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FORMULATION AND IN-VITRO EVALUATION OF NEEM AND KARANJ TOOTHPASTE: A COMPARATIVE STUDY WITH LEADING HERBAL BRANDS

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ABSTRACT

The present study seeks to develop herbal toothpaste from neem and karanj twigs. The plant extract constituents have antimicrobial, anti-inflammatory, and anti-caries properties, which are useful in the prevention of bad odors through dental hygiene. Three formulations of herbal toothpaste were prepared by using Pongamia pinnata twig powder and *Azadirachta indica* twig extract. All the prepared toothpaste formulations (TF1, TF2, TF3) and marketed formulations (MF1, MF2) were evaluated for pH, extrudability, spreadability, foamability, homogeneity, cleaning action, and antibacterial activity, with comparisons to marketed formulations. Herbal toothpaste preparations satisfy all of the requirements for maintaining the mouth fresh and preventing the bacteria that cause tooth decay. The antimicrobial study against E. coli (Escherichia coli) shows that prepared herbal toothpaste has significant antibacterial action. The results showed that the herbal toothpaste performed comparably or better in terms of antibacterial activity and cleansing effect of marketed formulations. The Research results indicate that such herbal toothpaste may represent a beneficial, environmentally friendly alternate to currently available synthetic oral care products.

Keywords: Toothpaste, herbal, antibacterial activity, pH, tooth decay, oral care product, cleansing activity

INTRODUCTION

Toothpaste is a semisolid dentifrice that contains abrasives, surfactants, humectants, binding agents, and other oral health-promoting ingredients. The product can be opaque, transparent, colored, or white and packaged in a container for continuous extrusion **[1-2]**. Components of toothpaste include abrasives for removing substances adhering to the teeth, binders to prevent separation of solid and liquid ingredients, humectants for preventing loss of water and hardening of toothpaste, solvents to dissolve all the ingredients, foaming agents to enhance cleansing activity and others such as coloring agents, flavoring agents, preservatives and sweeteners. Most commercially available toothpastes contain artificial excipients in their formulations, whereas this article discusses about herbal toothpaste made from natural extracts of neem and pongamia twigs **[3]**.

This article mentions about the herb Pongamia pinnata, which is used for maintaining dental hygiene and this work is first to report the use of pongamia twig extract in dental preparations.

The herbal ingredients used in this formulation include pongamia and neem. Pongamia pinnata commonly called as seashore mempari, pongam, Indian beech tree, poonga oil tree. It contains chemical constituents such as flavones and furanoflavones – karanjin, pongapin, demethoxykanujin, kanujin, pinnatin, gamatin. Pongamia pinnata twigs are tradionally used as toothbrush since ancient years and plays a vital role in anticaries activity **[4]**. The powder derived from dried karanja twigs can be used to clean teeth. Pongamia twigs have been traditionally utilized for dental cleansing; however, this article represents the first instance of formulating a toothpaste using pongamia twigs.

Azadirachta indica, commonly called Neem Tree. The chemical constituents of neem used for cleaning teeth include azadirachtin, nimbin, quercetin, salannin, tannins, and essential oils, all of which have anti-inflammatory, antibacterial, and antimicrobial effects. Extracts from several parts of the neem tree are mostly known for its antimicrobial, anti-fungal and anti-inflammatory effects.

While, trikatu powder is used in toothpaste for its anti-caries and antimicrobial activities. Fenugreek powder helps to reduce gum irritation and treat gingivitis. It improves gum health and maintains oral freshness. Clove is high in volatile compounds and antioxidants, including eugenol, α-humulene and β-caryophyllene. The oil of clove is used extensively in the perfume, cosmetic, health, medical, flavoring, and food preparation sectors.

Figure 1: Pongamia twig powder

Figure 2: Neem twigs

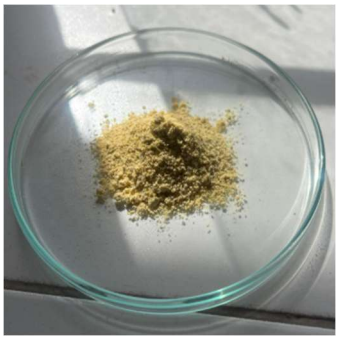

Figure 3: Fenugreek powder

MATERIALS AND METHODS

Plant based source (Karanj and Neem) twigs were collected from the surroundings of Gokaraju Rangaraju College of Pharmacy. The other compounds carried out were of analytical quality.

Method of preparation:

The method of preparation involves the extraction of active constituents from neem and karanj twigs using an organic solvent to obtain their beneficial properties.

Step 1: Extraction of neem

Neem twigs were collected, washed properly and shade dried for a week. After drying, the twigs were finely powdered and kept for extraction using an organic solvent i.e., methanol in the ratio 1:20(twig powder: methanol) in a conical flask. The set up was set aside undisturbed for 3 days and placed in an orbital rotary shaker for 3 days. After a week, the solution was filtered and washed properly with suitable organic solvent and the filtrate was collected. The obtained filtrate was placed on a water bath for evaporation to obtain the active constituents **[5-8]**.

Step 2: Extraction of Karanj

Twigs of karanj tree were collected, washed properly and shade dried for a week. After drying, the twigs were finely powdered and kept for extraction using an organic solvent i.e., methanol in the ratio 1:20 in a conical flask. The set up was set aside undisturbed for 3 days and placed in an orbital rotary shaker for 3 days. After a week, the solution was filtered and washed properly with suitable organic solvent and the filtrate was collected. The obtained filtrate was placed on a water bath for evaporation to obtain the active constituents **[9]**.

Preparation of toothpaste [10]

The polyherbal toothpaste was formulated by Wet gum method.

The ingredients are first weighed according to the formula. The liquid components, such as glycerin, water are then mixed together to create stage of liquid. The binding agent, guar gum, is then added to the liquid phase

and uniformly stirred to generate mucilage. The solid ingredients, which include pongamia, neem extract, fenugreek, trikatu, sodium CMC, sodium benzoate, CaCO3, NaHCO3, citric acid, and sodium saccharin (except the surfactant), are progressively added to the mucilage while stirring constantly in a mortar and pestle to make a uniform paste. Finally, the other ingredients, including SLS, peppermint oil, and clove oil, are mixed into the mixture.

Table 1: Formulation of Herbal Toothpastes

Ingredients	Uses	TF1	TF2	TF3
Pongamia (gm)	Anticaries	0.2	0.3	0.4
Neem (gm)	Anti-inflammation	0.05	0.1	0.15
Fenugreek (gm)	Prevents Gingivitis	0.5	0.5	0.5
Trikatu (gm)	Prevents Tooth decay	0.006	0.006	0.006
Clove oil (ml)	Antibacterial	0.01	0.01	0.01
SLS (gm)	Foaming agent	0.2	0.2	0.2
Sodium CMC (gm)	Stabilizer	0.2	0.2	0.2
Sodium Benzoate (gm)	Preservative	0.02	0.02	0.02
Guar gum (gm)	Thickening agent	0.4	0.4	0.4
$CaCo_3$ (gm)	Abrasive	10	10	10
$NaHCO_3$ (gm)	Whitening agent	0.02	0.02	0.02
Citric acid (gm)	Whitening agent	0.004	0.004	0.004
Glycerin (ml)	Humectant	6	6	6
Charcoal (gm)	Adsorbent	0.2	-	-
Sodium saccharin (gm)	Sweetener	0.06	0.06	0.06
Pippermint oil (ml)	Flavoring agent	0.01	0.01	0.01

EVALUATION TESTS:

1. Physical Examination: A physical examination evaluates color, odor, taste, and smoothness. The shade of the toothpaste was determined visually. The odor of the product revealed the presence of an aroma. The flavor of the formulation was assessed manually. Rub the paste formulation between fingertips to verify its smoothness **[11]**.

2. Homogeneity: The preparation is taken and smeared on a petridish, then observed for any presence of air bubbles, lumps, and separate parts.

3. Evaluation of sharp and abrasive particles: The formulation was placed on a petridish and pressed with a fingertip to recognize the presence of sharp and hard-edged grinding agents. Such particles should not be present in toothpaste.

4. pH: The pH of the established herbal toothpaste was determined using a pH meter. Two grams of toothpaste was placed in a beaker with a volume of 50 mL. 10 ml of water was added and quickly agitated to create a suspension.

5. Foamability [12]: To test the foamability of toothpaste, 2 grams of the formulation was mixed with 10ml of water in a test tube. The starting volume was observed and shaken ten times. The foamability of herbal toothpaste

was tested by comparing it to three toothpaste formulations and two marketed toothpaste brands. The final volume of foam was measured.

6. Viscosity: Viscosity of Herbal toothpaste was assessed using a viscometer manufactured by Brookfield at a temperature of 25 degrees Celsius and spindle number 64 at 50 rpm.

7. Assessment of moisture and volatile matter: 5g of the preparation was added in a porcelain dish that was 6–8 cm across and 2-4 cm deep. At 105^0 C, dry the sample in an oven.

$$\% \text{ of Moisture} = \frac{100\text{MI}}{\text{M}}$$

MI- Loss of mass(g) on drying

M- Mass (g) of the product

8. Spreadability: Two glass slides (10 x 10 cm) are put together with 1-2g of herbal toothpaste, and a weight of roughly 2kg is placed on top of each slide. Calculate how much toothpaste has spread (in centimeters) from its initial position after three minutes.

$$\textbf{spreadability} = \left[\mathbf{S = m} * \frac{\mathbf{L}}{\mathbf{T}}\right]$$

Where, S= Spreadability, M= weight in pan L= Length moved by slide, T= Time (sec) taken to separate

9. Extrudability: A collapsible aluminum tube was filled with the paste, and the end was crimped. After monitoring the tube weight, they were extruded up to 15 cm on butter paper, and their extrudability was examined **[13-14]**.

10. Fragnance test: Its acceptance had been determined based on user observations. Five people faced questions regarding fragrance reliability**.**

11.Cleansing ability [15]: After boiling 200 ml of water in a beaker, 15 ml of vinegar and 20 drops of red food coloring were added. For a period of five minutes, a hard-boiled egg was submerged in a food coloring solution, causing it to turn red. The eggshell was divided in two by drawing a line using a permanent marker. A pea-sized amount of toothpaste was put to the toothbrush after it had been washed with water. Ten strokes were used to brush half of the eggshell. After washing, the egg was checked for fading.

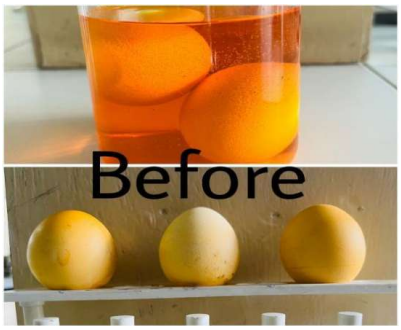

Figure 4: Before Cleansing

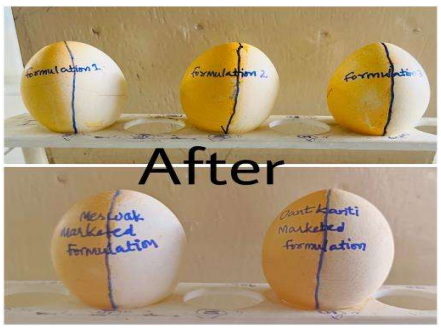

Figure 5: After Cleansing with tooth pastes

Shape retention: Toothpaste was squeezed from the tube and applied on a toothbrush. After 10 seconds, the toothpaste was rated using the following criteria **[16]**.

12. Anti-microbial activity: the cup plate method was used to conduct an in-vitro antibacterial research of the prepared paste against the pathogenic bacterial strain E. coli. Plates were then streaked with E. coli inoculum, and bores of 5mm diameter were bored into the medium with a sterile cork borer. The formed paste and commercialized formulations were then inserted in the bores on the cultured plates **[17]**. The plates were wrapped in paraffin, labeled, and incubated at 37^0 degrees Celsius for 24 hours. Each plate was evaluated following a 24-hour incubation period. The diameter of the zone of inhibition (ZOI) was measured using a ruler in millimeters (mm).

RESULTS

1. Physical Examination: The established toothpaste and marketed formulations was evaluated for its color, taste, odor and smoothness **(Table 2)**.

2. Homogeneity: The homogenicity of the prepared formulations were evaluated **(Table 3)**.

3. Determination of sharp and abrasive particles: The prepared formulations TF1, TF2, TF3 were evaluated for presence of any hard edged or sharp particles.

4. pH: The pH of the produced formulations was calculated using a pH meter and compared to marketed toothpaste. The typical toothpaste has a pH range from 7.3 to 8.2 **(Table 4)**.

5. Foamability: 2g of toothpaste was added with 10ml of water and jerked 10 times, then the volume of foam produced was compared to commercial formulation **(Figure 8, Table 5)**.

6. Viscosity: Using Brookfield viscometer with spindle no 64 at 50 rpm, the viscosity of the toothpaste was measured **(Table 6)**.

7. Evaluation of moisture content and volatile matter:1g of formulated Toothpaste was taken into petridish and kept in a hot air oven at 105^0 C **(Table 7)**.

8. Extrudability: The extrudability of the formulation was performed by extruding the paste upto 15cm on a butter paper **(Figure 9)**.

9. Spreadability: The Spreadability of the prepared formulations were found to be as shown in **Table 8**.

10. Fragnance test: The fragrance of the herbal toothpaste was analysed and the results were concluded as mentionedin **Table 9**.

11. Shape retention: This test has done by observing the shape of toothbrush after application of all prepared formulations TF1, TF2, TF3 and marketed formulations MF1 and MF2.

12. Microbiological evaluation: The antibacterial activity of the obtained formulations was determined using the cup plate method on nutrient agar medium infected with E. coli bacteria. The ZOI was measured and reported as can be seen in **Table 10, Figure 12, 13**.

Table 2: Physical evaluation of tooth pastes

Parameters	TF1	TF2	TF3	MF1	MF2
Color	Black	Light Greenish	Light Greenish	Brown	White
Odor	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant
Taste	Sweet	Sweet	Sweet	Sweet	Sweet
Smoothness	Smooth	Smooth	Smooth	Very smooth	Very smooth

MF1: Dantkanti; MF2: Meeswak

Table 3: Homogeneity of tooth pastes

Formulation code	TF1	TF2	TF3	MF1		MF2
Homogeneity	+++	+++	++	+++		+++

MF1: Dhantkanti; MF2: Meeswak+++ excellent, ++ clear, +turbid

Table 4: pH of tooth pastes

Formulation code	TF1	TF2	TF3		MF1	MF2
pH	7.88	8.08	8.07		9.5	8.9

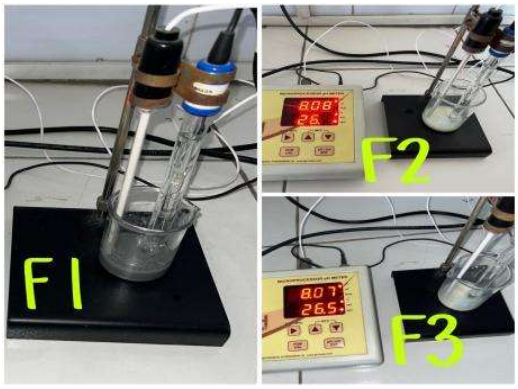

Figure 6: pH of Formulation

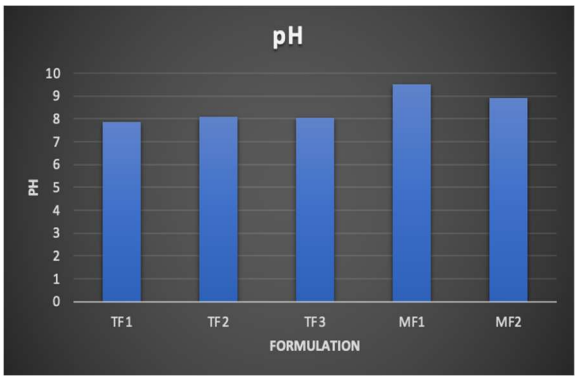

Figure 7: Graphical representation of pH

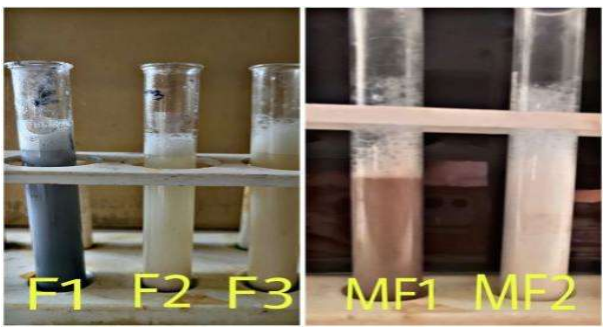

Figure 8: Foam test

Table 5: Foamability of tooth pastes

Formulation code	TF1	TF2	TF3	MF1	MF2
Fomability (cm)	2	1	1	4	3

Table 6: Viscosity of tooth pastes

Formulation code	TF1	TF2	TF3	MF1	MF2
Viscosity(cP)	11320	11820	17960	19940	19965

Table 7: Moisture contentand volatility of tooth pastes

Formulation code	TF1	TF2	TF3	MF1	MF2
Moisture content (%)	0.12	0.09	0.07	0.15	0.11

Figure 9: Extrudability of Herbal Toothpaste

Table 8: Spreadability of tooth pastes

Formulation code	TF1	TF2	TF3	MF1	MF2
Spreadability (cm)	2.22	1.11	1.6	4.2	4

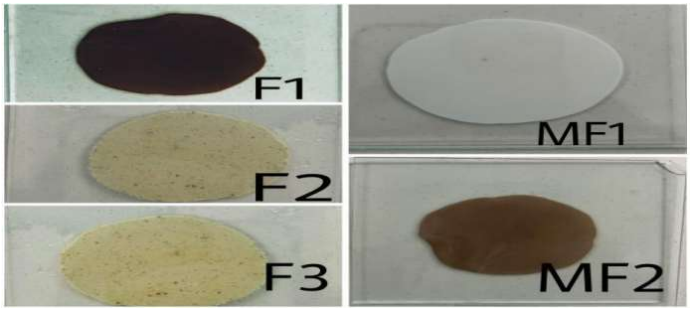

Figure 10: Spreadability of toothpaste formulations

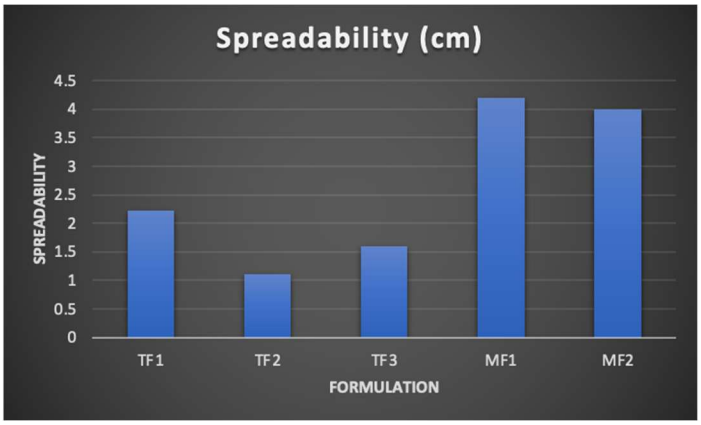

Figure 11: Graphical representation of spreadability

Table 9: Fragnance test of tooth pastes

Formulation	TF1	TF2	TF3	MF1	MF2
Fragnance	++	+++	+++	+++	+++

+++ excellent, ++ good, +bad

Table 10: Antimicrobial activity of tooth pastes

Formulation	TF1	TF2	TF3	MF1	MF2
ZOI (mm)	22.5	19	0	30	26

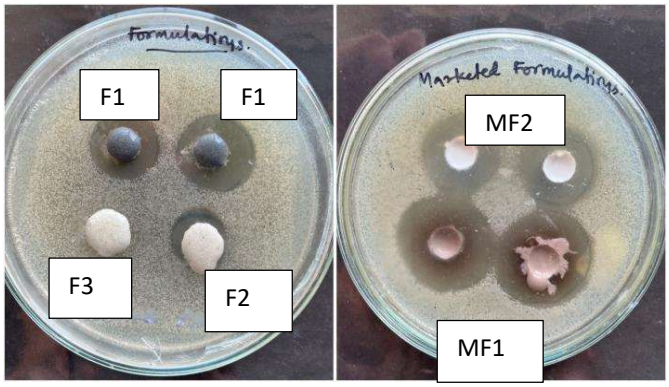

Figure 12: Antibacterial activity.

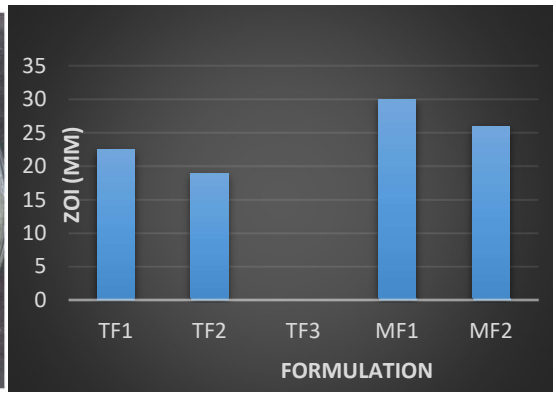

Figure 13: Antimicrobial activity (ZOI) of tooth pastes

DISCUSSION

1. Organoleptic character: Visual and sensory inspection were used to assess organoleptic properties. All the prepared formulations were of accepted color, odor, taste and smooth texture as mentioned in **Table 2**.

2. Homogeneity: All the formulations were found to have good characteristic extrudability as in **Table 3**.

3. Determination of sharp and abrasive particles: The prepared formulations TF1, TF2, TF3 does not have any sharp or hard edged particles.

4. pH: All the prepared formulations were exhibiting pH near to that in oral cavity as in mentioned in **Table 4**.

5. Foamability: The foamability of TF1 was found good compared to all the prepared formulations and closer to marketed formulations as that of mentioned in **Table 5**.

6. Viscosity: All the prepared toothpaste formulations exhibited good viscosity similar to that of marketed formulations as mentioned in **Table 6**.

7. Determination of moisture content and volatile matter: All the prepared formulations have good quantity if moisture content as in **Table 7**.

8. Extrudability: All the formulations were observed to have good characteristic extrudability.

9. Spreadability: TF1 formulation exhibited spreadability of 2.22cm which was good as that of marketed formulations than that of

other prepared formulation TF2 and TF3.

10. **Fragrance test:** All the prepared formulations were exhibiting good fragrance.

11. **Shape retention:** On application of all the prepared formulations TF1, TF2 and TF3 the shape of the toothbrush remained unchanged.

12. Anti-microbial activity: TF1 formulation was having greater ZOI which indicates good antibacterial activity due to the synergistic effect of neem, pongamia and charcoal.

13. **Cleansing activity:** All the prepared formulations were exhibiting good cleansing activity as indicated in **Figure 4 & 5**.

CONCLUSION

Herbal toothpaste is regarded as typically safe, readily available, and has equivalent antimicrobial and abrasive qualities same as that of regular toothpaste. The formulated herbal toothpaste is required for the treatment of variety of dental diseases, include gingivitis, tooth decay, and plaque. A total of three formulations of herbal toothpaste were prepared and evaluated for pH, homogenicity, spreadability, extrudability, foamability, viscosity, cleansing activity and antimicrobial activity. It was found that TF1 formulation exhibited acceptable organoleptic properties, pH (7.8), excellent homogenicity, foamability (2cm), viscosity (11320 cP), moisture content (0.12%), spreadability (2.22cm), good cleansing activity and antimicrobial activity with Zone of inhibition of 22.5mm. The activity of antimicrobial growth of TF1 was found to be greater than the other two formulations (TF2 and TF3). Results appeared close to that of marketed formulations thus proving the efficiency of formulated toothpaste. It was concluded that the desired objectives were met for the prepared toothpaste which has good cleansing and antibacterial activity.

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