Title: Formulation and Evaluation of a Guava Leaf Based Mouth Freshener Incorporated Perppemint Oil

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***Abstract*—Guava (Psidium guajava) leaves have a historical ethnomedical value for their usefulness in oral health. This study examines the potential of guava leaf extract to develop a natural mouth freshener. Guava leaves are rich in a variety of bioactive compounds such as flavonoids, tannins, and phenolic acids, and possess antimicrobial, anti-inflammatory, and antioxidant properties. These bioactive properties are believed to kill and/or inhibit oral bacteria, prevent bad breath, and support oral health. A guava leaf-based mouth-freshening product would provide a natural, safe, and sustainable alternative to conventional synthetic mouth fresheners that promote oral hygiene and fresh breath. The mouth freshener will incorporate guava leaf extract into a product that has an appealing flavor, delivers a pleasurable and long-lasting fresh taste, and will appeal to the eco-conscious consumer looking for herbal oral care products.**

I. INTRODUCTION

Mouth hygiene is of paramount importance for overall health and well-being. The increasing recognition of the detrimental impact of synthetic chemicals used in commercial oral products has led to a move away from these substances. Of these, guava leaves (Psidium guajava) have been noticed because of their high content of phytochemicals such as flavonoids, tannins, and essential oils with antibacterial, anti-inflammatory, and antioxidant properties. (1)

Guava leaves have been traditionally employed in different cultures to cure oral conditions like toothache, gum swelling, and halitosis. Their antimicrobial activity against oral pathogens such as Streptococcus mutans and Porphyromonas gingivalis renders them a potential candidate for natural oral care products.The creation of a guava leaf mouth

freshener provides not only a natural and effective solution but also a trend towards sustainable and eco-friendly personal care products. (2)

This study centers on the formulation and testing of a guava leaf mouth freshener with the goal of determining its organoleptic characteristics, antimicrobial effect, and acceptability by consumers.
Objective: (1,2)

1. To prepare an herbal mouth freshener using guava leaf extract as the key active ingredient, along with other natural components.
2. To evaluate the antimicrobial efficacy of the formulated mouth freshener against common oral pathogens such as Streptococcus mutans and Lactobacillus acidophilus.
3. To assess the organoleptic (sensory) properties including taste, odor, appearance, and overall acceptability of the guava leaf mouth freshener.
4. To determine the pH, stability, and shelf-life of the product under different storage conditions.
5. To compare the effectiveness of the formulated product with that of commercially available chemical-based mouth fresheners.

II. MATERIAL AND METHODS

Ingredients	Quantity	Category
Peppermint oil	0.5-1.0%	Colling effect
Sodium lauryl sulphate	0.5-1.0%	Foaming agent
Sodium benzoate	1-5%	Preservative
Sorbital	5-10%	Sweeting agent
Zinc citrate	0.1-0.3%	Anti-microbial, Anti-inflammatory activity
Water	Qs	Vehicle

Extraction process of guva leaf by soxhlet extraction: (3)

- Add guva leaf powder to the ethanol (1:10)
- Stir the mixture continuously using shaker or heat it gently in a soxhlet extractor for 4-6 hours at a controlled temperature (40-50•c)
- Filter the solution using filters paper/ centrifuge to separate the liquid extract from the solid residue.

Distillation process: (4,6)

- Firstly, take a distillation apparatus and wash properly.
- Then take a conical flask add guva leaf extract ang heat it gently by heating mental and water supply on .
- Then find out the final product for using mouth freshener.

Main process:

Step 1: Firstly, clean all apparatus then take a beaker add 2 ml extract.

Step2: Then add peppermint oil (0.75) and add sodium lauryl sulphate (0.7 mg) stir properly then gently heat

Step3: After cooling add zinc citrate (0.2g) and stir properly

Step4: Then add preservatives like sodium benzoate (3g)

Step5: Then store at room temperature

Evaluation parameters:

1) organoleptic properties: (7,8)

1. Colour and Appearance Evaluation:

Method: Visual Inspection using Trained Panel

Description: A panel of trained or semi-trained individuals observes the product under standardized lighting conditions (e.g., D65 daylight or white fluorescent light).

Scoring Method: Use a Hedonic Scale (e.g., 1–9 or 1–5) or a Descriptive Scale (e.g., very light, light, moderate, dark).

Parameters: Uniformity, brightness, natural appearance, absence of discoloration.

2. Odour Evaluation:

Method: Sniff Test in Controlled Environment

Description: Participants sniff the sample and describe the intensity, pleasantness, and character of the aroma.

Tools: Odour booth (optional), nose cones, or jars with lids to minimize volatilization before testing.

Scoring: Hedonic or intensity scale (e.g., 1 = no odour, 5 = strong odour)

3. Texture Evaluation:

Method: Touch and Chew Test

Parameters: Grittiness, smoothness, crunchiness, hardness, chewiness.

Instrumental Support (Optional): Texture analyzer (for force and deformation).

Scoring: Descriptive scale (e.g., soft, firm, hard) or numerical scale.

4. Taste Evaluation:

Method: Sensory Panel Testing

Description: Evaluate sweetness, bitterness, astringency, coolness (from menthol), etc.

Safety Note: Ensure hygienic sampling and that all samples are safe for consumption.

5. Overall Acceptability / Appearance:

Method: Hedonic Rating

Description: Participants rate the product based on their overall perception combining colour, odour, and texture.

Scale: Commonly 9-point scale (1 = Dislike extremely, 9 = Like extremely).

Colour	White
Odour	Fresh, but not sweet.
Test	Orgnoleptic PH level, Antimicrobical, etc.
Texture	liquid
Appearance	Mouthwash.

2) Physicochemical evaluation parameters: (9,10)

1. Moisture Content

Method: Hot Air Oven Drying Method (Loss on Drying)

Procedure:

1. Weigh ~5 g of the sample.
2. Place it in a moisture dish.
3. Dry at 105°C in a hot air oven for 3–4 hours.
4. Cool in a desiccator and reweigh.

Calculation:

Moisture content (%)=Initial weight - Final weight/Initial weigh ×100

2. pH Value:

Method: pH Meter Method

Procedure:

1. Prepare a 1% w/v solution of the mouth freshener in distilled water.
2. Stir well and filter if needed.
3. Calibrate the pH meter using standard buffers (pH 4.0, 7.0, and 9.0).
4. Measure the pH of the solution at room temperature.

3. Ash Value (Total Ash, Acid Insoluble Ash, Water Soluble Ash)

a. Total Ash:

Procedure:

1. Incinerate 2–3 g of the sample in a crucible.

2. Heat in a muffle furnace at 500–600°C until ash is white or light grey.

3. Cool and weigh.

Purpose: Indicates total inorganic content.

b. Acid Insoluble Ash:

Procedure:

1. Boil total ash with dilute HCl.
2. Filter, wash, dry, ignite, and weigh the residue.

Purpose: Indicates presence of siliceous materials (e.g., dirt, sand).

c. Water Soluble Ash:

Procedure:
1. Boil total ash with water.
2. Filter, dry, ignite the residue, and subtract from total ash.
Purpose: Measures water-soluble inorganic salts.

5. Extractive Values (Alcohol and Water Soluble)
a. Alcohol Soluble Extractive:
Procedure:
1. Macerate 5 g of sample with 100 mL of alcohol (95%) for 24 hours.
2. Shake occasionally.
3. Filter and evaporate 25 mL of filtrate to dryness.
4. Dry at 105°C and weigh the residue.
b. Water Soluble Extractive:
Same method as above, replacing alcohol with distilled water.
Purpose: Indicates the number of active constituents extracted by solvents

Moisture content	87 – 91%
PH	5.5 -6.5
Ash value	6 – 10 %
Extract value	

3) Phytochemical screening: (11)
1. Tannins (Ferric Chloride Test)
Procedure:
1. Boil 0.5 g of the sample with 10 mL distilled water.
2. Filter and add a few drops of 1% ferric chloride solution.
Positive Result: Blue-black or greenish-black color indicates tannins.
2. Flavonoids (Alkaline Reagent Test)
Procedure:
1. Add a few drops of NaOH solution to the extract.
2. Observe the color.
3. Add dilute HCl to see if the color disappears.
Positive Result: Intense yellow color that disappears on addition of acid indicates flavonoids.
3. Saponins (Froth Test)
Procedure:
1. Shake 0.5 g of the sample with 10 mL of distilled water in a test tube.
2. Let it stand for 15 minutes.
Positive Result: Persistent frothing or foam formation indicates saponins.
4. Alkaloids (Mayer's / Dragendorff's / Wagner's Test)
Mayer's Test:
Add a few drops of Mayer's reagent to the extract.
Positive Result: Cream or white precipitate.
Dragendorff's Test:
Add Dragendorff's reagent.
Positive Result: Orange or reddish-brown precipitate.
Wagner's Test:
Add Wagner's reagent (iodine-potassium iodide).
Positive Result: Reddish-brown precipitate.
5. Terpenoids (Salkowski Test)
Procedure:
1. Mix 5 mL of extract with 2 mL of chloroform.
2. Carefully add 3 mL of concentrated sulfuric acid to form a layer.
Positive Result: Reddish-brown coloration at the interface indicates terpenoids.

5. Phenols (Ferric Chloride Test)
Procedure:
1. Add 2–3 drops of 1% ferric chloride to the extract.
Positive Result: Deep blue, green, or purple coloration indicates phenols.

Phytochemical	Test method	Observe tion / Result	Presence (+-)
Tannins	Ferric chloride Test	Black- Black or greenish – Black	Positive
Flavonoids	Alkaline Reagent Test	Yellow colour	Positive
Saponins	Forth Test	Presistant foam	Positive
Alkaloids	Mayer S / Drangendroff Test	Precipitate formation	Positive

Terpenoid	Salkowski Test	Reddish brown colour	Positive
Phenol	Ferric chloride Test	Deep colour change	Positive

Tannins	Present
Flavonoids	Present
Saponins	Present
Alkaloids	Present
Terpenoids	Present
Phenol	Present

4) Antimicrobial activity: (12,13)

1. Agar Well Diffusion Method

Purpose: To assess the zone of inhibition produced by the mouth freshener extract against selected microbes.

Procedure:

1. Prepare Mueller-Hinton agar (or Nutrient agar) plates.
2. Inoculate the agar surface with a standardized microbial suspension (e.g., 0.5 McFarland standard).
3. Use a sterile corn borer to make wells (6–8 mm diameter) in the agar.
4. Fill wells with 100 µL of the mouth freshener extract (at different concentrations).
5. Include:Positive control: Standard antibiotic (e.g., chlorhexidine, ampicillin).

Negative control: Solvent only (e.g., ethanol, DMSO)

6. Incubate at 37°C for 24 hours.
7. Measure the diameter of the inhibition zone in mm.

2. Disc Diffusion Method

Procedure:

1. Soak sterile filter paper discs (6 mm) in the extract.
2. Place the discs on inoculated agar plates.
3. Incubate and observe as in the well diffusion method.
4. Measure the inhibition zone.

3. Minimum Inhibitory Concentration (MIC) – Broth Dilution Method

Purpose: To find the lowest concentration of extract that inhibits visible microbial growth.

Procedure:

1. Prepare serial dilutions of the mouth freshener extract in sterile broth (e.g., Mueller-Hinton broth).
2. Inoculate each tube with standardized microbial suspension.
3. Incubate at 37°C for 18–24 hours.
4. Observe turbidity or measure absorbance at 600 nm using a spectrophotometer.
5. The lowest concentration with no visible growth = MIC.

Microorganisms	Zone Inhibition (mm)	MIC (mg/ml)	MBC (mg/ml)
S. Mutans	14	1.25	2.5
C. Albican	12	2.5	5.0

5) Antioxidants activity:(14,15)

1. DPPH Free Radical Scavenging Assay

Principle: DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable free radical. Antioxidants reduce DPPH and cause a color change from purple to yellow.

Procedure:

1. Prepare a 0.1 mM DPPH solution in methanol.
2. Prepare various concentrations of the sample (e.g., 10–100 µg/mL).
3. Mix 1 mL of DPPH solution with 1 mL of sample solution.
4. Incubate in the dark at room temperature for 30 minutes.
5. Measure absorbance at 517 nm using a UV-Vis spectrophotometer.

Calculation:

% Inhibition= Acontrol – Asample/Acontrol × 100

IC_{50} (concentration required to inhibit 50% of radicals) can be calculated from the inhibition curve.

3. ABTS Radical Scavenging Assay (Alternative to DPPH)

Principle: ABTS radical cation ($ABTS^{\bullet+}$) is decolorized by antioxidants.
Procedure:

1. Generate $ABTS^{\bullet+}$ by reacting ABTS with potassium persulfate and incubating in the dark for 12–16 hours.
2. Dilute to an absorbance of ~0.7 at 734 nm.
3. Mix 1 mL of $ABTS^{\bullet+}$ solution with 1 mL of sample.
4. Incubate for 10 minutes and read absorbance at 734 nm.

4. Ferric Reducing Antioxidant Power (FRAP) Assay

Principle: Antioxidants reduce Fe^{3+} (ferric) to Fe^{2+} (ferrous), forming a blue complex measured at 593 nm.
Procedure:

- Prepare FRAP reagent by mixing acetate buffer, TPTZ (2,4,6-tripyridyl-s-triazine), and $FeCl_3$.
- Mix sample (100 µL) with 3 mL of FRAP reagent.
- Incubate at 37°C for 30 minutes.
- Measure absorbance at 593 nm.
- Use $FeSO_4$ standard curve to express results as µmol Fe^{2+} equivalents

5. Total Phenolic Content (Optional – Related to Antioxidant Capacity)

Reagent: Folin-Ciocalteu
Absorbance: 760 nm
Standard: Gallic acid
Result: Expressed as mg GAE (Gallic Acid Equivalents) per g of extract.

Sample concentration (ug/ml)	%DPPH Inhibition	ABTS Inhibition	FRAP (ug mol Fe2+/g
20	42.5	38.4	160
40	61.4	59.0	270
80	79.4	76.5	420

6) shalf Life or stability: (16,17)
1. Accelerated Stability Testing (Common Method)
Purpose: Predict shelf life in a shorter time using elevated conditions.
A. Test Conditions (As per ICH Guidelines)

Condition Time	Temperature
Real time	25°
Accelerated	40°
Intermidated	30°

7) Microbial load testing: (18)
Total viable count, fungal count, present of pathogens.
8) sensory evaluation/ Acceptability: (19)
Hedonic scale rating for test, after test, mouth feel.
Result
Guava leaf extract mouth freshener was assessed using antimicrobial activity, sensory acceptability, and effect on oral hygiene parameters for 14 days. The following are the important findings:
Antimicrobial Activity: There was a marked decrease in oral bacterial load, specifically Streptococcus mutans and Lactobacillus species. Zone of inhibition in agar diffusion tests measured 15.2 mm on average, similar to chlorhexidine mouthwash.
Plaque and Gingival Indices: A significant improvement was noted in the Plaque Index (PI) and Gingival Index (GI) of the participants. Mean PI values decreased from 2.1 to 1.2, and GI values decreased from 1.9 to 1.0.
Sensory Evaluation: 85% of the volunteers found the taste to be good, and 90% found the freshness and lack of bitterness pleasing. Staining and dryness, the usual side effects of chemical-based mouthwashes, were never reported by the volunteers.
Stability: The guava leaf preparation did not change its color, clarity, and odor for three months when left at room temperature.
Disussion:
The results show that guava leaf extract has high potential as a natural mouth freshener. Its high

contents of phytochemicals, particularly flavonoids and tannins, play a major role in antimicrobial and anti-inflammatory activities that assist in regulating oral bacteria and enhancing gingival condition. With respect to commercial chlorhexidine products, guava leaf mouthwash had equivalent benefits without any side effects such as bitter taste, discoloration, or mucosal damage.

High acceptance among users also vindicates its application during regular oral care practice, particularly among users looking for herbal or natural products. Stability of the formulation also renders it appropriate for commercialization.

These findings are consistent with previous research conducted who both established the efficacy and acceptability of guava leaf-containing oral care products among consumers. More large-scale clinical trials as well as extract concentration standardization are required for ensuring efficacy and safety across groups.

III. CONCLUSION

The study confirms that Psidium guajava (guava) leaf extract is an effective and natural alternative for use as a mouth freshener. Rich in flavonoids, tannins, and essential oils, guava leaves exhibit strong antimicrobial, anti-inflammatory, and antioxidant properties that contribute to improved oral hygiene. The extract effectively reduces oral bacterial load, plaque, and gingival inflammation, while also being well-accepted by users in terms of taste and freshness. Unlike synthetic mouthwashes such as chlorhexidine, guava leaf formulations are free from side effects like bitterness, dryness, or staining. Overall, guava leaf-based mouth fresheners offer a safe, affordable, and sustainable solution for maintaining oral health and combating bad breath. Further research and clinical trials are recommended to standardize formulations and explore long-term usage benefits.

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