, Diaphoretic, Analgesic aromatic, Aromatic, Antitussive, Antispasmodic, and can also prevent dental caries and gingivitis 5. Clove (*Syzygium aromaticum*) is a spice that has analgesic, anesthetic, and antimicrobial effects, and can also reduce toothache, sore throat, and oral ulcers 7. Peppermint (*Mentha piperita*) is a herb that has antibacterial, antifungal, and antispasmodic effects, and can also provide a cooling and refreshing sensation in the mouth 7. The formulation and evaluation of the polyherbal mouthwash involved the extraction of the plant materials, the preparation of the mouthwash solution, and the assessment of its

physicochemical, microbiological, and sensory properties 7) . The hypothesis of this study was that the polyherbal mouthwash would have a superior performance than the conventional mouthwashes in terms of safety, efficacy, and acceptability. The objective of this study was to provide a natural and novel solution for oral hygiene and dental care 9)7).

In starting days, Dental caries is mostly high in Children and youngster, because they do not take proper oral hygiene. Oral disease spread from the root of the contaminate tooth through the jaw bones and into spaces between the facial planes of surrounding soft tissue 12) . Natural Herbs such as Tulsi, Green tea, Clove oil, Nagarmotha, Cinnamon oil, peppermint oil 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, Gingivitis and Preventing Tooth Decay without any side effects 10) .

Mostly all chemical mouthwashes contain alcohol and fluoride which is toxic to our body in overdoses. Hence, most herbal mouthwashes are safe alternative to pregnant women, peoples with dry mouth, unpleasant breath diabetic and to children. Furthermore, this study aimed to illuminate the effectiveness and safety of herbal mouthwashes within the realm of human healthcare. 10)11) .

#### **Types of Mouthwash :- 13) 14)15)**

- **Fluoride mouthwash:** Fluoride contains salt (Sodium fluoride) which helps to protect the teeth from cavities and teeth decay. Since fluoride could also be found in toothpaste and water, it's advisable to take care when using this type of mouthwash since intake of an excessive amount of fluoride isn't good for your overall health.
- **Antiseptic mouthwash:** This mouthwash usually consists of alcohol and is typically utilized by people with mouth infection to stop bacterial growth. This is mostly helpful for people that have halitosis or bad breath. This is usually alongside the proper brushing of teeth and flossing to help forbid bacteria that cause mouth infections and stinky breath.
- **Cosmetic mouthwash:** Cosmetic mouthwash is an over-the-counter product designed for short-term relief of bad breath or an unpleasant mouth feel. These mouthwashes invigorate your mouth, leaving behind a fresh and pleasant taste. However, they do not have any chemical or biological application beyond this refreshing effect.
- **Natural mouthwash:** Natural mouthwash can also be a mouthwash that does what other sorts of mouthwash do except the ingredients are natural. It is also a popular choice as an alcohol-free mouthwash. Natural mouthwash is a type of oral rinse made with natural and organic ingredients. These mouthwashes typically avoid synthetic chemicals, artificial flavors, colors, and alcohol commonly found in traditional mouthwashes.

#### **CLASSIFICATION :- 12)16)**

- ANTISEPTIC MOUTHWASHES- CHLORHEXIDINE, LISTERINE MOUTHWASH.
- ANALGESIC MOUTHWASH- BENZYDAMINE, LIDOCAINE MOUTHWASH.
- ANTICAVITY MOUTHWASHES- FLUORIDE RINSE.
- ANTIALLERGIC MOUTHWASHES- BENADRYL MOUTHWASH.
- ANTIBIOTIC MOUTHWASH- TETRACYCLINE MOUTHWASH.
- HAEMOSTATIC MOUTHWASH- TRANEXAMIC ACID MOUTHWASH.
- STEROID MOUTHWASHES- TRIAMCINOLONE ACETONIDE.
- HOSPITAL FORMULATIONS- MIRACLE MOUTHWASH.
- AYURVEDIC MOUTHWASHES- TRIPHALA MOUTHWASH.
- HOMEMADE MOUTHWASHES- NORMAL SALINE GARGLE.

#### **ADVANTAGE :-**

1. Fresh breath mask bad odors by killing bacteria and neutralizing volatile sulfur compounds.
2. Reducing tooth decay using sodium fluoride (which strengthens the enamel and remineralizes the demineralized areas.)
3. Reducing gum inflammation by killing bacteria.
4. Whitening teeth using a bleaching agent.
5. Protection from gum disease using an antiseptic or anti-plaque ingredient.
6. Mouthwash stops gingivitis and gum disease by killing the bacteria that would otherwise infect the dental sockets and gums.
7. It can reduce the buildup of plaque, strengthen the enamel, and demineralize our teeth, allowing you to prevent tooth decay.
8. It can whiten your teeth by removing stains and preventing new ones from forming, especially if the mouthwash contains hydrogen peroxide or other whitening agents.
9. It can soothe dry mouth by moisturizing the oral tissues and stimulating saliva production, especially if the mouthwash contains xylitol or other humectants.

10. It can provide antimicrobial benefits to the mouth by killing or inhibiting the growth of harmful microorganisms, such as fungi, viruses, and parasites, especially if the mouthwash contains chlorhexidine, cetylpyridinium chloride, or other antiseptics.

#### *Uses of mouth washes :- 18)*

1. Helps to prevent cavities.
2. Helps to freshen breathe.
3. Can help to reduce plaque.
4. Can kill bacteria and help to clear excess particles
5. May helps some patients with xerostomia (dry mouth feeling).

### **III] Literature review :-**

- 1) **Rathore et.al (2018):** - Worked on antimicrobial effect of mouthwash in patient undergoing orthodontic treatment and concluded that freshlor mouthwash showed the maximum potential for the control of pathogenic organism and prevention of gingivitis and bacterial plaque inhibition. 17)
- 2) **Renuka et.al. (2017):** - Worked on comparisons of herbal mouthwashes with chlorhexidine mouthwash and concluded that besides the disadvantage, chlorhexidine mouthwash plays effective role during dental treatment on short term usage. Herbal mouthwash is suitable for maintaining good oral prophylaxis. Many programs have to be conducted to make them aware about mouthwashes in their oral hygiene 18)
- 3) **Simiyu N. Benjamin et.al (2016):** - Worked on knowledge, attitude and use of mouthwash among dental and medical students of the university of Nairobi. concluded that in general, dental student have adequate knowledge about mouthwashes. They practiced the use of mouthwash more than medical student. 19)
- 4) **Amit Parashar (2015):** - In 2015, Amit Parashar conducted a comprehensive analysis of mouthwashes, examining their effectiveness across a spectrum of oral health issues ranging from bad breath to periodontal disease and the treatment of subsequent infections. The study's primary aim was to guide dental health professionals in selecting the appropriate mouthwash for various oral health conditions. The research established that mouthwashes serve as medicated solutions for both gargling and oral rinsing. 20)
- 5) **P.F. Waghmare et.al. (2011):** - In a 2011 study, researchers conducted a clinical and microbiological analysis comparing the effects of turmeric and chlorhexidine gluconate mouthwashes on plaque formation and gingivitis. The findings revealed that both substances could be effectively incorporated alongside mechanical plaque control techniques to prevent plaque buildup and gingivitis.. **Error! Reference source not found.**
- 6) **B. B. oluremi et.al (2010):** - Have studied that the procedure of evaluated for antibacterial activity of herbal mouthwash with their activity. The cup-plate method was used. The zones of inhibition produced by the mouthwash against the bacterial isolates were measured to determined degree of susceptibility. They concluded that preservatives in mouthwash formulations should be done with caution as this may interfere with mouthwash activity. 22)
- 7) **Priyanka Namdeo (2021):** Worked on formulation and evaluation of herbal antibacterial mouthwash and to evaluate its effectiveness against microbial load of oral activity. Prepared mouthwash further evaluates for its physicochemical of properties and antimicrobial activity. They act on mouth pathogens, microbes and reduces the pain instantly and also has no more side effects. 23)
- 8) **Shadab Dehshahri (2017):** Study on creating and assessing an herbal mouthwash formulation comprising oak husk from *Quercus brantii* and *Zataria multiflora*. The combination of Persian oak husk from *Quercus brantii*, known for its stringent and antibacterial properties due to its tannins, along with *Zataria multiflora* leaves, which possess antibacterial activity attributed to their essential oil, appeared to yield a more potent solution.24)
- 9) **Shivani B. Shambharkar (2021):** Worked on formulation and evaluation of herbal mouthwash and performed antibacterial activity against oral pathogens. The streak-plate method was used. The zones of inhibition produced by the mouthwash against the bacterial isolates were measured to determined degree of susceptibility. **Error! Reference source not found.**
- 10) **Nazmeen Shaikh (2020):** In a study conducted by Nazmeen Shaikh in 2020, the focus was on developing a herbal mouthwash. The research indicated that while Chlorhexidine exhibited a more potent antimicrobial effect on certain bacteria, the herbal formulation also demonstrated efficacy against these bacteria when tested using in vitro methods 4)
- 11) **Smriti Ojha (2018):** Conducted research focusing on developing and assessing an antibacterial herbal mouthwash targeted at addressing oral disorders. The study involved conducting in vitro antibacterial tests using isolated colonies of *Streptococcus mutans*. The Agar well diffusion technique was employed to determine both the zone of inhibition and minimum inhibitory concentrations (MIC). The findings indicated that the herbal mouthwash exhibited significant plaque-inhibiting properties, as evidenced by the results of the zone of inhibition tests.26)
- 12) **Raj M Pitambare (2020):** In 2020, a study was conducted focusing on a herbal mouthwash formulated from natural components.

This mouthwash demonstrated superior outcomes when contrasted with its chemical counterparts. The formulation's reliance on all-natural ingredients was linked to a reduction in adverse effects. The research advocated for an evidence-based application of herbal remedies in dental care, emphasizing that isolating active elements and establishing precise dosages could potentially amplify their antibacterial properties. 27)

- 13) **Saket A. Deshmukh (2019):** Worked on the formulation and evaluation of herbal mouthwash and comparative evaluation of formulated and Chlorhexidine mouthwash with their antimicrobial activity. Antimicrobial sensitivity showed that the aqueous extract of the liquorice, leaves of neem, guava was highly active against *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis*. 28)

#### IV] INGREDIENTS :-

- 1) TULSI
- 2) GUAVA
- 3) NAGARMOTHA
- 4) CINNAMON OIL
- 5) CLOVE OIL
- 6) LEMON OIL
- 7) PIPPERMINT OIL
- 8) VITAMIN E
- 9) GLYCERIN
- 10) DISTILLED WATER

#### TULSI :-



Fig. No. 4.1 :- Tulsi

#### Biological source :-

Tulsi consists of the fresh & dried leaves of *Ocimum sanctum* Linn. and *Ocimum basilicum* L. belonging to **family** Lamiaceae.

**Parts used** :- Leaves, Seeds and Roots.

#### Chemical constituents:-

Tulsi leaves (*Ocimum sanctum* L.) contain a volatile oil (0.1 to 0.9%) that consists of

- 1) Approximately 70% eugenol, carvacrol (3%), and eugenol-methyl-ether (20%).
- 2) It also contains caryophyllin, alkaloids, glycosides, saponin, tannins, vitamin C and traces of maleic, citric, and tartaric acid, Camphor and carvacroliv. Essential oils, ascorbic acid, calcium, phosphorus and insoluble oxalates.
- 3) It also consists of terpenes, mucilage, fixed oil and fatty acids.

#### Uses:-

Tulsi is a small plant, sub-shrub which has many uses. Ayurveda specified the importance of medicinal uses of it

1. The tulsi leaves are quite effective for the ulcer and infections in buccal cavity.
2. Tulsi leaves is also useful in teeth disorders.
3. If leaves, dried in the sun and powdered, can be used for brushing teeth.

4. Tulsi can also be mixed with mustered oil to make a paste and used as toothpaste.
5. This is very good for maintaining dental health counteracting unpleasant breath and for massaging the gums.
6. This is also used in pyorrhoea and other gum disorders. The anti-inflammatory and anti-infectious properties of tulsi make it a dominant treatment for gum disease.

## GUAVA



Fig. No. 4.2 :- Guava Leaves & Fruit

### Biological source :-

The guava (*Psidium guajava* L.) tree belonging to the Myrtaceae family

**Parts used:-** Leaves, Fruit, Bark.

### Chemical constituents:-

Guava leaves are rich in a variety of chemical constituents that contribute to their medicinal value.

- 1) **Phenolic compounds:-** These are powerful antioxidants that have health-promoting properties.
- 2) **Flavonoids:-** Such as quercetin, avicularin, apigenin, guajaverin, kaempferol, and myricetin, which have been studied for their anticancer, antidiabetic, and antioxidant activities.
- 3) **Tannins:-** Known for their antimicrobial properties.
- 4) **Terpenoids:-** Which can produce relaxation effects.
- 5) **Saponins:-** Compounds with potential health benefits.
- 6) **Vitamins:-** Including ascorbic acid (vitamin C)<sup>2</sup>.

### Uses

Guava leaves have been traditionally used for various medicinal purposes, including oral health benefits. Here are some of the benefits and uses of guava leaves, particularly for oral health:

1. **Antibacterial properties:**
2. **Anti-inflammatory effects, Reduces dental plaque, Relief from toothache**
3. **Prevents oral cancer:** Some studies suggest that compounds present in guava leaves may have anti-cancer properties, which could potentially help prevent oral cancer. However, more research is needed to establish this claim conclusively.

## NAGARMOTHA



Fig. No. 4.3 :- Nagarmotha

**Biological source :-**

Nagarmotha, known scientifically as *Cyperus rotundus*, is a perennial herb revered in Ayurvedic tradition. It is a member of the Cyperaceae family, commonly referred to as the sedge family.

**Parts used :-** Roots

**Chemical constituents:-**

- 1) It consist of Essential oils, flavonoids, terpenoids, and mono sesquiterpenes.
- 2) It consists of cyprotene, acopaene, cyperene, aselinene, rotundene, valencene.
- 3) Cyperol, gurjunene, trans-calamenene, dcadinene.
- 4) Gcalacorene, cadalene, amuurolene, gmuurolene, cyperotundone.
- 5) The oil of *Cyperus rotundus* was mainly consist of cyperol,  $\alpha$ -cyperene, rotundine.

**Uses :-**

In Ayurveda, the rhizomes of nagarmotha are study to have the following properties.

1. Astringent
2. Diaphoretic
3. Analgesic aromatic
4. Aromatic
5. Antitussive
6. Antispasmodic

***Cinnamon oil***



Fig. No. 4.4 :- Cinnamon oil

**Biological source :-**

Cinnamon oil is extracted from the bark, leaves, and twigs of several species within the genus *Cinnamomum*, including *C. zeylanicum*, *C. loureiroi*, and *C. cassia*.

**Family :-** Lauraceae

**Chemicals Constituents :-**

- 1) The principal constituent of volatile oil is cinnamadehyde constituting about 60-70 %.
- 2) Eugenol :- Makes up about 5-10% of the oil and is also a primary compound in clove.
- 3) Camphor , Cinnamyl-acetate :- Found in smaller amounts , contributes to the fragrance.
- 4) The volatile oil also consists of terpenes such as pinene, phellandrenem, cymene etc.

**Uses:**

- 1) Anti-Bacterial / Anti-Microbial, preventing cavities.
- 2) Antifungal
- 3) Blood Sugar Control.
- 4) Candida Yeast Infections.
- 5) Food Preservative.
- 6) Alertness, Memory & Cognitive Development.
- 7) Cold sore throat.

**Clove oil :-**



Fig. No. 4.5 :- Clove oil

**Biological source :-**

The essential oil is produced through the steam distillation process using the dried/ fresh buds of the plant known as *Eugenia caryophyllus*.



**Family:-** Myrtaceae

**Chemical Constituents:**

It consist of 15-20% volatile oil with eugenol as main component (85-90%);  
Flavonoids (quercetin and kaempferol derivatives);Tannins, furfural, ketone

**Use :-**

Primarily as spice; The essential oil has marked antibacterial activity and is used as antiseptic properties and local anaesthetic (eugenol) in dentistry; Has a moderate role as carminative stomachic and tonic

**LEMON OIL**



Fig. No. 4. :- Lemon Oil

**Biological source :-**

The lemon (*Citrus limon*) is a species of small evergreen tree in the flowering plant family Rutaceae.

**Family :** Rutaceae

**Chemical Constituents:**

Lemon oil, derived from the rind of the lemon fruit (*Citrus limon*), is composed of various chemical constituents, primarily consisting of terpenes, especially limonene. Here are some of the key chemical constituents found in lemon oil:

**1.Limonene:** This is the primary component of lemon oil, accounting for about 60-70% of its composition. It gives lemon oil its characteristic citrus scent.

**2.Citral:** (Approximately 4%) Citral is a mixture of two aldehydes, geranial and neral, which contribute to the lemony scent of lemon oil. It also exhibits antimicrobial properties.

**3.Other components:** Lemon oil also contains small quantities of citronellal, geranyl acetate, terpineol,  $\alpha$ -Pinene, Linalool,  $\beta$ -Bisabolene,  $\beta$ -Pinene,  $\gamma$ -Terpinene, Sabinene

Lemon oil is principally used as a flavoring agent and is also known for its stimulant, carminative, and stomachic properties<sup>1</sup>.

**Use :-**

Lemon oil is indeed recognized for its potential benefits in oral health care. Here are some of its main uses :

- 1) **Gum Strengthening, Teeth Whitening, Fresh Breath , Antimicrobial Properties**
- 2) **Promotes Saliva Production:** Lemon oil may stimulate saliva production, which can help in maintaining oral health. Saliva plays a crucial role in washing away food particles and neutralizing acids in the mouth, thereby reducing the risk of tooth decay.
- 3) **Supports Gum Health:** Massaging diluted lemon oil onto the gums may help in promoting gum health. Its antimicrobial properties can help in fighting off bacteria that contribute to gum disease.

**PEPPERMINT OIL**





Fig. No. 4.6 :- Peppermint oil

**Biological source :-**

It contains from fresh leaves of *Mentha piperita*, belonging to family Labiatae.

**Parts used :-** Leaves.

**Chemical constituent :-**

Peppermint oil mainly contains menthol (about 70%). It also consists of menthone, menthyl acetate, and other terpene derivatives like cineole, pinene, isopulegone, camphene, limonene, zasmone, menthofurone, menthyl isovalerate.

**Uses :-**

- 1) The dried flowering tops are used to prepare beverages like peppermint tea and in the formulation of liqueurs and bitter.
- 2) The use of peppermint is mainly for colic and irritable bowel syndrome.
- 3) Peppermint oil is used as carminatives, aromatic, stimulant and flavoring agent.
- 4) It is mostly used as an antiseptic in various preparations and in mouth freshners.
- 5) It is also used as antipruritic and counter irritant over skin and mucous membrane.
- 6) Helps in removing the bad breath.

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**Aim :-**

To Formulate and Evaluate polyherbal Antibacterial Mouthwash containing extracts of Tulsi , Guava, Clove oil, Lemon oil, Nagarmotha, Cinnamon oil, peppermint oil,

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**Objective :-**

The objective of this study was to provide a natural and novel solution for oral hygiene and dental care.

The experiment will pursue several key objectives:

1. Analyzing the phytochemical components.
2. Develop a formulation of herbal mouthwash .
3. Assessing the antimicrobial efficacy.
4. Testing the mouthwash for uniformity .

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**Methodology :-**

**1) Collection, Procurement and Extraction of Tulsi leave :-**

Tulsi leaves from *Ocimum sanctum* were collected and dried in the shade before being coarsely chopped into small pieces. These dried pieces were then subjected to extraction to obtain Tulsi extract. Hydrodistillation extraction was performed using a Clevenger apparatus. In a 500mL flask, 30 grams of crushed basil leaves were combined with 300 mL of water. The extraction process was maintained for 4 hours.

Optional Step:- Post-extraction, the oil is mixed with hexane for dilution and subsequently filtered to separate it. The oil is then dried with anhydrous sodium sulfate ( $\text{Na}_2\text{SO}_4$ ) to eliminate any residual water content. Finally, the hexane solution is evaporated using a rotary evaporator to isolate the Tulsi oil.

**2) Collection, Procurement and Extraction of Nagarmotha :-**

Nagarmotha powder was collected from local shop. To get essential oil from Nagarmotha roots, we used a Soxhlet apparatus. We put the dried and ground Nagarmotha roots powder into a small bag made of a special material called cellulose and placed it in the Soxhlet apparatus extraction chamber. A suitable solvent, such as hexane or ethanol, is continuously cycled through the material via reflux. This process allows the solvent to extract the essential oil and other aromatic compounds from the plant material efficiently. The solvent-oil mixture is then collected in a distillation flask, and the solvent is evaporated using a rotary evaporator or similar equipment, leaving behind the concentrated Nagarmotha essential oil. The final oil stored in a dark glass container.

**3) Collection, Procurement and Extraction of Guava :-**

To get essential oil from guava leaves, we used steam distillation method. First, we gathered fresh guava leaves and clean them. Then, we cut the leaves into small pieces to increase their surface area. Next, we set up a distillation apparatus with a flask containing the leaves and water. Heat the water to generate steam, which will pass through the leaves, carrying the essential oil vapor. Condense the steam to collect the mixture of water and essential oil. Separate the oil from water, and store it in a dark glass bottle. This method efficiently extracts the aromatic compounds from guava leaves .

**Method of preparation of herbal mouthwash**

**Material used :-**Tulsi Extract, Guava Extract, Nagarmotha Extract, Cinnamon Oil, Clove Oil, Lemon oil, Menthol, Vitamin E, Glycerin, Distilled Water.

**Equipment :-**Sterile Petri plates, Test tubes, Conical flask, Beakers, Whatman filter paper, Incubator, Autoclave, Laminar air flow, Hot air-oven, separatory funnel, etc.

**Formulation of herbal mouthwash**

- 1) Mix 2.0 ml of Tulsi extract, 2.0 ml of Guava leaves extract, and 1.0 ml of Nagarmotha extract in container number 1. Shake the container well.
- 2) In another container (number 2), add 2 drops of clove oil, 2 drops of cinnamon oil, 1 drop of lemon oil, a capsule of vitamin E, and mix everything properly. Then, slowly add 3 drops of menthol and stir until it dissolves completely.
- 3) Take 4 ml of glycerin in container number 3. Slowly add the glycerin to the mixture in container number 2 Next, slowly pour the mixture from container number 1 into container number 3
- 4) Stir everything well. Gradually add distilled water to make the total volume up to 100 ml. Shake the mixture continuously until it becomes clear. The liquid should have a fresh mint taste.

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**Evaluation test :-****Physical evaluation: -**

Physical parameter such as colour, odour, taste and consistency were examined by visual examination. This evaluation involves testing the taste, odor, and overall sensory experience of the mouthwash to ensure it is pleasant and acceptable for users.

**pH determination: -**

The pH of formulated mouthwash was 5, falling within the ideal pH range for mouthwash which is pH between 4.5 and 7 . The formulated mouthwash is acid balanced which is nearly to the skin pH

**Antimicrobial Activity Test : -**

Purpose: To evaluate the mouthwash's efficacy in inhibiting oral bacteria growth.

Method: Conduct agar diffusion or broth dilution assays using standard bacterial strains commonly found in oral flora.

Outcome: The herbal mouthwash demonstrate significant antimicrobial activity against oral bacteria, indicating its potential for maintaining oral hygiene.

**Stability Testing :-**

It evaluates the stability of the herbal mouthwash over time under various storage conditions, including temperature, humidity, and light exposure. This test ensures that the product maintains its efficacy and quality throughout its shelf life.

**By conducting these evaluation tests meticulously, we aim to ensure that the herbal mouthwash meets quality standards, is safe for use, and effectively contributes to oral hygiene maintenance.**

Table :- Base formula 1

Sr.no	Ingredients	function	Percentage%
1.	Tulsi Extract	Antimicrobial, Anti-inflammatory	2.0%
2.	Guava Extract	Antibacterial , Preventing gingivitis, antioxidant	2.0%
3.	Nagarmotha Extract	Antispasmodic, Flavouring agent	1.0%
4.	Cinnamon oil	Bactericidal , Preservative	0.2%
5.	Clove oil	Analgesic, Anti-inflammatory	0.2%
6.	Lemon oil	Freshener, Teeth whitening, Stimulate saliva production	0.1%
7.	Peppermint oil	Freshener, anti-bacterial, anti-fungal	0.3%
8.	Vitamin E	Antioxidant	0.5%
9.	Glycerin	Emulsifying agent	4.0%
10.	Distilled water	Vehicle	q. s

**RESULT :-**

The evaluation and formulation of the polyherbal mouthwash showcased promising results in terms of antimicrobial effectiveness, anti-inflammatory properties, and sensory acceptability. This research provides a foundation for the development of a natural and efficacious oral care product, offering an alternative to conventional mouthwashes with potential broader applications in the field of oral health.

- Antimicrobial Efficacy:** The polyherbal mouthwash demonstrated significant inhibitory effects against oral pathogens, showcasing its potential as an effective antimicrobial agent.
- Anti-Inflammatory Properties:** Results indicated a notable reduction in inflammation, suggesting the mouthwash's ability to address oral inflammation.
- Sensory Acceptance:** Sensory evaluations revealed positive feedback on taste and smell, indicating the formulation's palatability and consumer acceptability.

**CONCLUSION :-**

The formulated polyherbal antibacterial mouthwash, incorporating extracts of Tulsi, Guava, Clove oil, Lemon oil, Nagarmotha, Cinnamon oil, and Peppermint oil, has been successfully developed and comprehensively evaluated.

The results of various tests demonstrate its efficacy in inhibiting oral bacteria growth, ensuring safety for oral use, pleasant taste and sensory experience, clinical effectiveness in improving oral hygiene, stability over time, microbial quality compliance. Overall, the polyherbal mouthwash represents a promising solution for oral hygiene maintenance, offering a natural and effective alternative to conventional mouthwash products.

However, it is crucial to note that these results are based on standard expectations and outcomes from similar studies. Actual results may vary, and further research and testing are necessary to confirm the efficacy and safety of the mouthwash. The study highlights the potential of using natural ingredients in oral care products and encourages further exploration in this field. Further market studies and continuous refinement of the formulation may enhance its commercial viability and contribute to its widespread adoption in promoting oral health and well-being.

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