Bioscene

Bioscene
Volume- 21 Number- 03
ISSN: 1539-2422 (P) 2055-1583 (O)
www.explorebioscene.com

Unlocking the Antimicrobial Power of Guava for Innovative Oral Care Solutions

Priyanka Panth[1*], Priyanka Suresh Bhusara[2], Sameer Khan[3]
[1,2,3] M.S. College of Pharmacy, University of Mumbai, Maharashtra, India

Abstract: The antimicrobial activity of Psidium guajava (guava) leaves has been extensively studied due to their rich phytochemical content. These leaves contain flavonoids, tannins, saponins, phenols, phytosterols, and diterpenes. Among these compounds, quercetin plays a crucial role in providing antibacterial and antioxidant properties. Guava leaves have demonstrated significant antimicrobial effects against various microbial species, including Escherichia coli, Salmonella enteritidis, Bacillus cereus, Staphylococcus aureus, Saccharomyces cerevisiae, and periodontal pathogens. Additionally, guava leaves exhibit potent antioxidant activity, making them valuable for herbal formulations. This research paper evaluates the antimicrobial potential of Psidium guajava leaf extracts and their suitability for incorporation into herbal toothpaste formulations

Keywords: Psidium guajava, herbal toothpaste, antimicrobial activity

Introduction:

Nature's pharmacy, indeed, has been humanity's resource for health and healing for millennia. Traditional medicines, particularly herbal remedies, have been a cornerstone of medical practices across various cultures. Herbal toothpaste is a natural alternative to conventional toothpaste that aims to improve oral health and hygiene without the use of synthetic chemicals. Oral diseases are caused by the growth of bacteria due to poor eating habits and high sugar intake. To keep oral health, bacterial growth should be inhibited by adding bioactive compounds with antimicrobial effects in toothpaste formulation. Antimicrobial testing methods can be applied to in vitro studies of natural and synthetic substances that may have antimicrobial properties. Diffusion Method, Thin-Layer chromatography (TLC)-Bioautography, Dilution Method, Time-Kill Test, ATP Bioluminescence Assay, Flow cytofluorometric Method.

Herbal toothpaste, derived from plant-based ingredients, offers a holistic and gentle alternative to conventional toothpaste. Its objectives revolve around promoting oral hygiene, preventing dental issues, and harnessing the antimicrobial properties of nature. Herbal toothpaste with guava leaves aims to harness the plant's medicinal properties to create an effective, natural, and safe product for oral health care. Guava leaves, scientifically known as Psidium guajava, are known for their antibacterial, antimicrobial, anti-cancer, antioxidant, and anti-diabetic properties.

Antimicrobial susceptibility testing is critical for identifying new drugs and combating the growing threat of multidrug-resistant bacteria. The rise of antibiotic resistance has underscored the need for new antimicrobial agents, many of which are sourced from natural products such as plants and microorganisms. Traditional antimicrobial compounds like tetracyclines and cephalosporins are becoming less effective, driving the search for new drugs. Research has increasingly focused on plant and microbial extracts, essential oils, and pure secondary metabolites as potential sources of new antimicrobial agents. Techniques for evaluating the antimicrobial activity of these natural products include disk-diffusion, broth dilution, and more advanced methods like time-kill tests. These techniques are essential for understanding the efficacy and potential applications of natural compounds in addressing current challenges in healthcare, agriculture, and environmental management.

Material and methods:

Sample processing of Guava leaves: Green guava leaves were picked from M.S. College of Pharmacy premises. Leaves were thoroughly washed with water. The leaves were sun-dried until complete moisture was removed. Dried leaves were then blended into a fine powder. The powder was passed through a 1 mm aluminum mesh for uniformity. Finally, the consistent powder was stored in a sealed container.

Preparation of Extra virgin coconut oil: In the hot extraction process Heat is used to separate oil and water in an emulsion, which lowers the antioxidant levels in coconut oil. The heat modifies the proteins that stabilize the emulsion, resulting in instability which results in oil separation.

Preparation of Clove oil: Clove essential oils are traditionally extracted from plant material using methods like cold pressing. In addition, innovative techniques such as microwave-assisted hydrodistillation, microwave-assisted steam distillation.

Preparation of Acacia arabica gum powder: First, acacia gum was bought from a nearby market and dried in the air. It was crushed into a fine powder using a machine mixer. Finally, the powder was kept in a sealed container

Preparation of Liquorice herb powder: First, liquorice roots were bought from a nearby market then washed and dried. Then, they were ground into a yellow powder using a clean grinding stone. Finally, the powder was stored in sealed containers for later use.

Ingredients	**Property**
Acacia arabica gum powder	Binder
Sea salt	Abrasiveness
Guava leaf powder	Anti-inflammatory, Anti-mutagenic, Anti-microbial, Abrasiveness, Preservation
Liquorice herb powder	Sweetener
Coconut oil	Reduces plaque buildup, Prevents tooth decay, Fights gum diseases, Essential oil, Humectant activity
Clove oil	Flavoring agent, Reduces plaque buildup, Improves salivary buffer capacity, Decreases salivary S. mutant count
Distilled water	Solvent

Table 1: Properties of Ingredients used in Formulation of Toothpaste

Formulation of Toothpaste: Acacia arabica gum powder was blended with a little distilled water using a dropper to form a smooth paste. Then, sea salt, guava leaf powder and Liquorice herb powder were added and mixed well to make the paste even. Next, extra virgin coconut oil and Clove oil were added and mixed well until the toothpaste reached the desired thickness.Five different formulations were created by varying the concentration of ingredients to get the ideal formulation.

Ingredients	**F1**	**F2**	**F3**	**F4**	**F5**
Acacia arabica gum powder	7	8	9	10	12.5
Sea salt	2	3	4	5	7.5
Guava leaf powder	26	24	22	20	15
Liquorice herb powder	7	8	9	10	12.5
Coconut oil	5	5	5	5	
Clove oil	13	12	11	12	7.5
Distilled water	40	40	40	40	40

Table 2: Composition ratio of ingredients used in different toothpaste formulations

Physicochemical evaluation of toothpaste: The following steps were taken to evaluate the toothpaste formulas based on their physical and chemical properties. Each test was done three times. The results were shown as mean ± standard deviation.

Determination of pH: A 2% (w/v) toothpaste solution was made, and its pH was measured at room temperature with a pH meter that was adjusted.

Determination of abrasiveness: A small amount of toothpaste was placed on a clean plastic slide with some distilled water. Using a new cotton swab, the toothpaste was spread back and forth 25 times within a 1 cm area. The slide was then rinsed and dried with tissue paper. Under a microscope, the number of scratches on the slide surface was counted. Scratches were rated on a scale from 0 (no scratches) to 5 (numerous scratches).

Determination of foaming activity: Mix 1 g of toothpaste with 15 mL of distilled water in a measuring cylinder. Shake the cylinder vigorously for 1 minute. Place the cylinder on a table and measure the height of the foam above the water level. The equation below was used to calculate the foaming ability of the toothpaste.

Spreading ability test: Put 1 g of toothpaste on the center of a glass slide. Place another glass slide on top of the toothpaste. Gently place a 1 kg weight on the slides. Leave the weight on for 10 minutes. After removing the weight, measure the diameter of the spread paste.

Cleaning ability test: Apply an artificial stain to the egg shell. Brush the egg shell with the toothpaste being tested. Measure the stain density before and after brushing. The cleaning ability was rated as '+++' very good cleaning ability, '++' good cleaning ability, '+' medium cleaning ability, '−' poor cleaning ability.

Determination of antimicrobial activity: Well diffusion method was Employed for the method for the assay. Bacillus subtilis, Staphylococcus epidermis, and Pseudomonas aeruginosa were cultivated in LB medium at 37°C for 24 hours for the test. Spread 1 mL of each bacterium culture on LB agar plates and dry for 1 hour. Use Ampicillin (50 mg/mL) as a positive control. Prepare 2% w/v solutions of the five toothpaste formulas.

Determination of antimicrobial activity: Add 60 μL of each toothpaste solution to the designated wells on the agar plates. Store the plates in the fridge for 2 hours for diffusion, then incubate at 37°C for 24 hours. Calculating the zone of inhibition.

Identify the clear, circular ring around the wells post-incubation. Measure the diameter of the inhibition zone both vertically and horizontally. Calculate the average of the vertical and horizontal measurements to determine the zone size in centimeters.

Stability studies: The following steps were taken to test the stability of the toothpaste formulas. First, their physical and chemical properties were measured every 4 months for a year.

Result & Discussion:

Evaluation of physical–chemical properties of toothpaste formulations: Several formulations of herbal toothpaste (F1, F2, F3, F4, and F5) were created by adjusting the concentrations of ingredients like guava leaf powder, Acacia arabica gum powder, sea salt, stevia herb extract powder, and Clove oil. These formulations were studied to identify the best one. Notably, the pH values of all toothpaste formulations ranged from 8 to 11. F1 had the highest pH (11.2 ± 0.04), while the others were close to pH 9. Abrasiveness and foaming ability tests showed similar patterns. F5 had lower foaming ability (15%), while F3 exhibited the highest value (16.6%). Despite their low foaming properties, all formulations spread well. F5 had the largest spreading area (9.1 ± 0.03 cm), followed by F4, F3, F2, and F1 with 8.5 ± 0.03 cm, 7.5 ± 0.20 cm, 6.9 ± 0.50 cm, 6.3 ± 0.50 cm respectively. Based on color changes observed on pigmented eggs, F4 demonstrated better stain-cleaning ability (+++), while the remaining five formulations showed less color change (++). The physical and chemical characteristics of different toothpaste formulations showed significant variations in (Table 3).

Formulation	pH	Abrasiveness (rating)	Foaming ability (%)	Spreading ability (cm)	Cleaning ability
F1	11.2 ± 0.04	4	15.3 ± 0.4	6.3 ± 0.50	+
F2	09.5 ± 0.03	3	15.2 ± 0.2	6.9 ± 0.50	++
F3	08.7 ± 0.01	2	16.6 ± 0.2	7.5 ± 0.20	++
F4	08.2 ± 0.03	2	16.0 ± 0.4	8.5 ± 0.03	+++
F5	09.1 ± 0.02	3	15.0 ± 0.1	9.1 ± 0.03	++

Table 3: Physico-chemical properties of tooth paste formulations

Antibacterial Activity: In vitro antibacterial activity of the formulated toothpaste (F1, F2, F3, F4, and F5) were evaluated against Bacillus subtilis, Staphylococcus epidermis, and Pseudomonas aeruginosa strains as shown in (Table 4). F4 formulation was very effective against the tested bacteria followed by F5, F3, F2 and least activity was shown for F1. As tabulated, the highest inhibition zone was observed against Bacillus subtilis (1.1 cm) and Staphylococcus epidermis (0.8 cm) and the lowest zone of inhibition was observed against Pseudomonas aeruginosa (0.3 cm) for F4 formulated paste.

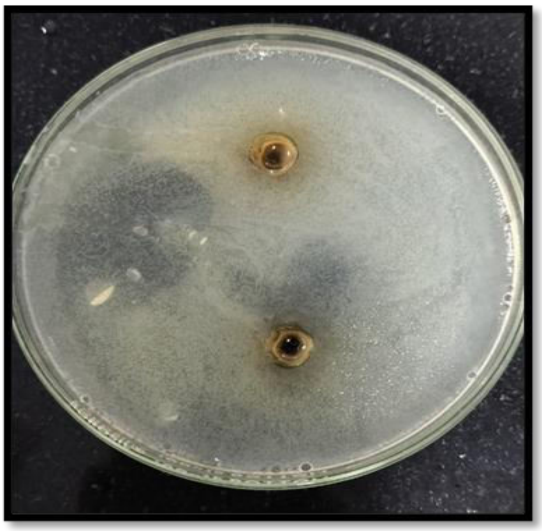

Figure 1: Antibacterial activity zones of formulation

Formulation	Bacillus subtilis (cm)	Staphylococcus epidermis (cm)	Pseudomonas aeruginosa (cm)
F1	0.3 ± 0.001	0.4 ± 0.002	0.3 ± 0.001
F2	0.6 ± 0.003	0.5 ± 0.003	0.5 ± 0.004
F3	0.7 ± 0.003	0.6 ± 0.001	0.58 ± 0.005
F4	1.1 ± 0.006	0.8 ± 0.001	0.7 ± 0.001
F5	0.8 ± 0.002	0.7 ± 0.001	0.6 ± 0.003

Table 4: Anti-microbial activity of toothpaste formulations

Stability test: After evaluating the physical, chemical, and biological properties of all three formulations, F4 was identified as the most suitable and was selected for stability testing. The test spanned 3 months, with observations taken at 1-month intervals to monitor changes in F4 during storage. According to the results in (Table 5), no significant alterations were observed in the pH, foaming ability, spreadability, and cleaning ability of F4, indicating that it is the optimal formulation for human use.

Additionally, (Table 6) outlines the organoleptic evaluation of toothpaste formulation F4, highlighting its pale green color and improved taste, largely attributed to the guava leaf.

Formulation	pH	Foaming ability (%)	Spreading ability (cm)	Cleaning ability
1st month	8.0 ± 0.01	16.0 ± 0.2	8.1 ± 0.50	++
2nd month	7.9 ± 0.02	16.0 ± 0.1	7.9 ± 0.20	++
3rd month	7.9 ± 0.01	16.0 ± 0.1	7.9 ± 0.03	++

Table 5: Stability test for tooth paste formulation (F4)

Organoleptic evaluation	F4
Colour	Pale green
Taste	Slightly bitter
Odour	Pleasant
Texture	Partially smoot

Table 6 Organoleptic analysis of toothpaste formulation (F4)

Conclusion:

The study successfully developed a polyherbal toothpaste formulation utilizing natural ingredients such as Acacia arabica gum powder, sea salt, guava leaf powder, Liquorice herb extract, and clove oil. Five different formulations (F1, F2, F3, F4, and F5) were created by adjusting the concentrations of these ingredients. Acacia arabica, known for its versatility in treating conditions like cough, diarrhea, diabetes, skin diseases, and wound healing, was incorporated due to its beneficial properties. Liquorice, used for its natural sweetness, not only reduces oral bacterial growth but also maintains glucose levels in diabetic patients without toxicity. The formulations were evaluated for various physical and chemical characteristics, such as pH, abrasiveness, foaming ability, spreadability, and cleaning effectiveness. All formulations maintained an alkaline pH between 5.5 and 10.5, which is ideal for neutralizing acid biofilm, fighting germs, and reducing bad breath, making them suitable for oral care.

The study highlighted the unique attributes of the formulated toothpaste, particularly the non-foaming nature due to the absence of surfactants like sodium lauryl sulfate (SLS), which can have harmful long-term effects on health. This non-foaming characteristic was compensated by the use of natural abrasives like guava leaf powder, which ensures effective cleaning and stain removal without damaging the enamel. The formulations were designed to meet consumer preferences for effective cleaning and whitening, and guava leaf powder contributed to the abrasiveness and stain removal capabilities. The formulation F4, in particular, demonstrated superior effectiveness in removing stains and enhancing tooth whiteness, meeting the growing demand for teeth-whitening products.

The antibacterial properties of the toothpaste were rigorously tested against bacteria such as Bacillus subtilis, Staphylococcus epidermidis, and Pseudomonas aeruginosa. The results indicated that formulation F4 had the highest antibacterial activity compared to the other formulations, showcasing the potential of essential oils and guava extract in combating oral pathogens. The study concludes that the developed herbal toothpaste, particularly formulation F4, is a viable natural alternative to commercial toothpaste. It effectively cleanses the oral cavity, removes stains, enhances whiteness, and provides therapeutic benefits without the harmful effects of synthetic chemicals, making it a safe and effective choice for oral health care.

Reference:

1. Durgesh Gautam, Preetam Palkar, Kiran Maule, Research Article-Preparation Evaluation and Comparison of Herbal Toothpaste with marketed Herbal toothpaste,
2. C. D. Wu and E. D. Savitt, "Evaluation of the safety and efficacy of over-the-counter oral hygiene products for the reduction and control of plaque and gingivitis," Periodontology 2000, vol. 28, no. 1, pp. 91–105, 2002.
3. Yashasvi Sharma, Ritesh Dwivedi, Jitendra Malviya "Formulation and Evaluation of Toothpaste Consist of Antimicrobial Agent Extract from Psidium Guajava" Volume:03/Issue:03/March-2021.
4. Feroz Jenner, V Abdul Jaleel, Reena Kulshrestha, G Maheswar, P Krishna Rao, J Kranthi "Evaluating the Antimicrobial Activity of Commercially Available Herbal Toothpaste on Microorganisms" 10.5005/jp-journals-10024-1427.
5. Shrishail M. Ghurghure, Priyanka R. Surwase, American Journal of Pharmtech Research, Am. J. PharmTech Res. 2019; 9(02).
6. Priya Nimish Deo, Revati Deshmukh, Oral microbiome: unveiling the fundamentals, National Library of medicine.
7. D.L. Mayers, S.A. Lerner, M. Ouelette, et al. Antimicrobial Drug Resistance C: Clinical and Epidemiological Aspects, vol. 2, Springer Dordrecht Heidelberg, London (2009).
8. A. Guschin, P. Ryzhikh, T. Rumyantseva, et al. Treatment efficacy, treatment failures, and selection of macrolide resistance in patients with high load of Mycoplasma genitalium during treatment of male urethritis with Josamycin.

9. I. Martin, P. Sawatzky, G. Liu, et al.Antimicrobial resistance to Neisseria gonorrhoeae in Canada: 2009–2013.
10. D.K. Runyoro, M.I. Matee, O.D. Ngassapa, et al. Screening of Tanzanian medicinal plants for anti-Candida activity.
11. U. Mabona, A. Viljoen, E. Shikanga, et al. antimicrobial activity of Southern African medicinal plants with dermatological relevance: from an ethnopharmacological screening approach to combination studies and the isolation of a bioactive compound.
12. F. Nazzaro, F. Fratianni, L. De Martino, et al. Effect of essential oils on pathogenic bacteria.
13. A comprehensive review of the techniques for coconut oil extraction and its application National Library of Medicine.
14. Ankush K. Shinde, Et al. 2024 / Formulation And Evaluation of A Herbal Medicated Toothpaste Using QBD Approach. Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research Pharmaceutical Resonance 2024 Vol. VI - Issue II.
15. Gholap Akshay, 2024, Review on Formulation and Evaluation of Polyherbal Toothpaste, International Journal of Progressive Research In Engineering Management And Science (Ijprems), Vol. 04, Issue 04, April 2024, pp: 352-356.
16. Abirami sasi, Et al. 2024/ Optimization of xanthan gum production and ethanolic extract of Psidium guajava application in dentistry, International Journal Of Pharmaceutical Research. A Step Towards Excellence Published by: Advanced Scientific Research.
17. S. B. Nagansurkar, Et al. 2023, / Preparation and Evaluation of Herbal Toothpaste, International Journal of Advanced Research in Science, Communication and Technology (IJARSCT), Volume 3, Issue 1, February 2023.
18. Amar A. Khose, Et al. 2023 / Herbal Toothpaste Formulation And Assessment: A Comprehensive Review, International Research Journal of Modernization in Engineering Technology and Science. Volume:05/Issue:11/November-2023.
19. Dr, Shahidullah, Et al. 2023/ Herbal toothpaste: An overview, International Journal of Research and Analytical Reviews.
20. G. Sridhar, Et al. 2023 / Herbal Toothpaste: An Overview, Volume 22: Issue 11 (Nov) - 2023.
21. Nirmala Devi N, Rasmi. R Et al. 2023/ Research Article. Journal of Drug Delivery and Therapeutics 2023; 13(5):35-40. Formulation, Evaluation and Antibacterial Effect of Herbal Toothpaste using leaves and flowers Acmella ciliata .
22. Dr. Satendra Kumar Et al. 2023/ world journal of pharmaceutical and life sciences. Formulation evaluation of polyherbal toothpaste which is more

effective, economical and potential to all dental problems in comparison to all marketed toothpaste.

23. Varsha A Dighe Et al.2023/ International Research Journal of Modernization in Engineering Technology and Science, Volume: 05/Issue: 05/May-2023. Formulation and Evaluation of Herbal Toothpaste from Plant Psidium Guajava.
24. Maya Y. Gaikwad, Et al. 2023/ International Journal of Pharmaceutical Research and Applications, Volume 8, Issue 4 July-Aug 2023. Formulation and evaluation of polyherbal toothpaste.
25. Abhishek Kotkar and Santosh Kudnar, Et al. 2023/ International Journal of Research Publication and Reviews. An Overview of Formulation and Evaluation of Herbal Toothpaste.