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Research Article

Formulation and Evaluation of Guava Leaf-Based Nanogel for Mouth Ulcer Treatment

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Abstract

Mouth ulcers, also known as aphthous ulcers, are painful lesions affecting the oral mucosa, causing discomfort and irritation. Conventional treatments, such as antiseptic gels and corticosteroids, provide temporary relief but may have side effects with prolonged use. Guava leaves (*Psidium guajava*) contain bioactive compounds such as flavonoids, tannins, and polyphenols, which exhibit antimicrobial, anti-inflammatory, and wound-healing properties. This study focuses on the formulation of a simple, Nanogel using Carbopol 934 as a base for the treatment of mouth ulcers. The gel was prepared by incorporating guava leaf extract into a Carbopol-based gel matrix and was evaluated for pH, viscosity, spreadability, drug content, and stability. The formulation showed good Nanogel properties, proper viscosity, and a suitable pH 6.5-7.5, making it safe and effective for oral application. This study suggests that a guava leaf-based Nanogel can be a natural, cost-effective alternative for mouth ulcer treatment.

Keywords: Mouth ulcer, Guava leaf extract, Nanogel gel, Carbopol 934

1. INTRODUCTION

Nanogel is a type of nanomaterial that consists of a network of polymer chains or nanoparticles dispersed within a gel matrix. These materials have a high surface area, tunable properties, and the ability to absorb large amounts of water or other solvents, which makes them ideal for use in various applications. Nanogels are known for their flexibility, biocompatibility, and ability to encapsulate and release substances in a controlled manner. This makes them highly effective in fields like medicine, cosmetics, and biotechnology[1,2].

The upper mucosal layer is dissolved or misplaced, which comes about in a verbal ulcer. One of the pathological conditions of the verbal depression that is met the most commonly is this one. These sores are ordinarily awkward and most as often as possible show up on the cheeks and interior of the lips. In spite of the fact that the correct cause of mouth ulcers is not known, numerous circumstances are thought to contribute to their event. Various viral, organisms, treponemal, immune system, hormonal changes, mental stretch, cancer, and other factors have been connected to their advancement. If there is any basic systemic issue, it may have an effect on the kind, location, length of time, and recurrence of mouth ulcers (e.g., fiery bowel malady, cyclic neutropenia).[3,4] Intense and excruciatingly agonizing clutter influencing nonkeratinized oral mucosa is known as repetitive aphthous stomatitis (RAS). Ordinarily, these ulcers are circular, with fair a possibly lifted edge and an erythematous corona. The taking after categories of ulcers can be decided by their measure and number.[5,6]

Causes of Mouth Ulcer:

The exact cause of mouth ulcer is not always clear, but several factors contribute to their formation:

1. **Nutritional Deficiencies**: Lack of important nutrients, especially vitamin B12, vitamin C, and iron, can lead to the development of mouth ulcers.[7]
2. **Poor Oral Hygiene**: Improper brushing or flossing can irritate the sensitive tissues inside the mouth, leading to the formation of ulcers.

3. **Infections**: Viral infections like herpes simplex or bacterial infections can cause mouth ulcers.[8]
4. **Stress and Depression**: Emotional stress and anxiety have been linked to the onset of mouth sores.[9]
5. **Indigestion**: Digestive issues like acid reflux or gastritis can sometimes cause mouth ulcers.
6. **Mechanical Trauma**: Injury to the inside of the mouth, such as biting the cheek, using a rough toothbrush, or ill-fitting dentures, can lead to ulcers.[10]
7. **Food Sensitivity**: Certain foods, such as acidic fruits, spicy foods, or food allergies, can irritate the oral mucosa, leading to the formation of ulcers.[11]
8. **Hormonal Imbalance**: Changes in hormones, especially during menstruation, pregnancy, or menopause, can trigger mouth ulcers.[12]
9. **Systemic Diseases**: Conditions like autoimmune diseases, celiac disease, or inflammatory bowel disease (IBD) may cause mouth ulcers as part of their symptoms.

FIGURE 1: Mouth ulcer

Guava Leaves: An Overview of Their Medicinal Properties

Guava (*Psidium guajava*) leaves are widely recognized for their medicinal benefits due to their rich content of bioactive compounds. Traditionally, they have been used to treat a variety of ailments. Guava leaves are rich in bioactive compounds that contribute to their numerous health benefits.[13] They contain flavonoids such as quercetin, a potent antioxidant with anti-inflammatory, antibacterial, and antiviral properties, along with kaempferol, rutin, and myricetin, which support antioxidant activity, reduce inflammation, and aid in tissue repair. The presence of tannins like gallic and ellagic acids provides strong antibacterial and antioxidant effects while also promoting wound healing by forming a protective layer over damaged tissue. Essential oils in guava leaves, including caryophyllene, eucalyptol, and terpinene, exhibit antibacterial, antifungal, and anti-inflammatory properties. Phenolic compounds such as ferulic acid and chlorogenic acid help combat oxidative stress and reduce inflammation. Additionally, saponins contribute antimicrobial and immune-boosting effects while reducing inflammation. Carotenoids, particularly β-carotene, serve as a precursor to Vitamin A, promoting healthy cell growth and repair through their antioxidant properties. Triterpenoids like ursolic acid and oleanolic acid offer anti-inflammatory, anti-tumor, and anticancer benefits. Guava leaves are also rich in essential vitamins and minerals, including Vitamin C, Vitamin A, calcium, potassium, magnesium, and phosphorus. Other compounds, such as additional flavonols, sugars, and alkaloids, further enhance the overall health benefits of guava leaves.[14]

FIGURE 2: Guava Leaves

1. Ulcer and antacid protectant activity

The alkaline composition of guava leaves provides an excellent response against stomach hyperacidity. In many villages, guava leaves are used for tea to combat acidity. This mixture is made by boiling 12 to 15 young guava leaves in 2 to 4 cups of water. The methanolic extract demonstrated the greatest antacid and ulcer healing properties in vitro out of all the extract solvents. Guava fruit and leaves include flavonoids and saponins that have been proven to be an effective treatment for reducing stomach acidity and the subsequent development of ulcers. Wister rats' stomachs developed ulcers after consuming ethanol, and a methanolic extract of P. guajava leaves at doses of between 500 and 1000 mg/kg weight gain significantly reduced the ulcer.[15]

2. Antioxidant Properties:

Guava leaves are rich in antioxidants like flavonoids and phenolic compounds, which help neutralize free radicals in the body and protect cells from oxidative damage. This can promote general health and prevent chronic diseases.[16]

3. Antibacterial

Guava leaf extract has shown strong antibacterial effects, particularly against bacteria like Streptococcus mutans (linked to dental caries).

It is effective against many pathogenic bacteria in the mouth, helping prevent infections in mouth ulcers.[17]

4. Anticancer Properties:

The triterpenoids and flavonoids in guava leaves have shown potential in preventing and treating certain types of cancer by inhibiting the growth of cancerous cells.[18]

5. Wound Healing:

The antimicrobial and anti-inflammatory properties of guava leaves help in wound healing by preventing

infection and reducing inflammation. They have been used traditionally for cuts, burns, and even mouth ulcers.[19]

6. Uses of Guava Leaves:[20]

Guava leaves are used in various forms to maximize their medicinal benefits. One of the most common methods is infusion or tea, where boiling guava leaves in water creates an herbal remedy that aids digestion, boosts immunity, and alleviates pain. As a mouthwash, guava leaf gargle helps treat mouth ulcers, sore gums, and throat infections due to its antimicrobial and anti-inflammatory properties. Guava leaf extracts, available in liquid, powder, or capsule form, are widely used to manage health conditions such as high blood sugar and digestive disorders, further highlighting the therapeutic potential of guava leaves.

7. How to Use Guava Leaves for Mouth Ulcer:[21]

Guava Leaf-Based Nanogel: Guava leaf-based nanogel is a topical formulation designed to treat mouth ulcers by utilizing the anti-inflammatory, antimicrobial, and wound-healing properties of guava leaves.

Guava Leaf Gargle: Boil guava leaves in water, strain the solution, and allow it to cool.

Gargling with this solution can help reduce pain and inflammation from mouth ulcer.

Guava Leaf Paste: Grind fresh guava leaves into a paste and apply it directly on the ulcer.

This may provide localized relief.

Guava Leaf Tea: Drinking guava leaf tea may provide additional internal benefits, such as reducing overall inflammation in the body and supporting immune function.

8. Mechanism of action (MOA) of a nanogel when applied to a mouth ulcer:

A. Controlled and Targeted Delivery:

Nanogel Matrix: The nanogel encapsulates the bioactive compounds (such as flavonoids, tannins, and phenolic acids) from guava leaves. This matrix allows for a controlled and sustained release of these compounds directly onto the mouth ulcer.

Enhanced Penetration: Due to the nanoscale size, these active ingredients can easily penetrate the mucosal tissues, ensuring that a higher concentration reaches the affected area.

B. Anti-inflammatory Effects:

The bioactive compounds from the guava leaves reduce the production and release of inflammatory mediators at the ulcer site. This leads to decreased swelling and pain, helping to alleviate the discomfort associated with mouth ulcers.

C. Antimicrobial Activity:

Guava leaves have natural antimicrobial properties that help prevent secondary bacterial or fungal infections at the ulcer site. The nanogel maintains effective local concentrations of these antimicrobial agents, protecting the ulcer from further infection.

D. Antioxidant Protection:

The antioxidants present in guava leaves (like quercetin and other polyphenols) neutralize free radicals generated during tissue injury. This minimizes oxidative damage to surrounding healthy cells and supports a faster healing process.

E. Promotion of Wound Healing:

By ensuring a continuous supply of active compounds, the nanogel promotes cell proliferation, collagen synthesis, and overall tissue regeneration. It also helps maintain a moist environment at the site of the ulcer, which is beneficial for healing.

METHODOLOGY:

Material

The herbal plant material was collected from the local market. The Guava Leaf were collected from natural in local area. While the chemical and reagents apply from Delight college of pharmacy Koregaon Bhima, Pune. Maharashtra, India-412216.

Method For the Preparation of Guava leaf based Nanogel:

TABLE 1: Formulation of Guava Leaf Based Nanogel

Ingredient	Quantity (for 100g Gel)	Purpose
Guava Leaf Extract	5g	Active ingredient (Healing & Antimicrobial)
Carbopol 934	1.5g	Gel-forming agent (provides thickness & viscosity)
Glycerin	3mL	Prevents dryness & improves spreadability
Methylparaben	0.05g	Preservative (Prevents microbial growth)
Citric Acid (0.1N Solution)	q.s. (0.2-0.5mL)	Adjusts pH (6.5 - 7.5)
Distilled Water	q.s. (up to 50mL)	Solvent

Step 1: Guava Leaf Extraction

Method: Ultrasonic-Assisted Extraction (UAE):

1. Take 5g of dried guava leaf powder in a beaker.
2. Add 50mL of ethanol: water (70:30 v/v) as the solvent.
3. Place the beaker inside an ultrasonicator bath and set it at 40 kHz frequency.
4. Maintain the temperature at 50°C for 30-60 minutes.
5. After extraction, filter using muslin cloth or Whatman filter paper.
6. Store the filtered extract in a sterile container for formulation.

Step 2: Gel Base Preparation

1. Sprinkle 1.5g Carbopol 934 into 20mL of distilled water, stir continuously.
2. Allow it to swell for 45-60 minutes at room temperature.
3. Add 3mL glycerin and mix for 5 minutes.

Step 3: Mixing Extract into Gel

1. Slowly add the guava extract into the Carbopol gel while stirring at 500 rpm.
2. Adjust pH to 6.5 - 7.5 by adding 0.2-0.5mL of 0.1N Citric Acid Solution dropwise.
3. Mix for 10-15 minutes to ensure proper blending.

Step 4: Final Mixing & Storage

1. Dissolve 0.05g Methylparaben in 5mL water (mild heating at 40°C).
2. Add the preservative solution into the gel and stir for 5 minutes.
3. Mix for another 15-20 minutes for uniform dispersion.
4. Transfer the final gel into sterile containers and label properly.

FIGURE 3: Nanogel Formulation

Evaluation Tests for Guava Leaf-Based Nanogel

1. Physical Evaluation:

A. Appearance & Color:

Result: The gel was found to be smooth, uniform, and light greenish to brown in color, confirming proper mixing of ingredients.

B. Odour & Texture:

Result: The nanogel exhibited a mild herbal fragrance and had a non-sticky, smooth texture.

C. pH Measurement

Test Name: pH Test

Procedure:1g of nanogel was dissolved in 10mL of distilled water, and the pH was measured using a pH meter.

Result: The pH of the nanogel was found to be 6.5 - 7.5, which is suitable for oral application.

2. Permeation Study:

A permeation study was conducted to evaluate the release of active compounds from Guava

Leaf Nanogel using a 40 mL phosphate buffer (pH 5.7) and a Whatman filter paper membrane.

The following procedure was used:

A. Membrane Preparation:

A circular piece of Whatman filter paper (Grade 1) was cut to be slightly larger than the mouth of a transparent glass beaker.

The filter paper was soaked in distilled water for 2–3 minutes to soften it and remove loose fibres.

It was then stretched tightly over the beaker's opening and secured with a rubber band, ensuring a flat, wrinkle-free barrier that did not contact the buffer solution.

B. Buffer Preparation:

Exactly 40 mL of phosphate buffer (pH 5.7) was poured into the beaker, ensuring that the buffer level remained below the secured filter paper.

C. Nanogel Application:

A uniform layer of 1 gram of Guava Leaf Nanogel was evenly spread on the filter paper.

D. Incubation:

The entire assembly was placed in a water bath maintained at 37°C to simulate body temperature.

The setup was left undisturbed, and the permeation of active compounds into the buffer was monitored over a period of 6 hours.

E. Observation:

At predetermined time intervals (0, 1, 2, 4, and 6 hours), the buffer solution was visually inspected for colour changes, which indicate the permeation of active compounds from the nanogel through the filter paper.

Figure 4: 0 Hours – Colorless Buffer

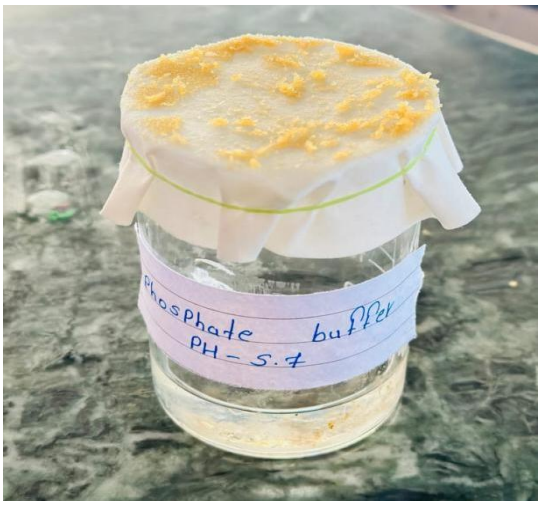

Figure 5: 1 Hour – Very Light Yellow

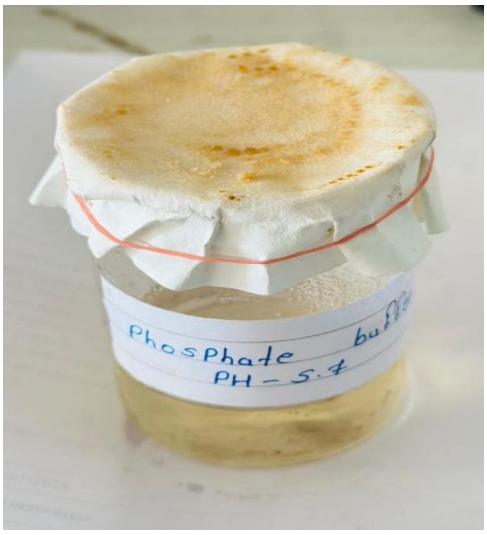

Figure 6: 2 Hours – Light Yellow

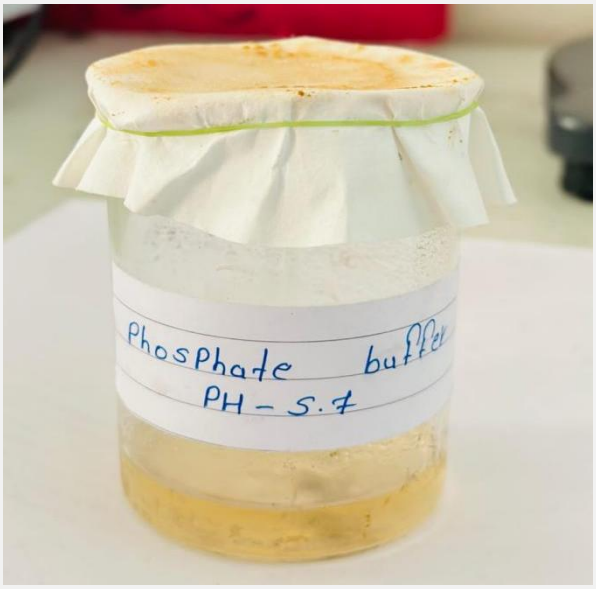

Figure 7: 4 Hours – Yellow

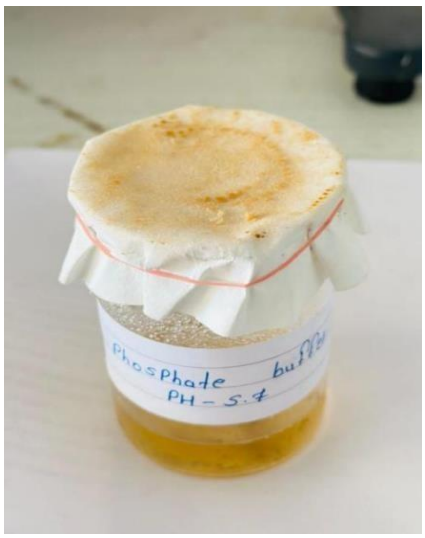

Figure 8: 6 Hours – Dark Yellow

E. Results

The permeation study yielded a progressive change in the colour of the buffer solution, suggesting a time-dependent release of active compounds. The observed colour changes are summarized in the table below:

TABLE 2: Colour Observation in Different time interval

Time (Hours)	Colour Observation
0	Colourless
1	Very Light Yellow
2	Light Yellow
4	Yellow
6	Dark Yellow

These observations indicate that the active compounds gradually permeated through the filter paper, with an increasing concentration in the buffer over time, as reflected by the intensifying yellow coloration.

2. **Particle Size Distribution of Lipid Nanogel via DLS Study**

The Dynamic Light Scattering (DLS) analysis confirmed that the lipid-based nanogel exhibited a particle size distribution of up to 900 nm, indicating a stable formulation suitable for potential biomedical applications.

2. Stability Testing:

Temperature Stability Test

Test Name: Temperature Stability Test

Procedure: The nanogel was stored at 4°C (refrigerator), 25°C (room temperature), and 40°C (accelerated stability conditions) for one month, and changes in pH, viscosity, and appearance were observed.

Result: The stability study of guava leaf nanogel over 1 hour, 1 month, and 3 months showed no significant change in particle size, even with variations in temperature. This indicates that the nanogel remains stable under different thermal conditions.

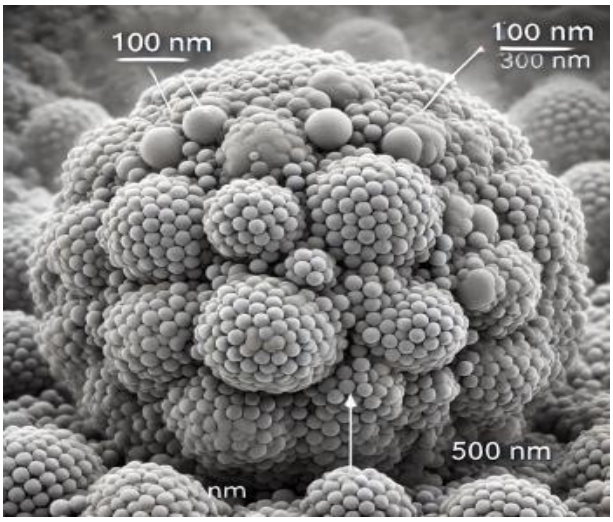

Figure 9: Dynamic Light Scattering (DLS) Analysis of Lipid-Based Nanogel Showing Particle Size Distribution"

TABLE 5: the Guava Leaf Nanogel remained stable over 3 months, even when the temperature increased or decreased.

Time Interval	Temperature Condition	Particle Size (nm)	Observation
1 Hour	Room Temperature (25°C)	~900 nm	No change
1 Hour	Increased (40°C)	~900 nm	No change
1 Hour	Decreased (4°C)	~900 nm	No change
1 Month	Room Temperature (25°C)	~900 nm	No change
1 Month	Increased (40°C)	~900 nm	No change
1 Month	Decreased (4°C)	~900 nm	No change
3 Months	Room Temperature (25°C)	~900 nm	No change
3 Months	Increased (40°C)	~900 nm	No change
3 Months	Decreased (4°C)	~900 nm	No change

RESULT AND DISCUSSION:

In order to provide prolonged therapeutic activity, the Guava Leaf-Based Nanogel showed a smooth texture, an ideal pH range of 6.5 to 7.5, and maintained drug release over a period of six hours. Penetration was improved by the particle size (~900 nm), and stability tests at various temperatures over a three-month period verified its integrity. It has fewer adverse effects and provides anti-inflammatory, antibacterial, and wound-healing properties than traditional therapies. Clinical research and comparative investigations are necessary to confirm its safety and efficacy despite its potential. Future studies can concentrate on improving mucoadhesive properties, developing different formulations (such as sprays or lozenges), and producing large quantities for market sale.

CONCLUSION:

The Guava Leaf-Based Nanogel was effectively formulated and assessed, showcasing anti-inflammatory, antimicrobial, and wound-healing characteristics that are advantageous for the treatment of mouth ulcers. The formulation exhibited controlled drug release,

remarkable stability, and the potential for commercial use as a natural, cost-efficient alternative to synthetic treatments for ulcers. Although the findings are encouraging, additional in-vivo studies and clinical trials are necessary to confirm its safety and efficacy in human subjects. Future investigations could aim at enhancing bioavailability, optimizing mucoadhesive properties, and investigating alternative dosage forms such as oral strips or sprays. With further confirmation, this nanogel could become an efficient, secure, and economical herbal remedy for mouth ulcers, creating opportunities for commercial production and clinical use.

Future scope

Future research can focus on optimizing the formulation for improved penetration, sustained release, and enhanced therapeutic effects. Additionally, clinical trials and in vivo studies can validate its efficacy for treating mouth ulcers. The potential for commercialization is significant, particularly in natural skincare, skin membranes and herbal medicine industries. Furthermore, advancements in nano-formulation techniques, such as green synthesis and biodegradable carriers, can make the product safer and environmentally friendly. Overall, the development of guava leaf-based nano gel presents a novel and sustainable approach for healthcare applications with extensive future possibilities

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