

# Formulation of Antimicrobial Mouthwash and Comparison with Commercial Mouthwashes

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## **Abstract:**

Dental caries is one of the most common infectious diseases affecting human beings. The most common methods for maintaining good oral hygiene and preventing dental caries are brushing and washing the mouth with tooth pastes and mouthwashes that have antimicrobial properties. The present research was therefore undertaken to formulate an antibacterial mouthwash using the most commonly used vegetables and spices in India so that the mouthwash can be prepared easily at home and is also highly economical. Antimicrobial activity of the formulated mouthwash was compared with the most commonly used commercial mouthwashes.

**Keywords:** Caries, Mouthwash, Antimicrobial activity.

## **Introduction:**

Mouthwash, mouth rinse or oral rinse is a liquid which is held in the mouth passively or swilled around the mouth for about half a minute and then spit out [1] (Matthews, 2003). Usually mouthwashes are antiseptic solutions which are supposed to reduce the microbial load in the oral cavity, although there are other mouthwashes which may be used for other reasons such as for their analgesic or anti-inflammatory.

The most common use of mouthwash at home is a part of an oral hygiene routine. Some manufacturers of mouthwash state that antiseptic and anti-plaque mouth rinse kills the microorganisms which causes cavities, gingivitis, and bad breath. Anti-cavity mouth rinse uses fluoride which gives protection against tooth decay. It is, however, generally agreed that the use of mouthwash does not eliminate the need for both brushing and flossing [2], [3](Gunsolley, 2006; Tal, 1990). Some natural products (clove oil, vegetable glycerine) are effective as an adjunct in improving the oral health [4](Chen et al., 2014).

## **Typical Formulation Of Commercial Mouthwashes**

The different commercial brands of mouthwashes have different ingredients. The active ingredients are usually alcohol, chlorhexidine gluconate, cetylpyridinium chloride hexetidine, benzoic acid (acts as a buffer), methyl salicylate, triclosan, benzalkonium chloride, methylparaben, hydrogen peroxide, domiphen bromide and sometimes fluoride, enzymes, and calcium [5], [6], [7], [8](Ribeiro *et al.*, 2007; John *et al.*, 2005; Levy, 2003; Rosenberg *et al.*, 1992). They can also possess essential oil constituents which have some antibacterial properties, like phenol, thymol, eugenol, eucalyptol or menthol [9] (Stoeken *et al.*, 2007). Ingredients also include water, sweeteners such as sorbitol, sucralose, sodium saccharin and xylitol [10](Giertsen *et al.*, 1999).

Commercial mouthwashes also contain a preservative such as sodium benzoate to preserve freshness once the container has been opened. Many newer brands are alcohol-free and contain agents that eliminate odour such as zinc ion. It helps to keep future bad breath from developing.

Minor and transient side effects of commercial mouthwashes are very common; these mainly include taste disturbance, tooth staining and sensation of a dry mouth. Alcohol-containing mouthwashes may make dry mouth and halitosis worse since it dries out the mouth. Soreness, ulceration and redness may also occur if the person is allergic or sensitive to mouthwash ingredients such as preservatives, coloring agents, flavors and fragrances. Such effects might be reduced or eliminated by diluting the mouthwash with water, using a different mouthwash or foregoing the mouthwash entirely [11](Doreen, 2012).

### Home-Made Mouthwashes

**There are multiple benefits for preparing mouthwash at home. Here is a list of benefits:**

- Inexpensive - Homemade mouthwashes are less expensive when compared to store bought mouthwashes with the same or better performance.
- No harmful chemicals - Manufacturers often use dyes, preservatives, artificial sweeteners, and other chemicals that are harmful to human beings and the environment also.
- Alcohol-free - It is very hard to find quality mouthwash that does not contain alcohol which leads to dry mouth and also causes bad breath.
- The quality of ingredients - When we make our own mouthwash we choose to use ingredients of only the finest quality. In order to get more profits some manufactures may choose to cut costs by using ingredients of cheap quality.

### Common Homemade Mouthwash Ingredients

#### Herbs/ Plants

**Aloe Vera Gel** - Aloe vera extracts have antibacterial and antifungal activities.

**Anise** - Anise contains anethole which has potent antimicrobial properties, against bacteria, yeast, and fungi.

**Basil** - Basil oil has potent antioxidant, anticancer, antiviral, and antimicrobial properties.

**Bergamot essential oil** - Bergamot can help calm inflamed skin and also has antiseptic properties which help ward off infection and aid recovery.

**Cardamom** - Cardamom is used to treat infections in teeth and gums, to prevent and treat throat troubles, and is a decongestant.

**Cinnamon** - Cinnamon is high in antioxidants. The essential oil of cinnamon also has antimicrobial properties.

**Clove** - Clove oil, applied to a cavity in a decayed tooth, relieves toothache. It also helps to decrease infection in the teeth due to its antiseptic properties. The buds have anti-oxidant properties.

**Coriander** - Both the leaves and seeds contain antioxidants, but the leaves were found to have a stronger effect.

**Eucalyptus** - Eucalyptus oil has antibacterial effects on pathogenic bacteria.

**Fennel** - It has potent antimicrobial properties, against bacteria, yeast, and fungi.

**Ginger** - Has antibacterial properties and has a sialagogue action which stimulates the production of saliva.

**Goldenseal** - It is anti-inflammatory and antimicrobial and may remove canker sores when gargled.

**Lemon essential oil** - The acidity of lemons enables the juice to be used to deodorize, remove grease, bleach stains, and disinfect.

**Peppermint** - Peppermint has a high menthol content, and is often used as tea and for flavouring ice cream, confectionery, chewing gum, and toothpaste.

**Rosemary** - Contains a number of potentially biologically active compounds, including antioxidants, such as carnosic acid and rosmarinic acid.

**Sage** - It is used as an antibiotic, antifungal, astringent.

**Spearmint** - Has a minty flavor. The essential oil of spearmint has some antifungal activity. Spearmint has also been described as having excellent antioxidant activity.

**Tea tree oil** - Tea tree oil has beneficial medical properties when applied topically, including antiseptic, antibacterial, antifungal, and antiviral action, and is also believed to have beneficial cosmetic properties.

**Thyme** - The essential oil of common thyme (*Thymus vulgaris*) is made up of 20-54% thymol. Thymol is an antiseptic and is the main active ingredient in Listerine mouthwash.

**Turmeric** – It is an antimicrobial, anti-inflammatory, and an antiseptic agent.

Sweeteners

**Raw honey** - Although honey contains sugar, some people want a more natural sweetener. Honey contains more complex carbohydrates than regular table sugar, so they break down more slowly and are less likely to cause problems in the oral cavity. Honey has a potent broad spectrum antimicrobial activity.

**Stevia** - It is about 30 times as sweet as sugar. Stevia inhibits the growth and reproduction of harmful bacteria that cause gum disease and tooth decay.

**Xylitol** – It is a sugar alcohol sweetener which is used as a naturally occurring sugar substitute. The bacteria are unable to feed on xylitol so it does not cause dental cavities or bad breath.

Others

**Baking soda** - Sodium bicarbonate or baking soda works as a mechanical cleanser on the teeth and gums. It neutralizes the production of acid in the mouth and also acts as an antiseptic which help to prevent infections.

**Glycerin (Glycerol)** – It is a sweet-tasting humectant (keeps foodstuff moist), thickening agent, solvent, sweetener, preservative, and 60% as sweet as sucrose. It cannot be used by the bacteria that form plaques and cause dental cavities.

**Gum Arabic** - Gum arabic reduces the surface tension of liquids and is also an emulsifier (encourage the suspension of one liquid in another, improves consistency therefore helping your mouthwash stayed mixed).

**Hydrogen peroxide** – It is a disinfectant, antiseptic, oxidizer, and bleach (whitener) odor. Hydrogen peroxide can be used as toothpaste when mixed with correct quantities of baking soda and salt. It should be stored in an opaque container because it breaks down quickly when exposed to light. The FDA has approved using up to a 3% hydrogen peroxide solution as mouthwash. Using a higher concentration than that might irritates the mouth and may do more harm than good.

**Salt** - Sodium chloride acts as a preservative by absorbing water. It is used in soaps, detergents, and cooking. Salt works as a preservative because it absorbs water out of bacteria therefore killing them or at least inhibiting their growth.

**Water** - Water is an essential ingredient in mouthwashes. Boiled, mineral, soda (carbonated), or distilled water can be used in the preparation. Tap water contains chlorine, mercury, fluoride, nitrates, and may even contain some carcinogens such as chromium-6. So use of tap water should be avoided. Filtered, bottled, distilled, or mineral water instead of tap water can be used.

## Methodology:

### Basic Mouthwash Preparation Procedures

For making homemade mouthwash, there are a couple of basic ways. Using these procedures one can try alternate ingredients in order to create different mouthwashes.

- **Boil it** - Bring water to boiling and then add herbs to water, infuse for 20 minutes, strain, cool and store in a bottle.
- **Steep it** - Place herbs in bottle then pour boiling water on them, steep overnight, strain. Steeping the ingredients for an entire week, while shaking them well once a day and then straining can also be followed.
- **Mix it** - Place all the ingredients into a bottle and shake well. It works well when we use essential oil extracts of herbs.

Generally heating is required to bring out the properties of raw or dried herbs so it is best to boil and steep the solution. In case mouthwash is prepared with essential oil extracts or non-herbal ingredients that do not require heat (such as salt, glycerin, baking soda) then only mixing of the ingredients is sufficient.

### Formulation Of Mouthwash Using Vegetable And Spice Extracts

A mouthwash was formulated using aqueous extracts of vegetables. Carrot, beetroot, cucumber, mint leaves, coriander leaves, tomato, lemon, ginger and cloves were used for preparing the mouthwash. All the vegetables and spices used were bought from the local market. The crude extracts were prepared.

#### Preparation of extracts:

Aqueous vegetable extracts were prepared according to methods previously reported by Onyeagba *et al.*, [12]. Fresh tomatoes, ginger, carrot, beetroot, cucumber, cabbage, coriander leaves and mint leaves were washed under running tap water and then rinsed with sterile distilled water. Aqueous extracts were prepared by macerating 100g of each vegetable in a sterile, ceramic mortar and then filtering off the homogenate with a sterile, muslin cloth. Similarly, lime extract was prepared as previously described by Onyeagba *et al.*, and Aibinu *et al.*, [13]. A juice extractor was used to obtain the juice from 200 g fresh, clean lime fruits. This was subsequently filtered with a sterile, muslin cloth.

Boiled decoction of cloves was prepared by grinding 100 grams of cloves and boiling it in 200ml sterile distilled water for 10 minutes. After the decoction got cooled, it was filtered with a sterile muslin cloth.

Carrot, beetroot, cucumber, mint leaves, coriander leaves and tomato extracts were taken in equal quantities (10ml each). Lemon juice, ginger extract and clove decoction were taken in the ratio 2:1:2 (4ml, 2ml, 4ml) respectively. This formulation was made up to 100ml by adding 30ml of sterile drinking water. Xylitol was used as a sweetener. For 100ml of the mouthwash, 10g of Xylitol was added and mixed well. The formulation was filtered through Millipore filters to remove any bacteria if present.

Two different mouthwashes were prepared using aqueous extracts of vegetables, herbs and spices, one containing preservative (Sodium benzoate) and the other lacking preservative. A portion of the prepared mouthwashes were stored at room temperature, refrigeration temperature, pasteurized at 75°C for 30 minutes and kept at room temperature.

**Comparison Of Formulated Mouthwash With Commercial Mouthwashes**

The antimicrobial activity of the formulated mouthwash was compared with the most commonly used commercial mouthwashes such as Colgate and Lysterine [14] (Prashant Shetty *et al.*, 2013).

**Antimicrobial Activity:**

Antimicrobial activity was determined by agar well diffusion method. Muller Hinton agar plates were inoculated with 100µl of the mixed culture isolated from oral cavity and was kept at room temperature for 15 minutes for absorption to take place. Wells of 6mm size were made with sterile borer in the inoculated agar plates and loaded with 50µl of the mouthwashes. Prior to incubation at 37°C for 24 hours, the Petri dishes were kept at room temperature for 15minutes in order to promote diffusion of the extracts into the agar [15], [16](Khokra *et al.*, 2008; Rios *et al.*, 1988). All the tests were made in triplicate and the mean diameter of the zone of inhibition in millimeters and standard deviation was measured after appropriate incubation.

The above antimicrobial activity was studied every third day for 30 days.

**Results And Discussion:**

Table: 1 show the antibacterial activity of the formulated mouthwashes containing preservative along with the activity of commercial mouthwashes (Colgate and Lysterine). Commercial mouthwashes did not show any antibacterial activity.

Formulated mouthwash had ingredients with antimicrobial properties, which may be the reason for the antibacterial activity.

The maximum antibacterial activity was exhibited by Formulation with added preservative. The activity exhibited by the fresh formulation stored at room temperature was same when compared to the formulation that was pasteurized (19.66±0.57 mm mean zone of inhibition) on the first day but we could observe a gradual decrease in the activity of all the formulations with time (day 1 to day 31).

When the activity of the formulations kept at room temperature, refrigerator and pasteurized formulations stored at room temperature were compared; it was observed that the pasteurized formulations showed slightly higher activity when compared to the other two formulations with exposure of time period.

Table: 2 show the antibacterial activity of the formulated mouthwashes without preservative along with the activity of commercial mouthwashes (Colgate and Lysterine). Antibacterial activity of the formulations without preservative was studied for seven days only as the formulations got spoiled. The highest activity was observed with Formulated mouthwash that was pasteurized and stored at room temperature (19.00±0.00 mm mean zone of inhibition). The formulations lacking preservative also showed a gradual decrease in the antibacterial activity.

**Table 1 : Comparison of antibacterial activity of the formulated mouthwashes containing preservative with that of commercial mouthwashes.**

S/No.	Day	Formulated Mouthwash			Colgate	Lysterine
		RT	R	P		
1	1	19.66±0.57	19.33±0.57	19.66±0.57	-	-
2	4	18.66±0.57	19.00±0.00	19.33±0.57	-	-
3	7	17.66±0.57	17.33±0.57	17.66±0.57	-	-

4	10	16.00±0.00	16.33±0.57	16.66±0.57	-	-
5	13	15.33±0.57	15.00±1.00	15.66±0.57	-	-
6	16	14.00±1.00	14.66±0.57	15.00±0.00	-	-
7	19	13.33±0.57	14.33±0.57	14.66±0.57	-	-
8	22	12.33±0.57	13.66±0.57	14.33±0.57	-	-
9	25	11.66±0.57	12.33±0.57	14.00±0.00	-	-
10	28	11.00±0.00	11.66±0.57	13.33±0.57	-	-
11	31	10.33±0.57	11.00±0.57	12.66±0.57	-	-

RT- indicates room temperature, R- indicates refrigeration, P- indicates pasteurized and -indicates no antimicrobial activity.

**Table 2 : Comparison of antibacterial activity of the formulated mouthwashes lacking preservative with that of commercial mouthwashes.**

S/No.	Day	Formulated Mouthwash			Colgate	Lysterine
		RT	R	P		
1	1	18.66±0.57	18.33±0.57	19.00±0.00	-	-
2	4	16.66±0.57	17.00±0.00	17.66±0.57	-	-
3	7	15.33±0.57	16.00±1.00	16.33±0.57	-	-

RT- indicates room temperature, R- indicates refrigeration, P- indicates pasteurized and -indicates no antimicrobial activity.

**Conclusion:**

Dental caries is one of the most common infectious diseases affecting human beings. It is highly prevalent among children. All age groups of children are prone to this infection involving both primary and permanent teeth. *Streptococcus mutans* and *Lactobacillus acidophilus* are the main opportunistic pathogens of dental caries. Other micro flora like *Fusobacteria*, *Actinomyces viscosus*, *Candida albicans*, *Bacillus cereus*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* are also associated with dental caries.

The most common methods for maintaining good oral hygiene and preventing dental caries are brushing and washing the mouth with tooth pastes and mouthwashes that have antimicrobial properties. Commercially available dentifrices are not only expensive but also contain chemical agents, which are known to produce harmful side effects on prolonged use. Hence, dentifrices that contain extracts of medicinal plants and herbs are becoming popular.

Since ancient times, medicinal plants have been employed for prophylactic and curative purposes. The present research was therefore undertaken to formulate an antibacterial mouthwash using the most commonly used vegetables and spices in India so that the mouthwash can be prepared easily at home and is also highly economical.

Carrot, beetroot, cucumber, mint leaves, coriander leaves, tomato, lemon, ginger and cloves were used for preparing the mouthwash. Carrot and beetroot provide good colour to the mouthwash.

Formulations were prepared with and without preservative and each formulation was stored at room temperature, refrigeration temperature and pasteurized at 75°C for 30 minutes and kept at room temperature.



Antimicrobial activity of the formulated mouthwash was compared with the most commonly used commercial mouthwashes (Colgate and Lysterine).

Commercial mouthwashes (Lysterine and Colgate) did not show any antibacterial activity.

The formulated mouthwashes which have been proved to be effective than the commercial mouthwashes contain natural ingredients, these are easy to prepare, highly economical, effective, free from unwanted synthetic chemicals (dyes) and other additives and safe for children, pregnant women, people with dry mouth and diabetics. This homemade mouthwash can be used by children without the supervision of elders.

Thus the formulated mouthwash using vegetables and spices was proved to be effective in the inhibition of the growth of caries causing bacteria. So, regular use of mouthwash which could be easily prepared at home using vegetables and spices could control dental caries.

Further research can be done on the action of the formulated mouthwashes on the biofilm present on the surface of the tooth and clinical trials would prove the potency of the formulated mouthwash.

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