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# (Research Article)

Formulation and evaluation of moringa and neem herbal toothpaste for comprehensive oral care

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### Abstract

The demand for natural and effective antimicrobial agents in oral care products has led to the exploration of botanical extracts such as Moringa and Neem. In this study, we aimed to prepare extracts from Moringa and Neem leaves and incorporate them into toothpaste formulations to evaluate their antimicrobial properties against oral pathogens.

The antimicrobial activity of the extracts was assessed using well-established agar diffusion assays against a panel of Gram-positive and Gram-negative oral bacteria. Additionally, the pH and consistency of the toothpaste formulations were optimized for usability and stability. Our results demonstrated significant antimicrobial activity of the Moringa and Neem extracts against a range of oral bacteria, including common pathogens associated with dental caries and periodontal diseases.

The formulated toothpaste containing these extracts exhibited promising inhibitory effects, suggesting their potential as natural alternatives or adjuncts to synthetic antimicrobial agents in oral hygiene products. This research work contributes to the ongoing exploration of plant-derived compounds for oral health applications.

Keywords: Antimicrobial activity; Oral bacteria; Periodontal diseases; Dental carries

# 1. Introduction

*Azadirachta indica*, often known as Neem, and *Moringa oleifera*, sometimes known as Moringa, are two incredibly extravagant trees that are well-known for their restorative rates. The monogenous plant Moringa, often known as the horseradish tree or drumstick, is one of the cultivated and wild variants of the genus Moringa. It is part of the Moringaceae family. Functional bioactive substances like flavonoids, phenolic acids, alkaloids, phytosterols, natural sugars, vitamins, minerals, carbs, protein, glycosides, and organic acids are found in moringa. Moringa exhibits a wide spectrum of pharmacological characteristics, including antibacterial, hepatoprotective, immunomodulatory, antioxidant, and anti-inflammatory effects, that may have therapeutic applications.

The plant *Azadirachta indica*, often known as neem, is a member of the Meliaceae family and has been used for medicinal purposes for a very long time. Several phytochemicals, such as quercetin and azadirachtin, and limonoids, like nimbin, nimbinin, and nimbidin, have been identified from different plant sections. Additionally, the leaves also comprise a combination of substances, including 6–desacetylnimbinene, nimbanene, Ascorbic acid, n-hexacosanol, nimbiol, nimbolide, nimbandiol, and other amino acids, together with a variety of other components. Neem has anti-inflammatory, antiviral, anti-microbial, antioxidant, anti-bacterial, and treatment of dental care qualities [1,2,3].

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Toothpaste is a dental product used to clean, preserve, and improve dental health. In addition to acting as an abrasive to aid in the removal of food particles and dental plaque from teeth, toothpaste can help to eradicate or disguise foulodour. Toothpaste is mainly used to promote oral cleanliness of the mouth. It protects, cleans and polishes enamel. It makes oral hygiene greater effective and has a clean flavour and smell and freshen the breath. Brushing two times an afternoon with toothpaste is crucial to keeping a wholesome mouth [4].

While the majority of toothpaste formulations sold in stores use artificial excipients, our formulation uses natural extracts. Herbal toothpaste is made by mixing ingredients like sodium bicarbonate and calcium carbonate, sorbitol and glycerine, binding agent sodium CMC, detergent and foaming agent sodium Lauryl sulphate, flavoring ingredient peppermint oil, preservatives sodium benzoate and methyl paraben, and sweetener sodium saccharine[5]. The homogenization process is used in the manufacturing of herbal toothpaste. In this technique, firstly, the base is prepared through homogenization using a mortar and pestle, and then herbal ingredients such as sorbitol and peppermint oil are added. Natural toothpastes are those that do not have either fluoride or triclosan. The composition of herbal toothpaste may include a combination of herbal extracts, abrasives, humectants, detergents, sweetening agents, preservatives, and flavouring agents[6]. The primary goal is to create a herbal toothpaste and assess its antimicrobial effectiveness. The ideal properties should have a good abrasive effect, non-irritant and non-toxic, will not cause stains on teeth, provide freshness in the mouth and keep it clean, and will have a prolonged effect while being cheap and easily available.

This investigation primary goal is to assess the formulation of herbal toothpaste. The strategy is to create herbal paste using readily available herbs like Moringa and Neem, also known as *neem*. These herbal blends have antibacterial properties[7].

# 2. Materials and methods

### 2.1. Chemicals And Reagents

The plants of Moringa and Neem was purchased from Shree Siddhivinayak Agro Center and Services, Thane, Maharashtra. and it was dried, and fine powder was prepared using mixer for extraction purpose. Calcium carbonate, sodium lauryl sulphate, sorbitol, sodium CMC, methyl paraben, sodium benzoate, sodium saccharine and peppermint oil were purchased from Research-Lab Fine Chem Industries, Mumbai, 400 002 (India). The plant materials were authenticated by Dr. Harshad M Pandit, Ph.D in Botany, Mumbai.

### 2.2. Experimental procedure

### 2.2.1. Sample collection and pre-treatment

The samples of Moringa and Neem were obtained from Shree Siddhivinayak Agro Center and Services in Thane, Maharashtra. Following collection, the fresh leaves were air-dried at room temperature before being ground using a milling machine.

### 2.2.2. Moisture content and ash value

### Moringa oleifera

- The fresh moringa leaves were dried in an oven at 103 °C at 5-minute intervals for a total of 30 minutes.
- The ash value analysis as it provides valuable insight into the inorganic content of the sample.

By subjecting the sample to complete incineration, we were able to quantify the mineral residue left behind, which is crucial for assessing its purity and composition

### Azadirachta indica

The fresh Neem leaves were dried in an oven at 103 °C at 5-minute intervals for a total of 30 minutes. The ash value analysis as it provides valuable insight into the inorganic content of the sample. By subjecting the sample to complete incineration.

### 2.3. Soxhlet Extraction

#### 2.3.1. Extraction of Moringa oleifera

Extractions were conducted in aseptic conditions at Lokmanya Tilak Institute of Pharmacy, Kharghar. To ensure sterility, a conical flask was sterilized using an autoclave at 121 °C for 15 minutes. The leaves were then washed with tap water to eliminate any dust particles and subsequently dried at room temperature. Next, the powdered samples were weighed using an electronic scale and transferred into four conical flasks. The extraction process for moringa followed the Soxhlet extraction method. Specifically, 10 gm of plant material were weighed and placed in a reflux apparatus. A solution of 200 ml of ethyl acetate solvent was added to the apparatus, which was heated to a temperature of 30 °C. The extraction process continued for a duration of 8-9 hours until the solvent became clear, indicating the completion of extraction. The extract was collected by evaporating the solvent using a water bath and then transferred to an airtight container.

#### 2.3.2. Extraction of Azadirachta indica

The Soxhlet extraction method was employed to conduct the extraction process. The extractions were performed in a sterile environment, with 50 gm of plant material weighed into a reflux apparatus setup. The set-up contained 300 ml of ethanol solvent, and the extraction was conducted at a temperature of 50 °C. The extraction process lasted for a duration of 8-9 hours, until the solvent became clear through refluxing. To collect the extract, the solvent was evaporated using a water bath. The resulting extract was then transferred into an airtight container and stored in a refrigerator.

#### 2.4. Phytochemical Analysis

#### 2.4.1. For Moringa oleifera

The qualitative and quantitative analysis of Moringa was conducted using various standard methods to determine the secondary metabolites present in the sample. These include alkaloids, tannins, saponins, beta-cyanins, anthocyanin, flavonoids, phenols, protein, amino acid, and sterols.

#### Determination of alkaloids

Following Wagner's test system, 2 ml of Extract was pipetted into a glass test tube. One- two drop of Sulphuric sharp was included to the extract Potassium iodide was also included and at that point shaken. The arrangement of a brownish accelerate shows the nearness of alkaloids.

#### Determination of Carbohydrates

Extract was added to 5 ml of distilled water to be dissolved and being filtered subsequently., Molisch Reagent was used to treat the precipitated. This was done to check if any sugars were present. Based on the observation, a violet ring conformation showed that the test was successful.

#### Determination of glycosides

Add 2 ml of distilled water to 2 ml of extract. To the extract, a few drops of sodium hydroxide were added. When a colour changes from yellow to orange, glycosides are present.

#### Determination of saponins

20 minutes were spent shaking 2 ml of the solvent extract and 5 ml of distilled water in a test tube. Saponin is present when a persistent foam layer forms on the surface, measuring 2 cm.

#### Determination of flavonoids

The extract was mixed with 2ml of ammonium hydroxide. In addition, add 2 ml of sodium hydroxide was added for the formation of orange colour as an indicator of the presence of flavonoids.

#### Determination of tannins

To the 2 ml of extract, 1 ml of distilled water was added. Then 2–3 drops of FeCl<sub>3</sub> was added to the extract. The formation of black or blue green precipitate shows the presence of tannins[10,11,12].

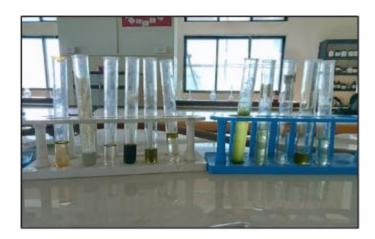


Figure 1 Phytochemical tests of Moringa extract

**Table 1** Phytochemical Screening of Moringa oleifera Leaf extract

| Sr. No. | Test              | Reagents used/ Test Preformed | Ethyl Acetate extract |
|---------|-------------------|-------------------------------|-----------------------|
| 1       | Alkaloid test     | Hager's reagent               | +                     |
| 2       | Carbohydrate test | Molisch test                  | +                     |
| 3       | Glycoside test    | Legal's test                  | +                     |
| 4       | Saponins test     | Foam test                     | +                     |
| 5       | Flavonoids test   | Alkaline reagent test         | +                     |
| 6       | Tannins test      | Ferric chloride test +        |                       |

(+: Present; -: Absent)

### 2.4.2. For Azadirachta indica

### Alkaloids test

Extract was dissolved separately in a dil. HCl acid and later, was filtered. Mayer's, Wagner's, and Dragendroff's Reagent were used to treat the filtrates individually to identify the alkaloids.

### Mayer's test

2 drops of Mayer's reagent were added in the side of the test tube of filtrate. The test is positive if there is an existence of white or creamy precipitate.

### Saponins Test

To thoroughly mix the liquids, 0.5 ml of neem extract was added to 2.5 ml of distilled water and vigorously shaken. After that, the mixture was held for a little while. The mixture was later kept for a few minutes. Based on the observation, the existence of saponins was confirmed because of the advancement of foam on the surface of the mixture.

### Carbohydrates Test:

5 ml of distilled water were mixed with neem extract, which was then allowed to dissolve and filter. The filtrates were then treated with Molisch Reagent. The purpose of this was to detect the presence of sugars.

### Cardiac glycosides Test:

A mixture of 0.5 ml of Neem extract, 1 ml of Iron (III) chloride reagent, and few drops of concentrated  $H_2SO_4$ , was done to examine for the existence of cardiac glycosides. Based on the observation, the existence of cardiac glycosides is confirmed when a greenish-blue colour precipitate appears. The test is positive.

### Tannins Test

0.5 ml of Neem was added to 2 ml portion of the 0.1% FeCl<sub>3</sub> to examine for the existence of Tannins. The test is positive when a precipitate appears in brownish-green or blue-black colour.

### Steroids Test

5 ml of chloroform were added in the 0.5 ml of extract. The presence of steroids was then investigated by adding 5 ml of sulfuric acid to the test tube's sidewalls. Based on the observation, the test is considered positive once the upper layer changes to red and the layer of the sulphuric acid turn to yellow with green fluorescent[10,13].

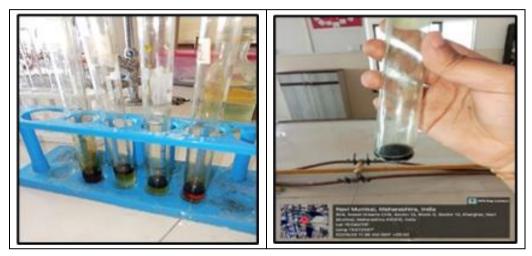


Figure 2 Phytochemical tests of Azadirachta indica extract

**Table 2** Phytochemical Screening of Methanolic Extract of Azadirachta indica

| Sr. No. | Tests                  | Reagents used/ Test performed | Methanolic extract |
|---------|------------------------|-------------------------------|--------------------|
| 1       | Alkaloids test         | Mayer's reagent               | +                  |
| 2       | Saponin test           | Foam test                     | +                  |
| 3       | Carbohydrate test      | Molisch test                  | +                  |
| 4       | Cardiac glycoside test | Legal's test                  | +                  |
| 5       | Tannins test           | Ferric chloride test          | +                  |
| 6       | Steroids test          | Salkowski's test              | +                  |

(+: Present; -: Absent)

| Sr.No. | Ingredients               | Batch A     | Batch B     | Batch<br>C  | Batch<br>D  | Batch<br>E  | Uses                           |
|--------|---------------------------|-------------|-------------|-------------|-------------|-------------|--------------------------------|
| 1      | Moringa                   | 1.0 gm      | 1.5 gm      | 2.0 gm      | 2.5 gm      | 2.5 gm      | Anti-Bacterial                 |
| 2      | Neem                      | 0.5 gm      | 1.0 gm      | 2.0 gm      | 1.5 gm      | 2.0 gm      | Anti-Microbial                 |
| 3      | Calcium Carbonate         | 9.141<br>gm | 9.141<br>gm | 9.141<br>gm | 9.141<br>gm | 9.141<br>gm | Abrasive                       |
| 4      | Sorbitol                  | 6.0 gm      | Humectant                      |
| 5      | Sodium Lauryl<br>Sulphate | 0.3 gm      | Detergent and Foaming<br>Agent |
| 6      | Sodium CMC                | 0.4 gm      | 0.3 gm      | 0.2 gm      | 0.1 gm      | 0.2 gm      | Binding Agent                  |
| 7      | Methyl Paraben            | 0.04 gm     | 0.04 gm     | 0.04<br>gm  | 0.04 gm     | 0.04<br>gm  | Preservative                   |
| 8      | Sodium Benzoate           | 0.02 gm     | 0.02 gm     | 0.02<br>gm  | 0.02 gm     | 0.02<br>gm  | Preservative                   |
| 9      | Sodium Saccharine         | 0.04 gm     | 0.03 gm     | 0.02<br>gm  | 0.01 gm     | 0.02<br>gm  | Sweetening Agent               |
| 10     | Sodium Bicarbonate        | 0.2 gm      | Abrasive                       |
| 11     | Glycerin                  | q. s        | Humectant                      |
| 12     | Peppermint oil            | q. s        | Flavoring Agent                |

Table 3 Trial Batch Formula

Table 4a Final Batch Formula a) Active Ingredients

| Sr. No. | Ingredients | Quantity | Uses           |
|---------|-------------|----------|----------------|
| 1       | Moringa     | 2.5 gm   | Anti-Bacterial |
| 2       | Neem        | 2.0 gm   | Anti-Microbial |

Table 4b Final Batch Formula b) Base

| Sr. No. | Ingredients            | Quantity | Uses                        |
|---------|------------------------|----------|-----------------------------|
| 1       | Calcium Carbonate      | 9.141 gm | Abrasive                    |
| 2       | Sorbitol               | 6.0 gm   | Humectant                   |
| 3       | Sodium Lauryl Sulphate | 0.3 gm   | Detergent and Foaming Agent |
| 4       | Sodium CMC             | 0.2 gm   | Binding Agent               |
| 5       | Methyl Paraben         | 0.04 gm  | Preservative                |
| 6       | Sodium Benzoate        | 0.02 gm  | Preservative                |
| 7       | Sodium Saccharine      | 0.02 gm  | Sweetening Agent            |
| 8       | Sodium Bicarbonate     | 0.2 gm   | Abrasive                    |
| 9       | Glycerin               | q. s     | Humectant                   |
| 10      | Peppermint oil         | q. s     | Flavoring Agent             |

# 2.5. Collection and isolation of bacteria

During the course of this research, various infections such as dental plaque, cavities, and periodontal diseases were identified in tooth samples collected from D.Y. Patil Dental College. The samples were carefully gathered in sterilized Eppendorf tubes containing 1 ml of nutrient broth and transported to Lokmanya Tilak College of Pharmacy laboratory. Subsequently, they were placed in a bacterial ––incubator overnight at 37 °C for optimal growth conditions. Pure bacterial cultures were isolated by streaking the samples on nutrient agar plates, which consist of Beef Extract (0.3 %), Peptone (0.5 %), and Agar (1.5 %) in water. The inoculated plates were then incubated in a thermal incubator for 24 hours at 37°C [21, 22].

# 2.6. Identification of bacterial strains:

Initially, the pure bacterial isolates underwent Gram staining, which comprises multiple steps.

- **Prepare Bacterial Smear:** Create a bacterial smear on a pristine microscope slide by transferring a small quantity of bacterial culture onto the slide using a sterile loop or swab.
- **Air Dry and Fix:** The bacterial smear is fully dried by allowing it to air dry completely. After it has dried, cautiously pass the slide through a flame multiple times to gently heat-fix the smear. This procedure will effectively attach the bacteria to the slide and prevent them from being washed off during the staining process.
- **Primary Stain (Crystal Violet):** Cover the slide completely with the crystal violet stain and allow it to sit for approximately one minute. Crystal violet serves as the primary stain in the Gram staining technique and imparts a purple color to all bacteria.
- **Rinse:** Rinse off the crystal violet with water.
- **Mordant (Gram's Iodine):** The slide then treated with Gram's iodine solution and left undisturbed for a duration of one minute. Gram's iodine functions as a mordant by creating a complex with the crystal violet present in the bacterial cells.
- **Decolourization:** The slide should be carefully rinsed off using a decolorizing solution, such as ethanol, to perform the decolourization process that allows for the differentiation of Gram-positive and Gram-negative bacteria.
- **Counterstain (Safranin):** After the process of decolourization, it is essential to promptly rinse the slide with water and subsequently immerse it in safranin stain. Safranin serves as a counterstain, imparting a pink or red colour to Gram-negative bacteria.
- Rinse and Dry: Rinse off excess safranin with water.
- **Examine:** Afterwards, analyse the slide with the aid of a microscope by employing oil immersion at a magnification of 10x. The identification of a purple or blue hue signifies the existence of Gram-positive bacteria[21, 22].

# 2.7. Antibacterial activities of Moringa oleifera and Azadirachta indica toothpaste

# 2.7.1. Agar Preparation

6.8 grams of nutrient broth and 20 grams of agar powder were dissolved in 1000 ml of distilled water. The solution was sterilized using an autoclave at 121 °C for 20 minutes. Afterward, the mixture was cooled down to 55 °C. A volume of 25 ml of the cooled media was poured onto the plate and allowed to solidify.

# 2.7.2. Streaking the bacterial sample:

The bacteria were streak on agar plate by in a bacterial incubator overnight at 37 °C for optimal growth conditions. Pure bacterial cultures were isolated by streaking the samples on nutrient agar plates, which consist of Beef Extract (0.3 %), Peptone (0.5 %), and Agar (1.5 %) in water. The inoculated plates were then incubated in a thermal incubator for 24 hours at 37 °C.

# 2.8. Assessment of Zone of Inhibition

In this study, we aimed to evaluate the antimicrobial activity of a newly formulated herbal toothpaste against common oral pathogens. The zone of inhibition assay was employed to assess the toothpaste's ability to inhibit bacterial growth, providing insights into its potential as an antimicrobial agent for oral hygiene.

### 2.8.1. Zone of Inhibition Assay:

Agar plates were prepared using appropriate media and inoculated with the bacterial strains. Wells were punched into the agar, and the herbal toothpaste was introduced into the wells. The plates were then incubated at optimal conditions

for bacterial growth. Following incubation, the zones of inhibition around the wells were measured using a calibrated ruler. The observed zones of inhibition indicate the potential antimicrobial activity of the formulated herbal toothpaste against common oral pathogens. The herbal ingredients present in the toothpaste formulation may contribute to its antimicrobial properties, possibly through its bioactive compounds known for their antibacterial effects[21, 22].

# 2.9. Method of Preparation

There are two different kinds of toothpaste formulation techniques, namely.

- Dry Gum Method
- Wet Gum Method

### 2.9.1. Wet Gum Method:

Preparation of Base

- This method involves initially mixing all the components to create a semisolid phase. The solid ingredients calcium carbonate, sodium lauryl sulphate, sodium benzoate, sodium saccharine, sodium CMC, methyl paraben were weighed accurately as mentioned in the formula.
- These ingredients were also mixed in a mortar and pestle and ground with accurately weighed sorbitol until a semi-solid was formed.
- Addition of herbal ingredients
- A precisely weighed Moringa and Neem extract was added to the base.
- Finally, peppermint oil was added as a flavoring agent.
- This method ensures the systematic integration of the various components, resulting in a uniform and wellmixed toothpaste formula[8,9].

### 2.10. Evaluation of Herbal Medicated Toothpaste

According to the guidelines, the standards were prescribed for each evaluation test of herbal medicated toothpaste.

### 2.10.1. Physical Examination (Colour, odour, taste, smoothness):

- Colour: The visually colour was checked.
- Odour: Smelling the product allowed one to detect its odour.
- Taste: The taste was checked manually by tasting the formulation.
- Smoothness: The Smoothness was tested by rubbing the paste formulation between the fingers[14].

# 2.10.2. Homogeneity:

Determination of homogeneity was studied by applying a normal force to containers at 27±2°C[14].

### 2.10.3. Determination of sharp and edge abrasive particles:

Extrude the ingredients onto the butter paper until it is 15-20 cm long. Continue this technique until you have at least ten collapsible tubes. Check for the presence of sharp, hard-edged abrasive particles by pressing the contents of the entire length with your fingertip. Such particles are not allowed in toothpaste[15].

# 2.10.4. Determination of pH:

To create a 50% aqueous suspension, dispense 10 g of toothpaste from the container into a 50 ml beaker and top it off with 10 ml of recently boiled and cooled water (at 27°C). To ensure a complete suspension, thoroughly stir[16].



Figure 3 Determination of pH

# 2.10.5. Determination of Spreadability:

Each sample weighed around 1 gram, which was then carefully placed in the centre of a 10 by 10 cm glass plate, and covered with another glass plate. Above the glass plates, 2-kilogram weight was placed at the centre of the plate to avoid sliding of the plate. The diameter of the paste in centimetres was measured, after 30 minutes for all samples. The experiment was repeated three times and the averages were reported for all samples[17].

$$S = M \times L T$$

Where, S= Spreadability M= Mass attached with the slide, L: Length T = T i m e required to travel a distance on the slide.



Figure 4 Spreadability Test

# 2.10.6. Determination of foaming power

Make sure that no more than 2 ml of foam is formed and that no lump paste enters the 250 ml graduated cylinder when you transfer the slurry from a beaker, stirring its contents with a glass rod. As you transfer the residue remaining in the breaker, keep going back and add another 5 to 6 ml of water at a time, making sure all of the contents in the beaker is transferred too. Once the cylinder's contents are at 30 °C, adjust the volume to 50 ml by adding enough water. To make sure that the suspension is consistent, agitate the contents of the cylinder using a glass rod or thermometer.

Stop the cylinder and give it twelve full shakes, each of which consists of motions in a vertical plane, upside down, and vice versa, as soon as the temperature of the cylinder's contents hits 30°C. Read the amounts of foam after the cylinder has been shaken 12 times and let to remain still for five minutes. The formula below is used to calculate foam power [17].

Foaming power, ml=  $V_1 - V_2$  Where,  $V_1$  = Volume in ml of foam with water and  $V_2$  = Volume in ml of water only.



Figure 5 Determination of foaming power

# 2.10.7. Extrudability

Using this approach, a conventional capped collapsible aluminium tube was filled with the prepared paste, and the end was sealed by crimping. The tubes' weights were noted. The tubes were clamped after being positioned between two glass slides. After covering the slides with 50 gm, the cap was taken off. Weighing was done on the amount of extruded paste that was collected. It was computed what percentage of the extruded paste was[18,19].

# 2.10.8. Antimicrobial Assay



Figure 6 Microbial Activity of formulated toothpaste

By using agar well diffusion method antimicrobial activity was performed. For growth of *Staphylococcus* (catalase-positive), *Simmons citrate agar* and *sabouraud dextrose* agar were used respectively. The agar was spread on the plate for solidification purpose. Spread 0.1  $\mu$ L overnight cultures of *Staphylococcus* (catalase-positive) onto the plate after solidification of agar. The 100  $\mu$ L of cell free supernatant was added in 6mm wells. Plates were kept in refrigerator for 4 hours for diffusion purpose and the plates were incubated aerobically at 37 °C for 24 hours *Staphylococcus* (catalase-positive). By recording zone of inhibition antimicrobial activity was studied[20].



Figure 7 Formulated Toothpaste

# 3. Result and discussion

### 3.1. Extraction Method with their % yield

The crude extracts of Moringa were obtained after the process of Soxhlet extraction were evaporated on water bath by evaporating the solvents. The yield of extracts obtained from the plants by using ethyl acetate and methanol as a solvent was found to be 15%

The crude extracts of *Azadirachta indica* was obtained after the process of Soxhlet extraction were evaporated on water bath by evaporating the solvents. The yield of extracts obtained from the plants by using methanol as a solvent was found to be 20%

### 3.2. Moisture Content and Ash Value

Ash value and moisture content of formulated toothpaste was performed.

The moisture content was determined to be 76.4%.

The ash value of the sample *Moringa oleifera* and *Azadirachta indica*. Was determined to be 10%, and 8% indicating the mineral content after complete incineration.

### 3.3. Phytochemical Analysis

Ethyl acetate extract of Moringa plant shows the presence of proteins, Carbohydrate, phenolic compounds, alkaloids, saponins, tannins, cardiac glycoside.

Methanolic extract of Neem plant shows the presence of alkaloids, saponins, tannins, carbohydrates, cardiac glycoside.

### 3.4. Evaluation Tests of Herbal Toothpaste

### 3.4.1. Physical Examination

The developed herbal toothpaste was assessed for its physical characteristics, including consistency, colour, odour, taste and pH.

Smoothness was tested by rubbing the toothpaste formulation between the fingers.

### 3.4.2. etermination of pH

The pH level of the in-house made herbal toothpaste was determined to be 7.06.

### 3.4.3. Determination of Foamability

The foam ability of formulated herbal toothpaste is initially 20 ml and then final volume is 40 ml.

### 3.4.4. Determination of Spreadability

The spreadibility of Formulated toothpaste was found to be 7.0 cm

3.4.5. Determination of Sharp and Edge Abrasive Particles

There is no sharp and abrasive particles observed in the contents.

### 3.4.6. Zone of Inhibition

The zone of inhibition for Batch E's herbal toothpaste formulation was found to be 29.00 mm.

The antimicrobial activity of the herbal toothpaste formulation has been examined in this study. So, it was observed that, formulated herbal toothpaste has good antimicrobial activity in Batch E concentration and shows optimisation effect. The formulated toothpaste was then tested against Gram Positive for antimicrobial activity by using different concentrations of toothpaste. The potency was assessed by the presence of a zone of inhibition values. The zone of inhibition is different in reading at different concentrations. The formulated toothpaste showed highly significant effect towards the tested bacteria.

### 4. Conclusion

Both Neem and Moringa toothpastes can meet the growing consumer demand for natural and effective oral care products. Their antimicrobial and health-promoting properties make them an attractive alternative to conventional toothpaste. However, continued research and validation are crucial for the full adoption and significant market position of these products. The use of Neem and Moringa in toothpaste formulations is a promising method for natural and effective oral care. Both ingredients offer unique benefits that fit the growing consumer demand for plant-based and sustainable dental care products.

In conclusion, Neem and Moringa toothpaste offers a range of potential benefits due to the unique properties of these natural ingredients. Neem, known for its antibacterial, antifungal, and anti-inflammatory properties, helps in reducing plaque, preventing cavities, and combating gum diseases. Moringa, rich in vitamins and antioxidants, contributes to overall oral health by strengthening teeth and gums and providing anti-inflammatory effects.

The combination of these two powerful natural ingredients can provide an effective and holistic approach to oral hygiene, promoting healthier teeth and gums with fewer chemical additives. Studies and anecdotal evidence suggest that regular use of neem and Moringa toothpaste can lead to significant improvements in oral health. For instance, neem has been extensively studied for its antimicrobial properties which help in controlling the bacteria responsible for dental plaque and gingivitis. Moringa, with its rich nutrient profile, supports overall oral health by providing essential vitamins and minerals necessary for maintaining strong teeth and healthy gums.

Incorporating neem and Moringa toothpaste into daily oral care routines could be a beneficial alternative to conventional toothpastes, especially for those seeking natural and effective solutions for their dental health.

# **Compliance with ethical standards**

### Disclosure of conflict of interest

No conflict of interest to be disclosed.

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