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ORIGINAL ARTICLE

TO DESIGN AND *IN-VITRO* EVALUATION OF NOVEL POLYHERBAL ORAL GEL FOR MOUTH ULCERS

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Abstract

Background: *The rational combination of botanical extracts into a crosslinked mucoadhesive hydrogel establishes an effective topical delivery system for managing oral mucosal lesions.* ***Materials and Methods:*** *Psidium guajava and Salvia officinalis primary extracts, together with six auxiliary herbs, were combined to create the gel using Carbopol 934. Using an in vitro scratch assay, it was assessed for physicochemical characteristics, in vitro release, antibacterial activities, and tissue regeneration.* ***Results:*** *With a physiological pH of 6.58, an ideal viscosity of 7935 cP, a realistic yield of 92\%, and strong 94.44\% DPPH radical scavenging and 71.21\% anti-inflammatory protection, the optimized formulation F3 was stable and transparent. It outperformed the commercial Marketed Control in combination with 94.25 % inhibition against Aspergillus niger and showed strong antibacterial activities against Pseudomonas aeruginosa (2.4 cm) and Proteus vulgaris (2.3 cm). The scratch assay, which achieved an 83.17% cellular wound closure rate within 24 hours compared to the control (39.10%), was crucial in confirming faster tissue repair.*

Conclusion: *The created polyherbal gel is a great composition that effectively combats oxidative damage, microbial invasion, and inflammation to accelerate mucosal healing.*

Keywords: *Polyherbal Oral Gel; Psidium guajava; Wound Healing; Scratch Assay; Mouth Ulcers.*

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INTRODUCTION

The biological process of oral wound healing involves hemostasis, inflammation, proliferation, and tissue remodeling. It is highly coordinated and dynamic. In contrast to skin wounds, the oral mucosa heals in a special physiological setting where it is constantly exposed to food particles, saliva, a dense microbial population, and repeated mechanical stress from speaking and chewing. Even though saliva contains a number of growth factors and antimicrobial proteins that aid in tissue regeneration, the ongoing irritation and microbial assault may impede healing and raise the risk of secondary infection. Because they make it difficult to eat, swallow, speak, and practice good oral hygiene, oral ulcers continue to be one of the most prevalent painful conditions affecting the oral cavity and severely lower quality of life. Oral ulcers are a major public health hazard, affecting 10–25% of the world's population at some point in their lives. [1,2,3,33]

Mouth ulcers, sometimes referred to as recurrent aphthous stomatitis or aphthous ulcers, are painful round or oval lesions with an erythematous inflammatory halo surrounding a yellowish-white fibrinous substrate. Local trauma, dietary inadequacies, psychological stress, hormonal imbalance, microbial infection, immunological dysregulation, and systemic disorders are all contributing factors to their complex development. At the molecular level, T-lymphocyte activation causes an overabundance of pro-inflammatory cytokines, such as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α), which leads to the death of inflammatory tissue. Concurrently, excessive production of reactive oxygen species (ROS) leads to oxidative stress, which results in DNA damage, lipid peroxidation, protein degradation, and delayed epithelium regeneration. These interrelated pathogenic processes suggest that rather than focusing on just one mechanism, effective treatment necessitates concurrent regulation of inflammation, microbial growth, oxidative damage, & tissue regeneration. [4,5,30,31,32]

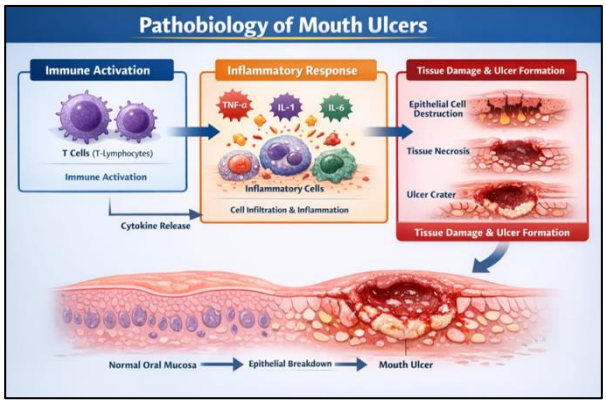


Figure 1. Pathobiology of Mouth Ulcers

Topical corticosteroids, mouthwashes containing chlorhexidine, local anesthetics, and antiseptic preparations are examples of conventional therapy approaches that primarily relieve symptoms by lowering pain or inflammation. While chlorhexidine may result in tooth discoloration, taste problems, and mucosal irritation, long-term corticosteroid use may induce mucosal thinning, delayed tissue healing, and increased susceptibility to opportunistic infections. Additionally, the majority of treatments now on the market are solely intended to address a single facet of ulcer pathology, which prevents them from offering complete disease management. These restrictions have prompted the creation of safer, multipurpose treatment strategies that can simultaneously reduce inflammation and infection and induce active tissue regeneration. [6]

Because they contain a variety of phytoconstituents with antioxidant, antibacterial, anti-inflammatory, analgesic, and wound-healing properties, such as flavonoids, tannins, alkaloids, phenolic compounds, terpenoids, and essential oils, medicinal plants have attracted a lot of scientific attention. The ability of several medicinal plants to work in concert on several disease targets to increase therapeutic

efficiency while reducing side effects makes polyherbal compositions very appealing. Many traditional medical systems, such as Ayurveda, which favors herb mixtures over single-plant medicines for chronic inflammatory illnesses, are based on this synergistic idea. [7]

Psidium guajava (guava) leaves are a rich source of flavonoids, such as quercetin and guaijaverin, as well as tannins, phenolic acids, saponins, and vitamin C. These phytochemicals have strong anti-inflammatory effects by inhibiting inflammatory cytokines, strong antioxidant activity by scavenging free radicals, antimicrobial activity against oral pathogens, and wound-healing qualities by promoting fibroblast proliferation, collagen synthesis, and epithelial regeneration.[8,9,10,11,12,13] Similarly, essential oils like cineole, camphor, and thujone are found in Salvia officinalis (sage), together with flavonoids and rosmarinic acid, which have broad-spectrum antibacterial, antioxidant, anti-inflammatory, and tissue-regenerative properties. Both plants efficiently lower the microbial burden, inhibit inflammatory mediators, and hasten the healing of damaged oral tissues, according to numerous experimental investigations. [14,15,16]

The current formulation additionally includes Triphala, Yashtimadhu (Glycyrrhiza glabra), Daruharidra (Berberis aristata), Khadir, Foeniculum vulgare (Fennel), and Aloe vera to further improve therapeutic efficiency. Strong antioxidant and antimicrobial properties are provided by triphala, [17,18] mucoprotective & anti-inflammatory effects provided by Yashtimadhu, [19,20] berberine-mediated antimicrobial action is provided by daruharidra; [21,22] Khadir functions as a natural astringent; fennel supports antimicrobial activity and plaque control; and Aloe vera encourages hydration, epithelial regeneration, and tissue repair. [24] A multi-target therapeutic approach that can concurrently reduce oxidative stress, control inflammation, limit microbial invasion, and accelerate wound healing is made possible by the sensible combination of various herbal constituents. [23]

By extending the duration of herbal components' residency at the ulcer site, the use of a mucoadhesive gel dosage form further improves therapeutic efficacy. Because of its superior mucoadhesive qualities, biocompatibility, and capacity to offer prolonged drug release, carbopol 934 was chosen as the main gelling polymer. Additionally, the formulation includes sodium benzoate as a preservative, menthol for cooling and patient comfort, propylene glycol as a penetration enhancer, glycerin as a humectant, and triethanolamine for pH adjustment. When combined, these pharmaceutical excipients reduce salivary washout and increase formulation stability, patient acceptance, and targeted drug administration. [25,26,27]

In order to treat mouth ulcers, a unique mechanism-based polyherbal mucoadhesive oral gel containing Psidium guajava, Salvia officinalis, and six complementary medicinal herbs was developed and scientifically evaluated in the current study. Using a scratch assay, the formulation was refined and thoroughly assessed for physicochemical characteristics, in vitro drug release, antibacterial, antifungal, antioxidant, anti-inflammatory, stability, and wound healing activities. The results of this study may offer scientific proof for the creation of a herbal remedy for oral mucosal ulcers that is efficient, safe, affordable, and multipurpose. [28,29,34]

MATERIALS

Fresh Psidium guajava leaves were gathered during the proper growth season in Mungusgaon, Ahilyanagar, Maharashtra, India. The Department of Botany at Ahilyanagar College, taxonomically verified the plant material to guarantee the botanical identity and purity of the gathered leaves. [3] A certified commercial herbal supplier provided the

dried Salvia officinalis leaves, which were authenticated before to use. [3] To retain their phytochemical components, all herbal materials were meticulously washed, shade-dried (if necessary), and kept in dry, airtight containers until further processing. [3,10]

The herbal ingredients used in the formulation included Triphala extract, Yashtimadhu (Glycyrrhiza glabra), Daruharidra (Berberis aristata), Khadir, Foeniculum vulgare (Fennel), and Aloe vera, all of which were obtained in pharmaceutical-grade quality from reliable commercial sources. These medicinal herbs were selected because of their reported antimicrobial, antioxidant, anti-inflammatory, mucoprotective, and wound-healing properties, making them suitable for the development of a polyherbal oral gel intended for the management of mouth ulcers. Pharmaceutical excipients used in the formulation included the mucoadhesive gelling polymer Carbopol 934, the humectant glycerin, the solvent and penetration enhancer propylene glycol, the neutralizing agent triethanolamine for pH adjustment, the preservative sodium benzoate, and the cooling and calming agent menthol. The study's chemicals and reagents were all of analytical reagent (AR) quality and were employed without additional purification. [26,8,9,14,15,20,21,23,24,26,27]

For comparative evaluation, diclofenac sodium was used as the reference standard for anti-inflammatory activity, ascorbic acid served as the standard antioxidant in the DPPH free radical scavenging assay, and a commercially available marketed oral gel formulation was employed as the reference product during physicochemical, antimicrobial, and wound-healing evaluations. Distilled water and phosphate buffer solutions were prepared according to standard laboratory procedures and used throughout the experimental study.

Extraction Methodologies

***Psidium guajava* Extract:** Fresh leaves of Psidium guajava were thoroughly washed with distilled water to remove dust, soil particles, and other impurities. The cleaned leaves were shade-dried at room temperature for 7–10 days until a constant weight was achieved in order to preserve thermolabile phytoconstituents. The dried leaves were then coarsely powdered using a mechanical grinder and stored in airtight containers until extraction.

Approximately 150 g of powdered leaf material was packed into a Soxhlet apparatus and extracted continuously with 300 mL of ethanol for 4–6 hours. Ethanol was selected as the extraction solvent because of its excellent ability to dissolve flavonoids, tannins, phenolic compounds, and other bioactive phytoconstituents responsible for antioxidant, antimicrobial, and wound-healing activities. After completion of extraction, the extract was filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator at a controlled temperature below 50°C. The concentrated extract was further dried to obtain a semi-solid mass, transferred into airtight amber-colored containers, and stored at 4°C until formulation. [1,10,11]

Salvia officinalis Extract: Salvia officinalis dried leaves were ground into a powder and extracted aqueously using the decoction method. To extract water-soluble phytoconstituents, such as rosmarinic acid, flavonoids, and phenolic compounds, about 50 g of powdered leaves were cooked with 500 mL of distilled water for 60 minutes under continuous heating. To get rid of insoluble plant remnants, the extract was allowed to cool to ambient temperature before being filtered through Whatman No. 1 filter paper. A concentrated aqueous extract was created by concentrating the filtrate under low pressure at about 50°C. This extract was then refrigerated until it was needed again. [11,12,13]

Supporting Materials: Standard aqueous extraction techniques were used to manufacture the supporting herbal constituents, which included Triphala, Yashtimadhu (Glycyrrhiza glabra), Daruharidra (Berberis aristata), Khadir, Foeniculum vulgare (Fennel), and Aloe vera. To guarantee consistent extraction of the active ingredients, each powdered herbal material was combined with distilled water in the proper quantity and well agitated. After removing insoluble components from the mixes using Whatman No. 1 filter paper, the filtrates were utilized to create the polyherbal oral gel. To maintain its biological activity, fresh aloe vera gel was extracted from healthy leaves, homogenized, filtered, and added right away. To preserve their stability and reduce the deterioration of bioactive components, all produced extracts were kept in sterile, airtight containers at 4°C until formulation. [25,26,29]

Formulation Design

Five experimental formulations (F1–F5) of the polyherbal oral gel were prepared by varying the concentrations of the herbal extracts and Carbopol 934 to obtain an optimized formulation with suitable physicochemical characteristics and biological performance. Carbopol 934 was dispersed in purified water and allowed to hydrate for 30 minutes to form a uniform gel base. Menthol was dissolved in propylene glycol and mixed with glycerin, while sodium benzoate was dissolved separately in purified water. Predetermined quantities of Psidium guajava, Salvia officinalis, Triphala, Yashtimadhu (Glycyrrhiza glabra), Daruharidra (Berberis aristata), Khadir, Foeniculum vulgare, and Aloe vera extracts were gradually incorporated into the hydrated polymer with continuous stirring to ensure uniform distribution of all herbal constituents. Finally, triethanolamine was added dropwise to adjust the pH and facilitate gel formation. The resulting formulations were smooth, homogeneous, and stable, making them suitable for further physicochemical and biological evaluation. [25,26,27,28,29] The composition of all experimental batches is presented in Table 1.

Table 1: Composition of Experimental Polyherbal Oral Gel Batches (per 10 g)

Ingredients	F1 (g)	F2 (g)	F3 (g)	F4 (g)	F5 (g)
Psidium guajava extract	0.03	0.04	0.05	0.05	0.05
Salvia officinalis	0.02	0.03	0.03	0.03	0.03

extract					
Triphala extract	0.03	0.04	0.04	0.04	0.04
Daruharidra extract	0.02	0.02	0.03	0.03	0.03
Yashtimadhu extract	0.02	0.03	0.03	0.03	0.03
Khadir extract	0.01	0.02	0.02	0.02	0.02
Fennel extract	0.02	0.02	0.025	0.025	0.025
Aloe vera extract	0.03	0.04	0.05	0.05	0.05
Carbopol 934	0.03	0.04	0.045	0.05	0.045
Glycerin	0.40	0.45	0.50	0.50	0.50
Propylene glycol	0.80	0.90	1.00	1.00	1.00
Menthol	0.01	0.012	0.015	0.015	0.015
Sodium benzoate	0.02	0.02	0.025	0.025	0.025
Triethanolamine	q.s	q.s	q.s	q.s	q.s
Purified Water	q.s to 10 g	q.s	q.s	q.s	q.s



Figure 2: Formulated Herbal Gel

Evaluation Parameters of Gel

1. Physical Evaluation:

The prepared formulations were evaluated for colour, odour, appearance, consistency, clarity, homogeneity, percentage yield, and pH using standard evaluation methods. These parameters were assessed to ensure the quality, stability, and suitability of the formulations for oral application. [26,27]

2. Spreadability:

Spreadability was determined using the slide-slip method to evaluate the ease of application of the gel over the mucosal surface. It was calculated using the following equation: [27,28]

$$S = (M \times L) / T$$

Where, S = Spreadability, M = Weight tied to the upper slide (g), L = Length of glass slide (cm), and T = Time taken (sec).

3. Extrudability:

Extrudability was evaluated by measuring the ease with which the gel was extruded from collapsible aluminium tubes under constant pressure. The test indicated the convenience of dispensing the formulation. [26,27]

4. Viscosity:

The viscosity of the formulations was measured using a Brookfield viscometer at room temperature and expressed in centipoise (cP). Appropriate viscosity is essential for adequate retention and patient acceptability. [26,27]

5. Gel Strength:

Gel strength was determined by recording the time required for a standard weight to penetrate a fixed distance into the gel. The test was performed to assess the mechanical strength and structural integrity of the formulation. [26,27]

6. Stability Study:

The optimized formulation was stored under open and closed conditions at room temperature for one month. Changes in colour, odour, pH, consistency, and homogeneity were monitored periodically to evaluate formulation stability. [26,27]

7. Drug Release Study (In-vitro Diffusion Method)

A Franz diffusion cell equipped with a pre-soaked dialysis membrane was used to assess the polyherbal oral gel's in vitro drug release profile. To replicate oral physiological conditions, the receptor compartment was filled with phosphate buffer (pH 6.8) and kept at 37 ± 0.5°C with constant magnetic stirring. The gel was precisely weighed and put in the donor compartment. To maintain sink conditions, 5 mL samples were taken out of the receptor compartment at predefined intervals (0–120 minutes) and promptly replaced with an equivalent volume of new phosphate buffer. [41,42] The cumulative percentage drug release was computed using the following formula after the collected samples were spectrophotometrically analyzed:

$$\%\ Drug\ Release = \left(\frac{Ammount\ of\ \ drug\ release}{Total\ drug\ content}\right) \times 100$$

8. Evaluation of Antibacterial Activity

The agar well diffusion method was used to assess the antibacterial properties of the prepared polyherbal oral gel against specific Gram-positive, Gram-negative, and anaerobic microorganisms frequently linked to oral and opportunistic infections. Bacillus subtilis, Staphylococcus aureus, Streptococcus mutans, Streptococcus mitis, Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumannii, Actinomyces israelii, Porphyromonas gingivalis, Fusobacterium nucleatum, and Proteus vulgaris.

Procedure:

To create a homogeneous microbial solution, fresh bacterial cultures were standardized to the 0.5 McFarland turbidity standard (about 1.5×10^8 CFU/mL). To create a confluent lawn of growth, a 100 µL aliquot of each standardized culture was uniformly distributed onto sterile Mueller–Hinton Agar (MHA) or Nutrient Agar plates using a sterile swab. Using a cork borer, sterile wells with a diameter of 6–8 mm were created in the agar medium and filled with the test samples, which included the developed polyherbal gel at the proper concentrations (10 mg for oral pathogens and 50 mg for general pathogens) and Marketed Control (50 mg) as the reference standard.

To help the samples diffuse into the agar medium, the infected plates were left at room temperature for half an hour. Depending on the test organism, the plates were then incubated for 24 hours at 37°C in either anaerobic or aerobic conditions. A Vernier caliper was used to measure the zones of inhibition (ZOI) in centimeters (cm) after incubation. Every experiment was conducted in triplicate, and the average results were noted for examination. [4,6,38]

9. Anti-inflammatory Activity (Albumin Denaturation Assay)

The anti-inflammatory potential was evaluated using the protein denaturation method with bovine serum albumin (BSA).

Procedure: The test sample (or standard Diclofenac sodium) and a 1% aqueous solution of BSA made up the reaction mixture. Phosphate-buffered saline was used to change the pH. To cause denaturation, the samples were heated to 51°C for 20 minutes after being incubated at 37°C for 20 minutes. Spectrophotometric measurements of the turbidity were made after cooling. [7,15,36,39]

Calculation: The percentage inhibition of protein denaturation was calculated using the formula:

$$\%\ Inhibition = \left(\frac{A0 - A}{A0}\right) \times 100$$

10. Antioxidant Activity (DPPH Free Radical Scavenging Assay)

The antioxidant capacity was determined by the sample's ability to bleach the stable DPPH (2,2-diphenyl-1-picrylhydrazyl) radical.

Procedure: A 0.1 mM DPPH solution in methanol was mixed with a certain concentration of the test sample and standard (such as ascorbic acid). After giving the mixture a good shake, it was allowed to stand for half an hour at room temperature in the dark. The decrease in absorbance at 517 nm was used to gauge the reduction of the DPPH radical. [5,40]

Calculation: The scavenging activity was expressed as:

$$\%\ Scavenging\ Activity = \left(\frac{A0 - A}{A0}\right) \times 100$$

11. Antifungal Assay (Poisoned Plate & Colony Count Method)

The poisoned plate method was used to assess the gel's antifungal activity against Aspergillus niger. The test sample was added to Potato Dextrose Agar (PDA), inoculated with fungal spores, and then incubated for 48–72 hours at 28°C. By comparing the colony forming units (CFU) of the test and control groups, the antifungal efficacy was assessed, and the percentage inhibition was computed. [2,15,23,43,44]

$$\%\ Inhibition = \left(\frac{Control\ CFU - Treated\ CFU}{Control\ CFU}\right) \times 100$$

12. Wound Healing Activity (In-vitro Scratch Assay)

The gel's capacity for tissue regeneration and cell migration was assessed using a common in-vitro scratch test. In 6-well plates, human fibroblast or epithelial cells were cultivated until an 80–90% confluent monolayer was obtained. A sterile micropipette tip was used to make a linear scratch across the cell monolayer, and detached cells were eliminated by washing with phosphate-buffered saline (PBS).

After that, the optimized polyherbal gel extract was added to the wells, while a control well was left untreated. Using an inverted microscope, pictures of the scratch area were taken at 0 and 24 hours, and the formulation's capacity to heal wounds was assessed by calculating the percentage of wound closure. [8,16,17,35,37,34]

Result and Discussion

1. Physicochemical Evaluation

All five formulations (F1–F5) exhibited acceptable physicochemical characteristics with minor variations depending on the concentration of Carbopol 934 and the herbal extracts. Among the prepared formulations, F3 demonstrated the most desirable organoleptic and physicochemical properties. It exhibited a dark brown colour, pleasant herbal odour, smooth consistency, clear appearance, and excellent homogeneity, indicating the successful incorporation of the herbal

ingredients into the gel base. Furthermore, F3 showed the highest percentage yield (92%), physiological pH (6.58), maximum spreadability (32.0 g·cm/sec), excellent extrudability, highest viscosity (7935 cP), and optimal gel strength (41 sec). Based on these findings, formulation F3 was selected as the optimized formulation for subsequent stability, in vitro drug release, antimicrobial, antioxidant, anti-inflammatory, antifungal, and wound healing studies.

2. Stability

Table 3: Stability of Gel Formulation

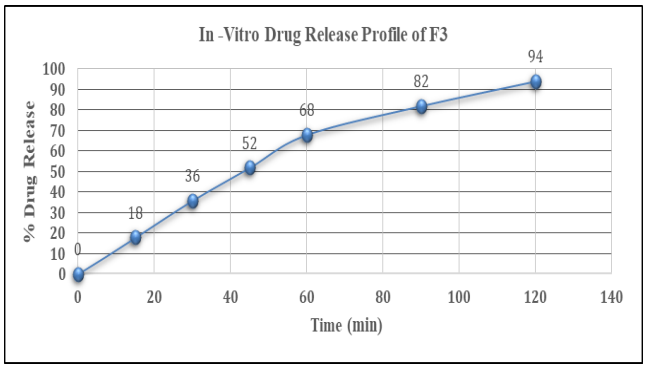
Formulation		Colour	Odour	pH	Consistency	Homogeneity
F3 (Opt)	Open	No change	Pleasant	6.58-6.55	No change	Uniform
	Closed	No change	Pleasant	6.58-6.55	No change	Uniform

Observation:

Under both open and closed storage circumstances, the improved formulation (F3) maintained its physical stability for the duration of the one-month storage period. Color, odor, consistency, and homogeneity did not significantly alter while being stored. The preservation of physicochemical stability was demonstrated by the pH remaining within the permissible physiological range (6.58–6.55). These results show that under the storage circumstances used, the optimized polyherbal oral gel maintained its quality and stability.

3. In-Vitro Drug Release Study (Franz Diffusion Cell Method)

***In-vitro* Drug Release Study Graph (Franz Diffusion Cell Method) Formulation**



Observation:

Over the course of the investigation, the improved formulation's (F3) in vitro drug release profile showed a steady and gradual release of the herbal components. From 18% at 15 minutes to 36% at 30 minutes, 52% at 45 minutes, 68% at 60 minutes, 82% at 90 minutes, and 94% at 120 minutes, the total drug release. The results show an effective and regulated release pattern, indicating extended availability of the active ingredients for topical treatment of mouth ulcers.

4. Anti-inflammatory Activity (Protein Denaturation Method)

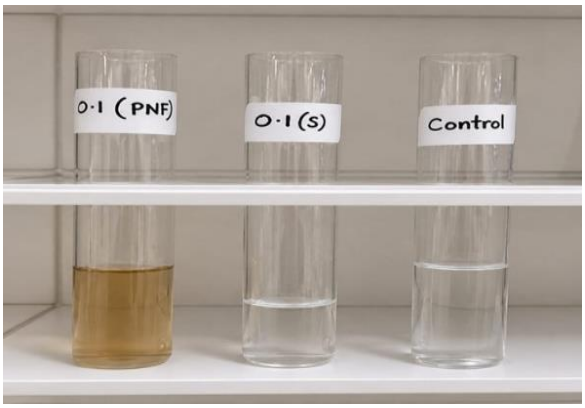
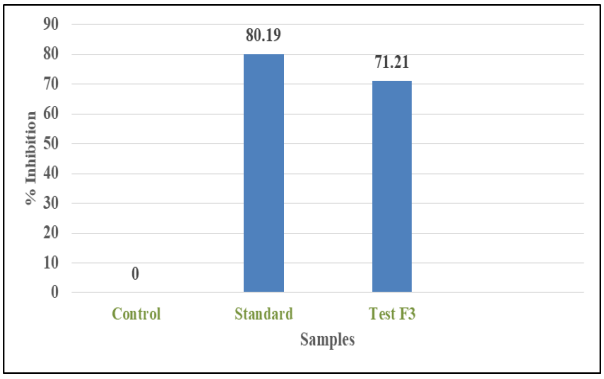


Figure 3: Anti-inflammatory Activity (Protein Denaturation Method

Graph of Anti-inflammatory Activity



Observation:

The anti-inflammatory activity of the optimized polyherbal oral gel (F3) was evaluated using the protein denaturation method. The formulation exhibited 71.21% inhibition of protein denaturation, while the standard drug (Diclofenac sodium) showed 80.19% inhibition. The control group showed no inhibitory activity. These findings indicate that the optimized formulation possesses considerable anti-inflammatory activity and demonstrates promising potential for the management of inflammatory conditions associated with mouth ulcers.

5. Antioxidant Activity (DPPH Assay)

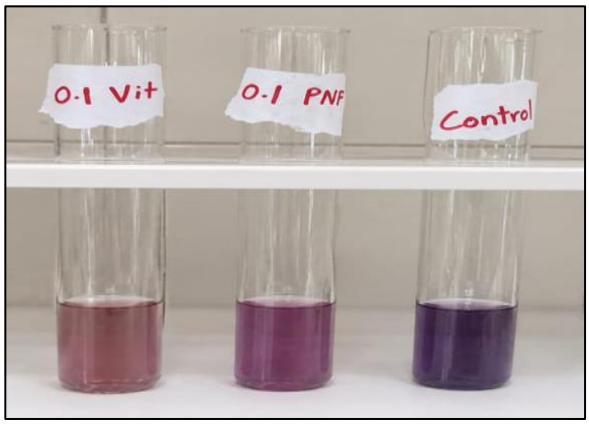
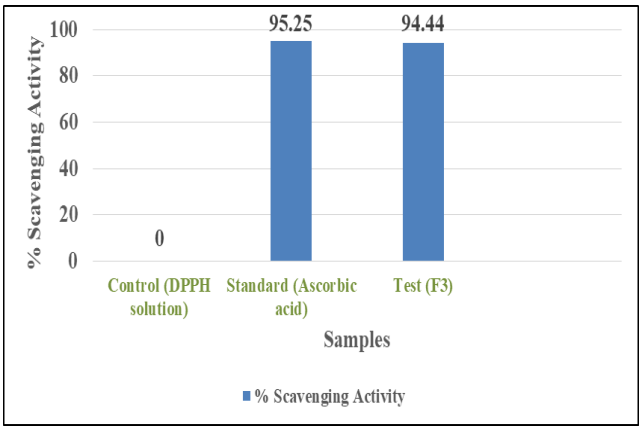


Figure 4: Antioxidant Activity (DPPH Assay)

Graph of Antioxidant Activity (DPPH Assay)



Observation:

Excellent antioxidant capability was demonstrated by the formed gel (F3), which showed 94.44% free radical scavenging activity in the DPPH assay, equivalent to ascorbic acid (95.25%). The presence of flavonoids and phenolic chemicals may be responsible for this effect. The gel's anti-inflammatory properties and their application in the treatment of mouth ulcers are further supported by the high antioxidant action.

6. Antifungal Activity (Poisoned Plate)

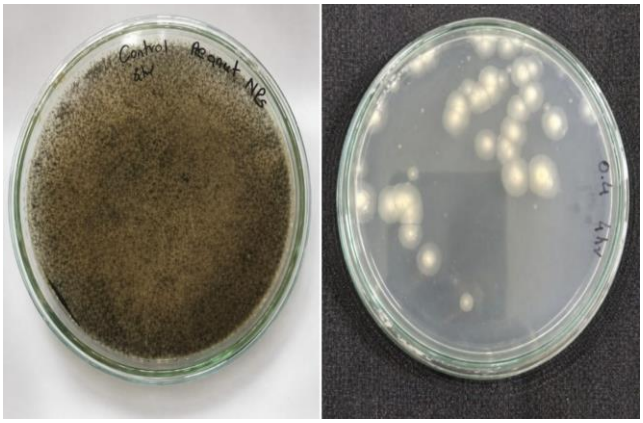


Figure 5: Antifungal Activity (Poisoned Plate)

Result Table

Table 4: Antifungal Activity of Gel Formulation

Sample	Colony Count (CFU)	% Inhibition
Control	400	—
Test (F3)	23	94.25%

Observation:

The optimized polyherbal oral gel's (F3) antifungal activity was assessed using the poisoned plate method. Fungal growth was inhibited by 94.25% in the improved formulation, with a colony count of 23 CFU as opposed to 400 CFU in the control group. The significant decrease in the number of fungal colonies shows the formulation's superior antifungal efficacy and suggests that it may prevent the growth of fungi linked to oral illnesses. These results imply that the optimized polyherbal oral gel can be useful in managing oral mucosal ulcers and preventing fungal colonization.

7. Evaluation of Antibacterial Activity

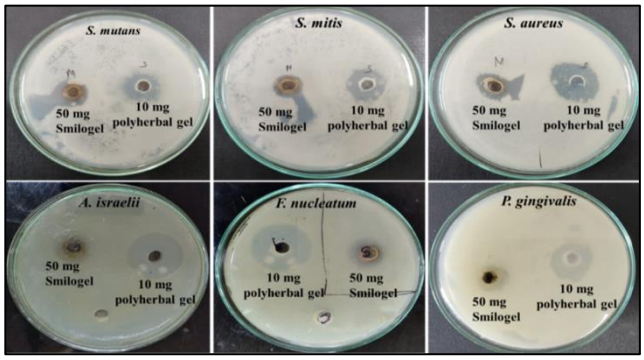


Figure 6: Antibacterial Activity of Gel Formulation

Bacteria	Smilogel (50 mg)	Polyherbal Gel (50 mg)
B. subtilis	1.6 cm	1.9 cm
S. aureus	1.1 cm	1.7 cm
E. coli	1.6 cm	1.5 cm
P. aeruginosa	1.8 cm	2.4 cm
A. baumannii	1.5 cm	2.2 cm
P. vulgaris	1.6 cm	2.3 cm

Bar graph of Antibacterial Activity

Bar graph showing zone of inhibition (cm) of Marketed Control and Polyherbal gel against different bacterial strains.

Observation:

The agar well diffusion method was used to assess the antibacterial activity of the optimized polyherbal oral gel (F3) against specific Gram-positive, Gram-negative, and oral pathogenic microorganisms. The formulation demonstrated broad-spectrum antibacterial action by showing distinct zones of inhibition against every tested pathogen. In addition to demonstrating inhibitory activity against oral pathogens such as Streptococcus mutans, Streptococcus mitis, Actinomyces israelii, Porphyromonas gingivalis, and Fusobacterium nucleatum, the optimized gel demonstrated notable antibacterial efficacy against Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumannii, and Proteus vulgaris. The findings show that the created polyherbal oral gel has strong antibacterial qualities and could aid in lowering the microbial load linked to oral infections and mouth ulcers.

8. Wound Healing Activity (In-vitro Scratch Assay)

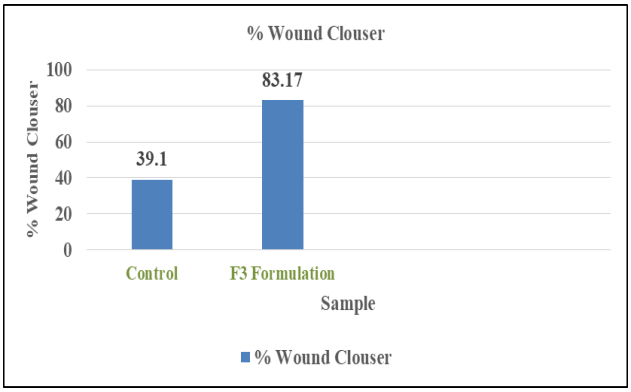


Figure 7: Wound Healing Activity (*In-vitro* Scratch Assay)

Bar graph of Wound Healing Activity

Observation:

The wound healing activity of the optimized polyherbal oral gel (F3) was evaluated using an in vitro scratch assay. The optimized formulation exhibited 83.17% wound closure after 24 hours, which was markedly higher than the control group (39.10%). Microscopic observations revealed enhanced cell migration and rapid closure of the scratch area in the treated group compared with the untreated control. These findings indicate that the optimized polyherbal oral gel possesses excellent wound healing potential and effectively promotes tissue regeneration, supporting its application in the management of oral mucosal ulcers.

CONCLUSION

The present study successfully formulated and evaluated a novel polyherbal mucoadhesive oral gel containing Psidium guajava, Salvia officinalis, and selected medicinal herbs for the management of mouth ulcers. Among all prepared formulations, F3 exhibited the most desirable physicochemical characteristics, including acceptable pH, smooth consistency, excellent homogeneity, optimum viscosity, satisfactory spreadability, superior extrudability, appropriate gel strength, and good stability under the tested storage conditions. The optimized formulation also demonstrated a sustained in vitro drug release profile, ensuring prolonged availability of the herbal constituents at the site of application.

Significant antibacterial, antifungal, antioxidant, anti-inflammatory, and wound-healing properties were also demonstrated by the optimized formulation, suggesting its potential to successfully manage a number of pathological variables linked to the development of oral ulcers. The combined effects of the chosen medicinal herbs may enable quicker healing of oral mucosal lesions by minimizing oxidative stress and inflammation, reducing microbial development, and promoting tissue regeneration.

Overall, the findings of the present study suggest that the developed polyherbal mucoadhesive oral gel is a safe, stable, and promising herbal formulation for the topical management of mouth ulcers. The optimized formulation demonstrated favourable physicochemical characteristics together with sustained drug release and significant biological activities, highlighting the therapeutic potential of the selected medicinal herbs. Psidium guajava, Salvia officinalis, and other complementary herbal ingredients work in concert to reduce microbial growth, control inflammation and oxidative stress, and promote tissue regeneration when combined with a mucoadhesive gel system. The proposed formulation is a viable herbal alternative for the treatment of oral mucosal lesions since it has the benefits of extended local retention, simplicity of application, and enhanced patient acceptability. The results of this study give polyherbal mucoadhesive formulations meant for oral healthcare applications a solid scientific foundation for future development and optimization.

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