



ISSN: 2320-2831

International Journal of Pharmacy and Analytical Research (IJPAR)

IJPAR | Vol.15 | Issue 2 | Jan - Mar -2026

www.ijpar.com

DOI: <https://doi.org/10.61096/ijpar.v15.iss1.2026.582-590>

Formulation and Evaluation of Herbal Toothpaste Containing Bioactive Ingredient Derived From *Ocimum Basilicum* Leaf Extract

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Published on:
24.04.2026

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Abstract

Background: The increasing concern over the adverse effects of synthetic ingredients in conventional dentifrices has led to a growing demand for herbal alternatives that are safer, biocompatible, and environmentally sustainable. *Ocimum basilicum* (basil) is a medicinal plant known for its antimicrobial, anti-inflammatory, and antioxidant properties, making it promising for oral care formulations aimed at preventing dental plaque and oral infections.

Objectives: This study aimed to formulate and evaluate a herbal toothpaste containing bioactive constituents derived from *Ocimum basilicum* leaf extract and to assess its physicochemical properties and antimicrobial efficacy against selected oral pathogens.

Methods: The leaves of *Ocimum basilicum* were dried, pulverized, and extracted using ethanol via Soxhlet extraction. The extract was incorporated into toothpaste formulations at different concentrations (0.5 g and 1.5 g) alongside standard excipients such as calcium carbonate, sodium lauryl sulphate, glycerol, sodium fluoride, and carboxymethylcellulose. The formulated toothpaste was evaluated for organoleptic properties, pH, viscosity, spread ability, and foamability. Antimicrobial activity was assessed using the agar well diffusion method against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans*. Minimum inhibitory concentration (MIC) of the extract was also determined.

Results: The formulated toothpaste exhibited acceptable organoleptic properties, including a leafy green colour, pleasant odour, and smooth consistency. The pH was 9.5, indicating suitability for maintaining oral alkalinity. Viscosity (6180 cP) and spread ability (2.2 cm) demonstrated appropriate consistency and ease of application. The toothpaste showed good foam ability and mild abrasiveness. Antimicrobial studies revealed that the formulation containing 1.5 g extract exhibited broader activity, with zones of inhibition up to 8.5 mm against *Candida albicans*, and measurable activity against *E. coli* and *P. aeruginosa*. MIC values indicated sensitivity of *E. coli* and *P. aeruginosa* at low concentrations.

Conclusion: The herbal toothpaste demonstrated satisfactory physicochemical properties and notable antimicrobial activity, supporting its potential as a safe and effective natural alternative to conventional dentifrices.

Keywords: *Ocimum basilicum*, Herbal toothpaste, Antimicrobial activity, Dentifrice formulation, Oral hygiene.

INTRODUCTION

Good oral health requires the removal of dental plaque and food debris, which also helps prevent caries and periodontal diseases.^[1] Chronic gingivitis caused by plaque is a common oral health issue that affects people of all ages worldwide. Dental plaque is a well-organized biofilm that was shown to be the main cause of chronic gingivitis.^[2]

Using dentifrices such as toothpastes and tooth powders to brush teeth is the most common oral hygiene practice worldwide. To enhance the effectiveness of dentifrices in removing dental plaque with mechanical brushing, they have been formulated with antimicrobial agents that can directly inhibit plaque growth.^[3,4]

However, modern dentifrices also contain chemical agents such as triclosan, sodium lauryl sulfate (SLS), and propyl paraben, as well as allergens, to boost their antibacterial properties.^[5, 6, 7] These ingredients have been shown to have adverse effects on long-term use, such as affecting taste and staining teeth.^[8]

More consumers are switching from fluoridated dentifrices to herbal (natural) dentifrices. In the last few years, there has been an increase in the demand for mouthwashes that contain natural compounds in the markets and among the professionals.^[9]

Many dentists also recommend toothpaste that has natural agents for better oral health. Herbal medicines, such as herbs, herbal materials, herbal preparations, finished herbal products that have plant parts or other plant materials as active ingredients, and various medicinal plants, alone or in combination, have been used for more than 2000 years to keep oral hygiene and to avoid inflammation.^[10, 11, 12]

Herbal extracts have natural antibacterial, antioxidant, anti-inflammatory, and anti-allergic properties that can also reduce side effects. Some of the dentifrices have been made with plant extracts such as green tea extract,^[13] clove and neem (*Azadirachta indica*), rosemary (*Rosmarinus officinalis*),^[14] and toothbrush tree (*Salvadora persica*).^[15]

Chemicals, mainly triclosan and chlorhexidine, have been added to mouth rinses and dentifrices to prevent plaque and gingivitis. But some of these substances have unwanted side effects such as tooth discoloration and taste alteration.^[16]

This has led to increasing attention on using natural ingredients in herbal dentifrices. Herbal ingredients have several advantages; chamomile has anti-inflammatory effect, echinacea has immune stimulatory property, sage and rhatany have anti-hemorrhagic properties, myrrh is a natural antiseptic, and peppermint oil has analgesic, antiseptic, and anti-inflammatory properties.^[17]

This study aimed to formulate herbal toothpastes containing bioactive ingredient derived from *Ocimum basilicum* leaf extract. Significance of this study lies in its potential to revolutionize oral care practices by introducing herbal toothpaste that combines efficacy with a natural approach. By offering a safer and more sustainable alternative to traditional toothpaste, this research contributes to the ongoing discourse on environmentally conscious and health-oriented consumer choices.

The current focus of toothpaste formulation is on delivering active ingredients that can prevent and/or treat oral diseases.^[18, 19, 20]

Many herbal formulations are very effective as they contain active chemical compounds such as polyphenols, gums, alkaloids, glycosides etc. These formulations have also been shown to have different biological activities.^[21, 22, 23]

METHODS

Table 1: Shows the reagents and solvents used

Reagent/Solvent	Source
Ethanol	JHD Chem., Guangdong, China
Chloroform	JHD Chem., Guangdong, China
Sulphuric acid	Sigma Aldreich Chemical, St. Louis, USA
Hydrochloric acid	Sigma Aldreich Chemical, St. Louis, USA
Ammonia	Sigma Aldreich Chemical, St. Louis, USA
Distilled water	Sigma Aldreich Chemical, St. Louis, USA
Mayer reagent	Sigma Aldreich Chemical, St. Louis, USA
Kedde reagent	Sigma Aldreich Chemical, St. Louis, USA
Hager reagent	Sigma Aldreich Chemical, St. Louis, USA
Fehling A & B reagent	Sigma Aldreich Chemical, St. Louis, USA

Culture Media

The culture media used in the study include; MacConkey agar (MAC), Nutrient agar (NA), Sabouraud dextrose agar (SDA), Muller-Hinton agar (MHA), Mannitol salt agar (MSA), Potato dextrose agar (PDA), Blood agar (BA).

Plant Material

Fresh Leaves of *Ocimum basilicum* were obtained from Choba market in Rivers state, Nigeria. The sample was authenticated by Taxonomist with voucher specimen deposited in the herbarium in the Department of Plant science and Biotechnology, University of Port-Harcourt. The sample was air-dried under room temperature for seven days. The dried sample was ground to fine powder using a domestic grinder and stored in air-tight container until further use.

Extraction Process

A 500g of the powdered sample of *Ocimum basilicum* was weighed using the weighing balance. The sample was extracted with 2 liters of absolute ethanol successively using a Soxhlet apparatus. The ethanol filtrate was filtered using Whatman No. 1 filter paper. The filtrate was concentrated using rotary evaporator and the yield obtained was noted. The weight of the extracts and its percentage yield was noted. The extract was transferred into a glass container and put inside an activated desiccator until when needed.

Collection, Identification and Standardization of the Test Microorganisms

Swab samples were obtained aseptically from the mouth of patients at the University of Port Harcourt Teaching Hospital, Choba, using sterile swab sticks already moistened with normal saline. The swab samples were transported to the laboratory and cultured on Nutrient agar, MacConkey agar and Sabouraud Dextrose agar respectively. The agar plates were incubated at 37°C for 24 hours and later examined for growth. The isolates were further purified through repeated sub culturing methods to obtain pure isolates. These pure isolates were then identified using Gram staining method and other biochemical test methods. They were then stored in agar slants, labeled appropriately and kept in the refrigerator at 4°C. The pure cultures of the test organisms; *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* were standardized to 1×10^6 cells/ml using Mcfarland standard.

Preparation of Stock Solution of Crude Extract and Dilutions of the Stock

One gram (1g) of the crude extract was reconstituted into 10ml of ethanol to obtain 100 mg/mL stock solution. The 100 mg/mL stock solution underwent a double serial dilution to obtain lower concentrations of the extract to determine the specific concentration of the extract where microbial growth inhibition started. The respective concentrations: 50 mg/mL, 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL and 3.125 mg/mL were obtained.

Antimicrobial Susceptibility Test of the Leaf Extract

Agar Diffusion Method

Bauer-Kirby Agar Diffusion method with slight modification was used to carry out this experiment. Sterile Petri dishes were labeled in duplicates for the various test organisms. A 0.1ml of each of the microorganisms was added aseptically to the prepared Mueller Hinton Agar pour in the universal bottle and properly mixed. The mixture was then poured into the corresponding Petri dish and allowed to solidify on the workbench. After the agar had solidified on the Petri dish, a sterile cork-borer was used to remove a disc of agar from the agar layer in order to produce a well in each agar plate. The well was labelled with stock concentration of extracts of 200mg/ml. Using a sterile Pasteur's pipette 0.1ml of the stock concentration was carefully dropped into the well and then left on the workbench for 15 minutes for proper diffusion. The plates (Petri dishes) were incubated at 37°C for 24 hours. The diameter of the resulting Zones of inhibition was measured in millimeter (mm) through the base of the plates using a meter rule.

Determination of Minimum Inhibitory Concentration (MIC) of Leaf Extract

Sterile Petri dishes were labelled in duplicates for the various test organisms. A 0.1ml of each of the microorganisms was added aseptically to the prepared Mueller Hinton Agar pour in the universal bottle and properly mixed. The mixture was then poured into the corresponding Petri dish and allowed to solidify on the workbench. After the agar had solidified on the Petri dish, a sterile cork-borer was used to remove 4 discs of agar from the agar layer in order to produce 5 wells in each agar plate. The Wells were labeled for the five (5) concentrations of leaf extracts. The concentration included 50 mg/mL, 25 mg/mL, 12.5 mg/mL and 6.25 mg/mL. Using a separate sterile Pasteur's pipette 0.1mL of each concentration of leaf extracts was carefully added to each of the wells and allowed to stand on the workbench for 15 min for proper diffusion of the extracts. All the plates (Petri dishes) were incubated at 37°C for 24 hours. The diameter of the resulting Zones of inhibition was measured in millimeter (mm) through the base of the plates using a meter rule.

Formulation of the Herbal Toothpaste

The oral toothpaste containing the ethanol leaf extract of *Ocimum basilicum*

Table 2: Ingredients Used in the Herbal Toothpaste Formulation

Ingredient	Amount (50 g)
Calcium carbonate	20g
Glycerol	5g
Sodium lauryl sulphate	1g
Methyl paraben	0.05g
Propyl paraben	0.05g
Sodium fluoride	0.5g
Sorbitol	1g
Menthol	0.1g
Sodium cmc	2g
Peppermint oil	0.5ml
Water	q.s
API	(0.5g, 1.5g)

Method of Formulation

A 20 g quantity of calcium carbonate was weighed and transferred into a ceramic mortar. A 2 g of sodium carboxymethylcellulose (CMC), a 0.05 g each of methyl and propyl parabens, and 0.5 g of sodium fluoride were measured and triturated using the doubling-up technique. A quantity of 5 g (6.2 ml) of glycerol and 0.1 g of menthol were measured and transferred into a beaker. The content was heated and transferred into the mixture in the mortar, and homogenized together. 1 g of sorbitol was added. 0.5 g of the extract was weighed and transferred into the mixture. 1 g of sodium lauryl sulfate was added, and 0.5 mL of peppermint oil was measured and transferred into the mortar. A sufficient amount of water was added. The content of the mortar was mixed vigorously and levitated until a homogeneous paste was formed. 50 g of the preparation was transferred into the dispensing container. The dispensing container was capped, polished, and appropriately labeled. The same procedure was repeated using 1.5 g of the extract.

Evaluation of the Formulated Herbal Toothpaste

Organoleptic Evaluation

The formulated herbal toothpaste was observed for its appearance, colour, odour and texture.

Determination of pH

A 0.5g amount of the formulated herbal toothpaste was weighed and transferred into a 150ml beaker. 50ml of freshly boiled and cooled potable water (at 30 °C) was measured and added into the beaker. It was stirred well to form a solution. The digital pH meter was placed inside the solution to determine the pH and the result recorded. ^[24]

Determination of Foam ability

The foam ability of the product was evaluated by taking small amount of preparation with water in a measuring cylinder, initial volume was noted and then shaken for 10 times. Final volume of foam was noted (Asha et al 2018).

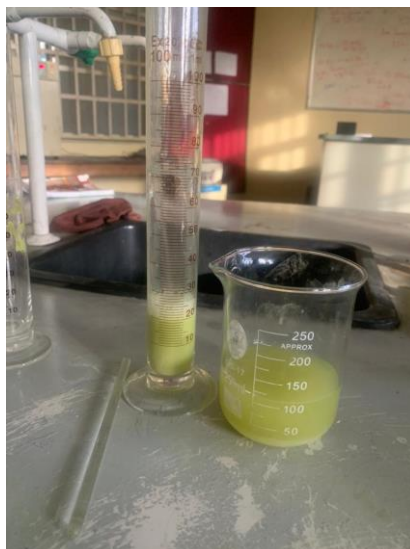


Figure 1: Foam ability Test

This diagram shows that the formulated toothpaste passed the foam ability test

Determination of Viscosity

Paste viscosity measurements were evaluated using a Brookfield digital viscometer (LV DV-II Ultra programmable Remoter, USA) using spindle no. 3 by applying increasing values of the shear rate, in order to reveal possible flow behavior of the pastes. All viscosities measurements were performed at controlled temperature of 300 c. [24]

Determination of Spreadability

A 0.5 g of the paste was weighed and kept at the center of the glass slide (10 x10 cm) and, another glass slide was placed over it carefully. 1kg weight was placed at the center of the plate (avoid sliding of the plate). The diameter of the paste in centimeter, after 15 min. was measured. [24]

The Spread ability (S) can be calculated using the formula;

$$S= M \times LT$$

S = spread ability, L= length moved on the lower glass slide

T =time taken, M = weight applied to the upper plate.

RESULTS

Percentage Yield of the Extract

Table 3: Percentage yield of leaf extract from *Ocimum basilicum*

Extract	Yield (g)	Yield (%w/w)
Ethanol	27.97	5.59

Key: Weight of dried sample, 500g

Physical Characterization of the Formulated Paste

Table 4: Physical Characterization of the Formulated Paste

Physical parameters	Observation
Colour	Leafy green
Odour	Characteristic
Stability	Stable
pH	9.5
Viscosity (cp)	6180
Abrasiveness	Mildly Abrasive
Spread ability	2.2
Foam ability	Very formable

ANTIMICROBIAL ASSAY OF THE CORRESPONDING TEST RESULTS

Table 5: Inhibitory Zone Diameter (IZD) of Ethanol Leaf Extract on Selected Micro-organisms (Antimicrobial Susceptibility Testing)

Sample	<i>Staphylococcus aureus</i> (oral)	<i>Escherichia coli</i> (oral)	<i>Pseudomonas aeruginosa</i> (oral)	<i>Candida albicans</i> (oral)
Ethanol Extract	-	5.0 mm	5.0 mm	-
Control (Negative)	-	-	-	-
Control (Positive)	13 mm	10 mm	8 mm	-

Positive control = Gentamicin

Negative control = Ethanol

Dilution = 100 mg/mL (1 g/10mL)

Table 6: Inhibitory Zone Diameter of the Ethanol Extracts on Selected Microorganisms

Name of Organism	Ethanol Extract(mm)			
	50 mg/ml	25 mg/ml	12.5 mg/ml	6.125 mg/ml
<i>Pseudomonas aeruginosa</i> (oral)	14	9	3	-
<i>Escherichia coli</i> (oral)	7	6	4	2
<i>Staphylococcus aureus</i> (oral)	-	-	-	-
<i>Candida albicans</i> (oral)	-	-	-	-

Table 7: Minimum Inhibitory Concentration (MIC) of the Ethanol Extracts

Name of Organism	Ethanol (mg/ml)
<i>Pseudomonas aeruginosa</i> (oral)	12.5
<i>Escherichia coli</i> (oral)	6.125
<i>Staphylococcus aureus</i> (oral)	-

Candida albicans (oral) -**Table 8: Inhibitory Zone Diameter (IZD) for the Formulated Herbal Toothpaste against the Selected Microorganisms (Antimicrobial Susceptibility Testing)**

Name of Organism	1.5g Ethanol Extract (mm)	0.5g Ethanol Extract (mm)	Control (mm)
<i>Staphylococcus aureus</i> (oral)	6.5	5.5	10
<i>Pseudomonas aeruginosa</i> (oral)	2	-	-
<i>Escherichia coli</i> (oral)	2.5	-	-
<i>Candida albicans</i> (oral)	8.5	4	11

Control: Colgate Toothpaste

DISCUSSION

The development and testing of herbal toothpaste with a bioactive component from the leaf of *Ocimum basilicum* (also called basil or tulsi) is a new and creative way to oral hygiene, as it blends the advantages of natural herbs with the features of standard toothpaste.

Bioactive ingredient successfully extracted from the leaves of *Ocimum basilicum* using ethanol showed a percentage yield of 5.59%. The herbal toothpaste was made by using various ingredients such as propyl paraben, calcium carbonate, sodium carboxymethylcellulose, sodium fluoride, glycerol, sorbitol, sodium laurel sulphate, peppermint oil and water, in addition to the basil leaf extract, which was acquired by the Soxhlet extraction process and filtration methods. The herbal toothpaste was assessed for various factors such as color, spread ability, foam ability, identification of sharp and abrasive particles, pH, viscosity, and antimicrobial activity against oral *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*.

The results revealed the potency of the produced herbal toothpaste against all the tested microorganism. The lowest value recorded for the Inhibitory zone diameter (IZD) was 2mm for *P. aeruginosa* using 1.5 g of the ethanol extract in the formulation, and the highest value was 8.5 mm for *C. albicans*. This corresponds partially with the results for the minimum Inhibitory concentration (mic) values of the ethanol leaf extract which shows antimicrobial activity only for *P. aeruginosa* and *E. coli* with MIC values of 12.5 and 6.125 mg/mL respectively.

It can be inferred from the herbal toothpaste IZD values that the herbal toothpaste containing the 1.5 g ethanol leaf extract seems to have the best antimicrobial properties, having recorded higher IZD values and also having activity against all the selected microorganisms as compared to the toothpaste containing 0.5 g ethanol leaf extract.

The results showed that the herbal toothpaste had a green colour, good spread ability and foam ability, no sharp or abrasive particles, a viscosity of 6180 cp, and a significant antibacterial activity against *C. albicans*, with a zone of inhibition of 8 mm. The toothpaste also recorded a pH of 9.5. This falls in line with the standard as the pH of toothpastes are supposed to be within the alkaline or slightly alkaline range (7-10), because the mineral of the enamel and dentine could be dissolved, and dental prosthesis corroded in an acidic condition. ^[25]

The results were good but less superior to the marketed toothpaste (Colgate), which had zone of inhibition of 10 mm and 11 mm for *S.aureus* and *C. albicans* respectively but unfortunately had no activity against *E. coli* and *P.aeruginosa*. Hence the formulated toothpaste with 1.5 g extract showed a broader spectrum of activity compared to the standard Colgate toothpaste, but the marketed toothpaste had greater zones of inhibition on *S.aureus* and *C.albicans*.

The findings showed that the herbal toothpaste could be useful in keeping oral hygiene, avoiding dental plaque and food particles from building up on the teeth, and removing or hiding bad breath. The findings also implied that the herbal toothpaste was harmless and compatible, as it lacked high amount of artificial additives that could lead to negative effects or allergies. ^[26, 27]

CONCLUSION:

This study successfully formulated and evaluated a herbal toothpaste incorporating bioactive constituents derived from *Ocimum basilicum* leaf extract, demonstrating its potential as a natural and effective oral care product. The formulation process yielded a stable and homogeneous toothpaste with acceptable organoleptic characteristics, including a characteristic green colour, pleasant odour, smooth texture, and good spread ability, all of which are essential for consumer acceptability and routine use. The physicochemical evaluation confirmed that the formulated toothpaste met key quality parameters required for dentifrice formulations. The pH value of 9.5 falls within the acceptable alkaline range for toothpaste, thereby supporting enamel integrity and reducing the risk of demineralization under acidic conditions. The viscosity (6180 cP) and spread ability values indicate a suitable

rheological profile, ensuring ease of extrusion from the container and effective application during brushing. The product also exhibited good foam ability, reflecting adequate surfactant performance necessary for efficient mechanical cleansing of the teeth. Importantly, the antimicrobial evaluation revealed that the herbal toothpaste possesses appreciable inhibitory activity against selected oral pathogens, particularly *Candida albicans*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The formulation containing a higher concentration (1.5 g) of the extract demonstrated broader antimicrobial activity, suggesting a dose-dependent effect of the bioactive constituents. Although the antimicrobial efficacy was comparatively lower than that of the commercial control for certain organisms, the herbal formulation exhibited a wider spectrum of activity, indicating its potential advantage in managing diverse oral microflora. The observed antimicrobial activity can be attributed to the phytochemical constituents of *Ocimum basilicum*, which are known to possess antibacterial, antifungal, and anti-inflammatory properties. These bioactive compounds contribute to plaque control, prevention of oral infections, and maintenance of overall oral hygiene. The developed herbal toothpaste represents a safe, effective, and environmentally friendly alternative to conventional dentifrices. The utilization of locally available plant materials enhances its economic viability and sustainability.

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