



# FORMULATION AND EVALUATION OF HERBAL MOUTHWASH

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**Abstract:** Medicinal plants play a predominant role in curing and preventing disease due to their antibacterial and antimicrobial activity against Human microorganism. The aim of present work is to formulate and evaluate antibacterial mouthwash and evaluate its effectiveness against microbes present in oral cavity. They act on mouth pathogens, microbes and reduces the pain and also has no more side effects. The various herbs and their extracts such as Tulsi, Green tea, Nagarmotha were selected for mouthwash and prepared formulation was further screened for antimicrobial activity against culture and further evaluated for physical properties. The presence mouthwash possesses a good antibacterial property. This solution can be used to reduce the microbial growth in the oral and may also be given for other reason like for analgesic action, gingivitis, anti-inflammatory activity.

**Index Terms - Herbal Mouthwash, Anti-microbial, *ocimum sanctum*, *camellia sinesis*, *cyperus rotundus*.**

## I. INTRODUCTION

Across the world, oral health is becoming a major concern. The world oral health report, 2003, highlighted oral health as an integral and essential component of general health<sup>[1]</sup>. Most of the chemical products contain an antiseptic that plays an important role in controlling plaque accumulation. The vehicles for delivery of chemical agents with antiplaque action are toothpaste, mouth washes, spray, irrigators, chewing gums and varnishes. However, mostly accepted method of delivering the antimicrobial agents after toothpaste is mouth wash. Mouth wash are an antiseptic solution which is used to reduce the microbial load in the oral cavity.

The mouth washes are concentrated aqueous anti-bacterial solutions that are used against oral microbes to counter oral infection, cleansing, to get rid of bad breath refreshing, anti-septic. The mouthwash plays a prominent role in the oral hygiene of an individual; it helps to relieve symptoms of inflamed gums gingivitis. And also it reliably used to destruct the pathogenic germs. The mouth washes are used by most of the dental patients to overcome sour mouth, ulcerated throat and sensitive teeth<sup>[2,3,4]</sup>. Dentists always use mouthwash as an antimicrobial agent before oral surgery of the patients, because they help to sterilize the surface of the inflamed gums and teeth, thereby the contamination of any other microorganisms can be avoided. Using a mouthwash that contain fluoride can help prevent tooth decay, but don't use mouthwash (even a fluoride one) straight after brushing your teeth or it'll wash away the concentrated fluoride in the toothpaste left on your teeth. Choose a different time to use mouthwash, such as after.

Using mouthwash is more effective than brushing alone. Although brushing your teeth is effective against plaque, adding mouthwash to the mix can be more effective that just brushing alone. Using mouthwash is effective because it help kill plaque and bacteria on all surfaces of your mouth and in between your teeth.

Mouth washes are liquids which contains anti-inflammatory, anti-microbial and analgesic action<sup>[5]</sup>. There are two types of mouthwashes- chemical and herbal mouthwash

Mouthwash is an aqueous solution which is most often used for its deodorant, refreshing and antiseptic properties or for control of plaque. It may contain alcohol, glycerine, synthetic sweetness, surface active agents, flavouring agents, colouring agents, etc. This can vary from breath fresheners to treatment of life threatening secondary infections such as oral mucositis<sup>[6,7,8]</sup> in patients undergoing bone marrow transplant

therapy. The use of mouthwashes requires a correct diagnosis of the oral condition and a thorough knowledge of the product to achieve effective treatment.

Major side effects for some marketed brand are: Skin irritation, Allergic Skin Reaction, Redness of skin, Acneiform eruptions, Thyroid imbalances, Tooth/tongue staining, Increased tartar, Mouth/throat irritation, Dry mouth, Unusual or unpleasant taste in your mouth, Decreased taste sensation, Tongue swelling, Gingivitis<sup>[8]</sup>. Almost all chemical mouthwashes contain alcohol and fluoride which is toxic to our body in overdoses.

Herbal mouthwashes are in excessive demand, because they act on oral pathogens and relieve the pain instantly and are also less side-effective<sup>[9,10]</sup>. Chemical mouthwashes have hydrogen peroxide a chlorine dioxide, and cetylpyridinium chloride, as an immediate whitener, sterilizer and pain reliever of teeth, but they tend to produce discoloration of teeth and may produce side effect, meanwhile they are cost effective.

Herbal products have helped to control dental plaque and gingivitis; they have been used for a short time and only as an adjunct to other oral hygiene measures such as brushing and flossing. Herbal mouthwashes have shown significant advantages over the chemical ones. Natural mouthwashes may offer significant advantages over the chemical ones. If such mouthwashes can be formulated which can be easily prepared and used safely by people at home using natural products, it may lead to improvement in the general dental health of the population. In starting days, Dental caries are high in Children and Adolescents, because they do not take proper oral hygiene. Oral infections spread from the root of the contaminate tooth through the jaw bones and into spaces between the facial planes of surrounding soft tissue.

Many herbal mouthwashes contain herbs with anti-microbial property such as neem, yavanisatva, nagavali, gandhapurataila, pilu, bibhitaka, ocimum, Echinacea, chameli leaves, etc.<sup>[10]</sup> 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, peppermint which gives cooling effect to the mouth. Natural Herbs such as Tulsi, Green tea, Clove oil, Nagarmotha, Cinnamon oil and many others<sup>[2,10,11]</sup> 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 side effects.

Hence, most herbal mouthwashes are safe alternative to pregnant women, peoples with dry mouth, diabetic and to children. The purpose of this study was to determine the prevalence of mouthwash use and not only the type of mouthwash but quantity of mouthwash to be taken for use is also important and also this study was performed to evaluate the efficacy and safety of herbal mouthwash for human medicines.

The objective to prepare Anti-bacterial Herbal Mouthwash from the alcoholic extract of *ocimum sanctum* (Tulsi) and aqueous extracts of 2 different powdered drugs namely *camellia sinensis* (Green tea), *cyperus rotundus*(Nagarmotha) that acts against the oral pathogens and to check the Anti-microbial activity by using Paper Disc method and comparing it with the Chlorhexidine Mouthwash I.P<sup>[12]</sup>.

## II. Literature Survey

### 1. 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 evaluate 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.

### 2. Shadab Dehshahri (2017):

Preparation and Evaluation of a Herbal Mouthwash containing Oak Husk of *Quercus brantii* and *Zataria multiflora*. Combination of Persian oak husk of *Quercus brantii* (Jaft) with a stringent and antibacterial properties of its tannins and *Zataria multiflora* leaves with anti-bacterial activity related to its essential oil seems to be more effective.

### 3. 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.

### 4. Nazmeen Shaikh (2020):

Worked on formulation of herbal mouthwash. In this study, Chlorhexidine showed higher levels of antimicrobial action against the selected bacterial species. However, the herbal mouthwash too was effective in these bacterial species in vitro method.

### 5. Yenny Lisbet Siahaan (2021):

Worked on the formulation of herbal mouthwash using bangun-bangun leaves (*Coleus amboinicus* Lour.) to prevent the dental plaque occurrence on the students of III A and III B classes in private Madrasah

Ibtidaiyah Annur Medan, North Sumatera, obtaining that 80% bangun-bangun leaves solution is the best treatment with OHI-S value of 0.76 (good criteria).

#### 6. Smriti Ojha (2018):

Worked on formulation and evaluation of antibacterial herbal mouthwash against oral disorders. In vitro antibacterial activity was performed on isolated colonies of Streptococcus mutans. The Agar well diffusion technique was used for determining the zone of inhibition and minimum inhibitory concentrations (MIC). The results of zone of inhibition also confirmed that this herbal mouth rinses was found to be a potent plaque inhibitor.

#### 7. Raj M Pitambare (2020):

Worked on the herbal mouthwash made up of herbal ingredient which gives the best result as compared to another chemical mouthwash. Use of all-natural ingredients so they can cause less harmful effects. It was cause less side effects. The use of herbs in dentistry should be based on evidence of effectiveness and safety. The anti-bacterial activities could be enhanced if active components are purified and adequate dosage determined for proper administration.

#### 8. 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.

### III. Drug Profile

#### 1) TULSI



Fig. 1 Tulsi

Taxonomical Classification	
<b>Kingdom</b>	Plantae
<b>Division</b>	Magnoliophyta
<b>Class</b>	Magnoliopsida
<b>Order</b>	Lamiales
<b>Family</b>	Labiatae
<b>Genus</b>	<i>Ocimum</i>
<b>Species</b>	<i>O. Tenuiflorum</i>
<b>Scientific Name</b>	<i>ocimum sanctum</i>

**Biological source**

Tulsi consist of the fresh & dried leaves of *ocimum sanctum L.* and *Ocimum basilicum L.* belonging to family Labiatae.

**Parts used**

Leaves, Seeds and Roots.

**Chemical constituents**

Volatile Oil-0.8%

- i. Eugenol, nerol, eugenol methyl ether.
- ii. Caryophyllene, terpinene-4-ol-decylaldehyde
- iii. Camphor and carvacrol
- iv. Essential oils, ascorbic acid, carotene, calcium, phosphorus and insoluble oxalates.
- v. It also contains terpenes, mucilage, fixed oil and fatty acids.

**Uses**

Tulsi is a small plant, sub-shrub which has multiple uses. ayurveda mentions the importance of medicinal uses of it.

1. The leaves are quite effective for the ulcer and infections in the mouth. A few leaves chewed will cure these conditions.
2. The herb is useful in teeth disorders.
3. Its leaves, dried in the sun and powdered, can be used for brushing teeth.
4. It 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 bad breath and for massaging the gums.
6. It is also used in pyorrhoea and other gum, disorders.
7. The anti-inflammatory and anti-infectious properties of tulsi make it a powerful treatment for gum disease

**2) Green Tea**

**Fig. 2 Green Tea**

Taxonomical Classification	
<b>Kingdom</b>	Plantae
<b>Division</b>	Magnoliophyta
<b>Class</b>	Magnoliopsida
<b>Order</b>	Theales
<b>Family</b>	Theaceae
<b>Genus</b>	Camellia L.
<b>Species</b>	Camellia Sinensis
<b>Scientific Name</b>	<i>Camellia Sinensis</i>

### Biological source

The green tea consists of leaves and leaf buds of *Camellia Sinensis* belonging to the Theaceae family.

### Parts used

Leaves, leaf buds

### Chemical constituents

The leaves of tea consist of these which is an enzymatic mixture containing an oxidase, which partly converts the phlobatannin into phlobaphene, as chemical constituent.

- i. Tannins, caffeine. (1-5% of tannin and 10-24% of caffeine.)
- ii. Theobromine is also present in small amount.
- iii. Theophylline and volatile oil.
- iv. Alkaloid content present in green tea leaves

### Uses

Green tea has multiple uses ayurveda mentions the importance of medicinal uses of it.

- i. Drinking green tea have beneficial effects on body.
- ii. It is non-alcoholic beverages.
- iii. It has strong antioxidant property due to presence of caffeine in addition with polyphenols.
- iv. It also has free radical scavenging properties.
- v. It helps in the inhibition of angiogenesis i.e. the process involving the growth of blood vessel essential for tumour growth and metastasis.
- vi. It is used to treat genetic haemochromatosis via inhibition of absorption of iron by tannates and other ligands.
- vii. It helps to treat blindness caused due to diabetes which is an angiogenic condition.
- viii. It helps to lower the risk of ischemic heart disease in older man.

### 3) Nagarmotha



Fig. 3 Nagarmotha Powder

Taxonomical Classification	
<b>Kingdom</b>	Plantae
<b>Division</b>	Magnoliophyta
<b>Class</b>	Liliopsida
<b>Order</b>	Poales (Cyperales)
<b>Family</b>	Cyperaceae
<b>Genus</b>	Cyperus
<b>Species</b>	Rotundus
<b>Scientific Name</b>	<i>Cyperus rotundus</i>

### Biological source

Nagarmotha is a classical ayurvedic herb of perennial origin that goes by the botanical name *Cyperus rotundus* and belongs to the sedge family or the Cyperaceae family.

### Parts used

Roots.

### Chemical constituents

Nagarmotha consists of

- i. Essential oils, flavonoids, terpenoids, and mono sesquiterpenes.
- ii. It contains cyprotene, acopaene, cyperene, aselinene, rotundene, valencene.
- iii. Cyperol, gurjunene, trans-calamenene, dcadinene.
- iv. Gcalacorene, cadalene, amuurolene, gmuurolene, cyperotundone.
- v. The oil of *Cyperus rotundus* was mainly composed of cyperol,  $\alpha$ -cyperene, rotundine.

### Uses

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

- i. Astringent
- ii. Diaphoretic
- iii. Analgesic
- iv. Aromatic
- v. Carminative
- vi. Antispasmodic
- vii. Antitussive
- viii. Litholytic (an agent that can dissolve stones)

## IV. Aim, Objectives and Need of research

### Aim

To Formulate and Evaluate Herbal Antibacterial Mouthwash.

### Objective

The experiment will be carried out to achieve the following

1. To develop a formulation of herbal mouthwash
2. To determine the antimicrobial activity.
3. To evaluate mouthwash for its consistency.

### Need of research

1. Mouthwash act on oral pathogen and also producing less side effects as compared to synthetic herbal products.
2. Herbal mouthwash is demanded because they instantly relieves the pain.
3. Herbal mouthwash can help to prevent from gingivitis and various infectious diseases.

## Materials and methodology

### 1) Collection, Procurement and Extraction of Tulsi leaves

The leaves of *ocimum sanctum* are collected dry under shadow and then coarsely cut into small pieces. The dried coarse piece was extracted to obtain a tulsi extract. The successive extraction was carried out by soxhlet method. The extract was filtered through muslin cloth and subsequently the filtrate was pass through filter paper and then packed in close container.

## 2) Collection, Procurement and Extraction of Nagarmotha powder

Nagarmotha powder was collected from local shop. Accurate quantity of powder was weighed and mix with desired quantity of alcohol and stirred well and kept it for 24 hours, the extract was filter and collected in a container.

## 3) Collection, Procurement and Extraction of Green tea

Green tea was collected from local shop. Accurate quantity of green tea was weighted and mix with desired quantity of water and stirred well and kept it for 24 hours, the extract was filter and collected in a container.

## 4) Equipments

Sterile Petri plates, Test tubes, Conical flask, Beakers, Whattmann filter paper, Incubator, Autoclave, Laminar air flow, Hot air-oven, etc.

## 5) Method of preparation of herbal mouthwash

### a. Material used

Tulsi Extract, Green Tea Extract, Nagarmotha Extract, Cinnamon Oil, Clove Oil, Menthol, Vitamin E, Glycerin, Distilled Water.

### b. Formulation of herbal mouthwash

1. Mix the Tulsi extract 2.0 ml, Green Tea extract 2.0 ml and Nagarmotha extract 1.0 ml in container (no.1) and shake it.
2. In a separate container (no.2), add 2 drops of clove oil, 2 drops of cinnamon oil, capsule of vitamin E and mix it properly. Then slowly add 0.04 gm of menthol, stir well to obtain solution.
3. Take 4 ml of glycerin in container (no. 3) and in that glycerine slowly add 2nd solution. The 1st solution is slowly added in the 3rd mixture, stir well and slowly add a distilled water to make a volume up to 100ml and then shake it continuously and clear liquid has a fresh mint taste.

**Table 1: The Herbal Mouthwash was prepared by the formula.**

Sr. No.	Ingredients	Function	Percentage (%)
1	Tulsi Extract ( <i>Ocimum Santum</i> )	Antimicrobial, Anti-inflammatory	2.0 %
2	Green Tea Extract ( <i>Camellia Sinensis</i> )	Preventing Gingivitis	2.0 %
3	Nagarmotha extract ( <i>Cyperus rotundus</i> )	Flavouring agent	1.0 %
4	Cinnamon oil	Bactericidal	0.2 %
5	Clove oil	Analgesic, anti-inflammatory	0.2 %
6	Menthol	Freshner, Flavouring agent	0.04 %
7	Vitamin E	Antioxidant	0.015 %
8	Glycerin	Emulsifying agent	4.0 %
9	Distilled water	Vehicle	q.s.

## Evaluation test

### 1. Physical evaluation:-

Physical parameter such as colour, odour, taste and consistency were examined by visual examination

### 2.pH determination:-

The pH of formulated mouthwash was 5, falling within the ideal pH range for mouthwash which is 5.5 or below. The formulated mouthwash is acid balanced which is near to the skin pH.



**Fig. 6 pH of Mouthwash**

### **3. Test for microbial growth in formulated mouthwash:-**

The formulated mouthwash was inoculated in the plate of nutrient agar media by streak plate method and a control was prepared. The plates were placed in the incubator and are incubated at 37°C for 24 hours. After the incubation period plate were taken out and checked for microbial growth by comparing it with the control.

### **Results and Discussion**

The pH of the formulation was found to be 5. As the skin is having an acidic pH around 5.5 this pH range of the formulation is suitable for oral disorders. The formulation was found to be free from heavy metals. The formulation was free from microbes as they have not produced any microbial growth when they got inoculated in the agar medium. This mouthwash is a purely herbal prepared without the addition of any kind of alcohol and any other additives as other products found in the market. The formulation was undertaken stability studies for physical and chemical change.

### **Anti-microbial Activity**

The formulation was free from microbes as they have not produced any microbial growth when they get inoculated in the agar medium<sup>[13,14]</sup>.

The antimicrobial activity was evaluated by paper disc method for different concentration of mouthwash. (Table: 3)

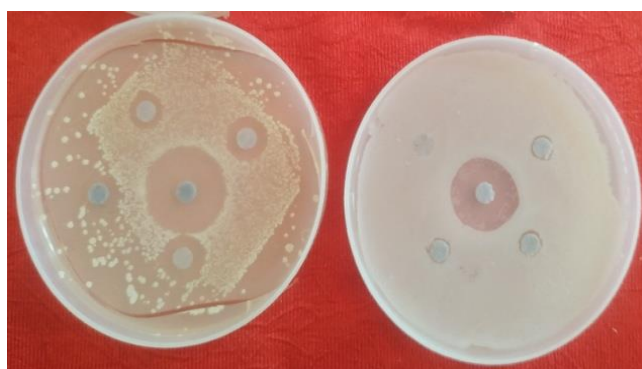
Herbal mouthwash mask bad odour and produce a pleasing flavour. Herbal mouthwash with therapeutic agents like anti-microbial, however may be effective for some long-term control of bad odour.

Alcohol consumption as well as alcohol and tobacco use are known risk factor for head and neck cancers. When used in mouthwashes antimicrobial ingredient like Tulsi, clove and other essential plant extracts have been found to reduce plaque and gingivitis.



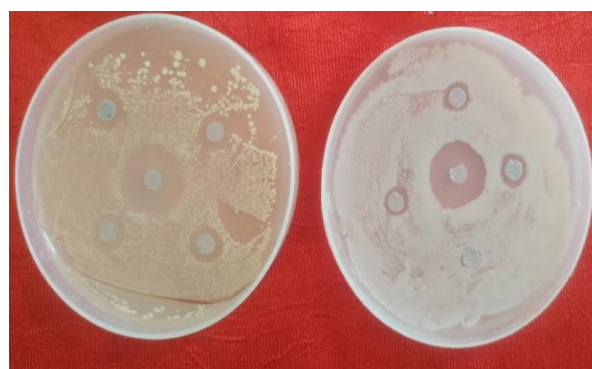
**Table 3: Zone of inhibition (in cm)**

Conc .	Zone of Inhibition					Control (Streptomycin )
	1	2	3	4	Average	
P	0.4	0.4	0.3	0.1	0.3	0.9
Q	0.4	0.4	0.4	0.4	0.4	0.9
R	0.5	0.4	0.4	0.4	0.4	0.9
S	0.5	0.4	0.4	0.5	0.5	0.9
A	0.4	0.3	0.3	0.4	0.3	0.9
B	0.4	0.4	0.4	0.4	0.4	0.9
C	0.4	0.5	0.5	0.5	0.5	0.9
D	0.4	0.5	0.6	0.5	0.5	0.9



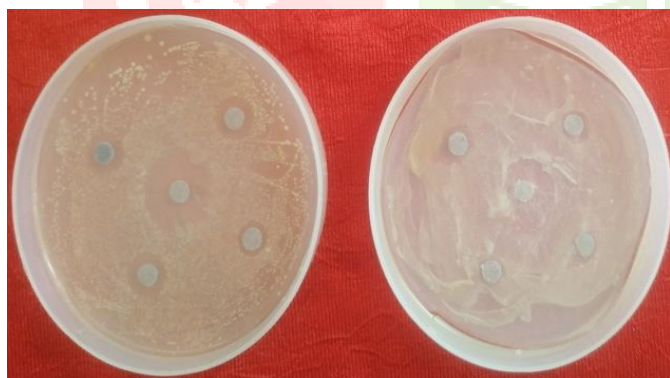
**A**  
(25 % conc. of Marketed Product)

**P**  
(25 % conc. of Formulated Product)



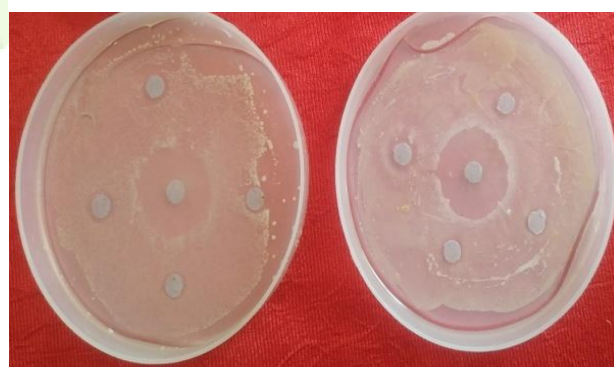
**B**  
(50 % conc. of Marketed Product)

**Q**  
(50 % conc. of Formulated Product)



**C**  
(75% conc. of Marketed Product)

**R**  
(75% conc. of Formulated Product)



**D**  
(100% conc. of Marketed Product)

**S**  
(100% conc. of Formulated Product)

### Conclusion

The data presented in this study, it was concluded that the developed herbal mouthwash possess significant, therapeutically efficacious, suitable vehicle for drug delivery. This study should be completed with more investigations and studies, to explore the product in long term follow-up and laboratory tests to improve all the effects and side effects of the new products, since it will be used as medical product.

Herbal mouthwash preparation have potent action and minimal side effects when compared with that of the other marketed mouthwashes, hence there is need for increased usage of herbal preparation to avoid the

adverse effects. Thus, there is a need to create awareness among prescribers and public about the use of herbal mouth washes and many other such studies should be encourage.

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