

# Formulation, Phytochemical Analysis, and Evaluation of Herbal Mouthwash Containing Pongamia pinnata Leaf Extract

### Shubham Pramanick<sup>1</sup>, Kamal Bhandari<sup>1</sup>, Mr. Praveen Semwal<sup>2</sup>

<sup>1</sup>Scholar, School of Pharmacy and Research, Dev Bhoomi Uttarakhand University, Dehradun, Uttarakhand, India <sup>2</sup>Assistant Professor, School of Pharmacy and Research, Dev Bhoomi Uttarakhand University, Dehradun, Uttarakhand, India Email ID: sopr.praveen@dbuu.ac.in

Cite this paper as: Shubham Pramanick, Kamal Bhandari, Mr. Praveen Semwal, (2025) Formulation, Phytochemical Analysis, and Evaluation of Herbal Mouthwash Containing Pongamia pinnata Leaf Extract. *Journal of Neonatal Surgery*, 14 (31s), 646-659.

#### **ABSTRACT**

Oral health plays a crucial role in overall well-being, and mouthwashes are widely used as adjuncts to mechanical oral hygiene. However, prolonged use of chemical mouthwashes such as chlorhexidine is often associated with side effects including tooth staining, taste alteration, and mucosal irritation. This study explores a natural alternative by formulating and evaluating a herbal mouthwash using *Pongamia pinnata* leaf extract, known for its traditional medicinal use and bioactive properties.

The dried leaf powder of *Pongamia pinnata* was extracted using Soxhlet apparatus with 70% hydroalcoholic solvent. The extract underwent preliminary phytochemical screening, confirming the presence of flavonoids, tannins, saponins, alkaloids, terpenoids, and cardiac glycosides. Six formulations (F1–F6) were developed with varying concentrations of the extract and supporting ingredients such as clove oil, peppermint oil, glycerol, PEG 400, Tween 80, sodium chloride, and saccharin.

The mouthwash formulations were evaluated for organoleptic properties, pH (6–7), viscosity (1–1.34 cP), foam height, and antimicrobial activity using the agar well diffusion method against oral bacteria isolated from human saliva. Formulations F2, F3, and F6 exhibited strong antimicrobial zones of inhibition up to 20 mm. All formulations showed stable physical characteristics with no phase separation or degradation during 7-day accelerated stability testing under alternating temperatures (4°C and room temperature).

The study concludes that *Pongamia pinnata*-based mouthwash formulations possess desirable physicochemical and antimicrobial properties, offering a promising natural, safe, and effective alternative to conventional chemical mouthwashes. These findings support further clinical research and development of plant-based oral hygiene products for public use.

**Keywords:** Herbal Mouthwash, Pongamia pinnata, Oral Hygiene, Antimicrobial activity

#### 1. INTRODUCTION

Oral health is an essential component of overall health, influencing nutrition, communication, and general well-being. The oral cavity harbors a diverse microbiota, and its imbalance can lead to common dental conditions such as caries, gingivitis, periodontitis, and halitosis. Mouthwashes are widely used as adjuncts to mechanical oral hygiene methods to control plaque, reduce microbial load, and freshen breath. However, conventional mouthwashes like chlorhexidine, although effective, are associated with side effects such as tooth staining, altered taste sensation, mucosal irritation, and potential microbial resistance with long-term use, limiting patient compliance and safety in routine oral care regimens [4,7,14,15,22]. To address these concerns, there is increasing interest in herbal mouthwashes formulated from medicinal plants that offer biocompatibility, safety, and multiple therapeutic actions. Herbal ingredients are famous for their antimicrobial, antiinflammatory, antioxidant, and wound-healing properties and have been widely used in traditional systems of medicine. Among them, *Pongamia pinnata* (Linn.), commonly known as Karanja or Indian Beech, is a leguminous tree traditionally used for treating skin disorders, wounds, inflammation, and microbial infections [1,2,17]. The leaves of *Pongamia pinnata* are rich in flavonoids, phenolic acids, tannins, saponins, and alkaloids. Notably, compounds like karanjin and quercetin contribute to the plant's strong antioxidant and antimicrobial properties [17,28,29,30]. Studies have shown its efficacy against oral pathogens such as Streptococcus mutans, Lactobacillus, and Porphyromonas gingivalis, suggesting its potential as an active ingredient in dental formulations. Despite its documented pharmacological benefits, its use in oral care remains underexplored. The present study aims to formulate and evaluate a herbal mouthwash containing *Pongamia pinnata* leaf

extract. The formulation was analysed for phytochemical constituents and assessed for organoleptic properties, physicochemical parameters, and antimicrobial efficacy against oral pathogens. This research supports the development of effective, natural alternatives to synthetic oral care products, aligning with the growing demand for herbal therapeutic solutions in dentistry [3,26].

## 1.1 Plant Profile: Pongamia pinnata:

Pongamia pinnata, commonly known as Karanja or Indian Beech. It's a medium-sized, quick-growing leguminous tree belonging to the family Fabaceae. Widely distributed across tropical and subtropical regions, especially in India, it typically grows along riverbanks, coastal zones, and degraded lands. This tree holds significant value in traditional medicine systems such as Siddha and Ayurveda, where its leaves, seeds, bark, and roots are used to treat various ailments including skin disorders, wounds, inflammation, and dental issues. The leaves are especially rich in bioactive compounds like flavonoids, tannins, alkaloids, saponins, and phenolic acids, which contribute to its antimicrobial, anti-inflammatory, and antioxidant properties [1,17,18,21,27,28,31]. Given its pharmacological potential and availability, *Pongamia pinnata* is increasingly being explored for use in herbal formulations, including natural mouthwashes.



Figure 1: Pongamia pinnata plant

### 1.2 Botanical Description:

The botanical description of *Pongamia pinnata* is summarized in the table below [17,31]:

Aspect	Description
Habit	Medium-sized evergreen or briefly deciduous glabrous tree or shrub, typically growing 15–25 meters tall with a straight or irregular trunk.
Trunk	Approximately 50–80 cm or more in diameter.
Crown	Broad and spreading, with drooping branches forming a wide canopy.
Bark	Dark-colored, smooth or with shallow vertical fissures; gray to dark brown.
Branchlets	Smooth (glabrous) with noticeable pale stipule scars.
Leaves	Alternate, imparipinnate with long slender petioles; young leaves are pinkish-red, mature leaves are glossy dark green above and dull green beneath.
Leaflets	Usually 5 to 9, nearly opposite, short-stalked, ovate to elliptic in shape, apex tapering, base cuneate or rounded; margins entire and slightly thick.
Inflorescence	Axillary, raceme-like, measuring 6–27 cm in length; flowers are aromatic and grouped in small clusters.
Flowers	Typically 2–4 per cluster, short-stalked, pea-shaped, measuring 15–18 mm long; flower clusters are often shorter than the leaves.
Calyx	Bell-shaped (campanulate), 4–5 mm long, truncate at the tip, with fine hairs.
Corolla	White to pink with a purple center and tannish veins outside; standard petal obovate, 1–2 cm long with basal auricles; outer surface has silky hairs.

#### Table 1: Botanical Description of *Pongamia pinnata*

#### 1.3 Traditional and Medicinal Uses:

In traditional medicine systems such as Ayurveda, Siddha, and Unani, *Pongamia pinnata* has long been recognized for its wide-ranging therapeutic applications. The leaves are frequently used topically to cure skin conditions such as eczema, scabies, and ulcers due to their anti-inflammatory and antimicrobial properties. Leaf poultices are applied to wounds and boils to promote healing. The seed oil, known as Karanja oil, is traditionally used for managing joint pain, rheumatism, and other inflammatory disorders. Additionally, it is incorporated into herbal soaps and ointments for its antiseptic effects. Bark decoctions are used in remedies for diarrhea, dysentery, and bleeding disorders, while root extracts are employed for dental issues and digestive problems [17,21,27,28,31].

#### 1.4 Mouthwash:

Mouthwash, also referred to as mouthrinse, is a liquid oral hygiene product used to rinse the mouth, teeth, gums, and throat. It serves as an effective adjunct to mechanical cleaning methods such as brushing and flossing. Mouthwashes can be categorized as therapeutic or cosmetic depending on their ingredients and intended effects. Therapeutic mouthwashes contain active agents such as chlorhexidine, fluoride, or essential oils that help in reducing plaque, gingivitis, dental caries, and oral microbial load. In contrast, cosmetic mouthwashes provide temporary relief from bad breath without targeting underlying causes [5,7,8,9,12,13,14,19].

Conventional mouthwashes, particularly those containing chlorhexidine, have shown proven efficacy but are also associated with adverse effects including tooth staining, mucosal irritation, and taste alteration, especially with prolonged use [15]. This has led to increased interest in herbal mouthwashes formulated from plant-based ingredients that offer comparable antimicrobial and anti-inflammatory benefits with fewer side effects. Herbal mouthwashes are often preferred for their biocompatibility, pleasant taste, and minimal toxicity. Studies have shown that extracts from plant such as *Pongamia pinnata* demonstrate significant efficacy in reducing oral microbial populations and maintaining oral health, supporting their use in natural oral care formulations [3,6,10,11,16,17,20].

## 1.5 Components of Herbal Mouthwash:

A herbal mouthwash system typically consists of active herbal extracts, essential oils, solubilizers/emulsifiers, humectants, preservatives, sweeteners, and a water base to ensure therapeutic efficacy and formulation stability [1,2,3,23,26,32,33,41,43,44,45,46,47].

Components	Role/Function	Examples
Active Ingredient	Provides antimicrobial, anti-inflammatory, antioxidant effects	Pongamia pinnata extract
Essential Oils	Enhances flavour, provides analgesic and antibacterial activity	Clove oil, Peppermint oil
Emulsifier	Solubilizes oils, ensures uniform distribution in aqueous base	Tween 80
Co-surfactant	Improves emulsion stability and solubility	PEG 400
Humectant	Retains moisture, enhances mouthfeel	Glycerol
Preservative	Prevents microbial contamination, improves shelf-life	Sodium chloride
Sweetener	Masks bitter taste, improves palatability	Sodium saccharin
Aqueous Phase	Acts as solvent and bulk vehicle	Distilled water

Table 2: Components of Herbal mouthwash and their role with examples

#### 2. MATERIALS AND METHODS:

### 2.1 Materials:

Component	Category	Function	Citation
<i>Pongamia pinnata</i> Leaf Extract	Active Ingredient	Antimicrobial, anti-inflammatory, antioxidant	[1,2,3,17]
Clove Oil (Syzygium aromaticum)	Essential Oil	Analgesic, antimicrobial, flavoring agent	[41,42]
Peppermint Oil ( <i>Mentha piperita</i> )	Essential Oil	Cooling effect, breath freshening, antimicrobial	[26,42]
Tween 80	Emulsifier	Solubilizes essential oils, stabilizes the formulation	[32,45]
PEG 400	Co-surfactant	Enhances solubility and emulsion stability	[24,33]
Glycerol	Humectant/Viscosity Agent	Retains moisture, improves texture and mouthfeel	[24,44]
Sodium Chloride	Preservative	Prevents microbial growth	[3,47]
Sodium Saccharin	Sweetener	Masks bitterness, enhances taste	[3,46]
Distilled Water	Solvent/Base	Vehicle for formulation, dilutes ingredients	[3,24,26]

Table 3: Composition of Herbal mouthwash

#### 2.2 Extraction Method:

The extraction of *Pongamia pinnata* leaves was carried out using the Soxhlet extraction method, which is well-suited for obtaining bioactive compounds from plant materials. Approximately 50 grams of dried *Pongamia pinnata* leaf powder, procured from a verified herbal supplier (Neotea India), was packed in a thimble made of muslin cloth and placed in the extraction chamber of a Soxhlet apparatus.

A 70% hydroalcoholic solution (ethanol: water = 70:30 v/v) was selected as the solvent system due to its efficiency in extracting both polar and moderately non-polar phytoconstituents. The solvent was heated in a round-bottom flask, evaporated, and condensed repeatedly to percolate through the plant material. Each extraction cycle was conducted continuously for 6-8 hours, and the process was repeated five times with fresh plant material and solvent for each cycle to ensure exhaustive extraction.

After each cycle, the extract was filtered and concentrated using a water bath maintained at 40–50°C to remove the solvent. The pooled semi-solid extract was then dried to a stable consistency and stored in a sterile, amber-colored container at 4°C for further phytochemical analysis and formulation development [17,38,39].

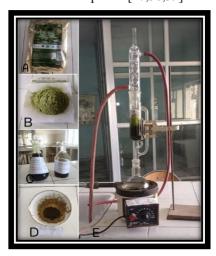


Figure 2: A & B – Pongamia pinnata leaf powder, C- Hydroalcoholic leaf extract, D- Dried leaf extract, E – Soxhlet apparatus

#### 2.3 Formulation Procedure:

The herbal mouthwash formulations (F1–F6) were developed using *Pongamia pinnata* leaf extract as the principal active ingredient, along with selected excipients to ensure physical stability, therapeutic efficacy, and user acceptability. The following standardized procedure was followed for each batch [1,2,3,24,26,32,33,34,35,36]:

### a) Preparation of Aqueous Phase:

Distilled water was measured and transferred into a clean beaker. For formulations F4–F6, glycerol (1.25 ml) was added to the water as a humectant, and stirred thoroughly until a uniform solution was formed. A separate solution of sodium chloride (2 ml in 100 ml water) was prepared to serve as a preservative and tonicity adjuster and was added to the main aqueous phase.

### b) Preparation of Oil Phase:

In a mortar and pestle, the essential oils- clove oil (5 drops) and peppermint oil (3 drops)- were triturated with Tween 80 and PEG 400 (used only in F1–F3) to form a homogenous emulsion. Tween 80 acted as the primary emulsifier, while PEG 400 served as a surfactant, enhancing solubility and emulsion stability.

## c) Incorporation of Herbal Extract:

A measured amount of *Pongamia pinnata* extract (250 mg to 1000 mg depending on formulation) was gradually added to the aqueous phase, followed by slow addition of the oil phase under continuous stirring to ensure uniform dispersion.

### d) Volume Adjustment and Mixing:

The formulation was adjusted to 100 ml with distilled water and stirred using a mechanical agitator for 15–20 minutes to achieve a clear and homogenous mouthwash solution.

#### e) Storage:

The prepared formulations were stored in sterile, amber-coloured containers at room temperature in a cool, dark place until further evaluation.

#### 2.4 Formulation table:

The table below presents the composition of the formulated mouthwash [24,25,26,34,35,36]:

Composition	Amount					
	F1	F2	F3	F4	F5	F6
Pongamia pinnata leaf extract	250 mg	500mg	1000mg	250 mg	500 mg	1000 mg
Clove oil	5 Drops	5 Drops	5 Drops	5 Drops	5 Drops	5 Drops
peppermint oil	3 Drops	3 Drops	3 Drops	3 Drops	3 Drops	3 Drops
Tween 80	3 ml	3 ml	3 ml	3.75 ml	3.75 ml	3.75 ml
PEG 400	2 ml	2 ml	2 ml	-	-	-
Glycerol	-	-	-	1.25 ml	1.25 ml	1.25 ml
Sodium Chloride	2 ml	2 ml	2 ml	2 ml	2 ml	2 ml
Sodium Saccharin	0.1 mg	0.1 mg	0.1 mg	0.1 mg	0.1 mg	0.1 mg
Distilled Water	Up to 100 ml	Up to 100 ml	Up to 100 ml	Up to 100 ml	Up to 100 ml	Up to 100

Table 4: Formulation Table



Figure 3: Final Formulations

# 2.5 Phytochemical analysis:

The preliminary phytochemical screening of the *Pongamia pinnata* leaf extract was carried out to identify the presence of major bioactive constituents using standard qualitative tests. The tests were performed as follows [1,2,40]:

Phytochemicals	Testing procedure	Observation	Result	Image
Tannins	Leaf Extract + Few drops of Ferric chloride	Brownish green or Blue-black coloration	+	
Terpenoids	Leaf Extract extract + 2ml of CHCl <sub>3</sub> + 3 ml Conc. H <sub>2</sub> SO <sub>4</sub> was added carefully	Reddish brown coloration was formed	+	
Flavonoids	1% NH <sub>3</sub> solution + Leaf extract	Yellow Coloration	+	
Saponins	Extract + Distilled water + Shake vigorously + 3 Drops of Olive oil	Formation of emulsion	+	
Cardiac glycosides	5 ml extract + 2 ml Glacial acetic acid + 1 drop of FeCl <sub>3</sub> (In one test tube), 1 ml of conc. H2SO4 (In another test tube) then mix together	Formation of a brown ring	+	

Alkaloids  1 ml of 1 % HCl + Leaf extract +	
---	--

**Table 5: Phytochemical screening** 

### 2.5 Evaluation parameters:

The formulated herbal mouthwash was subjected to a series of physicochemical and microbiological evaluations to ensure its quality, safety, efficacy, and stability. These evaluation parameters are crucial for standardizing the formulation and confirming its suitability for oral use [3,4,26,35,36,37].

## a) Organoleptic Evaluation:

Organoleptic properties such as colour, Odor, taste, appearance, and texture were assessed visually and sensorially. These attributes play a vital role in patient compliance and product acceptability in routine oral care.

## b) pH Determination:

The pH of the mouthwash was determined using pH indicator strips. An ideal mouthwash should maintain a pH close to neutral (5.5 to 7.0) to avoid mucosal irritation and protect tooth enamel from demineralization.

### c) Viscosity Measurement:

Viscosity, an important parameter influencing the ease of use and uniformity, was measured using an Ostwald viscometer. A mouthwash with appropriate viscosity ensures smooth flow, proper spreading in the oral cavity, and effective action of active ingredients.

## d) Foam Height Test:

The foam height test was performed to assess the surfactant action of the formulation. Foam production is associated with cleansing ability, though excessive foaming may reduce user comfort. A balance is necessary for optimal performance.

# e) Antimicrobial Activity:

The agar well diffusion method was used to evaluate the antimicrobial potential of the formulation against oral bacteria. This test involves inoculating culture media with microorganisms and measuring the zone of inhibition produced by the mouthwash.

## f) Stability Testing:

Short-term accelerated stability testing was carried out by exposing the formulations to varying temperature conditions (4°C and room temperature). Observations for changes in colour, Odor, clarity, phase separation, and pH were made over a fixed period to assess physical stability.

### 3. RESULT

The formulated herbal mouthwashes (F1–F6) were systematically evaluated through a comprehensive series of physicochemical and microbiological parameters to determine their overall quality, therapeutic efficacy, formulation stability, and user acceptability. These assessments were essential to establish whether the mouthwash formulations met the desirable standards for routine oral care products. The evaluation included analysis of organoleptic properties, pH, viscosity, foam height, antimicrobial activity, and short-term stability, each of which plays a significant role in determining the product's performance, safety, and shelf-life under practical usage conditions.

#### 3.1 Organoleptic Evaluation

All six formulations were found to be clear and homogenous liquids with a pleasant herbal or minty odor. The taste was acceptable, with a mild to slightly spicy flavor due to the presence of *Pongamia pinnata* extract, clove oil, and peppermint oil. The appearance and color varied from fluorescent yellow to dark brown depending on the concentration of the extract, but all remained aesthetically acceptable [3,36].

Formulation no.	Parameters	Observation
F1	Colour	Florescent yellow

	Odour	Pleasant (Cool Mint)
	Appearance	Clear and Homogeneous
	Texture	Liquid
	Taste	Minty & slightly spicy flavour
F2	Colour	Golden yellow
	Odour	Pleasant (Herbal/Mild Clove)
	Appearance	Clear and Homogeneous
	Texture	Liquid
	Taste	Minty & slightly spicy flavour
F3	Colour	Brown
	Odour	Pleasant Herbal Odor
	Appearance	Clear and Homogeneous
	Texture	Liquid
	Taste	Minty & Spicy flavour
F4	Colour	Yellow
	Odour	Pleasant (Cool Mint)
	Appearance	Clear and Homogeneous
	Texture	Liquid
	Taste	Minty & slightly spicy flavour
F5	Colour	Brown
	Odour	Pleasant (Herbal/Mild Clove)
	Appearance	Clear and Homogeneous
	Texture	Liquid
	Taste	Minty & slightly spicy flavour
Formulation no.	Parameters	Observation
F6	Colour	Dark Brown
	Odour	Pleasant Herbal Odor
	Appearance	Clear and Homogeneous
	Texture	Liquid
	Taste	Minty & spicy flavour

Table 6: Organoleptic evaluation Table

### 3.2 pH Measurement

The pH values of all formulations were in the ideal range of 6–7, which is close to the pH of natural saliva. This ensures that the formulations are safe for oral use and do not cause irritation or enamel erosion. pH values of all formulations were in the ideal range of 6–7, which is close to the pH of natural saliva. This ensures that the formulations are safe for oral use and do not cause irritation or enamel erosion [3,26,36,37].



Figure 4: pH paper

## 3.3 Viscosity

Using an Ostwald viscometer, the viscosity of the formulations was found to range between 1.00–1.34 cP, which is slightly above the viscosity of water (0.89 cP). This level of viscosity facilitates ease of rinsing and ensures even distribution in the oral cavity [3,37].



Figure 5: Ostwald viscometer

Sample name	Time Taken by Water (t <sub>1</sub> ) (sec)	Time Taken by Sample (t <sub>2</sub> ) (sec)	Viscosity of Water (η <sub>1</sub> ) (cP)		Density of Sample	Viscosity of Sample (η <sub>2</sub> ) (cP)
Distilled Water	31	-	0.83	1	1	0.83
F1	31	43	0.83	1	1.008	1.10
F2	31	45	0.83	1	1.007	1.21
F3	31	46	0.83	1	1.005	1.23
F4	31	45	0.83	1	1.03	1.24
F5	31	47	0.83	1	1.07	1.34
F6	31	46	0.83	1	1.08	1.33

Journal of Neonatal Surgery | Year: 2025 | Volume: 14 | Issue: 31s

### **Table 7: Viscosity table**

### 3.4 Foam Height

The foam height ranged from 2.1 to 3.1 cm across formulations. This indicated adequate surfactant activity from Tween 80 and PEG 400, contributing to cleansing without excessive foaming [3].

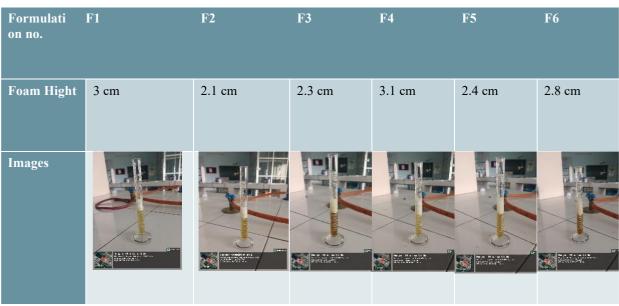


Table 8: Foam hight table

## 3.5 Antimicrobial Activity (Zone of Inhibition):

The agar well diffusion method revealed that formulations F2, F3, and F6 exhibited strong antimicrobial activity against oral bacteria isolated from human saliva, with zones of inhibition measuring up to 20 mm. Formulations F1, F4, and F5 showed moderate activity, while the control (distilled water) exhibited no inhibitory effect [4,26,35,36,48].

Sample Name	Test Organism	Zone of Inhibition (mm)	Remarks
F1	Oral bacteria (from human saliva)	16	Moderate antibacterial activity
F2	Oral bacteria (from human saliva)	20	Strong antibacterial activity
F3	Oral bacteria (from human saliva)	18	Strong antibacterial activity
F4	Oral bacteria (from human saliva)	17	Moderate antibacterial activity
F5	Oral bacteria (from human saliva)	15	Moderate antibacterial activity
F6	Oral bacteria (from human saliva)	20	Strong antibacterial activity
Distilled water	Oral bacteria (from human saliva)	0	No activity

**Table 9: Zone of Inhibition table** 

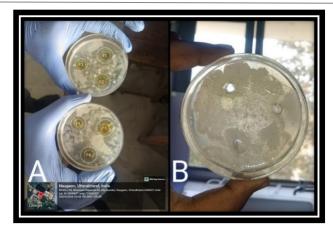


Figure 6: Zone of Inhibition Test

(A = Test with respected Mouthwash formulations, B = Test with Distilled water)

## 3.6 Stability Testing:

During short-term stability testing under alternating temperatures (4°C and room temperature), all formulations remained physically stable with no observable changes in color, odor, or phase separation. The pH also remained within the optimal range throughout the testing period, indicating good short-term stability of the mouthwash formulations [3,26].

Formulation no.	Parameters	In 4°C Temperature	In Room Temperature
F1	Change in colour	No Change	No Change
	Odour	No Change	No Change
	Physical Separation	No Change	No Change
F2	Change in colour Odour	No Change	No Change
	Odour	No Change	No Change
	Physical Separation	No Change	No Change
F3	Change in color	No Change	No Change
	Odour	No Change	No Change
	Physical Separation	No Change	No Change
F4	Change in colour	No Change	No Change
	Odour	No Change	No Change
	Physical Separation	No Change	No Change
F5	Change in colour	No Change	No Change
	Odour	No Change	No Change
	Physical Separation	No Change	No Change
F6	Change in color	No Change	No Change
	Odour	No Change	No Change
	Change in colour	No Change	No Change

Table 10: Stability Testing Table

## 4. DISCUSSION

This study successfully formulated and evaluated a series of herbal mouthwash preparations containing *Pongamia pinnata* 

leaf extract, aiming to provide a natural and effective alternative to conventional chemical-based mouthwashes. Soxhlet extraction using a 70% hydroalcoholic solvent was employed to ensure optimal recovery of phytoconstituents such as flavonoids, tannins, alkaloids, saponins, and glycosides, known for their antimicrobial and anti-inflammatory properties [3,17,38,39].

The formulations incorporated essential oils (clove and peppermint) for added antimicrobial activity and flavor enhancement, while Tween 80 and PEG 400 served as emulsifying agents to stabilize the oil-based ingredients. Glycerol was used as a humectant to improve texture and moisture retention, and sodium chloride acted as a preservative and tonicity agent [3,24,25,26,32,33,34,35,36,44].

All formulations demonstrated favourable physicochemical properties, with pH ranging between 6–7, viscosity within 1.00–1.34 cP, and foam height within acceptable limits—indicating suitability for oral use. The mouthwashes also showed good organoleptic properties, including pleasant aroma and appearance, which are crucial for patient compliance. Agar well diffusion tests revealed that F2, F3, and F6 had significant zones of inhibition, confirming the antimicrobial efficacy of *Pongamia pinnata* against oral pathogens isolated from human saliva [3,4,26,35,36,37].

Phytochemical screening confirmed the presence of multiple bioactive compounds that likely contribute to the observed antimicrobial activity. Furthermore, all formulations remained physically stable under accelerated stability testing, showing no signs of phase separation or degradation over the study period [3,26]. These findings support the potential of *Pongamia pinnata*-based mouthwash as a safe, stable, and effective herbal oral hygiene product suitable for further clinical validation.

### 5. CONCLUSION

The present study successfully formulated and evaluated a herbal mouthwash containing *Pongamia pinnata* leaf extract for oral hygiene application. The formulation was developed using hydroalcoholic Soxhlet extraction and optimized with key excipients including Tween 80, PEG 400, clove oil, and peppermint oil. Evaluation of the formulations revealed desirable properties such as a near-neutral pH (6–7), acceptable viscosity (1.00–1.34 cP), and stable foam height (2.1–3.1 cm), all indicating suitability for oral use. Phytochemical screening confirmed the presence of active constituents like flavonoids, tannins, and alkaloids. Antimicrobial testing demonstrated strong inhibition zones in selected formulations (F2, F3, F6), supporting the therapeutic potential of the extract. Stability testing under varying temperature conditions confirmed the physical integrity and storage suitability of the formulations, positioning *Pongamia pinnata* as a promising herbal alternative to conventional chemical mouthwashes.

## 6. ACKNOWLEDGEMENT

The authors sincerely thank the School of Pharmacy and Research, Dev Bhoomi Uttarakhand University, Dehradun, for providing the essential infrastructure and support required to conduct this research. They extend their gratitude to the faculty members and technical staff for their cooperation and assistance during the formulation and evaluation stages. The authors also deeply appreciate the continuous guidance, encouragement, and mentorship offered by their research supervisors throughout the duration of this study.

## REFERENCES

- [1] Mishra, R., Pounikar, Y., & Gangwar, M. (2019). Extraction, Phytochemical screening and quantitative determination of phenols and flavonoids in extract of Kalanchoe pinnata and Pongamia pinnata. *Journal of Drug Delivery and Therapeutics*, 9(4), 192-196.
- [2] Vaishnavi, V. V., & Priya, S. E. (2024). Phytochemical and Therapeutic Potential of Pongamia pinnata Linn.-A Comparative Analysis of Fresh and Dry Leaf Extracts. *South Asian Journal of Experimental Biology*, 14(4).
- [3] Chaudhary, P., Sharma, R., Rupanar, S., Jadhav, S., Bongade, A., Shinde, P., & Gavit, S. (2023). Preparation and evaluation of herbal mouthwash containing hydroalcoholic extract of Pongamia pinnata. *Asian Journal of Biological and Life Sciences*, 12(1), 173.
- [4] Nazeri, R., Ghaiour, M., & Abbasi, S. (2019). Evaluation of antibacterial effect of propolis and its application in mouthwash production. *Frontiers in dentistry*, 16(1), 1.
- [5] McGrath, C., Clarkson, J., Glenny, A. M., Walsh, L. J., & Hua, F. (2023). Effectiveness of mouthwashes in managing oral diseases and conditions: do they have a role?. *international dental journal*, 73, S69-S73.
- [6] Brookes, Z. L., McCullough, M., Kumar, P., & McGrath, C. (2023). Mouthwashes: implications for practice. *international dental journal*, 73, S98-S101.
- [7] Rodriguez-Archilla, A., & Muñoz-Martin, M. (2019). Evaluation of different mouthwashes for the treatment of halitosis. *International Dental & Medical Journal of Advanced Research*, 5(1), 1-7.
- [8] Sarvizadeh, M., Hemati, S., Meidani, M., Ashouri, M., Roayaei, M., & Shahsanai, A. (2015). Morphine mouthwash for the management of oral mucositis in patients with head and neck cancer. *Advanced biomedical*

- research, 4(1), 44.
- [9] Farah, C. S., McIntosh, L., & McCullough, M. J. (2009). Mouthwashes. Australian prescriber, 32(6).
- [10] Sparabombe, S., Monterubbianesi, R., Tosco, V., Orilisi, G., Hosein, A., & Ferrante, L. (2019). Efficacy of an all-natural polyherbal mouthwash in patients with periodontitis: a single-blind randomized controlled trial. Front Physiol. 2019 May; 10: 632.
- [11] American Dental Association. (n.d.). *Mouthrinse* (*Mouthwash*). https://www.ada.org/resources/ada-library/oral-health-topics/mouthrinse-mouthwash
- [12] Sahebjamee, M., Mansourian, A., Hajimirzamohammad, M., Zadeh, M. T., Bekhradi, R., Kazemian, A., ... & Doroudgar, K. (2015). Comparative efficacy of aloe vera and benzydamine mouthwashes on radiation-induced oral mucositis: a triple-blind, randomised, controlled clinical trial. *Oral Health Prev Dent*, 13(4), 309-15.
- [13] Raja, M., Saha, S., Reddy, V. K., Mohd, S., & Kumari, M. (2013). Mouthwashes-an overview of current knowledge. *Int J Oral Health Res Rev*, 1(2), 24-28.
- [14] Xiang, Y., Ren, X., Xu, Y., Cheng, L., Cai, H., & Hu, T. (2022). Anti-inflammatory and anti-bacterial effects of mouthwashes in intensive care units: a systematic review and meta-analysis. *International journal of environmental research and public health*, 20(1), 733.
- [15] MIhajlo, P., Olivera, T. P., Tamara, T., & Kiro, P. (2022). Side Effects Associated with Chlorhexidine Mouthwashes Use. *Maced. Pharm. Bull.*, 68, 377-378.
- [16] Świątek, I. M., & Adamska-Szewczyk, A. (2024). An overview of the potential use of plants in oral care products. *Medicinal Plant Biology*, 3(1).
- [17] Al Muqarrabun, L. M. R., Ahmat, N., Ruzaina, S. A. S., Ismail, N. H., & Sahidin, I. (2013). Medicinal uses, phytochemistry and pharmacology of Pongamia pinnata (L.) Pierre: A review. *Journal of ethnopharmacology*, 150(2), 395-420.
- [18] Cushnie, T. T., & Lamb, A. J. (2005). Antimicrobial activity of flavonoids. *International journal of antimicrobial agents*, 26(5), 343-356.
- [19] Richards, D. (2015). Chlorhexidine mouthwash more effective than dentifrice or gel. *Evidence-Based Dentistry*, 16(2), 59-59.
- [20] Salehi, B., Jornet, P. L., López, E. P. F., Calina, D., Sharifi-Rad, M., Ramírez-Alarcón, K., ... & Sharifi-Rad, J. (2019). Plant-derived bioactives in oral mucosal lesions: a key emphasis to curcumin, lycopene, chamomile, aloe vera, green tea and coffee properties. *Biomolecules*, 9(3), 106.
- [21] Ghumare, P., Jirekar, D. B., Farooqui, M., & Naikwade, S. D. (2014). A review of Pongamia pinnata—an important medicinal plant. *Current Research in Pharmaceutical Sciences*, 44-47.
- [22] Baldeón, V., Hernández, A., Tapia, S., & Rodriguez, A. (2025). Oral health-related conditions in Ecuador: A temporal trend analysis of hospital discharges from 2000–2023. *PloS one*, 20(1), e0317440.
- [23] Yazicioglu, O., Ucuncu, M. K., & Guven, K. (2024). Ingredients in commercially available mouthwashes. *international dental journal*, 74(2), 223-241.
- [24] Radzki, D., Wilhelm-Węglarz, M., Pruska, K., Kusiak, A., & Ordyniec-Kwaśnica, I. (2022). A fresh look at mouthwashes—what is inside and what is it for?. *International journal of environmental research and public health*, 19(7), 3926.
- [25] Alipour, S., Dehshahri, S., & Afsari, A. (2018). Preparation and Evaluation of a Herbal mouthwash containing Oak Husk of Quercus brantii and Zataria multiflora. *Jundishapur J Nat Pharm Prod*, 13(3), e13420.
- [26] Winarti, C., Hamaisa, A., Lesmayati, S., Misgiyarta, M., & Gusmaini, G. (2024). Emulsifier ratio and shock treatment effect on the characteristics of eucalyptus–peppermint oil-based mouthwash nanoemulsion. *Journal of Applied Pharmaceutical Science*, 14(12), 083-089.
- [27] Shirsat, R., Kengar, A., & Rai, A. (2021). HPTLC profile and screening of antimicrobial activity of pongamia pinnata pierre. *Journal of Pharmaceutical Research International*, 33(30A), 205-215.
- [28] Singh, A., Jahan, I., Sharma, M., Rangan, L., Khare, A., & Panda, A. N. (2016). Structural characterization, in silico studies and in vitro antibacterial evaluation of a furanoflavonoid from karanj. *Planta Medica Letters*, 3(04), e91-e95.
- [29] Shankar, M. (2020). Molecules of Interest-Karanjin-A Review.
- [30] Pandey, A. K., Bajpai, A. K., Kumar, A., Pal, M., Baboo, V., & Dwivedi, A. (2014). Isolation, Identification, Molecular and Electronic Structure, Vibrational Spectroscopic Investigation, and Anti-HIV-1 Activity of Karanjin Using Density Functional Theory. *Journal of Theoretical Chemistry*, 2014(1), 680987.
- [31] Nadeem, F., Inam, S., Rashid, U., Kainat, R., & Iftikhar, A. (2016). A review of geographical distribution,

- phytochemistry, biological properties and potential applications of Pongamia pinatta. *Int. J. Chem. Biochem. Sci*, 10, 79-86.
- [32] Carter, J. L., Kelly, C. A., & Jenkins, M. J. (2023). Processing optimization of PEDOT: PSS and PEDOT: PSS/Tween 80 films. *Polymer Journal*, 55(3), 253-260.
- [33] Benedini, L., Messina, P. V., Palma, S. D., Allemandi, D. A., & Schulz, P. C. (2012). The ascorbyl palmitate–polyethyleneglycol 400–water system phase behavior. *Colloids and Surfaces B: Biointerfaces*, 89, 265-270.
- [34] Yosephine, A. D., Wulanjati, M. P., Saifullah, T. N., & Astuti, P. (2013). Mouthwash Formulation of basil oil (Ocimum basilicum L.) and in vitro Antibacterial and Antibiofilm Activities Against Streptococcus mutans. *Majalah Obat Tradisional*, 18(2), 95-102.
- [35] Chaudhary, P., Dhingra, G., & Pingale, R. (2024). GC-MS Evaluation of Bioactive Constituents of Hydroalcoholic Leaves Extract of Pongamia pinnata with Antibacterial Potential of Prepared Mouthwash Formulations. *Research Journal of Pharmacy and Technology*, 17(10), 4811-4816.
- [36] Iskandar, B., Lukman, A., Syaputra, S., Al-Abrori, U. N., Surboyo, M. D., & Lee, C. K. (2022). Formulation, characteristics and anti-bacterial effects of Euphorbia hirta L. mouthwash. *Journal of Taibah University Medical Sciences*, 17(2), 271-282.
- [37] Stefani, R., Vanessa, G. E., & Fibryanto, E. (2023). Evaluation of pH and Viscosity of Juice of Zingiber officinale var. officinale Mouthwash Formulation. *Majalah Kedokteran Gigi Indonesia*, 9(2), 163-170.
- [38] Ghumare, P., Jirekar, D. B., Farooqui, M., & Naikwade, S. D. (2014). A review of Pongamia pinnata—an important medicinal plant. *Current Research in Pharmaceutical Sciences*, 44-47.
- [39] Mahanom, H., Azizah, A. H., & Dzulkifly, M. H. (1999). Effect of different drying methods on concentrations of several phytochemicals in herbal preparation of 8 medicinal plants leaves. *Malaysian Journal of Science*, 5(1 & 2), 47-54.
- [40] Menpara, D., & Chanda, S. (2014). Phytochemical and pharmacognostic evaluation of leaves of Pongamia pinnata L.(Fabaceae). *Pharma. Comm*, 4(2), 3-7.
- [41] Cortés-Rojas, D. F., de Souza, C. R. F., & Oliveira, W. P. (2014). Clove (Syzygium aromaticum): a precious spice. *Asian Pacific journal of tropical biomedicine*, 4(2), 90-96.
- [42] Krishna, S. G., Reddy, K. A., Kumar, M. S., Ramu, G., Rajeswari, B. U., & Kiranmai, M. EFFECT OF POLYHERBAL COMBINATIONS AND ESSENTIAL OILS AGAINST BIOFILM OF STREPTOCOCCUS MUTANS.
- [43] National Center for Biotechnology Information. (n.d.). *Glycerin*. PubChem Compound Summary. U.S. National Library of Medicine. https://pubchem.ncbi.nlm.nih.gov/compound/Glycerin
- [44] National Center for Biotechnology Information. (n.d.). *Polyethylene Glycol 400*. PubChem Compound Summary. U.S. National Library of Medicine. https://pubchem.ncbi.nlm.nih.gov/compound/Polyethylene-glycol-400
- [45] National Center for Biotechnology Information. (n.d.). *Polysorbate 80*. PubChem Compound Summary. U.S. National Library of Medicine. https://pubchem.ncbi.nlm.nih.gov/compound/Polysorbate-80
- [46] National Center for Biotechnology Information. (n.d.). *Sodium Saccharin*. PubChem Compound Summary. U.S. National Library of Medicine. https://pubchem.ncbi.nlm.nih.gov/compound/Sodium-saccharin
- [47] National Center for Biotechnology Information. (n.d.). *Sodium Chloride*. PubChem Compound Summary. U.S. National Library of Medicine. https://pubchem.ncbi.nlm.nih.gov/compound/Sodium-chloride
- [48] Nalawade, T. M., Bhat, K. G., & Sogi, S. (2016). Antimicrobial activity of endodontic medicaments and vehicles using agar well diffusion method on facultative and obligate anaerobes. International journal of clinical pediatric dentistry, 9(4), 335.