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## Original Research Article

Comparative evaluation of herbal toothpaste formulations: A preliminary *in-vitro* studyAfroj Ayyaj Shaikh <sup>1,\*</sup><sup>1</sup>Hon. Shri. Babanrao Pachpute Vichardhara Trust, Group of Institutions, Faculty of Pharmacy, Kashti, Maharashtra, India

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## ABSTRACT

**Background:** Toothpaste, available in gel or paste form, maintains oral hygiene through brushing. This research aims to develop herbal toothpaste formulations, avoiding potential side effects of chemical compounds found in conventional products.

**Aim & Objective :** This study assesses herbal toothpaste formulations for effective dental hygiene, specifically investigating natural ingredients like neem leaves, babul leaves, akkarkara leaves, guava leaves, jamun seeds, vidanga, Vajradanti, and clove fruit, traditionally used for dental care.

**Methods :** We assessed sensory and physical characteristics of the newly formulated herbal toothpaste, following Bureau of Indian Standards criteria. Comparative analysis showed laboratory-developed toothpaste performing similarly to Dantkranti, Babool, and Colgate toothpaste brands.

**Results :** The laboratory-created herbal toothpaste matched the essential toothpaste properties of three popular brands.

**Conclusion :** This preliminary *in vitro* study suggests herbal toothpaste's potential as an effective and safe alternative for oral hygiene. Further research and clinical studies are needed to validate these findings and explore their dental care applications.

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## 1. Introduction

Toothpaste, an indispensable component of oral healthcare, has a history dating back to ancient times. The formulation of toothpaste can be traced back to as early as 300-500 BC in China and India.<sup>1</sup> Dental caries and periodontal diseases, which are multifactorial in nature, find their primary causes in dental plaque. Maintaining good oral hygiene through the use of oral care products such as toothpaste, toothbrushes, mouthwashes, and antimicrobial toothpaste is crucial in preventing these conditions.

India boasts a rich tradition of indigenous medicinal practices. However, in contemporary times, there has been a shift towards the use of allopathic drugs.<sup>2</sup> Herbal toothpaste

offers an alternative by avoiding artificial colors, flavors, and fluorides commonly found in conventional products. Building upon this premise, this research aims to formulate a poly-herbal toothpaste and investigate its antimicrobial properties.<sup>3</sup>

Over the past decade, herbal remedies have gained popularity worldwide for both prevention and treatment of various ailments. These herbal medicines are often chosen for their perceived safety, as they tend to mitigate the side effects associated with traditional medicines.<sup>4</sup> These alternatives encompass dental products with natural ingredients and herbal formulations. Previous studies have demonstrated that dental plaque control can be achieved through physical removal and the use of pharmaceutical products like antimicrobial toothpaste and mouthwashes.<sup>4</sup>

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This research is geared towards formulating herbal toothpaste that incorporates natural ingredients such as neem leaves, babul leaves, akarkara leaves, guava leaves, jamun seeds, vidanga, Vajradanti, and clove fruit, all of which have a historical association with dental care.<sup>5</sup> The evaluation of the formulated herbal toothpaste will encompass its sensory and physical properties, benchmarked against the standards set by the Bureau of Indian Standards, as well as a comparison with commercially available toothpaste formulations.

With a long history and vital role in maintaining oral health, toothpaste<sup>6</sup> has a long and illustrious history. Ancient China and India, between 300 and 500 BC, are where the design of toothpaste first appeared. Crushed bone, powdered eggshells, and crushed clamshells were all used as abrasives during this time to clean teeth.<sup>6</sup> In the nineteenth century, new toothpaste formulas were developed that included soap and chalk as components. Following 1945, substantial developments in formulation led to the inclusion of a variety of detergents, with sodium lauryl sulfate playing a crucial role as an emulsifying agent.

As a means of both treating and preventing oral problems, toothpaste composition has recently turned its emphasis to maximizing the release of active chemicals. The major functions of toothpaste, also known as dentifrice, are oral hygiene maintenance and improvement. It serves as an abrasive substance that aids in the removal of food particles and dental plaque from teeth as well as a method of promoting oral hygiene. Toothpaste also helps to cover up or get rid of halitosis and contains active ingredients like fluoride to help prevent dental and gum problems like gingivitis.<sup>7</sup> Toothpaste excipients are essential for increasing the cleaning process even if the mechanical action of toothbrushes is essential for proper cleansing.

This investigation seeks to assess herbal toothpaste formulations and compare them to three popular commercial toothpaste products.

## 2. Materials and Methods

1. *Collection of plant material:* Babul leaves, Guava leaves, Neem leaves, Syzygium cumini seeds, Akarkara leaves, was collected from the local forest area of Gondia district. Clove oil, Barteia prionitis bark, Embelia ribes, was procured from local market of Shrigonda. The samples were stored at room temperature (37°C) until further use. Calcium carbonate, Sodium lauryl sulfate, sorbitol, Sodium benzoate was take from HSBPVT's, GOI, Faculty of Pharmacy.
2. *Drying:* Drying the Babul leaves, Guava leaves, Neem leaves, Syzygium cumin seeds, Akarkara leaves, Barteia prionitis bark, Embelia ribes, was done shade drying for one week. As all drugs contains volatile matters shade drying was preferred.

3. *Crushing:* Crushing of the leaves was done with the help of pestle and mortar at room temperature. Crude powder which was passed through sieve no – 6. The sample was stored at room temperature.

### 2.1. Preparation of extract

1. *Extraction Done by the Cold Maceration Process:* Crude powder of the above herbs Babul leaves, Guava leaves, Neem leaves, Akarkara leaves, Barteia prionitis bark, was defatted with petroleum ether and then the defatted material was subjected to extraction. The left marc was dried at room temperature and was similarly extracted with hydroalcoholic solution respectively. This simple widely used procedure involves leaving the plant to soak in a suitable solvent in a closed container. Simple cold maceration is performed at room temperature by mixing the fresh drug with the solvent (drug solvent ratio Water: Ethanol: 30:70) and kept the mixture for several days with occasional shaking or stirring. The extract was concentrated. The obtained residue was dried in desiccator.
2. *Preliminary Phytochemical Investigation tests of Extracts:* In these the various preliminary test was done to detect the presence of chemical constituent like alkaloids, flavonoids, tannins, glycosides etc.
3. *Formulation of Toothpaste:* All herbal ingredients were dried and grounded using domestic mixer. The required quantity of ingredients were weighed and taken in mortar. Calcium carbonate, Sodium lauryl sulfate, sorbitol, Sodium benzoate, honey was mixed in water. This solution was added drop wise into mortar containing herbal ingredients and triturated well until a paste consistency is formed. Table 1 shows plant extracts and composition of chemicals.<sup>8</sup>
4. *Agar Diffusion Test:* Utilizing the agar diffusion test—more specifically, the punch-hole technique described in Slack's<sup>9</sup> protocol—was necessary to assess the test isolates' susceptibility to the herbal solution.
5. *Inhibitory minimum concentration (MIC):* Using the tube dilution technique outlined by Cowan and Steel,<sup>10</sup> the minimal inhibitory concentration was calculated.
6. *Bactericidal/fungicidal minimum concentration test (MBC/MFC):* In accordance with Baron and Fingold's<sup>11</sup> description, the Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) Test was conducted.

**Table 1:** Chemical composition of formulation

Sr. No.	Composition	Quantity
1	<i>Azadirachta Indica</i>	3 g
2	<i>Anacyclus Pyrethrum</i>	4 g
3	<i>Guava Leaves</i>	3 g
4	<i>Barteia Prionitis</i>	3 g
5	<i>Embelia Ribes</i>	2 g
6	<i>Syzygium Cumini</i>	2 g
7	<i>Acacia Arabica</i>	4 g
8	Clove Oil	2 ml
9	Honey	10 ml
10	SLS	0.5 g
11	Calcium Carbonate	10 g
12	Sodium Benzoate	0.5 g
13	Sorbitol	1 g
14	Distilled Water	q.s

### 3. Evaluation and Comparison of Herbal Toothpaste<sup>12,13</sup>

#### 3.1. Physical examination

1. *Color:* The color of the cream was observed by visual examination.
2. *Odour:* The odor of cream was found to be characteristics.
3. *Taste:* Taste was checked manually by tasting the product. Relative density: Relative density was determined by weight in gram taken in 10 ml formulation and 10 ml distilled water using RD bottle
4. *Consistency:* The formulation was examined by rubbing cream on hand manually. The cream having smooth consistency. Cream did not leave greasy substances on skin surface after application.
5. *State:* The state of cream was examined visually. The cream having a semisolid state.

#### 3.2. Evaluation parameter

1. *Determination of sharp and edge abrasive particles:* The contents were taken on finger tip and scratched on the butter paper for 15-20 cm length to check for the presence of any sharp or abrasive particles. This was repeated 10 times. No sharp or abrasive particles were found.
2. *Spreadability:* A gram of toothpaste was placed on a glass slide (10 x 10 cm) and covered with another glass slide. Then 2 kg weight was placed (sliding, shall not take place). The spreading (in cm) of the toothpaste was measure after 3 minutes. The procedure was repeated 2 more times and average of three observations was noted.
3. *Foaming power:* A suspension of the material was taken in measuring cylinder and shaken for 12 times and the volume of the foam produced after shaking for

5 minutes was measured. Procedure: 5g of toothpaste was weighed in to a 100 ml glass beaker. 10 ml of water was added, covered with a watch glass and kept aside for 30 minutes. The suspension was heated gently to dissolve the detergent if present in it. The suspension was stirred with glass rods and transferred to 250 ml measuring cylinder. It was examined to check if no foam is produced (more than 2 ml). The residue retained in the beaker was transfer to measuring cylinder by adding of 5-6 ml of water. Then the volume was made up to 50ml of water. The contents were stirred with up-down movements to get uniform suspension at 30°C. After shaking, the cylinder was kept aside for 5 minutes. And note the final volume obtained with foam and water.

4. *pH Determination:* Dispense 10 gm of the toothpaste from the container in a 50 mL beaker and add 10 mL of freshly boiled and cooled water (at 27°C) to make 50 percent aqueous suspension. Stir well to make a thorough suspension. Determine the pH of the suspension within 5 min, using a pH meter.
5. *Washability:* Formulation was applied on the skin and then ease extends of washing with water was checked.
6. *Non-irritancy test:* Herbal cream formulation was evaluated for the non-irritancy test. Observation of the sites was done for 24 hr.
7. *Viscosity:* Viscosity of cream was done by using Brooke field viscometer at the temp of 25 °C using spindle no. 63 at 5 rpm.
8. *Phase separation:* The prepared cream was transferred in a suitable wide mouth container. Set aside for storage, the oil phase and aqueous phase separation were visualizing after 24h.
9. *Determination of moisture and volatile matter:* 5 g of formulation placed in a porcelain dish containing 6-8 cm in diameter and 2-4 cm depth in it. Dry the sample in an oven at 105°C.  
Calculation: % by mass = 100 MI/M  
MI- Loss of mass (g) on drying  
M- Mass (g) of the material taken for the test.
10. *Determination of Heavy Metals:* Accurately weigh 2 g of the sample in a kjeldahl flask. An acid mixture of HNO<sub>3</sub>:HClO<sub>4</sub> (4:1) was added in the flask and heated continuously till the solution becomes colorless. The sample was then transferred to a 25 ml volumetric flask and volume was made up with distilled water. A reagent blank was synchronously prepared accordingly to the above procedure. The standard of Lead (Pb) was prepared as per the protocol in the manual and then the sample was visually analyzed and compared with the standard solution of lead.

**Table 2:** Preliminary phytochemical investigation of extracts

Sr. No.	Test	A. Indica	A. Pyrethrum	B. Prionitis	A. Arabica
1	<b>Alkaloids</b>				
	Mayer's Reagent	+	+	+	+
	Dragendorff's Reagent	+	+	+	+
	Wagner's Test	+	+	+	+
	Hager Test	+	+	+	+
2	<b>Glycosides</b>				
	Legal Test	+	-	+	+
	Keller- Killani Test	+	-	+	+
3	<b>Triterpenoids</b>				
	Liebermann's- Burchardt's Test	+	+	+	+
	Salkaowaski Test	+	+	+	-
4	<b>Flavanoids</b>				
	Shinoda Test	+	+	+	+
	Alkali Test	+	+	+	+
	Lead Acetate Test	+	+	+	+
5	<b>Tannin</b>				
	Ferric Chloride Test	+	+	+	+
6	<b>Saponin</b>				
	Foam Test	-	+	+	+

Evaluation: The evaluation parameters were of the polyherbal and marketed paste were shown in tables below.

**Table 3:** Physical examination of formulations

Sr. No.	Parameters	Formulation	Dantkanti	Babool	Colgate
1	Colour	Slightly White Green	Brownish	White	White
2	Odour	Characteristics	-	-	-
3	Taste	Bitter	-	-	-
4	Relative Density	10.2	8.7	8.3	9.2
5	Consistency	Smooth	-	-	-
6	State	Semi- Solid	Semi- Solid	Semi- Solid	Semi- Solid

**Table 4:** Spreadability, Foaming ability & pH of lab made formulation and marketed formulation

	Formulation	Dantkanti	Babool	Colgate
Spreadability (cm)	7.4	6.9	6.5	7.2
Foaming Ability	54	45	48	52
pH	6.7	7.6	8.3	8.6

**Table 5:** Determination of hard and sharp edged abrasive particles, Washability and irritancy test of lab made formulation and marketed formulation

Product	Hard and sharp edged abrasive particles	Washability	Non- irritancy Test
<b>Formulation</b>	Absent	Easily	Non- irritant
<b>Dantkanti</b>	Present	Easily	Non- irritant
<b>Babool</b>	Absent	Easily	Non- irritant
<b>Colgate</b>	Absent	Easily	Non- irritant

**Table 6:** Stability Studies for three months

3 <sup>rd</sup> Month	Colour	Appearance	Spreadability	pH
At 25°C ±2°C/ 60% ± RH	Slightly White Green	Homogeneous	7.4	6.7
At 35°C ±2°C/ 65% ± RH	Slightly White Green	Homogeneous	7.4	6.7
At 40°C ±2°C/ 75% ± RH	Slightly White Green	Homogeneous	7.4	6.7

### 3.3. Anti-bacterial activity

#### 3.3.1. Growth and maintenance for bacteria

The strain from the plate was inoculated in the nutrient broth and then the inoculum was left for 1-2 days at 37°C in the incubator. After the growth of bacteria in the broth, it is used to perform the well diffusion method with the given sample.

#### 3.3.2. Procedure

1. *Preparation of sample:* The plant extracts are first allowed to dry in a petridish to get its concentrated form. Before performing the test, the dried plant extracts were added with little amount of distilled water to make a conc. solution.
2. *Preparation of plates:* Weigh all reagents and dissolved in 60 ml water. Heated with agitation to dissolve the constituents properly. Autoclaved at 121°C and 15 lbs. pressure. Immediately after autoclaving, allow it to cool in a 45 - 50°C. Pour the freshly prepared and cooled medium into petri plates. The agar medium should be allowed to solidify at room temperature.
3. *Reading of plate and interpretation:* After 15 to 16 hours of incubation, each plate was examined. If the plate satisfactory streaked, the inoculums were correct the result of ZOI should be uniformly circular and a confluent lawn of growth. After measure the diameter of ZOI the data was noted and interpreting the result.
4. *Stability study:* The stability study was performed as per ICH guideline. The formulated paste was filled in collapsible tube and stored at different temperature and humidity conditions, 25°C ± 2°C / 60% ± 5% RH, 30°C ± 2°C / 65% ± 5% RH, 40°C ± 2°C / 75% ± 5% RH for the period of 3 months and studied for appearance, pH and Spreadability.

## 4. Results

### 4.1. Anti-microbial activity observation

The formulated herbal toothpaste exhibited fairly good anti- *S.aureus* activity as compared to the standard drug Amoxicillin. The formulation exhibited an impressive ZOI of 7 mm at MIC of 25µg/mL, whereas Amoxicillin exhibited 10 mm ZOI at MIC of 6.25µg/mL. Therefore it may be concluded that formulated tooth paste have potential to exhibit anti-microbial activity.<sup>14,15</sup>

## 5. Conclusion

The herbal toothpaste is an emphasizing and more acceptable in dental research and they are safer with minimum side effects than synthetic preparation. The formulated tooth paste compiled the requirements of all evaluation tests and was found suitable for the teeth and oral hygiene. The formulated herbal toothpaste has good scope

in future in nature remedies research and dental health of public.

## 6. Source of Funding

None.

## 7. Conflict of Interest

None.

## 8. Acknowledgment

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