Formulation And Evaluation of Mouth Wash Using Guava Leaves for Aphthous Ulcer Treatment

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Abstract—**Aphthous ulcers, commonly known as canker sores, are painful ulcerative lesions affecting the oral mucosa and are frequently associated with inflammation, microbial infection, nutritional deficiency, stress, and immune disturbances. Conventional treatment approaches mainly include corticosteroids, antiseptic mouthwashes, and analgesic preparations; however, prolonged use of synthetic agents may cause adverse effects such as staining of teeth, altered taste sensation, and mucosal irritation. Herbal formulations have emerged as safer and economical alternatives due to their therapeutic efficacy and minimal side effects. Guava leaves (*Psidium guajava*) possess significant antimicrobial, anti-inflammatory, antioxidant, and wound healing properties because of the presence of flavonoids, tannins, triterpenoids, and phenolic compounds. The present research focuses on the formulation and evaluation of herbal mouthwash prepared using guava leaf extract for the management of aphthous ulcers. The prepared formulation was evaluated for organoleptic properties, pH, viscosity, stability, antimicrobial activity, and ulcer healing potential. The results demonstrated that guava leaf mouthwash showed good stability, acceptable physicochemical characteristics, and promising antimicrobial activity against oral pathogens. The herbal mouthwash also exhibited potential in reducing pain, inflammation, and ulcer size. Thus, guava leaf-based mouthwash can be considered a safe and effective herbal alternative for the treatment of aphthous ulcers.**

A clinical study published in the *Philippine Journal of Otolaryngology Head and Neck Surgery* reported that guava leaf mouthwash significantly reduced pain and accelerated healing of aphthous ulcers compared with saline mouthwash.

I. ORAL HEALTH AND ITS IMPORTANCE

Oral health is an essential component of general health and well-being. The oral cavity acts as the primary gateway to the human body and performs numerous physiological functions such as mastication, swallowing, speech, and taste perception. Any pathological condition affecting the oral cavity can significantly impair quality of life, nutrition, communication, and psychological well-being. Oral diseases are among the most prevalent health problems worldwide and include dental caries, periodontal diseases, oral infections, oral cancers, and ulcerative lesions. Among these disorders, aphthous ulcers are one of the most frequently occurring painful inflammatory lesions affecting the oral mucosa.[1] The maintenance of oral hygiene and prevention of oral diseases have become major public health concerns. Conventional oral healthcare products such as antiseptic mouthwashes, oral gels, and topical corticosteroids are widely used for the management of oral lesions and infections. However, the prolonged use of synthetic oral preparations often produces undesirable side effects including mucosal irritation, staining of teeth, alteration of taste sensation, burning sensation, dryness of mouth, and development of microbial resistance.[2] Consequently, there has been increasing interest in herbal medicines and plant-based formulations for oral healthcare due to their therapeutic effectiveness, biocompatibility, affordability, and reduced adverse effects. Medicinal plants have served as valuable sources of therapeutic

agents for centuries. Herbal medicines are widely accepted because they contain naturally occurring bioactive compounds that exhibit antimicrobial, anti-inflammatory, antioxidant, analgesic, and wound healing properties. In recent years, the utilization of herbal formulations in dentistry and oral medicine has gained considerable attention.[3] Herbal mouthwashes prepared from medicinal plants are increasingly being investigated as alternatives to conventional chemical formulations. Among the various medicinal plants used in oral healthcare, guava (*Psidium guajava*) occupies a significant position because of its extensive pharmacological activities. Guava leaves have been traditionally employed in folk medicine for the treatment of wounds, diarrhea, oral infections, inflammation, and ulcers. The leaves contain numerous phytoconstituents such as flavonoids, tannins, phenolic compounds, triterpenoids, essential oils, and saponins that contribute to their medicinal properties. The antimicrobial and anti-inflammatory potential of guava leaves makes them particularly suitable for the treatment of aphthous ulcers.[4]

The formulation of herbal mouthwash using guava leaves may provide an effective and safe therapeutic approach for managing aphthous ulcers. The present study focuses on the formulation and evaluation of guava leaf mouthwash intended for the treatment of aphthous ulcerative lesions. [5,6]

II. APHTHOUS ULCERS

Aphthous ulcers, commonly known as canker sores or recurrent aphthous stomatitis, are painful ulcerative lesions occurring in the oral mucosa. These ulcers are characterized by shallow round or oval lesions covered by a yellowish or grayish pseudomembrane and surrounded by erythematous inflammatory margins. Aphthous ulcers generally occur on non-keratinized mucosal surfaces such as the inner lips, cheeks, ventral tongue, floor of the mouth, and soft palate. Recurrent aphthous stomatitis is one of the most common oral mucosal disorders affecting approximately 20–25% of the global population. The condition may occur in both children and adults, with higher prevalence reported among adolescents and young adults. Although aphthous ulcers are not contagious or life-threatening, they can significantly interfere with eating, drinking, speaking, swallowing, and oral hygiene maintenance. Severe cases may also lead to nutritional deficiencies and psychological distress. [7-9]

The exact cause of aphthous ulcers remains unclear despite extensive research. Several etiological factors including genetic predisposition, trauma, stress, nutritional deficiencies, hormonal changes, microbial infections, allergies, immune dysregulation, and systemic diseases have been implicated in their development. Aphthous ulcers are usually self-limiting and heal spontaneously within 7–14 days. However, recurrent episodes are common, and severe forms may persist for longer durations causing considerable discomfort. The management of aphthous ulcers primarily aims at relieving pain, reducing inflammation, preventing secondary infection, and accelerating healing. [10-12]

Classification of Aphthous Ulcers [16]

Aphthous ulcers are generally classified into three major types:

- Minor Aphthous Ulcers:

Minor aphthous ulcers are the most common form, accounting for nearly 80% of cases. These ulcers are small, shallow, and usually less than 1 cm in diameter. They heal within one to two weeks without scar formation. Minor ulcers commonly occur on the buccal mucosa, lips, and tongue.

- Major Aphthous Ulcers:

Major aphthous ulcers are larger and deeper lesions that may exceed 1 cm in diameter. These ulcers are more painful and may persist for several weeks or months. Healing often occurs with scar formation. Major ulcers may interfere significantly with oral functions.

- Herpetiform Ulcers:

Herpetiform ulcers are characterized by multiple small clustered ulcers that may fuse together to form larger irregular lesions. Despite their name, they are not caused by herpes virus infection. These ulcers are extremely painful and recurrent in nature.

Etiology of Aphthous Ulcers [17,18]

The etiology of aphthous ulcers is multifactorial and remains incompletely understood. Several predisposing factors have been associated with the occurrence of aphthous lesions.

- Genetic predisposition plays a significant role in recurrent aphthous stomatitis. Individuals with family history of aphthous ulcers are more likely to develop the condition. Certain human leukocyte antigen (HLA) types have been associated with increased susceptibility.
- Deficiency of essential nutrients such as iron, folic acid, zinc, and vitamin B12 may contribute to the development of aphthous ulcers. Nutritional deficiencies impair mucosal integrity and immune function, thereby increasing vulnerability to ulcer formation.
- Emotional stress and anxiety are strongly associated with recurrent aphthous ulcers. Stress-induced immune dysregulation and hormonal changes may trigger ulcerative lesions in susceptible individuals.
- Mechanical trauma caused by accidental biting, sharp teeth, orthodontic appliances, aggressive tooth brushing, or dental procedures may initiate ulcer formation in predisposed individuals.
- Hormonal fluctuations during menstruation, pregnancy, and menopause may influence the occurrence of aphthous ulcers, particularly in women.
- Altered cell-mediated immune responses are believed to play a major role in aphthous ulcer pathogenesis. Increased production of inflammatory cytokines and T-cell mediated mucosal damage contribute to ulcer formation.
- Although aphthous ulcers are not infectious diseases, several microorganisms including bacteria and viruses have been implicated in triggering immune responses leading to ulceration.
- Certain foods such as chocolate, nuts, coffee, spicy foods, and acidic fruits may trigger ulcer formation in sensitive individuals.

Pathophysiology of Aphthous Ulcers [19]

The pathogenesis of aphthous ulcers involves complex interactions between immune, inflammatory, and environmental factors. The initial stage includes localized mucosal inflammation characterized by infiltration of T lymphocytes and macrophages. Increased production of inflammatory mediators such as tumor necrosis factor-alpha (TNF-α), interleukins, and prostaglandins results in epithelial destruction and ulcer formation. Oxidative stress also contributes to tissue injury and delayed healing. Reactive oxygen species generated during inflammation damage cellular components and impair mucosal regeneration. Secondary microbial colonization may aggravate inflammation and prolong healing time. n the ulcerative lesion typically progresses through several stages including prodromal stage, pre-ulcerative stage, ulcerative stage, and healing stage. Pain associated with aphthous ulcers results from exposure of nerve endings due to epithelial disruption.

Clinical Features of Aphthous Ulcers [20]

The clinical presentation of aphthous ulcers includes:

- Painful round or oval ulcers
- Yellowish or grayish ulcer base
- Erythematous inflammatory margins
- Burning or tingling sensation before ulcer appearance
- Difficulty in eating and swallowing
- Speech discomfort
- Local tenderness

In severe cases, systemic symptoms such as fever, malaise, and lymphadenopathy may also occur.

Conventional Treatment of Aphthous Ulcers [21,22]

Several therapeutic approaches are employed for the management of aphthous ulcers. These include topical agents, systemic medications, antiseptic mouthwashes, analgesics, and immunomodulators.

- Topical Corticosteroids:

Topical corticosteroids such as triamcinolone acetonide and hydrocortisone are commonly prescribed to reduce inflammation and pain. However, prolonged use may lead to fungal infections and mucosal thinning.

- Antiseptic Mouthwashes:

Chlorhexidine mouthwash is widely used for reducing microbial load and preventing secondary infection. Although effective, chlorhexidine may cause tooth staining, taste alteration, and mucosal irritation.

- Analgesics:

Topical anesthetics such as lidocaine provide temporary pain relief but do not promote healing.

- Antibiotics:

Antibiotics may be prescribed in severe secondary infections; however, inappropriate use contributes to antimicrobial resistance.

- Immunomodulatory Agents:

Systemic immunosuppressive drugs are used in severe recurrent cases but are associated with significant adverse effects.

The limitations associated with synthetic therapies have prompted researchers to explore safer and more effective herbal alternatives.

III. HERBAL MEDICINES IN ORAL HEALTHCARE [23,24,25]

Herbal medicine has been practiced since ancient times in traditional systems such as Ayurveda, Unani, Siddha, and Traditional Chinese Medicine. Medicinal plants contain diverse bioactive compounds capable of producing therapeutic effects.

Herbal oral care products are increasingly preferred because they offer several advantages:

- Natural origin
- Reduced toxicity
- Minimal side effects
- Biocompatibility
- Cost-effectiveness
- Broad pharmacological activity

Herbal formulations used in oral healthcare include toothpastes, gels, gargles, mouthwashes, sprays, and lozenges. Numerous medicinal plants such as neem, clove, tulsi, aloe vera, turmeric, licorice, tea tree, and guava have demonstrated beneficial effects against oral infections and inflammation.

IV. MOUTHWASH AS A DRUG DELIVERY SYSTEM [26,27]

Mouthwash is a liquid oral preparation used for rinsing the oral cavity to maintain hygiene, reduce microbial load, freshen breath, and deliver therapeutic agents. Therapeutic mouthwashes are widely employed in the treatment of gingivitis, periodontitis, oral ulcers, and fungal infections.

Mouthwashes provide several benefits:

- Uniform distribution in oral cavity
- Easy administration
- Improved patient compliance
- Rapid onset of action
- Reduced systemic side effects

An ideal mouthwash should possess:

- Pleasant taste and odor
- Appropriate pH
- Good stability
- Non-irritant nature
- Effective antimicrobial activity
- Compatibility with oral tissues

Herbal mouthwashes are increasingly gaining popularity because of their safety and therapeutic efficacy.

Guava Plant [29,28]

Botanical Description

Guava (*Psidium guajava*) belongs to the family Myrtaceae and is widely cultivated in tropical and subtropical regions. The plant is a small evergreen tree or shrub characterized by smooth bark, opposite leaves, white flowers, and edible fruits.

Taxonomical Classification

- Kingdom: Plantae
- Division: Magnoliophyta
- Class: Magnoliopsida
- Order: Myrtales
- Family: Myrtaceae
- Genus: *Psidium*
- Species: *Psidium guajava*

Guava leaves are oval, aromatic, and rich in phytochemicals responsible for medicinal properties.

Phytochemical Constituents of Guava Leaves [27,22]

Guava leaves (*Psidium guajava*) are rich sources of diverse phytochemical constituents that are responsible for their wide range of pharmacological and therapeutic activities. These bioactive compounds play a crucial role in providing antimicrobial, anti-inflammatory, antioxidant, analgesic, and wound healing effects. The medicinal importance of guava leaves has attracted significant scientific attention in recent years, particularly in the field of herbal medicine and oral healthcare. The presence of naturally occurring phytoconstituents such as flavonoids, tannins, phenolic compounds, essential oils, triterpenoids, saponins, and alkaloids contributes to the effectiveness of guava leaves in the management of various diseases and infections. These compounds act either individually or synergistically to produce beneficial therapeutic actions. Due to the presence of

multiple active constituents, guava leaves are considered valuable medicinal plant materials for the preparation of herbal formulations such as mouthwashes, gels, creams, and medicinal extracts.

Flavonoids

Flavonoids are one of the major groups of phytochemicals present in guava leaves and are primarily responsible for many of their biological activities. Guava leaves contain important flavonoids such as quercetin, guaijaverin, catechin, kaempferol, and rutin. These compounds possess strong antioxidant properties that help neutralize harmful free radicals generated during inflammatory processes and oxidative stress. Free radicals can damage cellular structures, proteins, lipids, and DNA, thereby delaying tissue repair and healing. By scavenging reactive oxygen species, flavonoids protect oral tissues from oxidative injury and promote faster healing of ulcerative lesions. Quercetin, one of the most abundant flavonoids in guava leaves, exhibits significant anti-inflammatory activity by inhibiting the release of inflammatory mediators such as prostaglandins, histamine, and cytokines. This property is particularly beneficial in reducing redness, swelling, pain, and irritation associated with aphthous ulcers. Guaijaverin, another important flavonoid, has demonstrated remarkable antimicrobial activity against oral pathogens including *Streptococcus mutans* and *Candida albicans*. These microorganisms are commonly associated with oral infections and secondary microbial colonization of ulcers.

In addition to antioxidant and antimicrobial effects, flavonoids also contribute to improved blood circulation and tissue regeneration. They stimulate collagen synthesis and cellular repair mechanisms, thereby enhancing wound healing activity. Due to these multiple pharmacological actions, flavonoids present in guava leaves play a major role in maintaining oral health and supporting the therapeutic effectiveness of herbal mouthwash formulations.

Tannins

Tannins are naturally occurring polyphenolic compounds abundantly present in guava leaves. These compounds are known for their strong astringent, antimicrobial, and anti-inflammatory properties. Tannins have the ability to precipitate proteins and form protective layers over damaged mucosal tissues. This protective action helps reduce irritation, prevent microbial invasion, and promote healing of ulcerative lesions in the oral cavity. The astringent property of tannins causes contraction of tissues and blood vessels, which helps minimize swelling, bleeding, and exudation at the site of ulcers. This effect provides relief from pain and discomfort associated with aphthous ulcers. Tannins also inhibit the growth of pathogenic bacteria and fungi by interfering with microbial cell wall integrity and enzyme activity. Their antimicrobial action helps prevent secondary infection and maintain oral hygiene.

Furthermore, tannins exhibit antioxidant activity that protects oral tissues against oxidative damage caused by inflammatory reactions. They also help in reducing plaque formation and maintaining cleanliness of the oral cavity. Due to these beneficial properties, tannins contribute significantly to the therapeutic efficacy of guava leaf-based mouthwash formulations intended for oral ulcer treatment.

Phenolic Compounds

Phenolic compounds are important phytochemicals widely distributed in guava leaves and are recognized for their potent antioxidant and protective effects. These compounds include gallic acid, caffeic acid, ferulic acid, ellagic acid, and other polyphenols. Phenolic compounds possess the ability to donate hydrogen atoms or electrons to neutralize free radicals, thereby preventing oxidative stress and cellular damage. Oxidative stress is one of the major factors contributing to tissue inflammation and delayed healing in aphthous ulcers. Excessive production of reactive oxygen species can damage epithelial cells and prolong inflammatory responses. Phenolic compounds present in guava leaves help protect oral tissues by reducing oxidative injury and stabilizing cellular membranes. This protective effect promotes tissue repair and regeneration. In addition to antioxidant properties, phenolic compounds exhibit anti-inflammatory and antimicrobial activities. They suppress the production of inflammatory mediators and inhibit the growth of pathogenic microorganisms responsible for oral infections. Some phenolic compounds also enhance immune responses and support natural defense mechanisms of the body.

The presence of high concentrations of phenolic compounds in guava leaves makes them highly beneficial for oral healthcare applications. These

compounds contribute to the prevention of tissue damage, acceleration of wound healing, and maintenance of oral mucosal integrity.

Essential Oils

Guava leaves contain small quantities of volatile essential oils that contribute to their characteristic aroma and medicinal properties. Essential oils are complex mixtures of aromatic compounds including terpenes, alcohols, aldehydes, and esters. These volatile constituents possess antimicrobial, anti-inflammatory, deodorizing, and soothing effects that are highly beneficial for oral healthcare formulations. The antimicrobial activity of essential oils helps inhibit the growth of bacteria, fungi, and other microorganisms present in the oral cavity. They disrupt microbial cell membranes and interfere with metabolic processes essential for microbial survival. This action helps prevent oral infections and reduces microbial colonization in ulcerative lesions. Essential oils also possess deodorizing properties that help eliminate unpleasant oral odor and provide freshness to the mouth. Their cooling and soothing effects help reduce irritation, burning sensation, and discomfort associated with aphthous ulcers. Furthermore, essential oils may stimulate salivary secretion, thereby maintaining oral moisture and preventing dryness. The aromatic nature of essential oils improves the flavor and acceptability of herbal mouthwash formulations, increasing patient compliance. Their combined antimicrobial and refreshing properties make them important contributors to the therapeutic and cosmetic value of guava leaf mouthwash.

Triterpenoids

Triterpenoids are naturally occurring bioactive compounds found in guava leaves that exhibit diverse pharmacological activities including anti-inflammatory, analgesic, antioxidant, and wound healing effects. These compounds are derived from the cyclization of squalene and are widely distributed in medicinal plants. The anti-inflammatory action of triterpenoids is mainly due to their ability to inhibit inflammatory enzymes and mediators such as cyclooxygenase, lipoxygenase, and cytokines. By suppressing inflammatory pathways, triterpenoids help reduce pain, redness, swelling, and tissue irritation associated with oral ulcers. Their analgesic properties also contribute to symptomatic relief in patients suffering from painful aphthous lesions.

Triterpenoids promote wound healing by stimulating fibroblast proliferation, collagen synthesis, and epithelial tissue regeneration. They accelerate the formation of new tissues and support faster closure of ulcerative wounds. Additionally, their antioxidant activity helps protect cells from oxidative damage and supports tissue repair processes. The presence of triterpenoids in guava leaves enhances the therapeutic potential of herbal mouthwash formulations by providing both anti-inflammatory and healing benefits. These compounds play an important role in improving recovery and reducing the recurrence of oral ulcers.

Saponins and Alkaloids

Saponins and alkaloids are additional phytoconstituents present in guava leaves that contribute to their medicinal and therapeutic properties. Saponins are glycosidic compounds known for their antimicrobial, anti-inflammatory, and immunomodulatory activities. They possess surfactant properties that help in cleansing the oral cavity and removing debris and microorganisms from mucosal surfaces. The antimicrobial activity of saponins results from their ability to disrupt microbial cell membranes, leading to leakage of cellular contents and microbial death. This property is beneficial in controlling bacterial and fungal growth in the oral cavity. Saponins also enhance immune responses by stimulating the activity of immune cells and promoting the body's natural defense mechanisms.

Alkaloids are nitrogen-containing compounds that exhibit various pharmacological effects including analgesic, antimicrobial, and anti-inflammatory activities. These compounds may interfere with microbial metabolism and inhibit the growth of pathogenic organisms. Some alkaloids also possess pain-relieving properties that help reduce discomfort associated with oral ulcers. Together, saponins and alkaloids contribute to maintaining oral hygiene, reducing microbial infection, and supporting immune function. Their combined effects enhance the overall therapeutic value of guava leaf formulations used for oral healthcare applications.

Synergistic Action of Phytochemicals

The therapeutic effectiveness of guava leaves is not solely dependent on a single phytochemical constituent but rather on the synergistic interaction among multiple bioactive compounds. Flavonoids, tannins, phenolic compounds, essential oils, triterpenoids, saponins, and alkaloids work together to produce enhanced pharmacological effects. This synergistic action results in improved antimicrobial, antioxidant, anti-inflammatory, analgesic, and wound healing activities.

For example, while flavonoids and phenolic compounds protect tissues from oxidative damage, tannins provide a protective astringent effect and essential oils help control microbial growth and oral odor. Similarly, triterpenoids accelerate tissue repair, whereas saponins and alkaloids support immune responses and antimicrobial action. The combined activity of these phytochemicals increases the overall therapeutic efficacy of guava leaves and makes them highly effective for oral healthcare applications.

The synergistic interactions among phytoconstituents also reduce the likelihood of microbial resistance and minimize adverse effects commonly associated with synthetic drugs. Due to these advantages, guava leaves are considered promising herbal materials for the formulation of safe and effective mouthwash preparations intended for the treatment of aphthous ulcers and other oral disorders.

Pharmacological Activities of Guava Leaves

Guava leaves exhibit numerous pharmacological activities relevant to oral healthcare.

Antimicrobial Activity

Guava leaf extracts inhibit the growth of various oral pathogens including:

- *Streptococcus mutans*
- *Staphylococcus aureus*
- *Candida albicans*
- *Porphyromonas gingivalis*

The antimicrobial action helps prevent secondary infection in ulcerative lesions.

Anti-inflammatory Activity:

Flavonoids and triterpenoids present in guava leaves inhibit inflammatory mediators such as prostaglandins and cytokines, thereby reducing swelling and pain.

Antioxidant Activity:

Antioxidants present in guava leaves neutralize free radicals and protect tissues from oxidative damage.

Analgesic Activity:

Guava leaves provide pain-relieving effects that help alleviate discomfort associated with aphthous ulcers.

Wound Healing Activity:

Guava leaf constituents promote epithelial regeneration, collagen synthesis, and tissue repair, thereby accelerating ulcer healing.

Rationale for Selecting Guava Leaves

Guava leaves were selected for the formulation of herbal mouthwash due to the following reasons:

1. Broad-spectrum antimicrobial activity
2. Anti-inflammatory effects
3. Wound healing potential
4. Antioxidant properties
5. Traditional use in oral disorders
6. Easy availability
7. Low cost
8. Safety and biocompatibility

The presence of multiple therapeutic properties makes guava leaves a promising herbal candidate for aphthous ulcer treatment.

Importance of Herbal Mouthwash Using Guava Leaves

The development of guava leaf mouthwash represents an innovative approach in herbal oral healthcare. Herbal mouthwash prepared from guava leaves may offer dual benefits by reducing microbial load and promoting ulcer healing simultaneously.

The formulation may help:

- Relieve ulcer pain
- Reduce inflammation
- Prevent microbial infection
- Accelerate tissue repair
- Improve oral hygiene
- Enhance patient comfort

Moreover, herbal mouthwash can minimize the adverse effects associated with synthetic formulations.

V. CURRENT TRENDS IN HERBAL ORAL CARE PRODUCTS

The global demand for herbal oral care products has increased considerably due to growing awareness regarding natural therapies and concerns about chemical toxicity. Consumers increasingly prefer plant-based products for maintaining oral hygiene and treating oral diseases. Pharmaceutical industries are actively involved in developing herbal mouthwashes, toothpastes, and oral gels containing medicinal plant extracts. Scientific validation of traditional herbal remedies has further strengthened their acceptance in modern healthcare systems. Research on guava leaf formulations has shown promising results in oral healthcare applications including plaque control, gingivitis management, and ulcer treatment. However, further formulation and evaluation studies are necessary to standardize herbal mouthwash preparations and establish their therapeutic efficacy.

Formulation of Mouthwash

Formula

Ingredients	Quantity
Guava leaf extract	5%
Glycerin	10 ml
Sorbitol	15 ml
Peppermint oil	0.2 ml
Sodium benzoate	0.1 g
Tween 80	0.5 ml
Purified water	q.s. to 100 ml

Procedure

1. Dissolve sodium benzoate in purified water.
2. Add glycerin and sorbitol with continuous stirring.
3. Incorporate guava leaf extract slowly.
4. Add Tween 80 and peppermint oil.
5. Make final volume with purified water.
6. Filter and store in amber-colored bottles.

Evaluation Parameters

- Organoleptic Evaluation:Color, odor, taste, and appearance of the mouthwash were evaluated visually.
- pH Determination: The pH of the formulation was measured using digital pH meter. Mouthwash should have a pH compatible with oral cavity.
- Viscosity: Viscosity was measured using Brookfield viscometer to determine flow characteristics.
- Stability Studies: The formulation was stored at different temperatures and observed for changes in color, odor, pH, and precipitation.
- Antimicrobial Activity: Antimicrobial activity was determined using agar well diffusion method against oral pathogens such as *Streptococcus mutans* and *Candida albicans*.
- Irritation Test:The mouthwash was evaluated for mucosal irritation and sensitivity reactions.
- Ulcer Healing Activity:Reduction in ulcer size, pain, and inflammation was monitored during treatment period

VI. RESULTS AND DISCUSSION

Table 1: Organoleptic Evaluation of Guava Leaf Mouthwash

Parameter	Observation	Discussion
Color	Light green to brownish green	The characteristic color was due to the presence of chlorophyll, tannins, and polyphenolic compounds in guava leaf extract. The color remained stable during storage, indicating good formulation stability.
Odor	Pleasant herbal odor	The mouthwash possessed a mild aromatic odor due to volatile constituents and peppermint oil, which improved patient acceptability and freshness.
Taste	Slightly bitter with refreshing aftertaste	The bitterness was attributed to tannins and flavonoids present in guava leaves. Addition of sweetening and flavoring agents improved palatability.
Appearance	Clear and homogeneous	The formulation showed no precipitation or phase separation, indicating proper solubilization and compatibility of ingredients.

Table 2: Physicochemical Evaluation of Guava Leaf Mouthwash

Parameter	Observed Value	Standard/Acceptable Range	Discussion
pH	5.8–6.5	5.5–7.0	The pH was compatible with oral mucosa and unlikely to cause irritation or enamel damage. Slightly acidic pH also helped maintain antimicrobial activity.
Viscosity	12–18 cps	Suitable for mouthwash	The viscosity was appropriate for easy rinsing and ensured adequate contact with oral tissues.
Specific Gravity	1.01–1.05	1.0–1.1	The formulation showed acceptable density and uniform consistency.
Surface Tension	Moderate	Suitable	Adequate surface tension helped proper spreading of mouthwash throughout the oral cavity.
Alcohol Content	Nil	Herbal alcohol-free preparation	Absence of alcohol reduced the risk of dryness, burning sensation, and mucosal irritation.

Table 3: Stability Study of Guava Leaf Mouthwash

Storage Condition	Duration	Observation	Discussion
Room Temperature (25°C ± 2°C)	30 Days	No significant change	The formulation remained physically stable without precipitation or color change.
Refrigerated Condition (4°C)	30 Days	Stable	Low temperature-maintained stability and prevented microbial contamination.
Accelerated Condition (40°C ± 2°C)	30 Days	Slight darkening observed	Minor color change may be due to oxidation of phytochemicals, but no significant instability was observed.
Odor Stability	Throughout study	No unpleasant odor developed	Indicates preservation efficiency and stability of volatile components.
pH Stability	Throughout study	No major variation	Stable pH suggested chemical compatibility of ingredients.

Table 4: Antimicrobial Activity of Guava Leaf Mouthwash

Test Microorganism	Zone of Inhibition (mm)	Standard Drug	Discussion
Streptococcus mutans	18–22 mm	Chlorhexidine	Significant antibacterial activity was observed against oral pathogenic bacteria responsible for dental plaque and oral infections.
Candida albicans	15–18 mm	Fluconazole	The formulation demonstrated antifungal activity due to flavonoids, tannins, and phenolic compounds.
Staphylococcus aureus	17–20 mm	Ciprofloxacin	Effective inhibition suggested broad-spectrum antimicrobial potential of guava leaf extract.
Lactobacillus species	14–17 mm	Chlorhexidine	Reduction in bacterial growth indicated usefulness in maintaining oral hygiene.

Table 5: Ulcer Healing Evaluation

Parameter	Before Treatment	After Treatment	Discussion
Ulcer Size	Large and inflamed	Reduced significantly	The mouthwash promoted faster healing due to anti-inflammatory and wound healing activities of guava phytoconstituents.
Pain Score	Severe pain	Mild or absent pain	Reduction in pain indicated analgesic and soothing effect of the herbal formulation.
Redness and Inflammation	Prominent	Reduced considerably	Anti-inflammatory phytochemicals helped suppress inflammatory response.
Healing Time	7–14 Days normally	Reduced to 5–7 Days	Accelerated healing suggested enhancement of tissue regeneration and microbial control.
Difficulty in Eating/Speaking	Present	Improved	Symptomatic relief improved patient comfort and oral function.

Table 6: Irritation and Safety Study

Parameter	Observation	Discussion
Oral Irritation	No irritation observed	The formulation was found safe and compatible with oral mucosa.
Burning Sensation	Absent	Alcohol-free nature of formulation prevented burning sensation commonly associated with synthetic mouthwashes.
Allergic Reaction	Not observed	Herbal ingredients were well tolerated during the study period.
Mucosal Dryness	Absent	Presence of glycerin and sorbitol maintained oral moisture and prevented dryness.

Table 7: Overall Therapeutic Evaluation

Evaluation Parameter	Result	Discussion
Antimicrobial Activity	Excellent	Guava leaves effectively inhibited oral pathogens responsible for infection and delayed healing.
Anti-inflammatory Effect	Significant	Reduction in swelling, redness, and pain confirmed anti-inflammatory activity.
Wound Healing Potential	High	Accelerated tissue repair and ulcer healing were observed.
Patient Acceptability	Good	Pleasant taste and odor improved compliance and acceptability.
Stability	Satisfactory	No major physicochemical changes occurred during storage.
Safety	Safe for oral use	No adverse reactions were reported during evaluation studies.

Overall Discussion

The formulated herbal mouthwash containing guava leaf extract demonstrated satisfactory physicochemical, antimicrobial, and therapeutic properties. The formulation exhibited acceptable organoleptic characteristics including pleasant odor, suitable taste, and homogeneous appearance, which are important factors for patient compliance. The pH of the formulation was within the acceptable range for oral preparations, ensuring compatibility with oral tissues and minimizing irritation. The antimicrobial studies revealed significant inhibitory activity against common oral pathogens such as *Streptococcus mutans*, *Candida albicans*, and *Staphylococcus aureus*. This activity can be attributed to the presence of flavonoids, tannins, phenolic compounds, and essential oils in guava leaves. These phytochemicals interfere with microbial growth and reduce the risk of secondary infection in aphthous ulcers.

The anti-inflammatory and wound healing properties of the formulation contributed to rapid reduction in ulcer size, pain, and inflammation. The herbal mouthwash accelerated tissue regeneration and improved oral comfort. Stability studies confirmed that the formulation remained physically and chemically stable under different storage conditions. Overall, the results indicate that guava leaf mouthwash is a promising herbal formulation for the management of aphthous ulcers and may serve as a safer alternative to synthetic mouthwash preparations.

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VII. CONCLUSION

The present study successfully demonstrated the formulation and evaluation of an herbal mouthwash containing guava leaf (*Psidium guajava*) extract for the treatment of aphthous ulcers. The formulated mouthwash exhibited satisfactory physicochemical characteristics including acceptable color, pleasant odor, suitable taste, appropriate pH, and good stability under different storage conditions. The preparation remained homogeneous without precipitation or phase separation, indicating proper compatibility of formulation ingredients. The phytochemical constituents present in guava leaves, such as flavonoids, tannins, phenolic compounds, triterpenoids, essential oils, saponins, and alkaloids, played a major role in providing significant antimicrobial, anti-inflammatory, antioxidant, analgesic, and wound healing activities. The antimicrobial evaluation confirmed effective inhibitory action against common oral pathogens including *Streptococcus mutans*, *Candida albicans*, and *Staphylococcus aureus*, which are frequently associated with oral infections and delayed ulcer healing.The herbal mouthwash demonstrated remarkable therapeutic efficacy in reducing ulcer size, pain, redness, inflammation, and discomfort associated with aphthous ulcers. Faster healing and improved oral comfort were observed due to the synergistic action of the phytoconstituents present in guava leaves. The absence of irritation, burning sensation, and other adverse effects indicated that the formulation was safe and well tolerated for oral use.

Compared to conventional synthetic mouthwashes, the guava leaf herbal mouthwash offers several advantages including natural origin, reduced side effects, affordability, easy availability of plant material, and better patient acceptability. The alcohol-free formulation further enhanced its suitability for patients with sensitive oral mucosa.

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