Research Article

Preparation and Evaluation of Herbal Mouthwash Containing Hydroalcoholic Extract of *Pongamia pinnata*

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ABSTRACT

Aim: To formulate and evaluate an herbal mouthwash containing hydroalcoholic extract of *Pongamia pinnata*. The oval shaped oral cavity contains surfaces covered with bacteria such as *Streptococcus mutans*, *Porphyromonas gingivalis*, *Staphylococcus* and *Lactobacillus* that causes various tooth problems such as decay, gingivitis, sensitive teeth, root infection, bad breath and enamel erosions. The role of herbal plants is significant in the development of many oral formulations. **Materials and Methods:** Phytochemical screening of the above-mentioned plant was done. Leaf extraction was carried out for preparation of mouthwash. Formulation and evaluation of all three formulations was done. **Results:** Preliminary phytochemical screening of hydroalcoholic leaves extract of *Pongamia pinnata* confirmed the presence of phenolic compounds, tannins, flavonoids, terpenoids and alkaloids. Three formulations of mouthwash were prepared by using *Pongamia pinnata*, mint oil, PEG 40, glycerol, benzoic acid etc. and evaluated for different parameters like colour, odour, pH, viscosity, turbidity and stability studies. **Conclusion:** In comparison to chemical products, herbal drugs are widely regarded as highly effective. The goal of the present research was to formulate and evaluate an herbal mouthwash. So, in this research article we have focused on *Pongamia pinnata* (Common name Pongame oil tree) plant that belongs to Fabaceae family. Herbal mouthwashes can be used in combination with other oral hygiene practices like brushing and flossing. Due to their effectiveness against oral pathogens, herbal mouthwashes are high in demand because it provides immediate pain relief and has few side effects.

Keywords: *Pongamia pinnata*, Mint oil, Oral hygiene, Mouthwash, *Streptococcus mutans*.

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INTRODUCTION

Mouthwash is an antiseptic aqueous solution used to clean the mouth and teeth or freshen up the breath.[1] It is most often used for control of plaque and other dental problems.[2] It is a medicated liquid that is held in the mouth and swished around by the perioral muscles to get rid of oral pathogens.[3] Mouthwashes come in two different varieties: Chemical and herbal.

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Natural compounds called polyphenols are found in herbal mouthwashes and have the desired anti-inflammatory and anti-microbial properties. The demand for herbal mouthwash is very high because they quickly relieve pain, fight oral pathogens, and have fewer side effects.[4] They work without alcohol, artificial preservatives and coloring agents. Chemical mouthwashes typically contain hydrogen peroxide and chlorhexidine, which instantly whiten, sterilize, and relieve tooth pain. However, they have the tendency to discolor teeth and may cause side effects, meanwhile they are economical.[5]

Chlorhexidine is a substance that adheres to the tooth surface and soft tissues in the mouth to prevent the growth of harmful bacterias. Like other medications, Chlorhexidine has negative side effects, such as increased

natural tooth staining (brown or yellowish stains on the teeth) and altered taste perception due to prolonged use.[6] The dentist suggests only using chlorhexidine for up to two weeks to thoroughly clean your soft tissues, gums, and teeth. Chlorhexidine mouthwash is not meant to be a long-term solution, but within the 2-week period, the staining side effect shouldn't be too bad as long as you brush and floss. So herbal mouthwashes can be used for longer period of time to control several dental problems.[7]

Benefits of Herbal Mouthwash

For the following reasons, herbal mouthwash has become more advantageous than chemical mouthwashes:

- Herbal mouthwashes are non-irritant and they have non-staining properties.
- They are less harmful and have very few or no side effects.
- Herbal mouthwashes are the better option for even the most sensitive mouth.
- Herbal mouthwashes have naturally antibacterial property as they have polyphenols.
- It doesn't contain any abrasive additives.
- Unlike chemical mouthwashes, herbal mouthwash does not cause dry mouth.[8]

Uses

Herbal mouthwashes are used:

1. To enhance oral hygiene.
2. To control dental plaque.
3. For eradicating bacterias present in oral cavity.
4. To cover bad breath and refresh the breath.
5. For gum disease prevention.
6. To relieve pain and inflammation.
7. To treat Mucositis (swelling and irritation in the mouth) and Halitosis (bad breath).[9]

Plant Profile of *Pongamia pinnata*

Pongamia pinnata is a medium-sized perennial tree with the common names Karanja in Hindi and India beech in English. It is a member of the Fabaceae family. It is extensively distributed in India, Bangladesh, China and Australia (Figure 1).[10]

Scientific Classification

Kingdom: Plantae
Sub Kingdom: Tracheobionta
Super Division: Spermatophyta
Division: Magnoliophyta
Order: Fabales
Family: Fabaceae

Figure 1: *Pongamia pinnata*.

Table 1: Equipments.

Sl. No.	Equipments	Company Name
1	Soxhlet Apparatus	Shambhavi IMPEX
2	Clevenger Apparatus	Shambhavi IMPEX
3	Weighing Balance	Dolphin
4	Water Bath	Gautam Lab

Genus: *Pongamia*
Species: *pinnata*[11]

Chemical Constituents

According to the several studies, *Pongamia pinnata* contains alkaloids, flavonoids, glycoside, tannins, saponins, fixed oil, resins and phytosterols.[12]

Uses

Different sections of the *Pongamia pinnata* have been utilized as traditional treatments for whooping cough, rheumatoid arthritis, bronchitis and diabetic twins. A hot infusion of the leaves is used to relieve rheumatic pains and to cleanse ulcers associated with gonorrhea and the scrotum.[13]

MATERIALS AND METHODS

Chemicals and Equipments

To formulate and evaluate the herbal mouthwash following equipments and chemicals were used (Tables 1 and 2):

Plants Collection and Authentication

In the month of January, *Pongamia pinnata* leaves were collected from the Nelkaranji village in the Atpadi Taluka of the Sangli District of Maharashtra, India, and verified by Dr. Rajendra D. Shinde, Principal and Head of the Department of Botany and Director of the

Table 2: Chemicals.

Sl. No.	Chemicals	Company Name
1	Ethanol	Research-Lab Fine Chem Industries
2	Saccharin	Ozone International Mumbai
3	PEG 40	Research-Lab Fine Chem Industries
4	Glycerol	Research-Lab Fine Chem Industries
5	Benzoic acid	Research-Lab Fine Chem Industries

Blatter Herbarium, St. Xavier's College, Mumbai, India (400001). He provided specimen no. (Shah- 183 of G. L. Shah.) for reference.

Preparation of Leaf Extracts

The leaves of *Pongamia pinnata* plant were collected, cleaned with sterile water, dried, coarsely ground, and stored in airtight bottles. About 132 grams of dried coarsely powdered leaves of *Pongamia pinnata* were filled in the thimble chamber of the Soxhlet apparatus and extraction process was carried out by using 800 mL of 70% (V/V) ethanol and water mixture for 48 hr. The solubility of both solvents is good for the majority of organic compounds. The solvents were heated in the round bottom flask, vaporised into the sample thimble, condensed in the condenser, and then dripped back into the flask. The procedure was resumed once the liquid content reached the syphon arm and drained back into the bottom flask. This process was repeated twice to obtain required quantity of extract. After being concentrated by evaporation at 70°C for 8 hr, the extract was dried. At last, % yield of the extract was calculated and kept it at room temperature prior to phytochemical screening.[14]

Isolation of Mint Oil by Using Clevenger Apparatus

The fresh leaves of *Mentha piperita* were hydro-distilled using a glass Clevenger apparatus. A pale greenish yellow essential oil was obtained. It was preserved in the dark at 4°C over anhydrous sodium sulphate drying.[15]

Phytochemical Screening

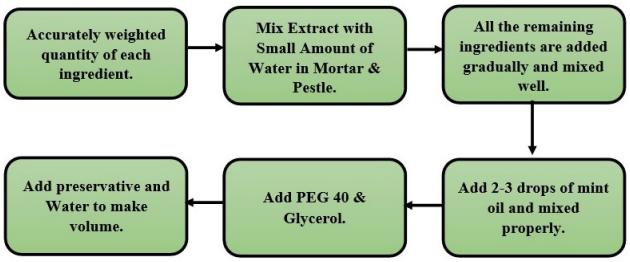
Standard procedures were followed to check for the presence of different phytoconstituents in the hydroalcoholic extract of *Pongamia pinnata*.[16]

Methods of Mouthwash Preparation

Three formulations of herbal mouthwash: Formulation 1 (F-1), Formulation 2 (F-2) and Formulation 3 (F-3) were prepared.

Each ingredient was taken in a weighted quantity. The extract was thoroughly combined with a small amount of water in a mortar and pestle. All of the remaining ingredients were added gradually and thoroughly mixed. Mint oil was added drop by drop and thoroughly combined, taking care to prevent lump formation. Then, drop by drop, PEG 40 and glycerol were added and thoroughly mixed. Finally, water was added to make up the volume, as well as a preservative, and the product was packaged in an attractive, well-closed container (Table 3).[17]

Methodology



Evaluation of Mouthwash

Physical Evaluation: Physical assessment (colour, odour, and consistency) was performed using sensory and visual examination and compared with the marketed mouthwash preparation (Chlorhexidine).

pH: A digital pen-style pH meter was used to measure the pH of prepared herbal mouthwash formulations. For the evaluation of pH, the electrode of the pH meter was immersed into the mouthwash formulations and kept it in the formulations until the displayed level was stable. Note down the readings from display.

Viscosity: Using an Ostwald viscometer, the viscosity of mouthwash formulations was measured. The

Table 3: Formulations of Mouthwash.

Ingredient	Function	Formulation		
		F_1	F_2	F_3
Pongamia pinnata	Active Drug	250 mg	500 mg	1000 mg
Mint Oil	Flavor	0.1 mL	0.1 mL	0.1 mL
Saccharin	Sweetener	0.1 mg	0.1 mg	0.1 mg
PEG	Surfactant	4 gm	4 gm	4 gm
Glycerol	Co-surfactant	8.5 mL	8.5 mL	8.5 mL
Salt (10gm/100mL)	Preservative	2 mL	2 mL	2 mL
Lemon Yellow Color	Coloring agent	1-2 drops	1-2 drops	1-2 drops
Purified Water	Up to 100 mL	Up to 100 mL	Up to 100 mL	Up to 100 mL

viscometer was mounted vertical position on a suitable stand. Mouthwash was filled in to the viscometer up to mark A. The time was counted for mouthwash to flow from A to mark B. Viscosity was measured in triplicate.

After Taste: The taste is strong and remains unchanged over the week except for the ambient temperature sample. Healthy male and female candidates, were selected to participate in the evaluation of taste. About 1 mL of herbal mouthwash was given to selected candidates and ask for the taste.

Foam Height: One mL of mouthwash was mixed in 50 mL distilled water. The mixture was poured into a 500 mL measuring cylinder. Water was added to the volume to make it 100 mL. The mixture received 25 strokes, after which it was kept aside. The height of the foam above the aqueous volume was observed.

Turbidity: Turbidity is the term used to describe a fluid's cloudiness or haziness as a result of individual particles (suspended solids or liquids) that are typically invisible to the unaided eye. The units of turbidity from a calibrated Turbidimeter are called Nephelometric Turbidity Units (NTUs). A formulation with an NTU of less than 12 (or about 12) is referred to as a clear formulation. Turbidity of all three formulations of mouthwash was measured by using Nephelometry (Turbidity meter).

Stability Studies: The aim of stability studies was to guarantee that the mouthwash formulations can be used and can continue to have the same properties over time. Prior to conducting antibacterial testing, stability studies were conducted on various mouthwash formulations.

Physical stability: The visual appearance, homogeneity and physical separation of the formulated mouthwash were measured throughout this test. Three formulations of mouthwash were then kept at different temperatures, including 12°C and 25°C. The appearance was then assessed at various temperatures, and the findings were recorded.

pH stability: A calibrated pH meter was used to record pH stability. To evaluate the changes in the pH readings, different mouthwash formulations were kept on the shelf of a room at 37°C and at 40°C. After that, the result was noted and compared for a period of six weeks.

RESULTS AND DISCUSSION

The % yield of hydroalcoholic leaves extract of *Pongamia pinnata* was calculated by using following formula:

$$\% \text{ yield} = \frac{\text{Weight of dry extract}}{\text{Weight of dry plant biomass}} \times 100$$

It was found to be 13.93%.

Mint oil was isolated by hydro-distillation using Clevenger Apparatus. A pale greenish yellow essential oil (3.45%) was obtained.

The preliminary phytochemical screening revealed that the hydroalcoholic leaves extract of *Pongamia pinnata* contains proteins, alkaloids, glycosides, saponins, flavonoids, and phenols (Table 4).

Preparation of Herbal Mouthwash Formulations

Three formulations of herbal mouthwash: Formulation 1 (F-1), Formulation 2 (F-2) and Formulation 3 (F-3) were prepared (Figure 2).

Evaluation Parameters

All prepared formulations (F-1, F-2 and F-3) of herbal mouthwash were subjected for the following evaluation parameters:

Physical Evaluation

All three formulations of mouthwash were evaluated for physical evaluation and the results were shown in the Table 5.

Table 4: Phytoconstituents Present in Hydroalcoholic Extracts of *Pongamia pinnata*.

Sl. No.	Chemical Constituents	Tests	*Pongamia pinnata*
1.	Alkaloids	Mayer's test	+
		Dragendorff's test	+
		Wagner's test	+
		Hager's test	+
		Molisch's test	-
2.	Carbohydrates	Benedict's test	-
		Fehling's test	-
3.	Glycosides	Modified Borntrager's	+
		Legal test	+
4.	Saponins	Foam test	+
		Froth test	+
5.	Phytosterols	Salkowski's test	+
		Libermann Burchard test	+
6.	Resins	Acetone-Water test	+
7.	Fixed oils	Filter Paper	+
8.	Terpenoids	Salkowski's test	+
9.	Phenols	Ferric Chloride test	+
10.	Tannins	Gelatin test	+
11.	Flavonoids	Alkaline Reagent test	+
		Lead acetate	+
		Zn-HCl acid reduction	-
		Shinoda test	+

Note: '+' sign denotes presence whereas '–' sign denotes absence.[18]

Figure 2: Herbal Mouthwash Formulations.

Table 5: Physical Evaluation.

Sl. No.	Formulation	Parameters	Observation
1	F-1	Color Odour Appearance Texture	Florescent yellow Pleasant (Cool Mint) Visual Appearance Liquid
2	F-2	Color Odour Appearance Texture	Gold yellow Pleasant Odour Visual Appearance Liquid
3	F-3	Color Odour Appearance Texture	Brown Pleasant Odour Visual Appearance Liquid

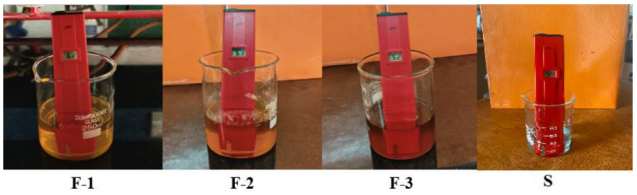


Figure 3: pH of Herbal Mouthwash Formulations.

Table 6: pH of Herbal Mouthwash Formulations.

Sl. No.	Formulation	pH
1	F-1	6.2
2	F-2	5.3
3	F-3	5.2
4	Chlorhexidine (S)	5.4

pH: The pH of all three formulations was recorded by digital pen style pH meter (Figure 3). The results of pH were shown in Table 6.

The pH of formulations F-1, F-2 and F-3 was found to be 6.2, 5.3 and 5.2 respectively.

Viscosity

We used an Ostwald viscometer to measure the viscosity of each of the three mouthwash formulations.

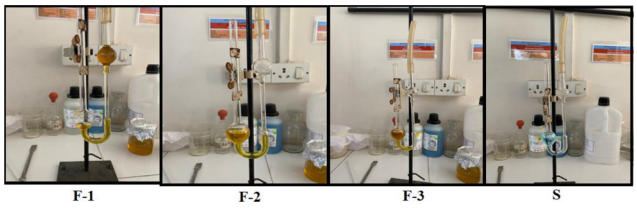


Figure 4: Viscosity of Herbal Mouthwash Formulations.

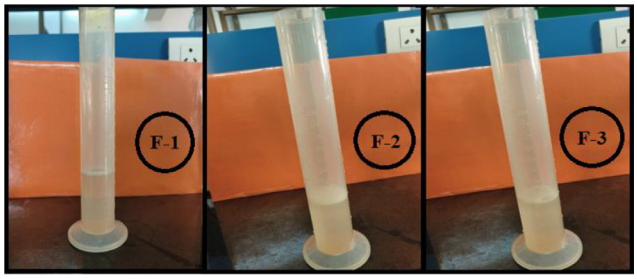


Figure 5: Foam Height of Herbal Mouthwash Formulations.

Figure 6: Turbidity of Herbal Mouthwash Formulations.

According to tests, F-1, F-2, and F-3's viscosities were 9.2, 8.5, and 8.2 CPS, respectively (Figure 4).

After Taste

For the evaluation of taste 10 healthy human volunteers were selected. 1 mL of each formulation of mouthwash was swished or gargled by all volunteers for about half a minute and then spat out. According to the feedback of all volunteers the taste of all three formulations on Day 1 and Day 7 was found to be similar.

Foam Height

The height of the foam of three formulation 1 (F-1), formulation 2 (F-2) and formulation 3 (F-3) was found to be 3 mL, 5 mL and 4 mL respectively (Figure 5).

Turbidity

Turbidity of mouthwash formulations was measured by using Nephelometry. The turbidity of F-1, F-2 and F-3 was found to be 004, 003 and 007 NTU respectively (Figure 6).

Stability Studies

The result of physical stability studies was shown in Table 7. Change in color, odour, physical separation and homogeneity of all three formulated mouthwash was observed at 12°C and 25°C.

Table 7: Physical Stability of Herbal Mouthwash Formulations.

Formulations	Parameters	Temperature (12°C)	Temperature (25°C)
F-1	Change in color Odour Physical Separation	No Change	No Change
F-2	Change in color Odour Physical Separation	No Change	No Change
F-3	Change in color Odour Physical Separation	No Change	No Change

According to the results of Stability studies, there was a slight change in the pH of formulations at 40°C.

DISCUSSION

The present study was designed to formulate and evaluate an herbal mouthwash and compare it with marketed mouthwash formulation chlorhexidine. Zoe L.S. Brookes *et al.* reported that, chlorhexidine is a chemical antiseptic commonly used in mouthwashes and dental products. It has some disadvantages compared to herbal mouthwashes. Chlorhexidine contains synthetic chemicals that may cause side effects such as staining, altered taste perception, and allergic reactions. It is also more likely to cause dry mouth, mouth ulcers, and other oral irritations than herbal mouthwashes.[7] Results of this study have shown that the test mouthwash formulation-2 (F-2) was better than F-1 and F-2. When compared, the evaluation parameters of the F-2 formulation produced findings that were highly similar to those of the standard but had less side effects. The formulation of F-2 mouthwash was found to have a pH of 5.3 and chlorhexidine to have a pH of 5.4. When the turbidity of the F-2 formulation was compared to that of standard chlorhexidine, it was found to be identical (003 NTU). The active metabolites of *Pongamia pinnata* plant extract like polyphenols, flavonoids, tannins impart antibacterial potential by attenuating bacterial pathogenicity.[19] Yadav R. D *et al.* showed that the *Pongamia pinnata*'s leaves are rich in polyphenols and flavonoids. These polyphenols, flavonoids and tannins precipitate microbial proteins and stop the growth of bacteria.[13] Other supporting studies include those done by Bodiba D. C *et al.*,[20] Panda K.S *et al.*,[21] and Nivethaprashanthi S *et al.*,[22] also found the antiplaque effect of herbalbased dentifrice (with similar extracts) comparable to the standard ones.

CONCLUSION

The formulated herbal mouthwashes can significantly work in long way to help people in eradicating dental issues such as decay, gingivitis, sensitive teeth, root infection, bad breath, enamel erosions and many other oral problems. Furthermore, we can assure that no harmful ingredients are present in mouthwash formulations. The herbal mouthwash is better than chlorhexidine mouthwash as it does not cause negative side effects, such as staining of the natural teeth, altered taste perception due to prolonged use and dry mouth. The phytochemical tests confirm the presence of flavonoids, phenolic compounds, tannins, alkaloids and terpenoids. It has been medically demonstrated that the natural herbs utilized in the current formulations may prevent the other problem of oral cavity such as tooth decay and gum diseases. This herbal mouthwash makes it simple for a person to rinse their mouth and avoid a variety of oral health problems. The present study is crucial for developing an affordable, efficient herbal oral health intervention for low socioeconomic communities. However, since this study was brief, longer studies with larger sample sizes are necessary.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

PEG 40: Polyethylene Glycol 40; **hr:** Hours; **°C:** Degree centigrade; **mL:** Millilitre; **NTU:** Nephelometric Turbidity Units; **%:** Percentage.

SUMMARY

The formulated herbal mouthwash is better than chlorhexidine mouthwash as it does not cause staining of the natural teeth. The formulated herbal mouthwashes are effective against several oral bacteria such as *Streptococcus mutans*, *Porphyromonas gingivalis*, *Staphylococcus* and *Lactobacillus*. According to the several researchers,

plant extracts with flavonoids and phenolic compounds are thought to be the source of the antibacterial activity.

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