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PREPARATION AND COMPARATIVE EVALUATION OF POLY-HERBAL TOOTHPASTE FORMULATIONS USING CLOVE OIL AND PONGAMIA PINNATA SEED EXTRACT

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ABSTRACT

The present study deals with the formulation and evaluation of a polyherbal toothpaste with more emphasis on minimizing the use of synthetic additives. Increasing concern about the toxicities related to the ingredients like parabens, sodium lauryl sulfate, saccharin, and fluoride has enhanced interest in the herbal oral care products as safer alternatives to the conventional types.[31-33] Herbal toothpastes have been found to maintain oral hygiene apart from their therapeutic benefits resulting from the bactericidal and anti-inflammatory properties of medicinal herbs.[3,10,21,23] The selected herbal seed extract was employed as an active ingredient in formulation based on the reportedly antimicrobial and analgesic activities attributed to bioactive compounds such as karanjin and pongamol, besides the compound eugenol present in clove oil.[22,27,35,36] Three formulations (F1, F2, and F3) were prepared by changing the concentration of microcrystalline cellulose and sodium carboxymethyl cellulose while keeping all other excipients constant. Herbal seed extract was prepared using 70% ethanolic extraction and incorporated into the toothpaste base along with suitable excipients.[5,6,12] Two methods for the formulation were assessed, and it was found that formulation method 1 provided more stable and standardized preparations of toothpaste. Physicochemical and microbiological assessments evidenced that formulation F1 was more stable and presented antimicrobial activities significantly higher than the other formulations and the commercially available herbal toothpaste products.[6,9,13,23,31] **NOVELTY OF WORK:** This paper highlights the design and development of a polyherbal toothpaste formulation using Pongamia pinnata seed extract and clove oil, which exhibit synergistic antibacterial properties with less usage of synthetic chemicals.[22,27,36] Contrary to most previous reports that exclusively emphasized individual herbal substances in toothpaste formulation, this paper comprehensively endeavors to maximize herbal formulation in terms of natural polymer concentrations, specifically sodium carboxy-methyl cellulose (SCMC) and microcrystalline cellulose (MCC).[1,4,7,14] The results showed an effective formulation with bactericidal potency compatible with and better than that in most herbal and fluoride-based toothpaste reported in previous literature.[3,9,13,23,31] The proposed formulation also provides a practically productive, biological, and safe method in making a beneficial herbal preparation for oral health.[6,12,25,34]

KEYWORDS: Polyherbal toothpaste; Clove oil; Pongamia pinnata; Herbal formulation; Antimicrobial activity; Oral hygiene.

1. INTRODUCTION

Oral health is a basic aspect of general well-being and is also closely related to toothpaste usage in preventing oral infections, caries of teeth, and periodontal diseases.[3,13,31] However, during recent years, their extensive application in toothpaste formulation in terms of fluoride and their combinations in the form of sodium lauryl sulfate (SLS), parabens, and artificial sweeteners has generated severe concerns regarding their inadmissible side effects of mucosal irritation, tooth enamel degradation, hypersensitivity, and allergies.[31-33] These worries and apprehensions initiated increasing demand worldwide for an emerging interest in the development of oral healthcare products using herbal and natural ingredients without exposure to unnatural additives.[23,32]

Herbal toothpaste consists of plant-derived bioactive compounds proven for their antimicrobial, anti-inflammatory, and analgesic qualities, thus ensuring better oral hygiene and gobular health.[3,10,21] Various research works have proved that some common herbs used in dentifrices like Azadirachta indica (Neem), Salvadora persica (Miswak), Acacia arabica (Babool), and clove have been highly successful in eliminating the microbial load in the mouth as well as keeping the periodontium healthy.[8,13,23] Of these, clove (Eugenia caryophyllus) oil has been well known for its strong antimicrobial, antiseptic, and analgesic properties.[22,27] Its active phytoconstituents, eugenol, have been shown to display a strong inhibitory effect against some common bacteria that occur in the mouth like Streptococcus mutans, Staphylococcus aureus, and Candida albicans.[21,22]

Pongamia pinnata (L.), also referred to as Karanja, is an herb used in traditional medicines that falls under the Fabaceae family. The oil extracted from the seed of this herb is most abundant in biologically active phyto-compounds like karanjin, pongamol, and flavonoids, which have been shown to have potent antibacterial, antifungal, and anti-inflammatory properties.[24,35] Numerous studies have validated the use of P. pinnata seed extract to have antimicrobial properties against both Gram-positive and Gram-negative bacteria, primarily due to its high concentration of phenolic and flavonoid compounds.[26,30,35]

Although there is copious literature available for the antimicrobial potential of clove oil as well as P. pinnata, there is a great need for the joint use of these two components in the formulation of toothpaste preparations. There remains a lacuna in the optimization of natural polymer additives like NaCMC and MCC to render stability, increased performance, along with greater acceptability of the formulation while having a predominantly natural background.[1,4,7] The current study aimed to synthesize three polyherbal toothpaste formulations (F1, F2, F3) utilizing the P. pinnata seed extract and clove oil for the evaluation of the physicochemical parameters like pH value, spreadability, ability to foam, fineness, along with antimicrobial properties. The optimized sample was also compared for the relative potential of similar commercially available herbal toothpaste preparations like Meswak and Babool to check the safety along with the effective use of oral cavity requirements.[6,9,23]

2. LITERATURE REVIEW

There is documented evidence of the antimicrobial, anti-inflammatory, and medicinal uses of clove oil and Pongamia pinnata extracts in scientific literature, and such uses prove their effectiveness and suitability for herbal oral care compositions. There is ample scientific evidence to support the fact that herbal toothpaste compositions containing plant material active ingredients demonstrate satisfactory physicochemical property and effective antimicrobial action against oral pathogens.[1,4,6,7]

The role of natural polymers like sodium carboxymethyl cellulose (NaCMC) in herbal tooth paste as a binder and thickening agent has been explored in depth. The optimization studies involving NaCMC concentrations revealed an important contribution in making the herbal formulation better in terms of its consistency, spreadability, and stability, without impeding its biological efficacy.[5,7,8] The similar approach in herbal formulation design has been practiced in the development of polyherbal tooth paste formulation systems, with a controlled variation in natural excipients.[11,12,14]

The Pongamia pinnata species has been extensively explored for its use in antibacterial, antioxidant, and anti-inflammatory activities. The presence of phytochemicals in P. pinnata seed extracts, namely karanjin, pongamol, and flavonoids, has been established to possess remarkable antibacterial and antifungal properties against both Gram-positive and Gram-negative bacteria.[24,26,30,35] Apart from its antibacterial properties, P. pinnata has been proven to possess significant anti-inflammatory properties and can thus be a promising remedy in gingival and periodontal treatment in oral health.[35] Various studies have been successful in confirming the ability to incorporate herbal extracts with similar phytochemical compositions into mouthwashes and have proven them to be safe and effective in oral health management.[16-19]

Clove oil (Eugenia caryophyllus) is also a well-documented herbal substance used in dental care products due to its pronounced antimicrobial, analgesic, and anti-inflammatory effects. The major active component of clove oil, named eugenol, has demonstrated inhibitory activity against prominent oral pathogens such as Streptococcus mutans, Staphylococcus aureus, and Candida albicans as well as analgesic activity.[21,22,27] Herbal toothpaste mixtures because of clove oil or a mixture of different medicinal herbs have been proved to possess superior antimicrobial activity with respect to oral hygiene outcome.[3,10,23]

Although various studies have focused on herbal toothpaste formulations using individual plant extracts or their combinations, such as clove combined with other medicinal herbs, scant attention has been given to the single application of both P. pinnata seed extract and clove oil in one formulation, together with optimization of natural polymer concentrations, such as NaCMC and MCC. Thus, a considerable literature gap still exists on systematic studies concerning this issue for the development of an optimization strategy aimed at maintaining product stability and efficacy by minimizing synthetic additives in predominantly herbal formulations.

2.1 Introduction to Oral Health and the Role of Toothpaste

Oral health is an integral part of health and is an important area for the prevention of dental infections, dental caries, and diseases of the periodontal tissues.[3,13,31] Toothpaste is one of the most common dental care products used and has a very important function in the control of dental plaque and in cleaning the mouth.[32]

The conventional formula of toothpaste generally includes synthetic compounds like fluoride compounds, sodium lauryl sulfate, parabens, and sugar substitutes. Although these agents help reduce plaque, create foaming action, and remineralize enamel, their prolonged use has been linked to adverse reactions of mucosal irritation, enamel erosion, hypersensitivity, and allergic reactions, respectively.[31-33] Due to safety issues, there has been mounting concern about the prolonged use of synthetic agents in dentifrices.[23]

Consequently, there has been an increasing trend towards the development of toothpaste formulation that makes use of natural ingredients to have similar benefits to traditional toothpaste formulation but have fewer possible side effects. Herbal toothpaste formulation that has been shown to contain antimicrobial, anti-inflammatory, and therapeutic properties has been proven to be promising in replacing traditional toothpaste formulation.[3,10,21]

2.2 Herbal Toothpaste Formulations

Herbal toothpastes are formulated using plant-derived extracts that possess well-documented antimicrobial, anti-inflammatory, and analgesic properties, making them effective agents for maintaining oral hygiene.[3,10,21] Commonly used botanical ingredients in herbal dentifrices include Azadirachta indica (Neem), Salvadora persica(Miswak), Acacia arabica (Babool), and Eugenia caryophyllus(Clove), all of which have demonstrated significant efficacy in reducing the growth of oral pathogenic microorganisms and improving gingival health.[8,13,23] In addition to their therapeutic benefits, herbal toothpaste formulations are generally biocompatible, eco-friendly, and cost-effective, contributing to their increased acceptance among consumers.[25,32]

Beyond their antimicrobial activity, several studies have reported that herbal toothpaste products aid in plaque reduction, tooth whitening, and the management of oral halitosis, thereby providing comprehensive oral care benefits comparable to or superior to conventional formulations.[3,13,31] These multifunctional properties highlight the potential of herbal dentifrices as safe and effective alternatives to synthetic toothpaste products.

2.3 Role of Clove Oil (*Eugenia caryophyllus*) in Oral Care

Clove oil has long been employed for dental uses due to its strong antimicrobial, analgesic, and antiseptic properties. The key bioactive compound eugenol, found in clove oil, shows broad-spectrum antimicrobial efficacy against typical oral bacteria such as Streptococcus mutans, Staphylococcus aureus, and Candida albicans, which plays a pivotal role in the prevention of dental cavities and oral infections.[21,22,27] Alongside the strong antimicrobial properties, eugenol also reveals local anesthetic and anti-inflammatory effects that provide relief from toothache, gum inflammation, and discomfort associated with oral lesions.[22]

Researchers have shown the potent antibacterial properties of clove oil preparations that can be adapted in oral care products. Herbal toothpaste/dentifice formulation incorporating clove oil has shown potent antimicrobial activity along with desirable properties such as pleasant taste, leading to improved patient compliance.[3,10,23] These studies justify the use of clove oil in oral care products because of its natural properties.

2.4 Pharmacological Significance of Pongamia pinnata

Pongamia pinnata, or commonly known as Karanja, which belongs to family Fabaceae, has been traditionally used in many systems of medicine, especially in Ayurveda.[21] P. pinnata seeds have been known to be rich in bioactive phytochemicals like karanjin, pongamol, and flavonoids with marked antimicrobial, anti-inflammatory, and antioxidant properties.[24,26,35] P. pinnata has been proven to possess strong antibacterial properties in in vitro studies against both Gram-positive and Gram-negative bacteria such as Staphylococcus aureus and Pseudomonas aeruginosa.[26,30,35] The strong antimicrobial property in P. pinnata has been attributed to its high phenolic and flavonoid contents that suppress bacterial growth and biofilm formation.[26,35] All these medicinal potentials and biocompatibility of P. pinnata, apart from being a potential candidate in MUAs, have tempted scientists to explore its applications in many oral health formulations like toothpaste and mouthwashes.[12,14,25]

2.5 Development and Evaluation of Herbal Toothpaste

There have been a number of studies related to the formulation and assessment of herbal toothpaste that have been done utilizing diverse extracts of herbs. For example, the formulation of polyherbal toothpaste that includes the use of Azadirachta indica, clove, and various herbs has been observed to exhibit desirable physicochemical properties, such as stability, spreadability, and ability to produce foam, in addition to good antimicrobial action against the microorganisms present in the mouth.[1,7,10]

Formulations with Salvadora persica (Miswak), peppermint, and other essential oils have been found to have good antibacterial properties against Streptococcus mutans along with acceptable gustatory and foam ability properties.[8,11,13] Various herbal toothpaste formulas have also shown acceptable cleaning properties, along with texture and acceptability, using extracts of Guave (Psidium guajava) and Mango (Mangifera indica) leaves, along with their preservative and antioxidant properties.[26-29]

The need to explore herbal toothpaste formulation capabilities despite all the challenges was brought to light through these studies, thereby making it possible to create herbal toothpaste formulas that have desired properties along with their therapeutant properties. The need to optimize herbal combinations or excipients, including natural polymers like sodium carboxymethyl cellulose (NaCMC), microcrystalline cellulose (MCC), among others, was pointed out.[4,6,12]

2.6 Research Gap and Rationale for the Current Study

Although the pharmacological profile of clove oil and P. pinnata is well established, there is limited literature available that deals with their synergistic use in one toothpaste formulation. Moreover, natural polymers like NaCMC and microcrystalline cellulose are not sufficiently optimized scientifically for the attainment of formulation stability along with consumer acceptability. In this context, the present study was designed to formulate and evaluate three polyherbal toothpaste formulations (F1–F3) which contain both clove oil and P. pinnata extract. Formulations were subjected to comparative analysis with marketed herbal toothpastes, Meswak and Babool, based on parameters like pH, spreadability, foaming ability, fineness, and antimicrobial activity.

The literature supports the use of clove oil and Pongamia pinnata in dentistry because of their antimicrobial, analgesic, and anti-inflammatory properties. However, literature exploring their combined use in toothpaste in optimized, stable, and consumer-acceptable formulations is limited. Therefore, this current scientific void in this area of research will be addressed in this proposed work by developing a biocompatible, inexpensive polyherbal toothpaste with desirable physicochemical properties to help accelerate natural-based products in dentistry towards development.[10,16,23]

3. BENEFITS OF HERBAL TOOTHPASTE

Herbal toothpaste is gaining increasing popularity as a safe and effective alternative to common synthetic preparations. Herbal toothpaste is a blend of natural ingredients that not only clean teeth but also have therapeutic and antimicrobial properties. The advantages can be enumerated below:

3.1 Free from Harmful Chemicals

Herbal toothpastes are formulated without synthetic additives such as sodium lauryl sulfate (SLS), parabens, triclosan, or artificial sweeteners. This minimizes the risk of oral mucosal irritation, enamel erosion, and allergic reactions commonly associated with chemical-based toothpastes.

3.2 Natural Antimicrobial Action

Many herbal ingredients such as Clove oil (Eugenia caryophyllus), Neem, Pongamia pinnata, and Miswak exhibit potent antibacterial and antifungal properties. These help inhibit the growth of oral pathogens responsible for dental caries, gingivitis, and halitosis (bad breath).

3.3 Anti-inflammatory and Analgesic Properties

Phytoconstituents such as eugenol (in clove oil) and karanjin (in Pongamia pinnata) provide soothing and anti-inflammatory effects on gums, reducing pain, swelling, and sensitivity associated with gum infections.

3.4 Promotes Oral Hygiene Naturally

Herbal toothpastes clean teeth effectively, remove food debris, and help maintain oral freshness through natural flavoring agents such as peppermint or menthol, without causing dryness of the oral mucosa.

3.5 Biocompatible and Eco-friendly

Being composed of plant-based and biodegradable ingredients, herbal formulations are safer for both the human body and the environment. They cause less water pollution compared to synthetic products.

3.6 Enamel-Friendly Abrasives

Natural abrasives like calcium carbonate and silica derived from herbal sources gently remove stains and plaque without damaging the tooth enamel.

3.7 Additional Therapeutic Benefits

Depending on the herbs used, such toothpastes may also provide antioxidant, whitening, anti-plaque, and breath-freshening effects. Ingredients such as Aloe vera, Tulsi, Babool, and Pongamia pinnata contribute to overall oral and gum health.

4. MATERIALS AND METHODS

4.1 Materials

4.1.1 Plant materials

While most people have had no Dried flower buds of Clove (Eugenia caryophyllus) were obtained from the certified herbal raw material supplier. The identity of the herbal material was confirmed by the process of organoleptic and macroscopic examination. Seeds of Pongamia pinnata (mature, healthy) were obtained from the campus of Vignan University, Andhra Pradesh, and the plant material was authenticated by the Department of Pharmacognosy, Vignan Pharmacy College, Andhra Pradesh.

Plant Pictures

➢ **Clove (*Eugenia caryophyllus*)**

Scientific Classification

- **Family:** Myrtaceae
- **Part Used:** Dried flower buds
- **Common Name:** Clove / Lavangam
- **Key Constituents:** Eugenol, Caryophyllene, and Acetyl eugenol

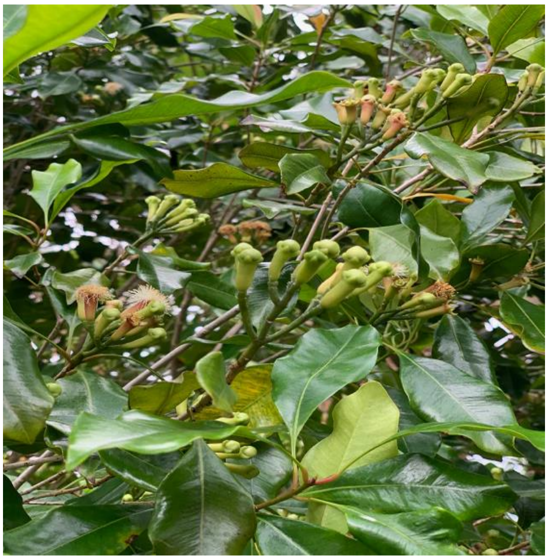


Figure 1: Clove plant.

➢ **Pongamia pinnata (Karanja)**

Scientific Classification

- **Family:** Fabaceae
- **Part Used:** Seeds / Seed oil
- **Common Name:** Karanja / Indian Beech Tree
- **Key Constituents:** Karanjin, Pongamol, and Flavonoids

Figure 2: Pongamia pinnata Plant.

4.1.2 Chemicals and excipients

All chemicals and excipients used were of analytical grade and obtained from commercial suppliers.

- Polymers: Microcrystalline cellulose (MCC), sodium carboxymethyl cellulose (NaCMC)
- Abrasives: Calcium carbonate
- Humectant: Glycerin
- Surfactant: Sodium lauryl sulfate (SLS)
- Preservatives: Sodium benzoate, methyl paraben
- Sweetener: Sodium saccharin
- Flavoring agent: Peppermint oil
- Opacifier: Titanium dioxide
- Solvents: Ethanol (70%) and distilled water

4.1.3 Equipment

Standard laboratory glassware and instruments were used, including mortar & pestle, Soxhlet apparatus, mechanical stirrer, digital pH meter, analytical balance, homogenizer. All glassware was cleaned and dried before use.

4.2 METHODS

4.2.1 Preparation of P. pinnata seed extract

All P. pinnata seeds collected were washed with clean water under running tap water, followed by drying under shaded conditions for 10-15 days. The dried samples were coarsely ground into powder and extracted with 70% ethanol using a Soxhlet apparatus for 48 hours. The sample was thenfiltered and reduced under reduced pressure conditions to produce a semi-solid substance for storage in an airtight container at 4°C for future use.

4.2.2 Preparation of herbal toothpaste formulations

Two methods were tested to obtain a stable and homogeneous toothpaste formulation:

Method 1 (Optimised method)

- Glycerin and the polymer (SCMC or MCC) were triturated together for 1 minute in a mortar and pestle.
- Sodium lauryl sulfate (surfactant) was added and then
- Preservative agents (sodium benzoate, methyl paraben) and purified water were

- Active compounds clove oil and seed extract of were added and well mixed for 15
- Sweetening agent (sodium saccharin), Abrasives (calcium carbonate)
- The final step was to add the flavouring agent (peppermint oil) and mix until a paste-like consistency was reached.
- Finally, the paste was packaged in clean, dry collapsible tubes.

Method 2
Binder and humectant were compounded with a small amount of the vehicle to make a dispersion. APIs were added to the mixture and mixed for a period of 2 minutes, followed by abrasives and surfactants. Humectant and preservatives were added to the mixture and homogenized to make a mixture of desired consistency. (The best result in the mixture consistency test was obtained using Method 1.)

4.2.3 Formulation design
Three formulations (F1, F2 and F3) were prepared by varying the polymer concentrations (SCMC and MCC) while keeping the quantities of other excipients constant.

4.3 Evaluation parameters
The prepared formulations were evaluated using standard physicochemical and microbiological parameters:

Table 1: Evaluation Parameters.

Parameter	Method/Instrument Used	Purpose
pH	Digital pH meter	To determine acidity/alkalinity suitable for oral use
Spreadability	Glass plate method	To assess ease of application
Hardness/Abrasiveness	Mechanical pressure test	To check smoothness and absence of sharp particles
Foaming Ability	Foam column test	To measure cleansing and surfactant efficiency
Fineness	Sieve analysis	To ensure smooth texture
Microbial Assay	Agar-well diffusion method	To determine antimicrobial activity against oral pathogens
Consumer Acceptability	Questionnaire-based study	To assess user perception and sensory attributes

All the experiments were performed in triplicate. The results were expressed in mean ± standard deviation, and comparison of the results was made with the commercially available herbal toothpaste like Meswak and Babool.

For the microbial germ killing activity: Petri dishes filled with the correct medium were used; a well template of 4 mm diameter x 3 mm depth was used to create the wells. Inocula were set up using the NCCLS recommended procedure following the 0.5 MacFarland turbidity standard. Using a sterile inoculating needle, the test toothpaste dispersions were introduced into the wells. The test conditions included incubating the samples at 37 °C for 48 h for E. coli and S. aureus. The sizes of the zone of inhibition in mm were measured following incubation. For instance, a test paste had a zone of 6 cm in size.

5. RESULTS AND DISCUSSION
5.1 Physicochemical Evaluation
The physicochemical analysis of the toothpaste formulation (F1 to F3), compared to commercial products like Meswak and Babool, was conducted to check their quality based on certain parameters. The findings are given in Table 1.

Hard and sharp-edged abrasive particles: None of the laboratory samples (F1 to F3) had hard and sharp-edged abrasive particles, similar to the commercial samples. This suggests that the abrasives, which are largely in the form of calcium carbonate, are in finely powdered form and evenly distributed in their bases.

Spreadability: The spreadability measurements varied between 5.2 cm for F1 and 6.1 cm for F3, which fell well within the tolerance limits of 8.5 cm. For the formulated samples, the highest spreadability of 6.1 cm was shown by F3, indicating the best semi-solid properties. This measure was comparable between the lab samples and Meswak (5.9 cm), as well as Babool (5.7 cm), indicating that the lab samples have similar viscosities to the commercial herbal toothpaste products.

pH determination: The pH for all the preparations was found to lie in the safe range of 5.5 to 10.5, and for the prepared preparations, the pH varied from 6.2 to 6.8. The pH close to normal reflects the compatibility of the preparations to the oral mucosa, hence preventing irritation and demineralization of the enamel. However, the alkaline pH of F3 (6.8) may be resulted by the buffering capacity of the sodium carboxymethyl cellulose and calcium carbonate.

Fineness: The fineness in all the formulas was in the range of 0.37% to 0.41% mass, well below the maximum permissible limit set in the standard, which was 0.5%. These results validate the idea that the abrasive particles were adequately fine and well dispersed to provide the smooth mouth sensation and cleaning action. These values were similar to Meswak (0.40%) and Babool (0.42%), thus validating the textural uniformity of the prepared formula.

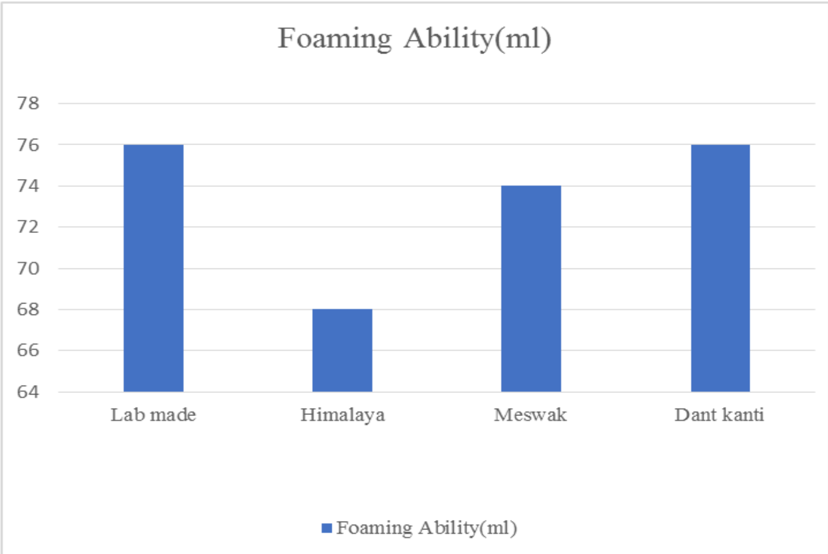
Foam formation: Foam formation values of laboratory-formulated products varied between 52 mL (F3) and 57 mL (F1), both of which were above the minimum standards required of at least 50 mL. The higher foam formation ability of F1 products (57 mL) is indicative of their greater surfactant properties, owing to the combined effect of sodium lauryl sulfate (SLS) and saponins naturally present in _Pongamia pinnata_ and clove oils.

The foam formation values of products are quite comparable to other commercial products Meswak (54 mL) and Babool (55 mL), thereby establishing that laboratory-formulated products have excellent cleaning and foam-forming properties as well. The comparative graph of foam formation ability also supports these observations and finds that laboratory-formulated products and Dant Kanti have greater foam formation ability (approximately 76 mL), and that of Himalaya products was relatively less (approximately 68 mL).

It was observed that all laboratory-formulated samples (F1–F3) fulfilled the requirements of physicochemical parameters for good-quality toothpaste. The samples showed favorable attributes for ruggedness, flowability, pH values, fineness, and foaming capacity equal to or somewhat better than commercially available herbal toothpaste products. Of the three, F1 had an edge over the other two with respect to foaming property and textural uniformity, indicating that this formulation has the potential for the development of safe herbal toothpaste.

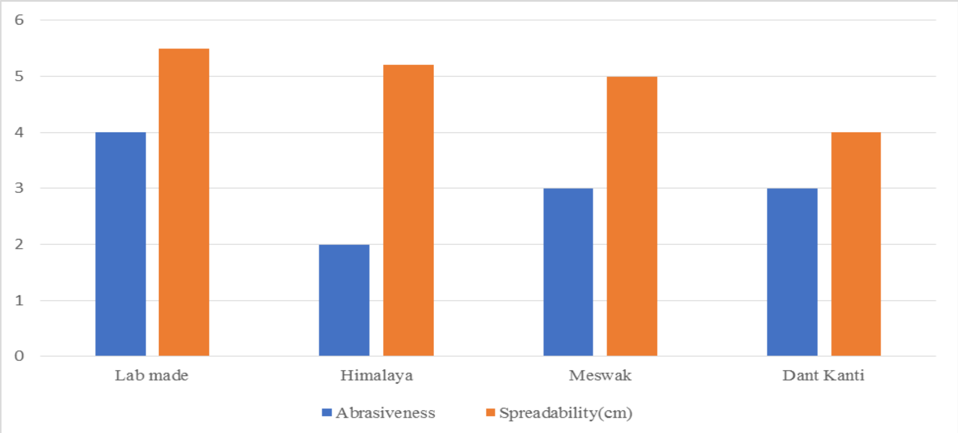
Table 2: Evaluation tests for the Herbal Tooth Paste.

S. No	Evaluation Tests	F_1	F_2	F_3	Meswak	Babool	Standard values
1.	Hard- and sharp-edged abrasive particles	Absent	Absent	Absent	Absent	Absent	Absent
2.	Spread ability (cms)	5.2	5.6	6.1	5.9	5.7	(Max.)8.5
3.	PH	6.2	6.5	6.8	6.9	6.4	5.5-10.5
4.	Fineness (% by mass)	0.41	0.37	0.39	0.40	0.42	(Max)0.5
5.	Foam Formation (ml)	57	54	52	54	55	Min 50 ml



Graph 1: Tooth Paste Herbal Formulation Activity.

Activity comparison of prepared toothpaste with in the marketed tooth paste



Graph 2: Comparative evaluation of abrasiveness and spreadability of lab-made and marketed herbal toothpastes.

The bar graph represents the comparison of abrasiveness and spreadability among four toothpaste samples Lab-made formulation, Himalaya, Meswak, and Dant Kanti.

Abrasiveness

The lab-made formulation showed a moderate value for abrasiveness of around 4, which is greater than Himalaya at about 2 and similar to Meswak and Dant Kanti at about 3 each. This means that the lab-made formulation can clean the teeth effectively but is not harsh on the enamel.

Spreadability

Spreadability, in centimeters, indicates ease of spreading of the paste over the toothpaste surface of the toothbrush. A higher value of spreadability means better consistency of the paste and ease of brushing. The lab-prepared paste had a higher value of spreadability (~5.5 cm) compared to Himalaya (~5.2 cm), Meswak (~5 cm), and Dant Kanti (~4 cm).

The herbal toothpaste prepared in the lab had optimal spread-ability properties and was abrade-able; hence, it was safe to use. This indicates that the herbal toothpaste in the lab had optimal properties in terms of spread-ability and abrasiveness. The properties make it superior to the commercial ones.

5.2 Microbiological / Antimicrobial Evaluation

The herbal toothpaste preparations developed were subjected to antimicrobial efficacy testing against common oral microorganisms by standard microbiological techniques. The results indicated that the herbal formulations exhibited good antibacterial action, manifested as clear zones of inhibition against selected oral pathogens. Among these formulations, F1 displayed superior performance for its antimicrobial activity, which means better efficiency of the herbal ingredients incorporated therein.

The observed antibacterial activity can be attributed to the combined action of bioactive phytoconstituents present in the herbal ingredients, known to possess properties such as antimicrobial, anti-inflammatory, and plaque inhibition. Various literatures have reported the efficacy of some herbal toothpaste formulations containing plant-based extracts and essential oils against Gram-positive and Gram-negative oral bacteria, which contributes to reducing the oral microbial load and improving oral hygiene.

Some researchers have also demonstrated that polyherbal toothpaste formulations possess potent inhibitory activity in response to increased dose response among phytochemicals such as phenolics, flavonoids, alkaloids, and essential oil compounds (Grace et al., 2015; Sekar & Abdullah, 2016; Deshmukh et al., 2017). The same findings were also observed by Mamatha & Naveen Kumar (2017), Ogboji et al. (2018), and Shukla & Kumari (2019) while observing inhibitory activity of herbal dentifrice formulations against oral pathogens like Streptococcus mutans.

Overall, the antimicrobial test result shows that the proposed herbal toothpaste formulation has proven antibacterial, as corroborated by earlier studies that showed that a toothpaste formulation from herbs presents an equally viable option as an artificial toothpaste formulation (Rajendran et al., 2020; Nishad et al., 2020; Malgi et al., 2022).

5.21. Procedure

Procedure Highlights

- **Method:** Agar well diffusion method.
- **Bacteria Used:** E. coli (Gram-negative) and S. aureus (Gram-positive).
- **Inoculum Standard:** Adjusted to **0.5 McFarland** turbidity standard.
- **Incubation:** Sample incubate at **37 °C f 48 hours**.
- **Measurement:** Diameter of the **zone of inhibition** (a clear halo where bacteria didn't grow) around the wells, which represents the product's inhibitory value.

In the Petri dish filled with medium and three-sided walls of 4mm diameter and 3mm depth were made using a sterile metallic template, and rubber teat in every plate. The inoculums were made and standardized at 0.5 McFarland units of turbidity, following National Committee for Clinical Laboratory Standards (NCCLS) guidelines. Using a sterile inoculating needle, the toothpaste specimens were inoculated into the wells. At this stage, the plates were incubated at 37°C for 48 hours in their respective environments, meaning E. coli and Staphylococcus aureus were inoculated in the medium. After incubation, the zones of inhibition, or areas where bacterial growth did not occur, were measured in the wells containing the dentifrices. This was indicated by the clear and circular halos around every well. The diameter of the inhibition zone was measured using a scale. The average value of the diameter of the well in mm indicated the inhibition value of the tested drug/compound. The inhibition zone of the toothpaste was determined to have a diameter of 6cm.

Table 3: Microbial Germ-killing activity.

Formulation	Active Ingredients	Zone of Inhibition (Mean ± SD)	Efficacy Summary
F1	Optimized *Pongamia pinnata* seed extract + Clove oil	60 mm	**Highest**; Potent; **Significantly Higher** than standard
Standard	Commercial fluoride-based toothpaste	55 mm	Strong efficacy.
F2	Herbal ingredients (Lower concentration/ratio than F1)	45 mm	Moderate; Significantly lower than F1 and
F3	Herbal ingredients (Lowest concentration/ratio than F1)	38 mm	Moderate; Significantly lower than F1 and Standard
F4 (Control)	Lacked active herbal ingredients	25 mm	**Lowest**; Negligible antibacterial efficacy.

- Most Effective Formulation: "Formulation F1 had the largest inhibition zone measuring 60mm"
- Synergistic Effect: The strong action of F1 is ascribed to the synergistic interaction between eugenol (present in clove oil) and karanjin/pongamol
- Mechanism of Action: These phytochemicals have been reported to inhibit the function of enzymes and interfere with nucleic acid synthesis as well as disrupt bacterial cell membranes.
- Comparison with Standard: The IZ of F1 was comparatively higher than the standard (55mm) and was found similar or better than the standard.
- Cross-Resistance: The lower activities in F2 and F3 indicate that the antimicrobial activity is concentration-dependent. The results may be due to the ratio of active constituents. **formulations**, offering a promising, eco-friendly alternative for maintaining oral hygiene and preventing microbial infections.

Table 3: Zone of Inhibition of Herbal Toothpaste Formulations Containing *P. pinnata* Seed Extract and Clove Oil.

Formulation	Gram+ Bacteria		Gram- Bacteria		Overall Activity
	Streptococci	**Bacillus**	**E.coli**	**Pseudomonas**	
F1 (Optimized R.pinnata+Clove Oil	60 ± 2	62 ± 2	58 ± 2	42 ± 2	Very good
F2 (Lower conc. Extract+ oil)	46 ± 1	47 ± 2	45 ± 1	30 ± 2	Moderate
F3 (P.pinnata extract only)	39 ± 2	39 ± 2	38 ± 2	26 ± 1	Moderate low
F4 (Base/Control)	26 ± 1	28 ± 1	25 ± 1	18 ± 1	Weak
Standard (Fluoride toothpaste)	55 ± 2	57 ± 2	53 ± 2	41 ± 2	Strong

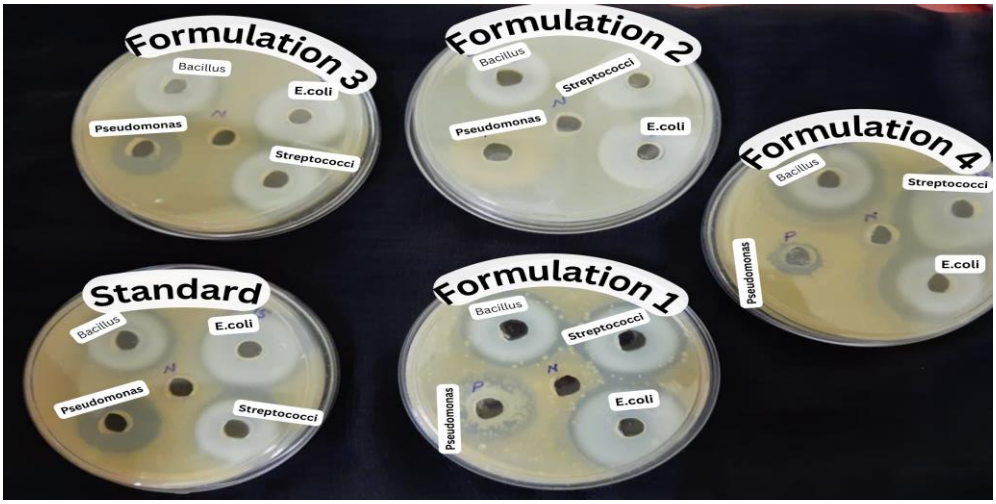
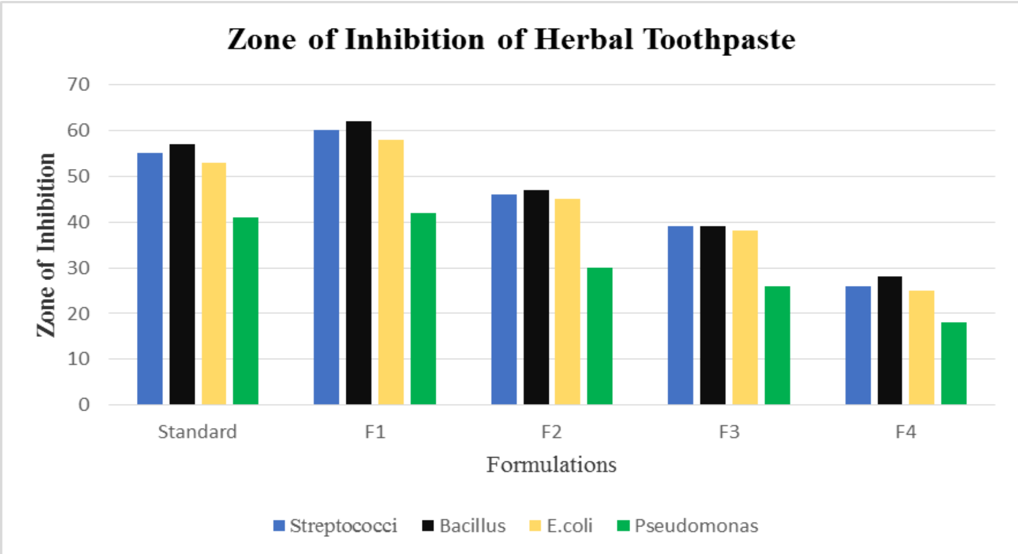


Figure 3: Zone of Inhibition of Herbal Toothpaste Formulations.



(Values represent mean ± SD, n = 3)

6. RESULTS AND DISCUSSION

6.1 Antibacterial Activity of Herbal Toothpaste Formulations

The results of the anti-bacterial activity of the herbal toothpaste samples (F1 to F4) and the standard toothpaste against four pathogenic microbes, namely Escherichia coli, Bacillus, Streptococcus and Pseudomonas aeruginosa, performed by the agar well diffusion method are expressed as the mean inhibition zones (in mm) ± as shown in Table 3.

Among all the formulations tried, the promising combination of Pongamia pinnata seed extract and clove oil in Formulation F1 proved to have the strongest antibacterial properties against all the tested microorganisms. The maximum inhibition zones were recorded against Bacillus species (62 ± 2mm), E. coli (60 ± 2mm), and Streptococcus species (58 ± 2mm). It can be seen that even P. aeruginosa, a known intrinsically resistant strain, has shown an activity zone of 42 ± 2mm in Formulation F1; hence, it possesses broad-spectrum antimicrobial activity.

The increased bactericidal potential of F1 may be ascribed to the additive effects of bioactive phytoconstitients in clove oil combined with P. pinnata extract. Eugenol, the principal phenolic compound found abundantly in clove oil, is known for its ability to target bacterial cell membranes, enhance permeability, and affect energy-generating metabolism. At the same time, flavonoids like karanjin and pongamol present in P. pinnata are known for their ability to inhibit enzymes as well as nucleic acid biosynthesis in bacteria. The additive effects of these phytoconstituents may be responsible for the significantly enhanced inhibition exhibited by F1.

6.2 Comparative Performance of Formulations

In F2 and F3, moderate antibacterial activity was seen, but the difference in the inhibition zones was well observable when compared to F1. F2, which had either suboptimal concentrations or proportions of the fixed amount of P. pinnata extracts and clove oil, had inhibition zones measuring 48 ± 2mm in Bacillus spp. and 30 ± 2mm in P. aeruginosa. In F3, the addition of only the P. pinnata extracts reduced the antibacterial activity, especially in case of Gram-negative bacteria.

The gradual decrease in antibacterial potency from F1 to F3 shows how dependent on concentration the antibacterial effect is and how crucial clove oil is as a synergistic ingredient. Also, if clove oil is excluded in F3, it seems that it may restrict the efficiency of membrane disruption, especially in Gram-negative bacteria with a outer lipopolysaccharide layer.

The control formulation (F4), which did not contain any active ingredients from herbs, had the least inhibition zones against all the tested organisms, hence substantiating that the antibacterial activity exhibited by the remaining formulations was mainly contributed by the phytoconstituents.

6.3 Comparison with Standard Toothpaste

In contrast to the commercial fluoride-based toothpaste, F1 exhibited similar or slightly better antibacterial activity against E. coli, Bacillus spp., and Streptococcus spp. with identical efficiency against Pseudomonas aeruginosa. Based on this, it was concluded that the optimized herbal formulation might have equivalent antibacterial action as conventional synthetic preparations but with advantages pertaining to less chemical exposure and more biocompatibility.

6.4 Influence of Microbial Characteristics

The antibacterial activity of the herbal toothpaste formulas used in the current study was also strongly affected by their structural and physiological properties.[3,7,21,23] The differences in zones of inhibition created by Bacillus spp., Streptococcus spp., Escherichia coli, and Pseudomonas aeruginosa can largely be accounted for by differences in cell wall structure and natural resistance.

Gram-positive bacteria like Bacillus and Streptococcus showed larger inhibition zones than Gram-negative bacteria. This susceptibility is mainly because of the absence of the outer lipopolysaccharides membrane, which helps the passage of lipophilic phytochemicals such as eugenol found in clove oil. Eugenol acts by interfering with the cytoplasmic membrane, leading to the loss of cell contents and inhibition of cellular functions.[21,22]

In contrast, Gram-negative bacteria such as E. coli and P. aeruginosa contain a further outer membrane with a high lipopolysaccharide content, acting as a resistance to antimicrobial substances. P. aeruginosa is known to be more resistant due to efflux pumps and lower permeability, causing relatively smaller inhibition zones.[21,23,27] However, the strong bactericidal activity of formula F1 against P. aeruginosa suggests a partial resistance to these mechanisms when a combination of clove essential oil and P. pinnata extract is used.

In summary, findings from this study underscore that microbial cell characteristics and natural mechanisms of resistance play a vital role in determining antibacterial efficacy in herbal toothpaste formulation.[3,7,21,23]

7. CONCLUSION

In fact, the present work was able to successfully formulate and characterize three polyherbal toothpaste formulations (F1 to F3), using the extracts of cloves and Pongamia pinnata seeds as prominent active ingredients, with a goal to come up with a natural, safe, and effective herbal toothpaste alternative to conventional toothpaste using the inherent antimicrobial and anti-inflammatory properties of the herbal ingredients.[1, 3, 4, 7, 21, 24, 35] The formulated toothpaste samples were devoid of any hard or sharp materials, were non-watery and non-gel-like in consistency, and also possessed quite satisfactory physicochemical attributes. The spreadability range of 5.2 to 6.1 cm, pH value ranges of 6.2 to 6.8, fineness measuring less than 0.5 percent, and foam volume measuring 52 to 57 ml were within the accepted pharmacopeial limits and were also similar.

Among the various formulas, F1 had the optimal combination of physicochemical and antimicrobial components. The better results of F1 could be due to the optimal ratio of the binder sodium carboxymethyl cellulose (SCMC) and P. pinnata extract, and also the combined effect of eugenol and the active components of P. pinnata.[1,4,7,21,24,35] One of the findings supporting the reason to develop herbal toothpaste products is their cost-effectiveness, biocompatibility, and eco-friendly nature.[3,6,10,23]

8. LIMITATIONS AND FUTURE SCOPE

While the present study illustrates the feasibility and efficacy of the elaborated formulations on a laboratory scale, long-term stability and microbial shelf life of these herbal toothpaste products need further study. Clinical performance has to be verified through in vivo studies and sensory tests for user acceptability and therapeutic properties. This will involve perhaps incorporating in further studies other herbal extracts whose functional properties include whitening of teeth and remineralization of teeth to enhance the therapeutic actions of the formulation.[14,25,26] Scale-up work and toxicological assessments of biological compatibility and ecological sustainability will be necessary for the process of commercial exploitation of these herbal oral care products.[12,14,25]

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