

# Comparative Evaluation of Herbal Waters with a Medicated Mouth Rinse by In Vitro Anti-Inflammatory Study

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## Abstract

Frequent usage of chemical mouthwashes cause discoloration of teeth, disturbance of taste, sores on the mouth to avoid these herbal mouthrinses are preferred. The objective of this study is to prepare and characterize Clove, Mint, Tulasi and Neem herbal waters and evaluate Anti-inflammatory activity of those herbal waters. Protein denaturation assay method was used and results showed Clove herbal water has 80.6% of Anti-inflammatory activity, Mint herbal water has 72.1% of Anti-inflammatory activity, Tulasi herbal water has 72.8% of Anti-inflammatory activity, Neem herbal water has 76.1% of Anti-inflammatory activity and Honey has 73.1% of Anti-inflammatory activity. We estimated the amount of herbal powders per 100ml (Clove, Mint, Tulasi and Neem) having the equal potency of Anti-inflammatory activity with chlorhexidine. Hence these herbal waters can be used in the preparation of herbal mouthrinses.

**Key Words:** *In Vitro, Clove, Mint, Tulasi, Neem, Honey, Chlorhexidine and Anti Inflammatory.*

## Introduction

With the exponential advancement in the field of dentistry, various preventive measures have emerged targeting the causative factors of the oral diseases. Plaque accumulation is one such factor which predisposes the individual to both dental caries and periodontal disease. Salivary microfloras like *Streptococcus mutans* and other predisposing factors lay an important role in the initiation and progression of dental diseases such as dental caries. Chemotherapeutic and antimicrobial agents aiming at these predisposing factors, therefore play, a significant role in prevention of these oral diseases and have a dramatic impact on improving the oral health of the individual.

Among the plethora of oral hygiene products available, chlorhexidine has been the mouthwash of choice owing to its dramatic therapeutic effect, but its various side effects like taste alteration, supragingival calculus formation, and desquamation of oral mucosa have restricted its usage in pediatric age group. Moreover, it also causes extrinsic staining by

attaching to the polyphenolic and tannin group of beverages like tea and coffee. therefore agents based on herbal extracts are therefore of particular interest.

### **Herbs Used In Oral Health:**

Herbs are characterized as unrefined pharmaceuticals derived from plants that are used to cure illnesses, frequently chronic ones, or to achieve and sustain better health. Nature has provided herbs to heal human ailments. Traditional medicine has been using herbal extracts since ancient times. The use of dietary and herbal supplements (HDS) by customers of all ages has been well-documented. Numerous plants contain a variety of beneficial qualities, including antioxidants, astringent action, antibacterial activity, and anti-inflammatory effects. Dental conditions are being treated with these qualities.

### **Oral Disorders:**

#### **Gingivitis:**

The gingiva will often be red, swollen, and bleeding when it has gingivitis. It is brought on by the buildup of bacteria in the gingival crevice, which causes the gingival tissues to become inflamed. Gingivitis is one of the most common diseases that affect people, and it can lead to periodontitis. Gingivitis can result from viral infections. The most prevalent ones are brought on by Varicella zoster, the virus that causes chicken pox, and Herpes simplex 1 and 2, which are linked to genital warts and cold sores, respectively. Herpes simplex primary infection typically strikes children and can result in "herpetic gingivostomatitis," a severe case of gingivitis that is painful but eventually goes away and is accompanied by fever.

#### **Dental Caries:**

Dental caries is the most common long-term illness. It is an infectious illness that slowly deteriorates tooth hard tissues due to a complex and multifactorial path. Dental caries is a condition that gradually worsens over time as a result of intricate biological interactions between acidogenic bacteria, fermentable carbohydrates, and host elements including saliva and teeth. The disease arises from a complex interplay of factors, ranging from biological to societal. Dental caries is a post-eruptive bacterial infectious illness that affects the mineralized dental tissues and is typified by a gradual demineralization process. It is thought to be the primary cause of tooth loss and the most common oral illness in the globe.

#### **Dental Plaque:**

The varied population of microorganisms present on the surface of teeth as a biofilm, embedded in an extracellular matrix of polymers originating from both the host and the microorganism, is known as dental plaque. Whereas bacteria are always found in nature in groups, biofilms have characteristics not shared by the same organisms living in liquid (planktonic) culture. Dental plaque preferentially builds in areas that are stationary and shield the teeth from the strong forces that remove it from the mouth.

#### **Mouth Washes:**

Mouthwashes, sometimes known as mouth rinses, oral rinses, mouth washes, or simply mouth rinses, are aqueous liquid compositions that are primarily used to prevent, treat, and cure oral health conditions like dental caries, dental erosion, halitosis, gingivitis, periodontitis, mucositis, and to lower the oral microbiota.

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### Characteristics Of Mouthwash:

It reacts rapidly and has sufficient strength to provide the intended effect at a given dilution. Robust enough taste to mask unpleasant breath odors. It should have a passable flavor. It shouldn't irritate the mucous membranes or the mouth cavity. It can't be irritating or dangerous.

### Uses Of Mouth Wash:

1. Mouthwashes are concentrated, transparent, aqueous solutions with a pleasant flavor that are used to clean and deodorize the mouth and buccal cavity.
2. They assist in treating oral infections and maintaining good oral hygiene. Mouthwashes and mouth rinses that contain hydroalcoholic solutions are used in dental hygiene to clean and freshen cavities.
3. A new inhalation.
4. Guarding against dental decay using sodium fluoride.
5. It helps prevent tooth decay by demineralizing your teeth, strengthening the enamel, and halting the creation of plaque.

### Advantages Of Chlorhexidine:

Gingivitis and other inflammatory dental disorders are treated with topical chlorhexidine, a broad-spectrum antibacterial cationic bisbiguanide. One of the most efficient antimicrobial agents for lowering the quantity of dental plaque and periopathogenic microorganisms for an extended length of time is association as an antibacterial and anti-inflammatory agent. Additionally, using chlorhexidine can promote recovery following periodontal surgery.

### Disadvantages Of Chlorhexidine:

1. Disturbance of taste
2. Discoloration of teeth
3. sores on the mouth
4. Reversible oral epithelium desquamation
5. A change in taste perception and more deposits, particularly in the subgingival area.

### Description of Clove:



Cloves consist of dried flower buds of *Eugenia caryophyllus*, (Family: Myrtaceae). It is indigenous to Amboyna and Penang Molucca islands. It is now cultivated chiefly in Zanzibar, Pemba, Madagascar, West Indies, Sri Lanka and India. In India, cloves are grown in Nilgiri, Tenkasi-hills and in Kanyakumari district of Tamil Nadu state. It is also cultivated in Kottayam and Quilon districts of Kerala. It should contain not less than 15 % (v/w) of clove oil. acid), resin, chromone and eugenin. The volatile oil contains eugenol (about 70 to 90 %), eugenol acetate, methylamylketone, caryophyllenes and small quantities of esters and alcohols. Bio active compounds such as eugenol and caryophyllene contain anti-inflammatory activity. It has medicinal properties such as antimicrobial, anti inflammatory,

anti-stress, antioxidant, antiviral hepatoprotective, antinoceptive activities .Clove is also used as a dental analgesic, carminative, stimulant, flavouring agent, an aromatic and antiseptic.

### Description Of *Mentha Piperita*:



*Mentha piperita* is popularly known as “pudina.” It belongs to the family Lamiaceae. *Mentha piperita* is an aromatic herb, known for its essential oils. Pharmaceutical grade oil produced by distilling the fresh aerial parts of the plant at the beginning of the flowering cycle is standardized to contain no less than 44% menthol, 15%–30% menthone, and 5% esters, in addition to various terpenoids. Other compounds found in it are flavonoids (12%), polymerized polyphenols (19%), carotenes, tocopherols, betaine, and choline. The oil contains terpenoids such as  $\alpha$ -pinene or  $\beta$ -pinene,  $\alpha$ -phellandren, and also ester-connected with menthol or free acetic acid and isovaleric acid. Bio active compounds such as Methanol, Menthone, and peppermint oil contain anti-inflammatory activity. It has wide medicinal/pharmaceutical applications due to its antimicrobial, anti inflammatory, anti-emetic, anti spasmodic, carminative, diaphoretic and analgesic properties and it is used to treat bronchitis, anorexia, flatulence, colitis, nausea, migraines, headaches, anesthetic, myalgia and liver complaints.

### Description of *ocimum sanctum*



Tulsi consists of fresh and dried leaves of *Ocimum sanctum* Linn., belonging to family Labiatae. The medicinal plants are widely used by the traditional medicinal practitioners for curing various diseases in their day to day practice. In traditional system of medicine, different parts (leaves, stem, flower, root, seeds and even whole plant) of *Ocimum sanctum* Linn. Bio active compounds such as caryophyllene, Ursolic acid, Rosmarinic acid and linalool contains anti-inflammatory activity. It has been recommended for the treatment of bronchitis, malaria, diarrhea, dysentery, skin disease, arthritis, eye diseases, insect bites and so on. The *O. sanctum* L. has also been suggested to possess anti-fertility, anticancer, antidiabetic, antifungal, antimicrobial, cardioprotective, analgesic, antispasmodic and adaptogenic actions. It has medicinal properties such as Anti- stress, antioxidant,

hepatoprotective, immuno modulating, anti-inflammatory, antibacterial, antiviral, antifungal, antipyretic, antidiuretic, antidiabetic, antimalarial and hypolipidemic.

Description of *Azadirachta indica*



It consists of dried leaves of *Azadirachta Indica* belongs to family Meliaceae. Bio active compounds such as quercetin and beta-sisterol contains anti-inflammatory activity.v : Neem tree has numerous medicinal properties by virtue of its chemical compounds. Seeds of the Neem tree contain the highest concentration of Azadirachtin. Apart from Azadirachtin , salannin, gedunin, azadirone, nimbin, nimbidine, nimbicidine, nimbinol, etc are other important liminoids of neem. : Neem tree has numerous medicinal properties by virtue of its chemical compounds. Seeds of the Neem tree contain the highest concentration of Azadirachtin. Apart from Azadirachtin , salannin, gedunin, azadirone, nimbin, nimbidine, nimbicidine, nimbinol, etc are other important liminoids of neem.

Traditional Ayurvedic uses of neem include the treatment of fever, leprosy, malaria, ophthalmia and tuberculosis. Various folk remedies for neem include use as an anthelmintic, antifeedant, antiseptic, diuretic, emmenagogue, contraceptive, febrifuge, parasiticide, and insecticide.

### Materials And Methods

Collection of Plant Materials:

Leaves of Clove, Mint, Tulasi, and Neem were collected from local market and washed with sterile water and dried in shades.

Collection Of Mouthwash:

Chlorhexidine (CHX) is collected from local market.

HERBALS:

Clove (Advaitha Natural Foods).

Mint (Advaitha Natural Foods).

Tulasi (Advaitha Natural Foods).

Neem (Advaitha Natural Foods).

Honey (Dabur).

Egg Albumin Solution:

Store-bought egg albumin powder can be used to generate a 1% egg albumin solution by adding 1 mL of the translucent part to 100 mL of distilled water with a thorough swirling motion. Egg albumin is the name of the transparent part of the egg. When creating the solution, the water needs to be cold. When water reaches a boil, it will coagulate.

Egg Albumin Assay:

It is possible to assess the anti-inflammatory properties of unidentified crude extracts In-vitro by observing how they inhibit the denaturation of egg albumin (a protein). A reaction

mixture with a total volume of 5 mL was created by combining 0.2 mL of 1-2% egg albumin solution (from commercially available egg albumin powder), 2.8 mL of phosphate buffered saline (pH 7.4), and 2 mL of distilled water. 2 mL of triple-distilled water, 0.2 mL of 1-2% egg albumin solution, and 2.8 mL of phosphate buffered saline were combined to make a total volume of 5 mL of the control. Triple distilled water was used as the blank in a suitable UV/Vis spectrophotometer to measure the absorbance at 280 nm.

**Preparation Of Phosphate Buffer Solution:**

Weigh 0.68g of Potassium dihydrogen phosphate and 0.156g of Sodium hydroxide and dissolve in 100ml of distilled water which has a PH of 7.4.

## Method:

### In-Vitro Anti-Inflammatory Activity

**Inhibition of protein denaturation assay:**

Protein denaturation causes protein molecules to lose their biological characteristics. Inflammatory diseases such as cancer, diabetes, and rheumatoid arthritis have been linked to protein denaturation. Thus, a substance's ability to stop protein denaturation might also aid in the prevention of inflammatory diseases.

Protein used in this assay is egg albumin. Protein denaturation is achieved by incubating the reaction mixture for ten minutes at 70°C in a water bath. Plant extract in different quantities (1000 µL; 100-500 µg/ml), egg albumin (200 µL) or bovine serum albumin (450 µL; 5% w/v aqueous solution), and phosphate buffered saline (1400 µL) make up a reaction mixture. As a negative control, distilled water is utilized rather than the extracts with the above mixture. The mixture is then heated to 70°C for five minutes after being incubated for fifteen minutes at 37°C. At 660 nanometers, their absorbances are measured after cooling under flowing tap water. As a positive control, acetyl salicylic acid, diclofenac sodium, ibuprofen, or indomethacin are administered. The experiment is carried out in triplicates and percent inhibition for protein denaturation is calculated using following equations:

% Inhibition of denaturation =  $(1-D/C) \times 100$ .

Where D is the absorbance of test sample and C is the absorbance of negative control (without the test sample or reference drug).

## Results

Table No 1: PHYSICAL, ORGANOLEPTIC PROPERTIES OF HERBAL WATERS

Samples	Colour	Odour	Taste	pH
Clove	Dark Brown	Aromatic	Spicy	4.4
Mint	Light green	Aromatic	Sweet	6.9
Tulasi	Green	Spicy scent	Astringent	6.3
Neem	Light green	Pleasant	Bitter	7.8
Honey	Dark Brown	Pleasant	Sweet	6.1



## PRELIMINARY STUDIES

Table No 2: Phytochemical Analysis of Clove, Mint, Neem, Tulasi Herbal waters

Phytochemical Test	Clove	Mint	Neem	Tulasi
Alkaloids	+	—	—	+
Flavonoids	+	+	+	+
Tannins	+	+	+	+
Saponins	+	+	—	+
Steroids	+	+	+	—
Glycosides	+	—	+	—
Terpenoids	+	+	+	+
Phenolics	—	+	—	+
Cardiac Glycosides	—	+	—	+
Carbohydrates	—	+	—	—
Proteins	—	+	—	—
Fats	—	—		—
Phlobatannins	—	—	+	—
Resins	—	—	—	+
Steroids	—	—	—	+

Table No 3: Protein Denaturation Assay of Anti-inflammatory Activity

S.NO	Samples	Amount in of Taken 100ml Distilled Water	Absorbance at 660nm	% Inhibition
1	Control	—	0.336	—
2	Chlorhexidine (CHX)	—	0.063	82.8%
3		3g	0.082	77.6%

4	Clove	6g	0.071	80.6%
5		3g	0.141	61.4%
6	Mint	6g	0.102	72.1%
7		3g	0.126	65.6%
8	Tulasi	6g	0.099	72.8%
9		3g	0.117	68.0%
10	Neem	6g	0.104	71.6%
11		3g	0.142	61.2%
12	Honey	6g	0.098	73.3%

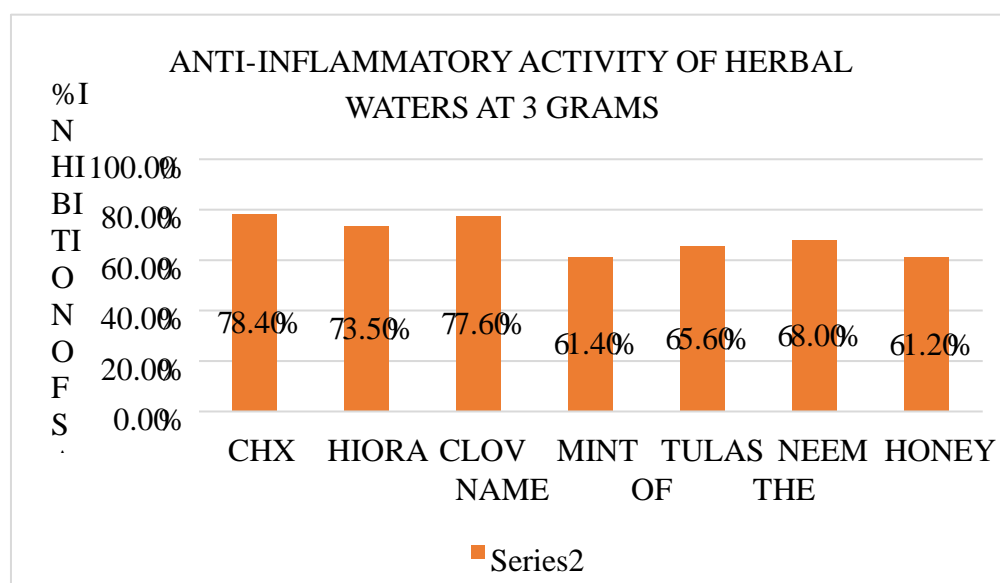


Fig:1 – Anti-Inflammatory Activity Of Herbal Waters At 3 grams



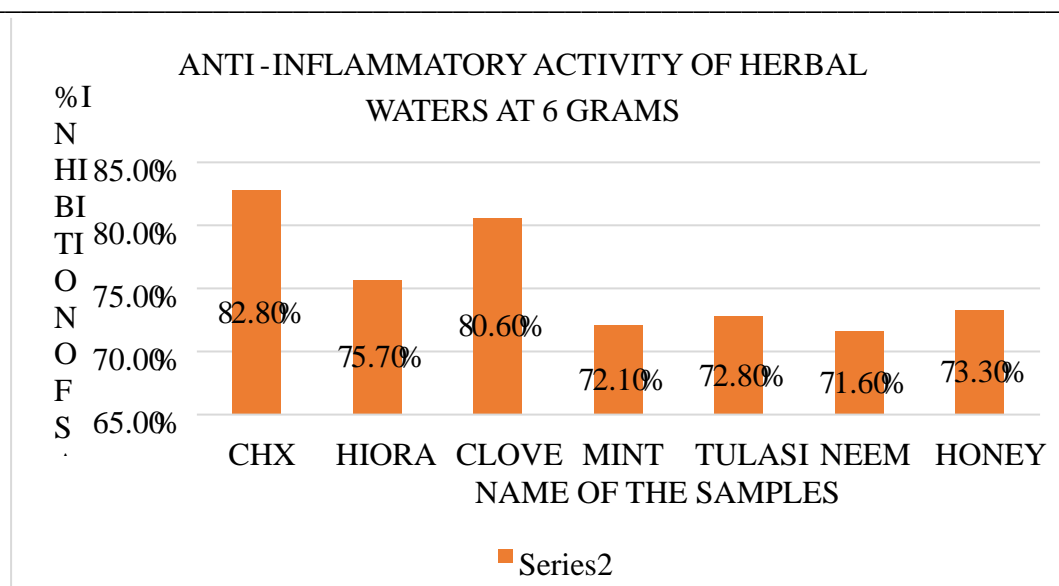


Fig:2 – Anti-Inflammatory Activity Of Herbal Waters At 6 grams

### Discussion

According to this Study, Clove Herbal Water has Dark Brown colour, Spicy taste, Aromatic odour, and pH of 4.4. Mint Herbal Water has Light green colour, Sweet taste, Aromatic odour, and pH of 6.9. Tulasi Herbal Water has Green colour, Astringent taste, Spicy odour, and pH of 6.3. Neem Herbal Water has Lightish Green colour, Pleasant smell, Bitter in taste, and Ph of 7.8.

Phytochemical studies indicate that Alkaloids, Flavonoids, Tannins, Saponnins, Steroids, Glycosides, and Terpenoids were present in Clove Herbal water.

In this Study we also found that Saponnins, Steroids, Terpenoids, Flavonoids, Carbohydrates, Glycosides, Tannins, Proteins, and Phenolics were present and Fats and Alkaloids were absent in the Mint Herbal water.

In this Study, we also found that Plobatannins, Tannins, Terpenoids, Glycosides, Flavonoids, and Steroids were present and Saponnins were absent in the Tulasi Herbal water.

In this Study, we also found that, Flavonoids, Alkaloids, Cardiac glycosides, Phenols, Resins, Tannins, Steroids, and Saponnins were present in the Neem Herbal water.

Anti-inflammatory activity of Clove herbal water was determined by using Diclofenac sodium as a standard. In this Study, We Evaluated the anti inflammatory activity of Clove herbal water by taking Chlorhexidine Mouthwash as a standard by using Protein denaturation assay method. 3g/100ml of Clove herbal water exhibited 77.6% of Inhibition and 6g/100ml of Clove herbal water exhibited 80.6% of Inhibition. According to this study, 6.16g/100ml of Clove herbal water may have the same potency with Chlorhexidine (82.8%).

Anti-inflammatory activity of Mint herbal water was determined by using Diclofenac sodium as a standard. In this Study, We Evaluated the anti-inflammatory activity of Mint herbal water by taking Chlorhexidine Mouthwash as a standard by using Protein denaturation assay method. 3g/100ml of Mint herbal water exhibited 61.4% of Inhibition and 6g/100ml of Mint herbal water exhibited 72.1% of Inhibition. According to this study 6.89g/100ml of Mint herbal water may have the same potency with Chlorhexidine (82.8%).

Anti-inflammatory activity of Tulasi herbal water was determined by using Diclofenac sodium as a standard. In this Study, We Evaluated the anti-inflammatory activity of Tulasi herbal water by taking Chlorhexidine Mouthwash as a standard by using Protein denaturation

assay method 3g/100ml of Tulasi herbal water exhibited 65.6% of Inhibition and 6g/100ml of Tulasi herbal water exhibited 72.8% of Inhibition. According to this study, 6.82g/100ml of tulasi herbal water may have the same potency with Chlorhexidine (82.8%).

Anti-inflammatory activity of Neem herbal water was determined by using Diclofenac sodium as a standard. In this Study, We Evaluated the anti-inflammatory activity of Neem herbal water by taking Chlorhexidine Mouthwash as a standard by using Protein denaturation assay method. 3g/100ml of Neem herbal water exhibited 68.0% of Inhibition and 6g/100ml of Mint water exhibited 71.6% of Inhibition. According to this study, 6.93g/100ml of Neem herbal water may have the same potency with Chlorhexidine (82.8%).

Anti-inflammatory activity of honey herbal water was determined by using Diclofenac sodium as a standard. In this Study, We Evaluated the anti-inflammatory activity of honey herbal water by taking Chlorhexidine Mouthwash as a standard by using Protein denaturation assay method. 3g/100ml of honey herbal water exhibited 61.20% of Inhibition and 6g/100ml of honey water exhibited 73.3% of Inhibition. According to this study, 6.93g/100ml of honey herbal water may have the same potency with Chlorhexidine (82.8%).

### Conclusion

The herbal waters of Clove, Mint, Tulasi, and Neem have shown Anti -inflammatory activity as Chlorhexidine (CHX) mouthwash. As per the study, 6.16g/100ml of clove water may have equal potency with chlorhexidine in anti-inflammatory activity. 6.89g/100ml of Mint water may have equal potency with chlorhexidine in anti-inflammatory activity. 6.82g/100ml of Tulasi water may have equal potency with chlorhexidine in anti-inflammatory activity. 6.93g/100ml of Neem water may have equal potency with chlorhexidine in anti-inflammatory activity. This study calls for further investigation to evaluate Anti-microbial and Anti-oxidant activities of these herbal waters which are required to act as herbal Mouth rinses.

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