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Original Research Article

Exploring the efficacy of herbal toothpaste formulated with karanj, miswak and banana peel

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ABSTRACT

Background: Oral health is essential for maintaining overall well-being, as poor oral hygiene has been associated with various systemic diseases. Common issues such as halitosis, dental caries, and gum inflammation adversely affect quality of life. Conventional toothpastes often contain synthetic ingredients that may cause side effects, creating a growing need for natural alternatives. This study aimed to develop an herbal toothpaste using karanj (*Millettia pinnata*), miswak (*Salvadora persica*), and banana peel (*Musa spp.*), known for their antibacterial, anti-inflammatory, and antimicrobial properties.
Methods: Active compounds were extracted from the botanicals using standard solvent extraction techniques. The extracts were characterized for their phytochemical constituents and incorporated into a toothpaste base optimized for pH, stability, texture, and consistency. The antimicrobial activity of the formulation was tested against common oral pathogens, *Staphylococcus aureus* and *Escherichia coli*. Additionally, a sensory evaluation was conducted to assess taste and foaming ability.
Results: Phytochemical screening confirmed the presence of alkaloids, flavonoids, saponins, and phenolic compounds responsible for antimicrobial action. The herbal toothpaste exhibited significant antibacterial activity, showing inhibition zones comparable to some commercial formulations. Sensory evaluation results indicated favorable responses in terms of taste, texture, and overall satisfaction. The formulation remained stable in consistency and pH during the testing period.
Conclusions: The herbal toothpaste formulated demonstrated strong antimicrobial efficacy. This study supports the potential of plant-based ingredients as safe, cost-effective, and eco-friendly alternatives to synthetic oral care products, promoting sustainable and holistic oral hygiene.

Keywords: Karanj, Miswak, Antimicrobial, Banana peel, Herbal toothpaste, Oral health, Oral hygiene, Natural ingredients

INTRODUCTION

Oral health is essential to overall well-being, influencing both physical and mental health. Poor oral hygiene can lead to various diseases, including dental caries, periodontal diseases, and tooth loss, significantly impacting quality of life.[1,2] Moreover, oral health is linked to systemic conditions like cardiovascular disease, diabetes, and respiratory infections, as the oral cavity serves as a portal for pathogens that may exacerbate other health issues.[3] Routine mechanical cleaning and the use of toothpastes are the most effective preventive measures against these conditions.[4-6] An alternate choice for oral hygiene that may benefit oral health while treating

periodontal disease is a toothpaste with antibacterial and improved healing properties.[7] As the best defence for our oral cavity, tooth paste serves as a dosage to keep our mouths healthy and shields us from illnesses and oral disorders. According to Janvi et al, tooth paste is semi-solid in nature and can be found in the form of paste, gel, or emulsion.[8] While chemical-based toothpastes are common, natural alternatives are gaining popularity due to their antimicrobial and anti-inflammatory properties often derived from plant-based ingredients known for their minimal side effects.[9-12]

This study focuses on the formulation of an herbal toothpaste using the stems of karanj (*Milletia pinnata*), miswak (*Salvadora persica*), and banana peel. Miswak, traditionally used for oral care, has proven antimicrobial, plaque-controlling, and enamel-remineralizing properties, offering a sustainable alternative, particularly in areas with limited access to conventional oral products. Banana peels, rich in bioactive compounds like saponins and flavonoids, exhibit antibacterial activity and may contribute to teeth whitening due to their potassium and manganese content.[13,14] These peels also act as cationic bio-sorbents, which may help brighten tooth color, with saponins' foaming properties enhancing their cleansing effect.[15]

Karanj, known for its antimicrobial, anti-inflammatory, and antioxidant properties, has shown potential in promoting oral health by inhibiting bacteria responsible for periodontal diseases and reducing gum inflammation and redness.[16-18] Karanj also exhibits anti-cariogenic and wound-healing properties, making it a promising candidate for oral health applications.[19,20]

Herbal extracts that contain a range of phytochemicals, including tannins, terpenoids, flavonoids, and saponins, give them antimicrobial qualities and can either kill or stop the growth of various bacterial communities.[21] Hence, together, these ingredients present a promising natural solution for improving oral hygiene.

METHODS

Study type

This was an experimental laboratory-based study conducted to evaluate the phytochemical constituents and antimicrobial potential of selected herbal extracts used in the formulation of a herbal toothpaste.

Study place and period

The study was carried out in the department of life science, ITM University and Acube Lab-Rapture Biotech, Lucknow, from June 2023 to October 2024. All experimental procedures, including extraction, phytochemical analysis, microbial isolation, and antimicrobial testing, were performed under aseptic conditions in the institutional laboratories.

Selection criteria of study samples

The plant materials were selected based on their traditional use in oral hygiene and their medicinal properties:

Karanj (*Milletia pinnata*) (Figure 1c)- known for its antimicrobial and anti-inflammatory activity.

Miswak *(Salvadora persica*) (Figure 1b) traditionally used as a natural toothbrush with proven oral health benefits.

Banana peel (*Musa paradisiaca*) (Figure 1a)- contains bioactive compounds with potential antimicrobial and cleansing properties.

Sample collection

Stem of karanj (*Milletia pinnata)*, twigs of miswak (*Salvodara persicca),* and peel of banana were collected from local markets of Lucknow*,* washed and stored at room temperature (37°C) until further use. Only clean, intact plant parts were selected for further processing.

Figure 1: a) Banana peel; b) Miswak bark; c) Karanj stem.

Sample preparation

The samples collected were dried for 3-4 days at room temperature in aseptic conditions. The dried plant part was powdered mechanically and stored in air tight containers. The extraction was carried out using the Soxhlet. About 10 gm of powder was extracted with 100 ml saline water (any polar solvent). The extract was concentrated to dry under controlled temperature 40-50°C then preserved in the refrigerator.

Phytochemical tests

Test for saponin

1ml sample was dissolved in 5 ml distilled water. It was shaken well, froth formation took place. Stability of froth confirms the presence of saponin.[22]

Test for tannins

1ml sample was dissolved in 1ml 5% $FeCl_3$. Appearance of dark blue, black or dark green confirms presence of tannin.

Test for flavonoids

1ml sample was dissolved in 2 ml 1% NaOH. The appearance of yellow colour indicates the presence of flavonoid.

Test for carbohydrate

1 ml Fehling A and 1 ml Fehling B was dissolved in 2 ml sample. Test tube was heated in water bath for 20 minutes. The appearance of red precipitate confirms the presence of carbohydrate.

Biuret test for protein

1 ml of 1% $CuSo_4$ and 1ml of 1% NaOH was dissolved in 2 ml sample. The appearance of purple color confirms the presence of protein.[23]

Test for alkaloid

1ml of iodine was dissolved in 1 ml sample. The appearance of reddish-brown precipitate confirms the presence of alkaloid in sample.

Iodine test for starch

1ml of iodine was dissolved in 1ml sample. The appearance of blue or black color confirms the presence of starch in sample.[24]

Isolation of oral bacteria

15 ml nutrient agar was prepared. Saliva sample was collected with a sterile cotton swab was for the isolation of oral micro flora. The swab was spread slowly on a nutrient agar plate and incubated for 24-48 hours at 37°C in an inverted position. The bacterial colony morphology was then observed.

Identification of isolated microorganism

Gram staining

Gram staining was performed by the standard procedure and observed.

Colony forming unit (CFU) was calculated for isolated oral bacteria using formula.

$$\frac{\text{CFU}}{\text{ml}} = \frac{No.\ of\ colonies \times dilution\ factor}{\text{Vol. of sample spread (ml)}}$$

Agar well diffusion method

The agar plate surface was inoculated with *Staphylococcus aureus*. A well with diameter of 6-8 mm was punched aseptically and a volume (20-100 µl) of the antimicrobial agent or extract at desired concentration was introduced into the well. The agar plates were incubated under suitable conditions. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested.

Table 1: Composition of herbal toothpaste.

Ingredient	Standard (100 ml)	Quantity (20 ml)
Calcium carbonate ($CaCO_3$)	20 ml	4 ml
Glycerine	5 ml	1 ml
Sodium lauryl sulfate (SLS)	1 ml	0.2 ml
Sodium chloride (NaCl)	0.5 ml	0.1 ml
Sucrose	0.5 ml	0.1 ml

Ethical approval

Ethical approval was not necessary for this study as it involved only plant-based materials and did not include human or animal subject.

All the experiments and analysis were performed in replicates.

RESULTS

The banana peel, miswak, and karanj samples were collected, dried, extracted, and subsequently analyzed for the presence of various phytochemicals. The miswak extract exhibited the presence of protein, starch, and carbohydrates, while both banana peel and karanj extracts tested positive for alkaloids, flavonoids, saponins, and phenols (Table 2).

The extracts were evaluated for their antibacterial activity. Both karanj and miswak extracts exhibited antibacterial activity against Staphylococcus aureus, with zones of inhibition (ZOI) measuring 8 mm and 11 mm, respectively (Table 3). Bacteria isolated from saliva swabs were gram-stained, revealing a purple coloration, and were identified as gram-positive oral cocci.

The minimum inhibitory volume (MIV) of the extracts was assessed against both *Staphylococcus aureus* and the isolated gram-positive oral cocci. Miswak and karanj extracts exhibited inhibitory activity at volumes of 50 µl and 75 µl, respectively, against the gram-positive oral cocci and *S. aureus*. Additionally, banana peel extract inhibited the growth of *S. aureus* at a volume of 75 µl, resulting in a zone of inhibition (ZOI) of 10 mm (Table 4).

Table 2: Phytochemical test of banana peel, karanj and miswak.

Sample name	Alkaloid test	Flavonoid test	Saponin test	Protein test	Starch test	Phenolic test	Carbohydrate test
Banana peel	+	+	+	+	+	+	+
Karanj	+	+	+	+	+	+	+
Miswak	-	-	-	+	+	-	+

Table 3: Antibacterial activity against *S. aureus*.

Sample	Zone of inhibition (ZOI)
Positive control (Ciprofloxacin)	15 mm
Karanj	8 mm
Miswak	11 mm
Banana peel	0 mm

Table 4: Minimum inhibitory volume (MIV) of extracts against oral bacteria.

Volume of extracts	Gram positive oral cocci			*S. aureus*		
	Karanj (ZOI)	Miswak (ZOI)	Banana peel (ZOI)	Karanj (ZOI)	Miswak (ZOI)	Banana peel (ZOI)
Positive control (10 µl)	15 mm	20 mm	10 mm	18 mm	18 mm	8 mm
100 µl	10 mm	18mm	15 mm	15 mm	11 mm	0 mm
75 µl	6 mm	13 mm	10 mm	18 mm	18 mm	0 mm
50 µl	3 mm	5 mm	5 mm	0 mm	0 mm	0 mm

Table 5: Evaluation of physical parameters of the formulated toothpaste.

Parameters	Result
Colour	Light green
pH	7.56pH
Odour	Muddy smell
Texture	Rough texture
Appearance	Opaque

Figure 2: Formulated toothpaste: excipients along with the extracts of miswak, banana peel and karanj triturated to a paste like formulation.

The herbal toothpaste was formulated by incorporating excipients, including calcium carbonate, glycerine, sodium lauryl sulfate (SLS), NaCl, and saccharin, into 20 ml of water, as outlined in Table 1. The excipients were triturated using a mortar and pestle along with the extracts (Figure 2). The resulting formulation was subsequently evaluated for various physical parameters, as detailed in Table 5.

Table 6: MIV of formulated toothpaste against isolated oral bacteria.

Volume of the formulated toothpaste	Isolated oral bacteria	*E. coli*	*S. aureus*
Positive control	12 mm	10 mm	10 mm
100µl	10 mm	5 mm	8 mm
75µl	6 mm	5 mm	5 mm
50µl	0 mm	3 mm	4 mm

Table 7: Comparison of different toothpastes with the formulated herbal toothpaste against oral bacteria.

Sample	ZOI (mm)
Positive control	15
Colgate	5
Dabur	0
Formulated toothpaste	2

The herbal toothpaste formulation was evaluated for its antimicrobial activity and minimum inhibitory volume (MIV) against the isolated oral bacteria *Staphylococcus aureus* and *Escherichia coli*. *Staphylococcus aureus* and *Escherichia coli* were potential pathogens commonly associated with poor oral hygiene or compromised immune systems. The MIV of the formulated toothpaste was found to be 50 µl against both *S. aureus* and *E. coli*, with zones

of inhibition (ZOI) of 4 mm and 3 mm, respectively. At a volume of 75 µl, the toothpaste exhibited a ZOI of 6 mm against the isolated oral bacteria (Table 6).

In a comparative analysis, the efficacy of the formulated herbal toothpaste was assessed against commercially available toothpastes, namely Dabur and Colgate. The results indicated that Colgate exhibited the highest antimicrobial activity with a ZOI of 5 mm, followed by the formulated herbal toothpaste with a ZOI of 2 mm. Dabur showed no detectable antibacterial activity against the isolated oral bacteria (Table 7). These findings suggest that the formulated herbal toothpaste demonstrates comparable efficacy to commercially available products, indicating its potential as a more sustainable and effective alternative.

DISCUSSION

The present study aimed to evaluate the phytochemical constituents and antibacterial activity of miswak (*Salvadora persica*), karanj (*Millettia pinnata*), and Banana peel (*Musa paradisiaca*) extracts and to formulate an herbal toothpaste incorporating these plant-derived materials. The results revealed the presence of multiple bioactive compounds and significant antibacterial activity against *Staphylococcus aureus* and oral cocci, supporting the potential of these natural ingredients in oral hygiene formulations.

Phytochemical composition

Phytochemical screening confirmed the presence of alkaloids, flavonoids, saponins, tannins, carbohydrates, proteins, and phenols in the plant extracts. Miswak exhibited the presence of proteins, starch, and carbohydrates, whereas both banana peel and karanj tested positive for alkaloids, flavonoids, saponins, and phenols.

These findings are in accordance with earlier studies reporting the rich phytochemical composition of *Salvadora persica*, which contains tannins, flavonoids, alkaloids, and saponins that contribute to its antimicrobial, anti-inflammatory, and antioxidant.[25-27] Likewise, *Millettia pinnata* has been shown to contain flavonoids, alkaloids, and phenolic compounds with demonstrated antimicrobial and wound-healing effects.[28,29]

The presence of alkaloids, flavonoids, and phenolic compounds in banana peel was also reported by Anhwange et al and Akinmoladun et al, supporting its antioxidant and antibacterial potential.[30,31] These compounds are known to disrupt microbial cell walls, chelate metals, and inhibit nucleic acid synthesis, which may explain the observed antimicrobial activity in this study.

Antibacterial activity of extracts

Both miswak and karanj extracts exhibited notable antibacterial activity against *Staphylococcus aureus*, with inhibition zones of 11 mm and 8 mm, respectively, while *Banana peel* extract showed inhibition only at higher concentrations.

This pattern mirrors previous findings by Sofrata et al, who demonstrated that *Salvadora persica* extracts were effective against *S. aureus* and *Streptococcus mutans*, two major oral pathogens.[32] The antibacterial activity of *Miswak* has been attributed to its content of silica, salvadorine, sulfur, and benzyl isothiocyanate compounds that exert bactericidal effects.[33]

Similarly, the antibacterial activity of *Millettia pinnata* observed in this study aligns with reports by Gogoi et al, who found significant inhibition of Gram-positive and Gram-negative bacteria due to the plant's flavonoids and phenolic acids.[34] The relatively weak activity of banana peel observed here may result from the use of aqueous extracts, as organic solvent extracts generally yield higher antimicrobial potency.[30,31]

Thus, the results support the idea that these herbal extracts, particularly miswak and karanj can serve as natural antimicrobial agents for oral care.

Formulation and evaluation of herbal toothpaste

The formulated herbal toothpaste exhibited desirable physicochemical properties, including a light green color, pH of 7.56, and acceptable consistency. The pH value lies within the ideal neutral range for oral applications, preventing enamel erosion and mucosal irritation.

The toothpaste demonstrated antibacterial activity against both *S. aureus* and *E. coli*, with inhibition zones of 4-6 mm depending on concentration. These results indicate moderate antimicrobial potential, comparable to other herbal formulations reported in the literature.[33,35]

Although the activity of the formulated toothpaste was slightly lower than that of the commercial brand Colgate, it was superior to that of Dabur in the present assay. This finding is consistent with studies by Prabu et al, who reported that herbal toothpastes containing plant extracts such as miswak and neem demonstrated comparable efficacy to commercial formulations without the drawbacks of synthetic chemicals such as triclosan or fluoride.[36]

The observed antibacterial effects can be attributed to the combined action of flavonoids, saponins, and phenols, which are known to cause bacterial membrane disruption and inhibit protein synthesis.[26,31]

The results highlight the phytochemical potential of the selected plant materials; however, some methodological and analytical constraints must be considered when interpreting these findings. Although the study revealed the presence of various phytochemicals, biological or pharmacological assays (e.g., antioxidant, antimicrobial, or cytotoxic activity tests) were not conducted to correlate

chemical composition with bioactivity. The phytochemical content of plants can vary with factors such as soil type, climate, and season of collection. Since the study was conducted at one time point, such variations were not accounted for. Further, the study focused on preliminary phytochemical screening to detect the presence or absence of compounds. Quantitative estimation of individual phytochemicals (e.g., total phenolic or flavonoid content) was not performed, limiting the depth of interpretation.

Interpretation

Overall, the results indicate that combining miswak, karanj, and banana peel extracts into a single formulation enhances their synergistic antimicrobial action. Similar synergistic interactions have been reported in herbal formulations containing multiple phytochemical-rich ingredients.[36]

The antibacterial potential observed here is in agreement with prior studies highlighting miswak's strong activity against oral pathogens and karanj's antimicrobial activity.[27,29,32] The incorporation of banana peel adds antioxidant benefits and natural abrasiveness, contributing to cleaning efficacy and sustainability of the formulation.

The findings suggest that the herbal toothpaste developed in this study can serve as a natural, eco-friendly alternative to commercial toothpastes, aligning with the global shift toward sustainable personal care products. Future studies using solvent-based extractions, formulation optimization, and sensory evaluation could enhance its efficacy and consumer acceptance.

CONCLUSION

The formulated herbal toothpaste demonstrated effective antimicrobial activity against isolated oral bacteria, *S. aureus* and *E. coli*, with comparable efficacy to commercially available products. Despite Colgate showing the highest antibacterial activity, the herbal toothpaste formulation still displayed considerable efficacy, suggesting its potential as a viable alternative. Additionally, the herbal formulation offers a more sustainable option, given the natural sources of its active ingredients. These findings highlight the potential of the formulated herbal toothpaste as an effective and eco-friendly oral hygiene product, warranting further investigation into its long-term benefits and broader applicability.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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