JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2024/34575

SHORT COMMUNICATION

Plants as Regular Phytochemical Sources of Dentistry - Formulation and Evaluation of Polyherbal Tooth Paste

Surya Prabha Matangi[1*], Ramu Samineni[2], Kanhaiya Kumar[1], Ashutosh Kumar[1], Shantanu Kumar[1] and M. Salmon Raju[3]

[1]Department of Pharmaceutical Sciences, School of Biotechnology and Department of Pharmaceutical Sciences, VFSTR, Vadlamudi, Guntur - 522213, Andhra Pradesh, India; prabhalsurya@gmail.com

[2]School of Pharmaceutical Sciences, Department of Pharmaceutics, Sandip University, Nashik - 422213, Maharashtra, India

[3]Andhra Hospital, Eluru - 534001, Andhra Pradesh, India

Abstract

Different plant components have been used in medicinal preparations in indigenous systems of medicine to clean teeth or treat oral diseases such as periodontal disease. Herbal toothpaste is equally as good at controlling plaque and gingivitis as dentifrices made according to standard formulas. *Streptococcus* mutants, out of all the examined bacteria, were shown towards the most susceptible to the created dentifrice, as evidenced by a zone inhibition (7-8 mm), followed by *Escherichia coli* (7 mm), as well as oral micro-biota (8mm). The created Poly-herbal dentifrice was successfully tested using a variety of industry-recognized standards to verify its high quality and physio-chemical qualities. According to the findings, the created Poly-herbal dentifrice shows promise for having antibacterial properties on both gramme-positive and gramme-negative organisms. Compared to completely synthetic dentifrice, it must be safer. For the development of Poly-herbal dentifrice to be proven safe and effective, more research is required. According to the study's findings, herbal toothpaste is safer and has fewer side effects than synthetic preparations. It is also more commonly used in dentistry research. The toothpaste that is designed for oral hygiene and teeth shows antimicrobial activity against pathogens. The market preparation was contrasted with the formulation. Therefore, it demonstrates an equal amount of patronizing and engrossing passion for the promoted formulations. The development of herbal toothpaste has a promising future in the research of natural cures and general dental health.

Keywords: Herbs, Microbicidal Activity, *Mimusops elengi*, Periodontal Diseases, Toothpaste

1. Introduction

Oral diseases are among the most prevalent chronic illnesses that afflict people and are a serious public health concern. Since they have fewer adverse effects than synthetic antimicrobials and because it can be challenging to overcome both primary or secondary antibiotic resistance while receiving treatment, the use of herbal items in the treatment of oral illnesses is considered to be an intriguing alternative. Research on the antibacterial effectiveness of a combined effect of these phytoconstituents against dental caries as well as gingival microbes is urgently needed, and such research will help develop a novel, ground-breaking technique that can concurrently restrict two of humankind's most prevalent dental diseases while also preventing the emergence of drug resistance. In certain nations, treating illnesses of the mouth is the fourth most expensive disease.

Chlorhexidine use may have several unfavourable effects, including changed taste perception, tooth

*Author for correspondence

Article Received on: 28.07.2023 Revised on: 03.10.2023 Accepted on: 24.11.2023

discolouration, and the emergence of bacteria with bacterial resistance, which make it impossible to use it over an extended period. There is a requirement to create some novel approaches that combat periodontal and dental caries concurrently. Studying the many medicinal plants that nature has to offer would be one such tactic. Plant medicines restore health with the least amount of side effects and the greatest amount of effectiveness because of their "naturally occurring" active substances. The usage of natural goods is a complete treatment that incorporates health promotion and prevention techniques.

Plant medicines restore health with the least amount of side effects and the greatest amount of effectiveness because of their "naturally occurring" active substances. The usage of natural goods is a complete treatment that includes health promotion and prevention techniques. Natural herbs are both safe and efficient when used alone or in combination to treat a variety of oral health issues, including gingivitis, gum disease, mouth sores, and tooth decay. Herbal medicines don't include alcohol or sucrose, which are the two common components, in addition to having fewer side effects than other over-the-counter drugs. These components are used by some bacteria, which then produce the by-products that lead to halitosis. As a result, using herbal remedies locally may also enhance the effectiveness of periodontal therapy[1].

Additionally, a variety of leaf extracts, including aqueous, acetone-water, methanolic, spray-dried extracts, and essential oil, may have inhibitory effects on both Gram-positive and Gram-negative bacteria and fungus[2-6].

1.1 Natural Herbs

1.1.1 Clove (Syzygium aromaticum)

Various species, including *S. aureus*[7], *L. monocytogenes,* and *Aspergillus*[8], are inhibited by clove oil[9]. As a well-known pain reliever, cinnamon oil has long been used to treat toothaches. Clove oil can be used to ease discomfort and aid in the abscess's infection removal[10]. The germicidal properties of the oil make it very effective for relieving dental pain, toothache, sore gums and mouth ulcers[11].

1.1.2 Miswak (Salvadora persica)

The mechanical action of Miswak produces its medicinal effects[7]. Some people think that topical fluoride comes from a natural source[8]. Significant amounts of calcium and phosphorus, which are necessary for tooth demineralization, were released by *Mangifera* and Miswak[12-14]. This revealed a significant inhibitory effect on human collagen-induced platelet aggregation and a moderate antibacterial activity. The benzylisothiocyanate isolated from the Miswak showed antiviral activity against HSV-1[15] and acts as an agent for controlling dental caries[16]. The principal active components in bloodroot are alkaloids, including sanguinarine. Due mainly to their capacity to suppress oral bacteria development, they are extensively used for periodontitis and gingival disease as well as in toothpaste and other oral sanitary products.

1.1.3 Mango (Mangifera indica)

The leaves of *Mangifera indica* are reducing the total cholesterol levels very significantly[16]. *Mangifera* has been demonstrated to prevent bone resorption induced by parathyroid hormone in mice[17]. The MI leaf extracts were found to be active against the deadly *Clostridium tetani*, according to Godfrey SB *et al.,* in 2007. At MICs of 6.25 and 12.5 mg/ml, correspondingly, ether as well as ethanolic leaf extracts demonstrated anti-*Clostridium tetani* efficacy[18]. An analysis of the anti-toothache effects of *M. indica* revealed that its extract reverses the process by which *S. mutans* develop tooth decay. It also helps in reducing the bacterial count and plaque index[19,20].

1.1.4 Betel (Piper betel)

Moreover, several studies have shown that betel leaves have stronger antibacterial action than other herbs, hence they are used in toothpaste and mouthwash to avoid dental caries[21]. Betel leaf extract, a well-known antibacterial agent, may be used to treat a variety of conditions, such as toothaches, bleeding, and abscesses[22]. The killing kinetics of ethyl acetate extract against *Streptococcus mutans* and *Streptococcus intermedius* were time and dose-dependent. Scanning electron microscopy micrographs showed the morphological changes and depletion of the tested pathogens indicating cell destruction after exposure to this extract[23].

1.1.5 Papaya (Papaya carica)

According to a clinical study, using a dentifrice containing *Carica papaya* leaves extract decreased periodontal bleeding and swelling[24]. In a 2.5% papaya leaf extract solution, research found that the impact on plaque index and gingival index in mild gingivitis was the same as 0.2% chlorhexidine[25]. A clinical trial concluded that the *Carica papaya* leaf extract dentifrice is effective in the reduction of gingival bleeding and inflammation[26].

1.1.6 Peppermint (Mentha piperita)

The presence of potassium, magnesium, calcium, iron and phosphorus in mint leaves is essential for the maintenance and formation of bone density in tooth and jaw. The vitamins and minerals work together to fortify enamel and ensure strengthening teeth and gums. In addition to menthone (20 – 31 %), and the different isomers of menthol in addition to other constituents *M. piperita* is one of the most promising species with antibacterial potential against cariogenic bacteria such as *Streptococcus mutans*[27].

1.1.7 Guava (Psidium guajava)

The antimicrobial activity of guava is mainly attributed to flavonoids, guaijaverin and quercetin[28].

1.1.8 Bakul (Mimusops elengi)

The proven properties of *M. elengi* and the scope of advancement for using it as an effective suture coating material projecting its use in decreasing bacterial load after periodontal surgery[29]. Tannins present in this plant provide astringent and haemostatic properties to any compound. They interact with carbohydrates, proteins, and therefore enzymes polysaccharides, and bacterial cell membranes. Hence, it interferes in plaque accumulation and acts as an anti-plaque agent[30].

2. Materials and Methods

2.1 Sample Collection

Plant materials like Bakul (*Mimusops elengi*), Peppermint (*Mentha piperita*), Miswak (*Salvadora persica*), Clove (*Syzygium aromaticum*), Betel (*Piper betel*), Guava (*Psidium guajava*), Mango (*Mangifera indica*), and Papaya (*Papaya carica*) were collected from local market of Guntur, Andhra Pradesh, India. The plant materials were taxonomically identified and authenticated by The Department of Botany, Acharya Nagarjuna University, Guntur, Andhra Pradesh.

2.1.1 Drying

A week at room temperature was spent drying the Bakul (*Mimusops elengi*), Peppermint (*Mentha piperita*), Clove (*Syzygium aromaticum*), Betel (Piper betel), as well as Guava (*Psidium guajava*).

2.1.2 Crushing

We used a pestle and mortar at room temperature to crush the various plant pieces. The sample fragments were kept at room temperature.

2.2 Extraction Procedure

2.2.1 Soxhlet Extraction or Hot Continuous Extraction

In this method, finely ground samples are placed in a porous bag or "thimble" made from a strong filter paper or cellulose, which is placed, is in the thimble chamber of the Soxhlet apparatus. Extraction solvents heated in the bottom flask, vaporizes into the sample thimble, condenses in the condenser and drips back. When the liquid content reaches the siphon arm, the liquid contents are emptied into the bottom flask again and the process is continued. Using ethanolic and hydro alcohol extracts (4:1 v/v) resulted in the highest extraction yield with the maximum presence of phytoconstituents (alkaloids, saponins, carbohydrates, tannins and flavonoids) compared to the other solvents such as petroleum ether, chloroform and water. The extract was tested for the presence of bioactive compounds by using the following standard methods[31-33].

2.3 Phytochemical Tests

- **Saponins:** 6 ml of distilled water was used to dissolve 2 ml of material, shaken vigorously; it started to foam up. The stability of the foam indicates that saponins are present in the samples.
- **Tannin:** The material was dissolved in 1ml of 5% $FeCl_3$. Tanning in the sample is confirmed by the presence of dark blue or greenish-black colour. Using a heating mantle to modify the colour is utilised if there are no colour changes.

- **Flavonoids:** 20 ml of NaOH received 2 µl of samples, which were added in drops. Another drop of Conc. HCL was added, and the emergence of a yellow tint proved that flavonoids were present in the sample.
- **Carbohydrates:** After heating for more than 20 minutes, 1ml of Fehling's reagent was dissolved in 2ml of sample. The presence of carbohydrates in the sample is confirmed by the appearance of red ppt.
- **Protein:** Both 500 µl of 1% CuSO4 and 500 µl of 5% NaOH were produced. mixed. The sample was added to the solution, and the appearance of a purple colour indicated the presence of protein in the sample.
- **Alkaloids:** Wagner's reagent and 500 µl of extract were added after it had been centrifuged. After a good shake, I walked away. When alkaloids are present, a reddish-brown tint appears.
- **Fats:** Place a tiny amount of extracts between two filters and press. One filter's paper strain revealed the presence of fixed oils.
- **Terpenoids test:** 250 µl of chloroform was used to dissolve a 500 µl sample. 625µl of concentrated H2SO4 was added to the mixture. The solution's reddish-brown precipitation confirms the presence of terpenoids.
- **Phenol test:** Distilled water was used to dissolve 500 µl of extract. $FeCl_3$ solution in 2 drops was added. Blue or green colouration suggests the presence of phenols.
- **Coumarins test:** To dilute the 1ml of crude extracts, 10% NaOH was added. When red or a blue-green colour was perceived, Quinone's were present.
- **Quinones test:** Observe the 10% NaOH that was added to the 1ml of phytochemical constituents to make it diluted. Quinones were present when the experienced colour was blue-green or red.
- **Fragrance test:** It was evaluated for acceptability based on personal observation. It was decided whether the aroma was acceptable after asking five persons for their opinions. The following criteria were used to evaluate fragrance:

(a) The scent was commensurate with that of a toothpaste of reference.
(b) The aroma wasn't great, but it was equivalent to the toothpaste used as a reference.
(c) The toothpaste's aroma was inferior to that of the standard toothpaste.

- **Shape retention:** Toothpaste was completely applied on a toothbrush after being squeezed out of the tube, and its condition was assessed after standing for 10 seconds using the criteria listed below:

(a) The toothbrush retains its shape after the toothpaste has been squeezed out of it.
(b) The shape of the toothbrush is nearly preserved immediately after the toothpaste is squeezed out.
(c) The toothpaste cannot keep its shape after being squeezed off the toothbrush.

2.4 Formulation of Toothpaste

All of the herbal components were dry and ground in a mixing. Measured quantities of the components were added to the mortar. Water was blended with methylcellulose, sodium lauryl sulphate, calcium carbonate, honey, and glycerine. This mixture was added to a mortar with herbal components and thoroughly ground to a paste-like consistency. The plant extract and chemical composition of formulations are represented in Table 1.

2.4.1 Procedure

In a mortar and pestle, 1 gram of para-hydroxybenzoic acid and 0.5 gram of sodium chloride were used to triturate 3 grams of Bakul bark powder, 2 grams of clove extort, 2 grams of peppermint extort, 3 grams of guava extort, 3 grams of betel extort, and 2 gram of turmeric extort (as a preservative). 1gm of sodium lauryl sulphate is used as a foaming agent, and stevia is further as a sweetening ingredient. The weight was increased to 100gm by adding 5 ml of glycerine as a humectant, 5 ml of triturated acacia gum as a binder, and 80 ml of demineralised water. The bitter taste is concealed by the addition of clove oil.

2.5 Evaluation of Toothpaste

2.5.1 Physical Evaluations

- **Colour:** The colour was tested visually.
- **Odour:** By smelling the product, odour was found.
- **Taste:** The formulation was carefully tasted to check for taste.
- **Relative Density (RD):** 10 ml solution and 10 ml of water were weighed in grams using a relative density container to determine the relative density.

Table 1. Plant extracts and chemical composition of formulations

S. No.	Ingredients	Quantity (grams and ml)		
		F1	F2	F3
1	*Mimusops elengi*	1	2	3
2	*Salvadora persica*	1	2	3
3	*Mangifera indica*	1	2	3
4	*Papaya carica*	1	2	3
5	*Syzygium aromaticum*	2	2	2
6	*Mentha piperita*	2	2	2
7	*Psidium guajava*	1	2	3
8	Betel extract	2	2	2
9	Sodium lauryl sulphate	1	1	1
10	Calcium Carbonate	10	10	10
11	Sodium Chloride	0.3	0.3	0.3
12	Stevia	0.5	0.5	0.5
13	Glycerine	3	3	3
14	Distilled water	70-80 ml	70-80 ml	70-80 ml

2.5.2 Evaluation Parameters

2.5.2.1 Abrasiveness

To create at least 10 collapsible tubes, extrude the material for 15 to 20 cm onto the butter paper. By pressing your fingertip along the length of the contents, you can feel any sharp or hard-edged abrasive particles. Some contaminants can't be in toothpaste.

2.5.2.2 Determination of Spreadability

The paste's slipping and dragging properties are incorporated in this process. The ground slide under investigation was coated with the produced paste (2g). This glass slide and another glass slide were sandwiched with the prepared paste for five minutes to remove air and create a consistent paste coating between slides. The edges had extra paste scraped off of them. The upper slide's time to move 7.5 cm was measured in seconds after the top plate was pushed with an 80g force using a thread that was attached to the hooks and shorter periods had higher spread ability.

Spreadability (S) = ML/T Eq.-1

Where S stands for spreadability, M for weight (attached to the top slide), L for distance travelled by the glass slide, and T for the time (in seconds) needed to separate the upper slide from the lower slide.

2.5.2.3 pH Determination

The pH of the composition of the herbal toothpaste was measured using a pH metre.10g of toothpaste should be put in a 150 ml beaker. Allow 10 cc of water that has been heated and then cooled. To create a suspension, stir ferociously.

2.5.2.4 Homogeneity

The collapse tube or any other suitable container must extrude the toothpaste in a homogenous bunch with normal force at a temperature of 27 ± 2 °C. Before being gradually rolled, the bulk of the contents must also protrude from the container's crimp.

2.5.2.5 Foaming

By half-filling and shaking a measuring cylinder with the formulation 10 times, the foaming ability of the toothpaste formulation was assessed. Foam volume was measured in its complete.

2.5.2.6 Determination of Foaming Power

Foaming power = V1–V2 Eq.-2

V1-Volume in millilitres of water and foam. V2-Volume in millilitres of water.

2.5.2.7 Stability

The strength study was conducted in accordance with ICH guidelines. The produced paste was stored for three months in a collapsible tube at a range of temperatures and humidity levels, including 25 ± 2 °C/ 60 % ± RH 5% RH, 35 ± 2 °C/ 65% ± RH, and 40 ± 2 °C/ 75% ± RH and its appearance, pH, and spreadability were assessed.

2.5.2.8 Evaluation of Volatile Matter and Moisture

5g of the mixture was put into a porcelain dish that was 68 cm in length as well as 2-4 cm deep. In an oven set to 105°C, dry the sample.

Mass-based calculation = 100*MI/M Eq.-3

M-Mass (g) of the substance intended for the test MI-Loss of mass (g) on drying.

2.6 Stability Studies

Stability analysis (storage stability) for 45 days, toothpaste was kept at 40°C and RH 75%, 5%. Some five

samples were collected following the initial estimate of flavonoids, which was done after nine days. To thoroughly extract the flavonoids, toothpaste (1 gram) was refluxed for 30 minutes with distilled water (75 ml), and then passed through a powdered glass funnel with a vacuum filtration system. A 100 ml volumetric flask was used to collect the supernatant after centrifuging the filtrate at 2000 rpm for 20 minutes. The remaining capacity was filled with water. Each sample underwent an identical technique, and the total flavonoid content of the solutions (100 ml) was calculated.

2.7 Antibacterial Activity

The sample from the dish was placed in the nutrient broth, and the inoculation was then maintained and allowed to develop for 1-2 days at 37°C in the incubator. Once bacteria have started to grow in the broth, the given sample is used to use the good diffusion procedure. *Staphylococcus aureus, Escherichia coli,* and oral microbial flora are the bacteria that were employed (*Streptococcus mutans*).

2.7.1 Procedure

The drugs used in the standard formulation were IP-grade ofloxacin and ciprofloxacin. A 24-hour culture of *S. aureus* and *S. mutans* was used to perform the antibacterial activity. In investigations on antibiograms, three concentrations 25, 50, and 100 mg/ml were employed for each isolated phytochemical. The addition of wells containing antibiotics to the agar surfaces right after inoculation with the organism under test is its key component. Inoculums should never be made up of undiluted overnight broth cultures. During 24 hours of incubation at 37°C, the plates were inspected to see whether there were any clear zones of inhibition surrounding the wells that had been impregnated with a particular drug concentration. It was measured how wide each wall's zone of inhibition was.

2.7.2 Preparation of Plates

Weigh all the chemicals, then dissolve them in 60 ml of water heated while being stirred to properly dissolve the ingredients. Then autoclaved at 121°C and 15lb of pressure. Let it cool in a 45–50 °C environment after autoclaving. Place petri plates with the recently prepared and cooled medium inside. Let the agar media harden at ambient temperature.

2.7.3 Spreading of Bacteria

A cotton swab that has been previously exposed to UV radiation should be dipped into a fresh and dried culture of *S. aureus*, *E. coli* and oral microbiota.

Removing the excess moisture in the sample before spreading the bacteria uniformly around the plate's surface, ensuring that some is present in each plate corner.

2.7.4 Incubation

At a temperature of 37 °C, plates should spend the night in the incubator.

2.7.5 Reading of Plate and Interpretation

Each plate was evaluated after 15 to 16 hours of incubation. If the plate was correctly streaked and the inoculums were precise, the result of Zones of inhibition should be equally round and a confluent lawn of growth. The data was recorded and the results were assessed after the Zones of inhibition's diameter was measured.

2.7.6 Comparison

Toothpaste made with herbs and sold as a product in terms of antimicrobial activity, spreadability, foam ability, pH measurement, and moisture content, the prepared herbal toothpaste was compared to commercially available products.

3. Results and Discussion

3.1 Phytochemical Screening

All the selected plant extracts contain Phyto secondary metabolites which are responsible for antimicrobial activity. Several parts of various plants have been utilised in medicinal formulations in indigenous medical systems to treat periodontal disease or to clean teeth. Plaque and gingivitis can be controlled just as effectively with herbal kinds of toothpaste as with dentifrices that are made according to standard formulas. The entire plant has a high concentration of bioactive components, according to a preliminary phytochemical investigation represented in Table 2. The extruded weight of toothpaste and percentage extrudability are represented in Table 3.

3.2 Physical Evaluations

The physical evaluations for prepared herbal toothpaste were recorded as light green colour, sweetish taste,

Table 2. Phytochemical screening

Test	Bakul	Miswak	*Mangifera indica*	Clove	Peppermint	Betel	Guava
Alkaloids	+	-	-	+	+	+	+
Saponins	+	+	-	+	+	+	-
Tannins	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	-
Proteins	+	+	+	+	-	-	+
Terpenoids	+	+	-	+	-	+	-
Carbohydrates	+	+	+	-	-	+	+
Coumarins	-	+	-	+	-	+	+
Quinone	-	-	-	+	-	-	-
Starch	+	+	+	+	-	+	+

+ indicates present; - indicates absent

Table 3. Extrudability

Extrudability	Average of three tubes*
Net weight of the formulation in a tube (g)	12.9
Extruded weight of toothpaste (g)	12.1
Extrudability % amount	89.2

*Mean (n=3)

pleasant odour having smooth appearance and a relative density value of 10. The physical evaluation parameters of prepared herbal toothpaste are represented in Tables 4 and 5.

The prepared herbal toothpaste paste having a pH value of 7.4, foaming power, abrasiveness, and homogeneity is good and it shows a spreadability value is 5.8 cm/sec. Herbal toothpaste having moisture content 15 and absence of microbial growth shows stability.

3.3 Stability Studies

The stability research showed that the toothpaste formulation had high stability. No significant change occurred in physicochemical changes during 3 months of stability studies represented in Table 6.

3.4 Antimicrobial Activity

3.4.1 Oral Bacteria

Based on the outcomes of morphological as well as gram-stained microscopic analysis, the test organisms were isolated, identified, and recorded.

Table 4. Physical evaluation of herbal toothpaste

S. No.	Parameters	Observation
1.	Colour	Light green
2.	Taste	Sweetish
3.	Odour	Pleasant
4.	Smoothness	Smooth
5.	Relative density	10

Table 5. Evaluation of parameters

S. No.	Parameters	Observations
1.	pH	7.4
2.	Foam	14 ml (good)
3.	Spreadability	5.8 cm/sec
4.	Moisture content	15
5.	Homogeneity	Good
6.	Microbial growth	No microbial growth
7.	Abrasiveness	Good abrasives
8.	Stability	Stable

Table 6. Stability studies

Colour	Appearance	Spread ability	pH
25 ± 2 °C/ 60% ± RH (3rd **month)**			
Light green	Homogenous	5.8	7.4
35 ± 2 °C/ 65% ± RH (3rd **month)**			
Light green	Homogenous	5.7	7.3
40 ± 2 °C/ 75% ± RH (3rd **month)**			
Light green	Homogenous	5.7	7.1

3.4.2 Anti-microbial Activity Observation

Comparing the formulation of the herbal toothpaste to the usual medication Amoxicillin, the herbal toothpaste showed rather good anti-microbial effectiveness. The formulation showed a remarkable Zone of inhibition of 8 mm at a minimum inhibitory concentration of 25 g/mL, while Amoxicillin showed a Zone of inhibition of 10 mm at a minimum inhibitory concentration of 6.25 g/mL against the oral bacterium *Streptococcus mutans*. As a result, it is possible to conclude that toothpaste that has been specifically designed may have antimicrobial properties. The prepared herbal toothpaste is more active against *Streptococcus mutans*. Zone of F1, F2 and F3 inhibition in opposition to oral bacteria, (A). *E. coli*, (B). *S. aureus*, and (C). *S. mutans* represented in Figures 1, 2 and Table 7.

3.5 pH, Spreadability and Foam Ability

Optimized herbal toothpaste (F3) has less pH and shows good foam ability and Spreadability characters when compared to the marketed products represented in Table 8 and Figure 3. Along with this herbal-based formulation, a comparison study of previously marketed herbal toothpaste was conducted to gain insight into key physical parameters, such as pH, stability, extrudability, spreadability, foam ability, and homogeneity, that can be used to create a formulation that is more successful, stable, and effective. In terms of all toothpaste evaluation qualities, this preliminary *in-vitro* investigation showed that herbal toothpaste was just as effective as commercially available popular toothpaste.

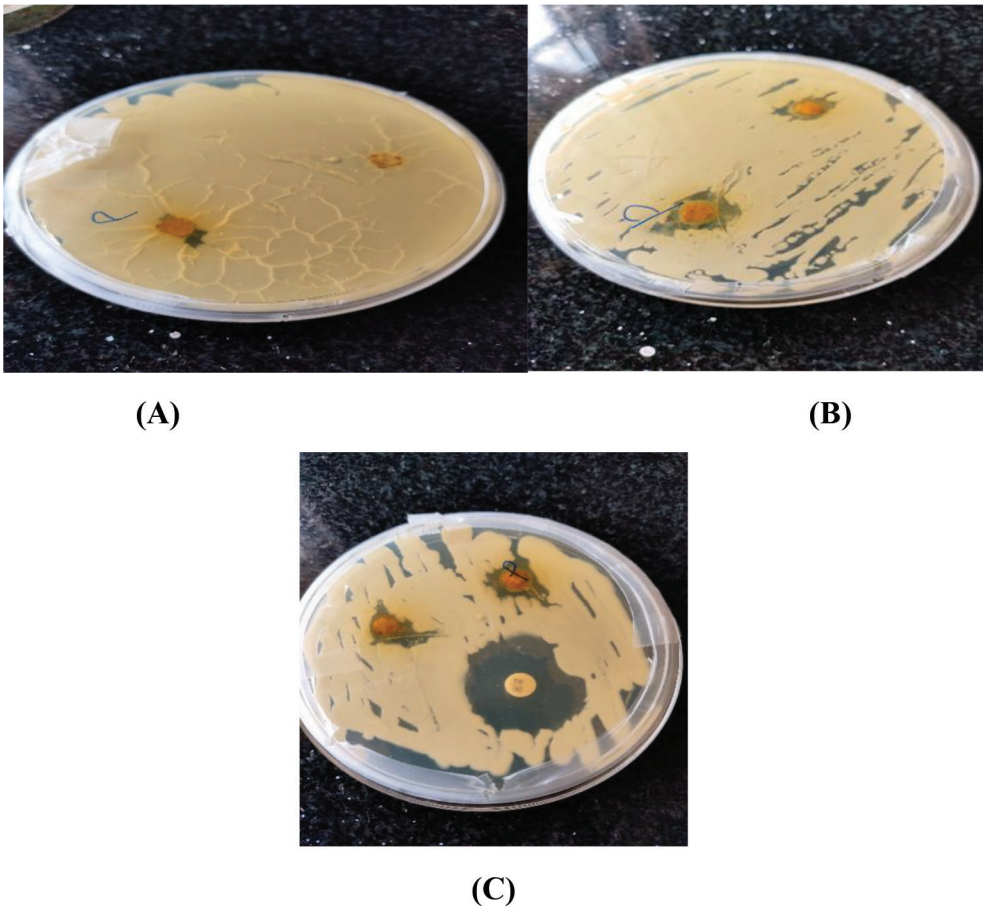

Figure 1. Zone of F1, F2 and F3 inhibition in opposition to oral bacteria, **(A).** *E. coli,* **(B).** *S. aureus,* **(C).** *S. mutans.*

Table 7. Toothpaste formulation's inhibition zone

Bacteria	Amoxicillin	F1	F2	F3
S. aureus	10	5	6	7
E. coli	13	6	6	7
S. mutans	11	6	7	8

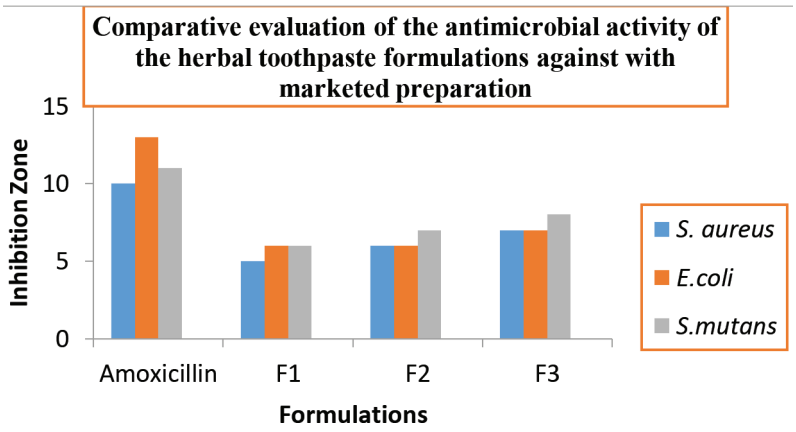

Figure 2. Comparative evaluation of the antimicrobial activity of the herbal toothpaste formulations (F1, F2, and F3) against with marketed preparation.

Table 8. Comparison between the prepared herbal tooth-paste with against marketed products in terms of the pH, spread ability, and foam ability

Formulation	pH	Spreadability	Foam ability
Colgate *tooth paste*	8.74	3	8
Dabur Red tooth paste	8.55	4	12
Dant kanti *tooth paste*	8.3	3	8
Herbal tooth paste (F3)	7.4	5.8	14

Toothpaste that has been specially formulated (F3) exhibits antimicrobial efficacy against microorganisms including *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus mutans* while also being able to preserve dental and oral hygiene. The herbal formulation (F3) exhibiting nearly the same antimicrobial activity as marketed products represented in Table 9 and Figure 4.

3.6 Comparison of Percentage Moisture Content

Prepared Herbal toothpaste (F3) has the same moisture content as Colgate. The percentage moisture content for prepared herbal toothpaste was compared with Dant Kanti, Colgate and Dabur Red was represented in Table 10.

Table 9. Inhibition zone of commercial toothpaste formulation

Bacteria	Colgate	Dabur Red	Dant kanti	F3
S. aureus	13	12	13	7
E. coli	11	10	11	7
S. mutans	15	14	13	8
Herbal tooth paste (F3)	7.4	5.8	14	

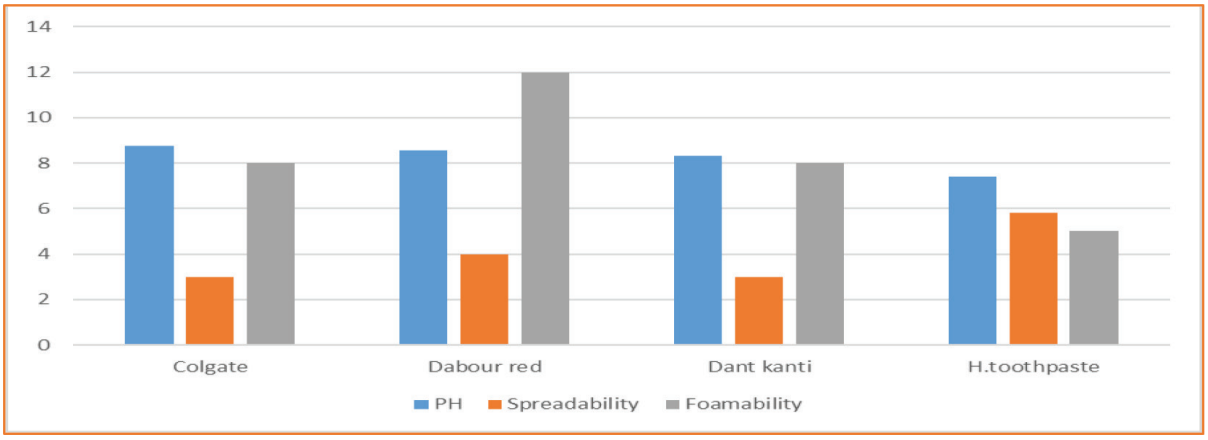

Figure 3. Comparison between the preparation that is marketed and the pH, spread ability, and foam ability.

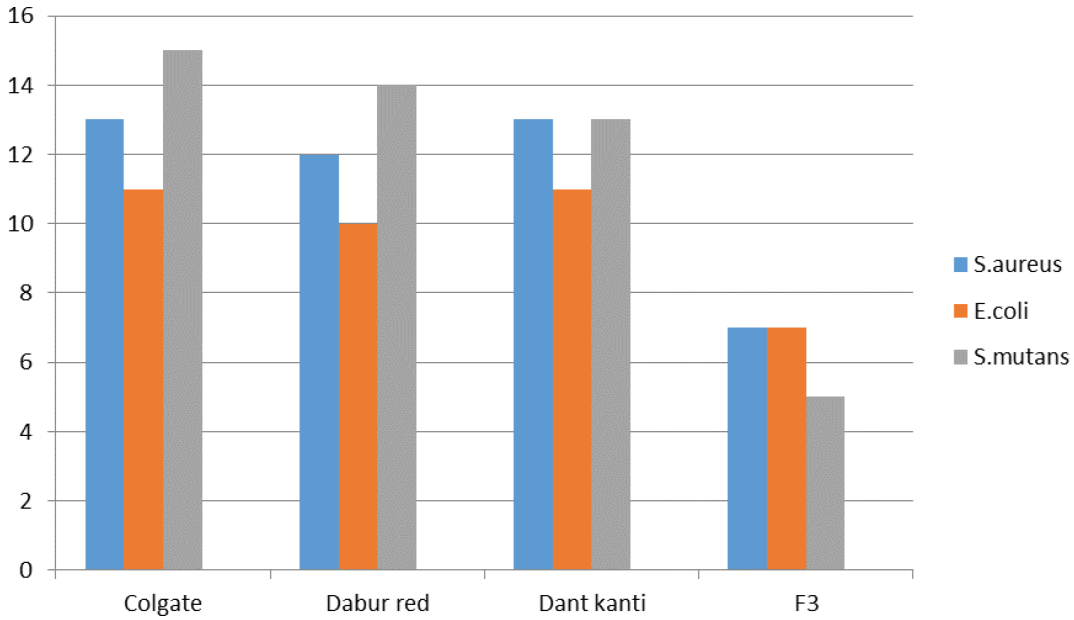

Figure 4. Comparative analysis of anti-microbial efficacy using commercially available formulations of Dant Kanti, Colgate, Dabur Red, and F3.

Table 10. Comparison of percentage moisture content

S. No.	Preparation	% Moisture content
1	Colgate	15.11
2	Dant Kanti	10.17
3	*Dabur Red*	25.0
4	Herbal toothpaste (F3)	15.15

4. Conclusion

The prepared herbal toothpaste has a prominent function in maintaining oral hygiene and preventing dental caries and are safer with minimum side effect than chemical-based synthetic toothpaste. Formulated herbal toothpaste was discovered to be of high quality. *Streptococcus mutans*, *Escherichia coli* and *Staphylococcus aureus* are among the microorganisms that the phytoconstituents of *Mimusops elengi* are demonstrating anti-microbial efficacy against. By adding more natural ingredients to the formulation of herbal toothpaste, more and safer natural therapies can be created for use in research and dental care for the general population, society, and country. So further research needs to be done on a long time and bigger samples.

5. Acknowledgement

The authors would like to express their deep appreciation to the VFSTR Management, Guntur and Department of Pharmaceutical Sciences for providing the facilities. The authors are acknowledging the Department of Botany, Acharya Nagarjuna University, Guntur, Andhra Pradesh for authenticating the plant materials.

6. References

1. Moro MG, Silveira Souto ML, Franco GCN, Holzhausen M, Pannuti CM. Efficacy of local phytotherapy in the nonsurgical treatment of periodontal disease: A systematic review. J Periodontal Res. 2018; 53:288–97. https://doi.org/10.1111/jre.12525 PMid: 29352465
2. Nair R, Chanda S. *In-vitro* antimicrobial activity of *Psidium guajava* L. leaf extracts against clinically important pathogenic microbial strains. Braz J Microbial. 2007; 38:452–8. https://doi.org/10.1590/S1517-83822007000300013
3. Dhiman A, Nanda A, Narasimhan B. *In-vitro* antimicrobial activity of methanolic leaf extract of *Psidium guajava* L. J Pharm Bio Allied Sci. 2011; 3:226–9. https://doi.org/10.4103/0975-7406.80776 PMid:21687350 PMCid:PMC3103916
4. Fernandes MRV, Dias ALT, Carvalho RR, Souza CRF, Oliveira WP. Antioxidant and antimicrobial activities of *Psidium guajava* L. spray dried extracts. Ind Crops Prod. 2014; 60:39–44. https://doi.org/10.1016/j.indcrop.2014.05.049
5. De Araújo AA, Soares LAL, Assunção Ferreira MR, de Souza Neto MA, da Silva GR, de Araújo RF, Guerra GCB, de Melo MCN. Quantification of polyphenols and evaluation of antimicrobial, analgesic and anti-inflammatory activities of aqueous and acetone-water extracts of

Libidibia ferrea, *Parapiptadenia rigida* and *Psidium guajava*. J Ethnopharmacol. 2014; 156:88–96. https://doi.org/10.1016/j.jep.2014.07.031 PMid:25124277
6. Nisha K, Darshana M, Madhu G, Bhupendra MK. GC-MS analysis and anti-microbial activity of *Psidium guajava* (leaves) grown in Malva region of India. Int J Drug Dev Res. 2011; 3:237–45.
7. Emslie RD. A dental health survey in the republic of Sudan, B. R. dent. 1966; 120:167-178.
8. Chan DCN, Dogman AU, Dugan MM. Sem XRF, Empa. Evaluation of middle eastern tooth brush, *Salvedora persica*. J Elec Micro. 1987; 5:145.
9. Snyder OP. Hospitality Institute of Technology and Management, Antimicrobial effects of spices and herbs. 1997.
10. Robbers JE, Tyler VE. 1999. Tyler's herbs of choice: The therapeutic use of phytomedicines: CRC. Saeed S.
11. Kaur D. *Syzygium aromaticum* L. (clove): A vital herbal drug used in periodontal disease. Indian Journal of Pharmaceutical and Biological Research. 2017; 5(02). https://doi.org/10.30750/ijpbr.5.2.9
12. Arjunajathesan. Antibacterial efficacy of *Mangifera indica* L. kernel and *Ocimum sanctum* L. leaves against *Enterococcus faecalis* dentinal biofilm. 2013; 16(5):454-7. https://doi.org/10.4103/0972-0707.117507 PMid:24082577 PMCid:PMC3778630
13. Sher H, Al-Yemeni MN, Wijeya L. Ethnobotanical and antibacterial potential of *Salvadora persica* L: A well-known medicinal plant in Arab and Unani system of medicine. Journal of Medicine Plants Research. 2011; 5(7):1224-9.
14. Andrew Joiner, Fred Shafer. Enhanced enamel benefits from a novel fluoride toothpaste. International Dental Journal. 2009; 59(4):244-253.
15. AI-Bagieh NH. Anti-herpes simplex virus type 1 activity of benzylisothiocyanate, Biomed Lett. 1992; 47:67-70.
16. AI-Bagieh NH, Weinberg ED. Benzylisothiocyanate: A possible agent for controlling dental caries. Microbios Lett. 1988; 39:143-51.
17. Dzink JL, Socransky SS. Comparative *in-vitro* activity of san-guinarine against oral microbial isolates. Antimicrob Agents Chemother. 1985; 27:663–5. https://doi.org/10.1128/AAC.27.4.663 PMid:4004199 PMCid:PMC180120
18. Hannah JJ, Johnson JD, Kuftinec MM. Long-term clinical evaluation of toothpaste and oral rinse containing sanguinaria extract in controlling plaque, gingival inflammation, and sulcular bleeding during orthodontic treatment. Am J Orthod Dentofacial Orthop. 1989; 96:199–207. https://doi.org/10.1016/0889-5406(89)90456-3 PMid:2788990
19. Shah KA, Patel MB, Shah SS, Chauhan KN, Parmar PK, Patel NM. Anti-hyper lipidimic activity of *Mangifera indica* leaf extract on rats fed with high cholesterol diet. Der Pharmacia Sinica. 2010; 1(2):156-61.
20. Chikaodili eziamaka A. The efficacy of extracts from mango (*Mangifera indica*) stem in the treatment of toothache. Asian Journal of Advances in Research. 2022; 15(1):15-26.
21. Li H, Miyahara T, Tezuka Y, Namba T, Nemoto N, Tonami S, *et al*. The effect of Kampo formulae on bone resorption *in-vitro* and *in-vivo*. Active constituents of Tsukan-gan. Biology and Pharmacology Bulletin. 1998; 21:1322-6. https://doi.org/10.1248/bpb.21.1322 PMid:9881647
22. Godfrey SB, Aloysius L, Nathan M, David B, Kyegombe Paul W. The activity of *Mangifera indica* leaf extracts against the tetanus causing bacterium, *Clostridium tetani*. African Journal of Ecology. 2007; 45:54-8. https://doi.org/10.1111/j.1365-2028.2007.00858.x
23. Pimpak puhmut. Effects of *Piper betle* fractionated extracts on inhibition of *Streptococcus mutans* and *Streptococcus intermedius*. Drug Discov Ther. 2018; 12(3):133-41. https://doi.org/10.5582/ddt.2018.01021 PMid:29998994
24. Méndez J. Carica papaya mouthwash for reducing dental plaque. Sun Text Review of Dental Sciences. 2020; 1(1):102. https://doi.org/10.36879/GoD.20.000133
25. Pratiwi R. Perbedaan daya hambat terhadap *Streptococcus mutans* dari beberapa pasta gigi yang mengandung herbal. Dent. J. 2005; 38(2):64-7. https://doi.org/10.20473/j.djmkg.v38.i2.p64-67
26. Moeljanto RD. Khasiat and manfaat daun sirih: Obat mujarab dari masa ke semasa. Agro Media. 2003.
27. Marwa AA Fayed. *Mentha piperita* L.: A promising dental care herb mainly against cariogenic bacteria. Universal Journal of Pharmaceutical Research. 2019; 4(3).
28. Prabu GR, Gnanamani A, Sadulla S. Guaijaverin: A plant flavonoid as potential antiplaque agent against *Streptococcus mutans*. J Appl Microbiol. 2006; 101:487–95. https://doi.org/10.1111/j.1365-2672.2006.02912.x PMid:16882158
29. Salve1 AV, Malik R, Ansari S. *Mimusops elengi* (Linn.)*:* An effective aid in dental care. Journal of Global Oral Health. 2022; 5:1-4. https://doi.org/10.25259/JGOH_4_2022
30. Ashok PK, Upadhyaya K. Tannins are astringent. J Pharmacogn Phytochem. 2012; 1:45-50.
31. Sofowra A. Ibadan, Nigeria: Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd.; 1993. p. 191-289.
32. Trease GE, Evans WC. Pharmacognosy, 11th edition, Bailliere Tindall, London; 1989. p. 45-50.
33. Harborne JB. London: Phytochemicals Methods. Chapman and Hall Ltd.; 1973. p. 49-188.