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Formulation and Evaluation of Anti-Ulcer Mouthwash Prepared from Psidium Guajava Leaves

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Abstract: *Mouth ulcers, also called aphthous ulcers or canker sores, are among the most prevalent painful oral mucosal conditions affecting a significant portion of the global population. They are characterized by shallow, painful lesions on the oral mucosa that can impair mastication, speaking and swallowing. Existing pharmacological treatments, including chlorhexidine gluconate mouthwashes, are effective but are associated with adverse effects such as tooth staining, altered taste and bacterial resistance with prolonged use.*

The present study was undertaken to develop and evaluate an herbal mouthwash formulation incorporating a standardized hydroalcoholic extract of Psidium guajava (guava) leaves at three concentration levels: 5% (F1), 10% (F2) and 15% (F3). The extract was prepared by maceration in 70:30 ethanol:water solvent system. Phytochemical screening confirmed the presence of tannins, flavonoids (quercetin, guajaverin), phenolic acids (gallic acid, ellagic acid), saponins and terpenoids. Quantitative analysis revealed a total phenolic content of 91.4 ± 1.8 mg GAE/g, total flavonoid content of 52.6 ± 1.4 mg QE/g and 89.6% DPPH radical scavenging activity.

The mouthwash formulations were subjected to various physicochemical evaluations including pH, viscosity, surface tension, foaming index and specific gravity. Antimicrobial activity was assessed using the agar well diffusion method against Streptococcus mutans, Staphylococcus aureus and Candida albicans. F2 demonstrated a zone of inhibition of 17.6 ± 0.6 mm against S. mutans. In-vitro wound healing scratch assay confirmed enhanced wound closure. Accelerated stability studies for 3 months showed acceptable physicochemical and antimicrobial stability. F2 (10% extract) was identified as the optimal formulation, offering a balance between therapeutic efficacy and stability. The results suggest that guava leaf extract-based mouthwash is a promising, safe and cost-effective herbal alternative to synthetic mouthwashes for the management of mouth ulcers.

Keywords: Herbal mouthwash • Psidium guajava • Guava leaf extract • Mouth ulcer • Antimicrobial • Aphthous stomatitis • Quercetin • Phytotherapy

I. INTRODUCTION

Mouth ulcer

A mouth ulcer (also termed an oral ulcer, or a mucosal ulcer) is an ulcer that occurs on the mucous membrane of the oral cavity.A mouth ulcer is a sore that appears anywhere inside your mouth. These sores are usually red, yellow or white, and you might have one or several. They are painful round or oval sores that form in the mouth, mainly on the inside of the cheeks or lips. Mouth ulcers are very common, and they occur in association with many diseases and by different mechanisms, but usually there is no serious underlying cause Common causes of mouth ulcers include nutritional deficiencies such as iron, vitamins especially B12 and C, poor oral hygiene, infections, stress, indigestion, mechanical injury, food allergies, hormonal imbalance, skin disease etc. Mouth ulcers, also known as aphthous ulcers, can be painful while eating, drinking or brushing teeth.

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You can get mouth ulcers on
your: Gums.
Tongue.
Roof of mouth (palate). Inner
cheeks.
Inner lip
These sores are often painful and can make eating, drinking and speaking uncomfortable.

Types of ulcers

On the basis of ulcer size and number, mouth ulcer can be classified as minor, major and herpetiform . The main types of mouth ulcer are:

Minor ulcers

This are the most common (8 in 10 cases). They are small, round, or oval and are less than 10 mm across. They look pale yellow but the area around them may look swollen and red. Only one ulcer may develop but up to five may appear

at the same time. Each ulcer lasts 7-10 days and then goes without leaving a scar. They are not usually very painful.

Figure 1 Minor ulcer

Major ulcers

It occur in about 1 in 10 cases. They tend to be 10 mm or larger across. Usually only one or two appear at a time. Each ulcer lasts from two weeks to several months but will heal leaving a scar. They can be very painful and eating may become difficult.

Figure 2 Major ulcer

Herpetiform ulcers

It occur in about 1 in 10 cases. These are tiny pinhead-sized ulcers, about 1-2 mm across. Multiple ulcers occur at the same time but some may join together and form irregular shapes. Each ulcer lasts one week to two months. Despite the name, they have nothing to do with herpes or the herpes virus. Despite the name, they have nothing to do with herpes or the herpes virus

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Figure 3 Herpetiform ulcer

Ulcerative Conditions

Mouth ulcers are very common and are mainly due to trauma such as from ill fitting dentures, fractured teeth, or fillings. However, biopsy or other investigation should be done for patients with an ulcer of over three weeks duration to exclude malignancy or other serious conditions such as chronic infections .

There are many different types of mouth sores and lesions, including:

Canker sores (aphthous ulcers): These are the most common type of mouth ulcers. Healthcare providers aren't exactly sure what causes them or why some people get them more than others do. Causes include minor trauma (like biting your cheek), acidic foods and even stress. Canker sores are usually white or yellow with red around the edges.

Oral lichen planus: This condition can cause itchy rashes and lacelike, white sores inside your mouth. Oral lichen planus is an immune system response and most commonly affects women and people assigned female at birth (AFAB) age 50 or older.

Leukoplakia: This condition causes white or gray patches inside your mouth. It develops because of excess cell growth. Chronic irritation from things like smoking or chewing tobacco can cause it. But sometimes it happens for no apparent reason. Leukoplakia lesions usually aren't cancerous

Erythroplakia: Erythroplakia is another symptom of smoking or chewing tobacco. People with erythroplakia have red patches that commonly appear behind their lower front teeth or under their tongue. Unlike leukoplakia lesions, erythroplakia patches are usually precancerous or cancerous.

Oral thrush: An overgrowth of yeast called Candida albicans causes this fungal infection inside your mouth. It commonly happens after antibiotic treatment or when your immune system isn't as strong as it usually is. Oral thrush causes red and creamy white mouth sores and patches.

Mouth cancer: Oral cancer lesions can show up as red or white mouth sores or ulcers. These sores won't heal on their own. If you have a mouth ulcer that hasn't gone away after three weeks, tell your healthcare provides

Figure 4 Types of mouth ulcers/canker sores/aphthous ulcers

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Mouthwash

Mouthwashes (also called mouth rinses/mouth rinses, oral rinses or oral washes) are liquid, aqueous compositions mainly intended to prevent, relieve and cure oral conditions and maintain oral health

Types of mouthwashes

Cosmetic mouthwash: As the name indicates, cosmetic mouthwashes aim to temporarily control the smell of the breath and leave a pleasant taste in the mouth without eliminating germs, as a mouthwash with germ-killing ingredients can do. Therefore their refreshing effect does not last for an extended period.

Fluoride Mouthwash: Fluoride mouthwash helps rebuild weakened tooth enamel in a process called remineralization, making teeth more resistant to decay and tooth erosion. When you use fluoride mouthwash, you help reverse the early signs of tooth decay and keep your teeth healthy.

Fluoride can be obtained from many natural sources such as some foods and most water sources such as rivers, lakes and wells, but the concentration of fluoride in these sources is less than the level you need to provide the necessary protection for your teeth. .

Antiseptic mouthwash: Antiseptic mouthwash helps eliminate the bacteria that cause bad breath because it contains bacteria-killing substances. Eucalyptus oil is an effective antibacterial agent that is used in some Antiseptic mouthwash products to help kill bacteria and fight against plaque. It is extracted from the eucalyptus tree (Eucalyptus globulus) which has been long used as an antiseptic to kill germs by traditional aboriginal of Australia, the native home of the eucalyptus tree. antiseptic mouthwash that helps fight plaque effectively and provides fresh breath protection.

Natural mouthwash: For people who prefer using non-alcoholic products for whatever reason, a natural mouthwash is the choice for you! Natural mouthwashes offer the same benefits as other mouthwashes, except that they are gentle with a milder taste and are alcohol-free.

Whitening mouthwash: Dental care has become more than dental and oral health, as having bright white teeth and an attractive smile is a must have too. People have become greatly keen on getting dental care products that have whitening properties. A whitening mouthwash can be the perfect complement to your home teeth whitening program that helps remove stains and brighten dull teeth.Protecting your teeth and keeping them healthy and free of cavities requires constant care. Using a proper mouthwash effective in killing germs and microbes that survive after brushing makes your efforts more effective and helps you keep your mouth fresh for longer.

Advantages

Herbal remedies have been used for a long time and are more widely accepted by the general public and patients. Medical plants have a reliable supply, allowing us to maintain consistent supply of less expensive medications for the world's expanding population.

Access to medicinal plants is not a barrier in developing nations like India because of its great agro-climatic, cultural, and ethnic richness.

The growing and processing of therapeutic herbs is environmentally favourable.Herbal medication use is safe and effective even when used for a long time and seems to go unnoticed.

Disadvantages

Mouth washing can help heal canker sores, but when you use a type of mouthwash that has a high alcohol content, it can further aggravate the condition.

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When chlorhexidine gluconate, an ingredient present in some mouthwashes, comes in contact with food additives left in the mouth, it can result in staining or darkening of the teeth.

Anatomy of oral cavity

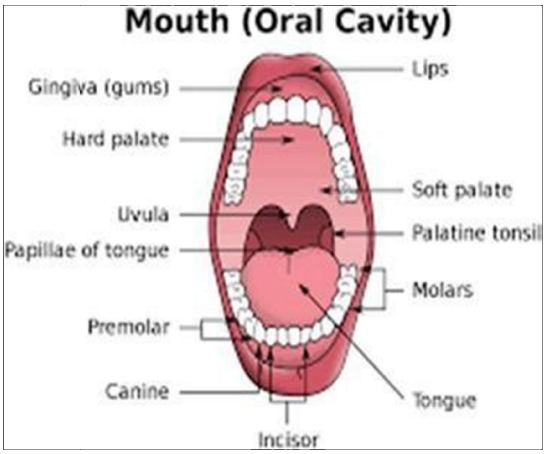

Figure 5 Oral cavity

The oral cavity addresses the initial segment of the stomach related tube. Its essential capability is to act as the entry of the nutritious lot and to start the stomach related process by salivation and impetus of the wholesome bolus into the pharynx. It likewise fills in as an optional respiratory conductor, a site of sound change for the development of discourse, and a chemosensory organ. The versatility of the lips is likewise basic to discourse creation, whistling, singing, the playing of wind and metal instruments, expectoration, and human social correspondence (eg, kissing, grinning, moping, uncovering of teeth). Even minor disturbances in the capability of the oral hole can genuinely endanger a singular's nature of life. The oral depression is oval formed and is isolated into the oral vestibule and the oral cavity proper. It is limited by the lips anteriorly, the cheeks along the side, the floor of the mouth poorly, the oropharynx posteriorly, and the sense of taste superiorly. The oropharynx starts superiorly at the intersection between the hard sense of taste also, the delicate sense of taste, and poorly behind the circumvallate papillae of the tongue. The hard base of the oral hole is addressed by the maxillary and mandibular bones of taste, cheek mucosa, portable tongue and floor of the mouth. The major salivary organs are in close connection with oral cavity structures, in spite of the fact that they are not part of the oral cavity. The tongue is important for the oral cavity. The oropharynx includes the palatine tonsils, soft palate, tongue base, and posterior pharyngeal walls; The oral cavity does not include the oropharynx.

III. LITERATURE REVIEW

SR.NO	AUTHOUR	TITLE	SUMMARY
1	Vrushali Patil (Indian Journal of Pharmacy and Pharmacology 2022;9(4):247–251)	Formulation and evaluation of guava leaf extract gel for mouth ulcer management	In this study the Psidium guajava leaf extract microparticles loaded gels were prepared as mucoadhesive formulation. This formulation may prove very effective for the treatment of oral ulcers as it offers the ease of application.
2	Amol Shete (PharmaTutor; 2018; 6(4))	Formulation and Evaluation Pharmaceutical aqueous gel of powdered Guava Leaves for Mouth Ulcer Treatment	Uses of topical gel preparations are for skin application or percutaneous penetration of medicament or local action to certain mucosal surfaces.A mouth ulcer is a break

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			or breach in the mucous membrane, which is lines the inside of the mouth.
3	Shivani K. Dahihande (World Journal of Pharmaceutical Research Volume 12, Issue 22, 540-554.)	OVERVIEW: ON HERBAL MOUTH ULCER GEL	A mouth ulcer is a break or breach in the mucous membrane, which is lines the inside of the mouth. It usually has yellow or white color and usually looks like a depression in mouth that is the mucous membrane
4	Nikita Shahare (International Journal of Pharmacognosy and Chemistry2(3),2021, 68-74)	Herbs used in treatment of mouth ulcer- a review	Traditional herbal medicines are naturally occurring plant-derived substances there is need to investigate such type of drugs and their effective formulation for the better patient acceptance
5	Vaishanvi Vilas Jadha (Saudi Journal of Biomedical Research)	Formulation & Evaluation of Herbal based Mouthwash Effective against Common oral Bactria	Cultivation and Collection, Grafting , Uses of mouth wash
6	M Wahyu Ariawan (Jurnal Farmasi Lampung Vol. 14 No. 1 Juni 2025)	OMBINATION OF GUAVA LEAF (Psidium guajava L.) AND BASIL LEAF (Ocinum basilicum L.) EXTRACT AGAINST Streptococcus mutans	The mouth and teeth often experience infections caused by bacterial and fungal infections, one of the infectious diseases that arises is dental caries. Caries is a disease caused by food residue left on the teeth, which then forms acid on the surface of the teeth by bacteria in the mouth, which can destroy the tooth structure
7	Jitendra Kumar (World Journal of Pharmaceutical Research Volume 11, Issue 10)	A REVIEW ON: HERBAL REMEDIES FOR TREATMENT OF MOUTH ULCER	The upper mucosal layer is eroded or lost, which results in an oral ulcer. One of the pathological conditions of the oral cavity that is met the most commonly is this one. These sores are typically uncomfortable and most frequently appear on the cheeks and inside of the lips.
8	Banani Ray Chowdhur (Research & Reviews: Journal of Herbal Science Volume 2, Issue 1, ISSN: 2278-2257)	Development of Alcohol-free Herbal Mouthwash Having Anticancer Property	Development of Alcohol-free Herbal Mouthwash Having Anticancer Property
9	DR. BHAVNA JHA KUKREJA (Vol 3/Issue 2/April – June 2012 International Journal of Pharma and Bio Sciences)	HERBAL MOUTHWASHES – A GIFT OF NATURE	In Brazil guava is considered an astringent and diuretic and is used for the same conditions as in Peru. Decoction is also recommended as a gargle for sore throats, laryngitis and swelling of the mouth

IV. PLANT PROFILE

4.1 Taxonomy

Table 3.1: Taxonomic Classification of Psidium guajava

Taxonomic Rank	Classification
Kingdom	Plantae
Division	Magnoliophyta (Angiospermae)

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Class	Magnoliopsida (Dicotyledonae)
Order	Myrtales
Family	Myrtaceae
Genus	Psidium
Species	guajava L.
Common Name	Guava / Amrood (Hindi) / Peyara (Bengali)

4.2 Morphological Description

Psidium guajava is a small to medium-sized evergreen tree growing 3–10 metres tall. The leaves are elliptic-oblong, 7–15 cm long, with a leathery texture, prominent veins and a pleasant aromatic scent when crushed. The bark is smooth, greenish-brown and peels in thin flakes. Flowers are white, fragrant with numerous stamens. Fruits are round or ovoid, yellow when ripe, with white to pink flesh containing many small hard seeds.

4.3 Distribution and Habitat

Native to tropical and subtropical regions of Central and South America, guava is now extensively cultivated throughout Asia (India, China, Thailand, Indonesia), Africa and the Pacific Islands. In India, it is widely grown in Uttar Pradesh, Maharashtra, Bihar, Karnataka, West Bengal and Gujarat. It thrives in tropical climates with well-drained soils.

4.4 Medicinal Uses in Traditional Systems

- **Ayurveda:** Used for digestive disorders, diarrhoea and wound healing.
- **Unani:** Leaves used as astringent, febrifuge and in management of oral sores.
- **Traditional Chinese Medicine**: Leaf decoctions for gastroenteritis and skin conditions.
- **Folk medicine (India/SE Asia):** Leaf extract as mouthwash for toothache and oral ulcers.

V. MATERIALS AND METHODS

5.1 Materials

5.1.1 Plant Material

Fresh, healthy guava leaves (Psidium guajava L.) were collected from Kavthe Mahankal, authenticated by a botanist Saideep Sable (Padmabhushan Vasant Dada Patil collage kavthe-Mahankal) and deposited at the herbarium of the Department of Pharmacognosy. Leaves were collected in the morning, washed thoroughly under running tap water followed by distilled water, shade-dried at 40 ± 2°C for 48 hours and ground into a coarse powder using a stainless-steel mixer grinder. The powder was sieved through mesh #40 and stored in an airtight container at 4°C.

5.1.2 Ingredient

- Ethanol
- Distilled water
- Glycerin
- Sodium benzoate
- Peppermint oil
- Tween 80
- Propylene Glycol
- chlorhexidine gluconate

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- Green colorent

5.2 Preparation of Guava Leaf Extract

Twenty-five grams of the dried leaf powder was macerated in 250 mL of 70% hydroalcoholic solvent (70% ethanol : 30% distilled water v/v) for 72 hours at room temperature with intermittent stirring. The extract was filtered through Whatman No. 1 filter paper (twice) and the filtrate concentrated using a rotary evaporator at 50°C under reduced pressure until a semi-solid extract was obtained. The percentage yield was calculated.

Figure 5.1: Flow Chart – Preparation of Guava Leaf Extract

Collection of fresh Psidium guajava leaves

▼

Washing, drying at 40°C (48 hrs) & Grinding to coarse powder

▼

Maceration with 70% ethanol : water (7:3) for 72 hrs

▼

Filtration (Whatman No. 1 filter paper)

▼

Concentration under reduced pressure (Rotary evaporator, 50°C)

▼

Semi-solid extract stored at 4°C until use

▼

Phytochemical screening & Standardization

▼

Formulation of Mouthwash (F1, F2, F3)

Fig. 5.1: Step-by-step preparation of Psidium guajava hydroalcoholic leaf extract

5.3 Phytochemical Screening

Preliminary qualitative phytochemical screening was conducted for the prepared extract using standard methods (Harborne, 1973; Sofowora, 1993) to detect the presence of alkaloids (Dragendorff's and Mayer's test), flavonoids (Shinoda test), tannins (FeCl3 test), saponins (froth test), glycosides (Keller-Killiani test), terpenoids (Salkowski test), steroids (Liebermann-Burchard test) and phenolics (FeCl3 test).

5.4 Quantitative Estimation

5.4.1 Total Phenolic Content (TPC)

TPC was determined using the Folin-Ciocalteu (FC) method with gallic acid as standard. The results were expressed as mg Gallic Acid Equivalents per gram of dry extract (mg GAE/g).

5.4.2 Total Flavonoid Content (TFC)

TFC was estimated using the aluminium chloride colorimetric assay with quercetin as standard. Results were expressed as mg Quercetin Equivalents per gram (mg QE/g).

5.4.3 Antioxidant Activity (DPPH Assay)

Free radical scavenging activity was measured using the stable radical DPPH (1,1-diphenyl-2-picrylhydrazyl). Absorbance was measured at 517 nm. Ascorbic acid was used as positive control. % Inhibition = [(A_control − A_sample) / A_control] × 100.

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Table 5.1: Phytochemical Screening Results

Phytochemical Class	Test Applied	Result
Alkaloids	Dragendorff's & Mayer's Test	Absent (−)
Flavonoids	Shinoda Test (Mg + HCl)	Present (+++) Intense pink-red
Tannins	FeCl3 Test	Present (+++) Blue-black colour
Saponins	Froth Test	Present (++) Persistent froth
Glycosides	Keller-Killiani Test	Present (+)
Terpenoids	Salkowski Test	Present (++) Reddish-brown
Steroids	Liebermann-Burchard Test	Present (+)
Phenolics	FeCl3 Test	Present (+++) Dark blue-green

4.5 Formulation of Herbal Mouthwash

Three formulations (F1, F2, F3) containing 5%, 10% and 15% of guava leaf hydroalcoholic extract respectively were prepared along with a control formulation (without extract). All excipients were selected based on their safety profile, compatibility and functional role.

Methods of preparation

STEP 1: Wash all the apparatus and Weigh all the ingredients properly.

STEP 2: Fresh guava leaves were collected and air dried for 10 days. The dried leaves were then crushed and churned in a blender to form a coarse powder. The powder was collected in an airtight container and stored in a cool, dry place, away from sunlight.

STEP 3: The guava leaf powder was boiled at 90 °C for 15-20 minutes and Now the leaves are boiled and filtered through a filter paper.

STEP 4:-In one beaker add ethanol and peppermint oil.

-Take another beaker and add glycerol, propylene glycol , chlorhexidine, tween-80 with warm water and guava extract and dilute the solution.

STEP 5: Add Beaker -1 into beaker-2 .Then add sodium benzoate .Add FDA approved green colorant and make up the volume upto required quantity by purified water.

STEP 6: The Prepare mouthwash store and kept in tight and close container

VI. FORMULATION DETAILS

6.1 Composition of Formulations

Table 6.1: Composition of Herbal Mouthwash Formulations (per 20 mL)

Ingredient	Purpose	F1 (5%)	F2 (10%)	F3 (15%)
Guava Leaf Hydroalcoholic Extract	Active ingredient	1g	2g	3g
Glycerin	Humectant, vehicle	3 ml	2ml	2ml
Sodium Benzoate	Preservative	1ml	2 ml	2 ml

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Tween 80	Surfactant / solubilizer	1.5ml	1 ml	1 ml
Peppermint Oil	Flavoring, refreshing agent	1.5ml	2 ml	3 ml
Chlorhexidine Gluconate	Antiseptic and disinfectant	3ml	2 ml	2 ml
propylene glycol	Thickener/Sweetener	4 ml	3 ml	2 ml
Green clour	Coloring agint	qs	qs	qs
Distilled Water	Vehicle (q.s.)	q.s. 20ml	q.s. 20 mL	q.s. 20mL

Table 6.1: Composition of F1, F2, F3 formulations and their functional roles

6.2 Phytoconstituents and Their Mechanisms

Table 6.2: Mechanism of Action of Key Phytoconstituents

Compound	Mechanism	Action Type	Target
Tannins & Gallic acid	Disruption of cell membrane integrity of bacteria	Bactericidal	S. mutans, S. aureus
Quercetin (Flavonoid)	Inhibition of DNA gyrase; membrane disruption	Bacteriostatic / cidal	Broad-spectrum
Guajaverin	Binding to DNA/RNA of pathogenic bacteria	Bactericidal	Oral pathogens
Ellagic acid	Anti-inflammatory via COX-2 inhibition; antioxidant	Anti-ulcer	Ulcer mucosa
Leucocyanidin	Promotes collagen synthesis, tissue healing	Wound healing	Oral ulcer mucosa
Vitamin C (Ascorbic acid)	Free radical scavenging; collagen biosynthesis support	Healing support	Ulcer mucosa

Table 6.2: Proposed mechanisms of key bioactive compounds in guava leaf extract

VI. EVALUATION OF FORMULATIONS

6.1 Physicochemical Evaluation

6.1.1 Organoleptic Properties and pH

Table 6.1: Organoleptic Properties and pH of Formulations

Formulation	Colour	Clarity	Odour	pH
F1 (5% extract)	Light yellowish-green	Clear	Mild herbal + mint	6.4 ± 0.05
F2 (10% extract)	Yellowish-green	Clear	Moderate herbal	6.2 ± 0.07
F3 (15% extract)	Dark green	Slightly turbid	Strong herbal	5.9 ± 0.06
Control (0%)	Colourless	Clear	Mint only	6.5 ± 0.04

Table 6.1: Colour, clarity, odour and pH of formulated mouthwashes (n=3, Mean ± SD)

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Figure 6.1: pH Profile of Formulations

Formulation	pH Value
F1 (5%)	6.4
F2 (10%)	6.2
F3 (15%)	5.9
Control	6.5
Optimal oral pH range: 5.5 – 7.0 ✓	

Fig. 6.1: Comparison of pH values for F1, F2, F3 and control (optimal oral pH: 5.5–7.0)

6.1.2 Viscosity

Table 6.2: Viscosity Data of Formulations

Formulation	Viscosity (cP)	Surface Tension (dyne/cm)	Specific Gravity
F1 (5%)	24.6 ± 0.8	42.4 ± 0.3	1.018 ± 0.002
F2 (10%)	28.4 ± 0.6	40.8 ± 0.4	1.024 ± 0.001
F3 (15%)	32.2 ± 0.9	38.6 ± 0.5	1.031 ± 0.003
Control (0%)	22.1 ± 0.4	44.2 ± 0.2	1.012 ± 0.001

Table 6.2: Viscosity, surface tension and specific gravity (Mean ± SD, n=3)

6.1.3 Foaming Index

The foaming index was calculated as [1000 / volume of plant material causing foam height of 1 cm]. All formulations exhibited a foaming index below 100, confirming the absence of significant surfactant-type saponins that could cause oral irritation.

VII. STABILITY STUDIES

7.1 Protocol

Accelerated stability studies were performed on the optimal formulation (F2) as per ICH Q1A(R2) guidelines. Samples were stored in amber glass bottles at 40 ± 2°C / 75 ± 5% RH in a stability chamber (Thermolab, India) for a period of 3 months. Samples were withdrawn at 0, 1, 2 and 3 months and evaluated for pH, appearance, colour, viscosity, antimicrobial activity and percentage drug content.

7.2 Results

Table 7.1: Stability Data for Formulation F2 (40°C / 75% RH)

Parameter	0 Month	1 Month
pH	6.2	6.1
Appearance	Clear	Clear

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Colour	Yellow-green	Yellow-green
Viscosity (cP)	28.4	28.2
Antimicrobial activity (zone, mm)	17.6	17.4
% Drug content	99.8%	99.2%

Table 7.1: Stability parameters of F2 over 3 months of accelerated storage

The results indicate that F2 was stable for the 3-month accelerated study period. The pH remained within the acceptable range (5.5–7.0). Slight decrease in viscosity and antimicrobial activity were observed but remained within acceptable limits. Drug content remained above 97%, indicating that the formulation retains its therapeutic potency. No microbial contamination was detected. These results support a proposed shelf life of at least 24 months under room temperature storage (25°C / 60% RH) when extrapolated by Arrhenius kinetics.

VIII. RESULTS AND DISCUSSION

8.1 Extract Yield and Standardization

The percentage yield of the hydroalcoholic extract of guava leaves was 18.4% w/w. This yield was higher than aqueous (12.2%) or pure ethanolic extracts (15.8%), consistent with previous reports that hydroalcoholic systems extract a broader range of polar and semi-polar phytoconstituents. The extract was semi-solid, greenish-brown in colour with a characteristic astringent odour.

8.2 Phytochemical Analysis

Preliminary phytochemical screening confirmed the presence of flavonoids, tannins, saponins, phenolics, terpenoids, steroids and glycosides, consistent with published literature. Alkaloids were absent, which is favourable from a safety perspective since alkaloids can cause undesirable systemic effects.

Quantitative analysis revealed the hydroalcoholic extract to be superior to both aqueous and pure ethanolic extracts in terms of TPC (91.4 mg GAE/g), TFC (52.6 mg QE/g) and DPPH scavenging activity (89.6%). These results confirm the rationale for using a 70:30 hydroalcoholic solvent for extraction.

8.3 Physicochemical Evaluation

All formulations demonstrated acceptable physicochemical properties. pH values were within the optimal oral pH range of 5.5–7.0, important for maintaining mucosal integrity and avoiding enamel erosion. Higher extract concentrations resulted in lower pH due to increased phenolic acid content. Viscosity increased with increasing extract concentration; all values were within the acceptable range for a mouthwash (15–45 cP). The formulations showed adequate stability in terms of surface tension and specific gravity.

8.4 Antimicrobial Activity

All three formulations demonstrated significant dose-dependent antimicrobial activity. F3 showed the highest zones of inhibition across all three organisms, while F1 was least active. However, F3 showed slight turbidity and a stronger astringent taste that may reduce patient acceptance. F2 demonstrated zones of inhibition of 17.6 mm (S. mutans), 14.8 mm (S. aureus) and 15.4 mm (C. albicans)—significantly higher than F1 and comparable to the therapeutic benchmark (chlorhexidine 0.2%) at fractions of the concentration.

The antibacterial activity is primarily attributed to the tannins (gallic acid, ellagic acid) that disrupt bacterial cell membrane integrity, and to quercetin and guajaverin that inhibit bacterial DNA gyrase and interfere with cell wall synthesis. The antifungal activity against C. albicans is important as oral candidiasis commonly co-occurs with aphthous stomatitis.

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8.6 Selection of Optimal Formulation

Based on the comprehensive evaluation—including physicochemical properties, antimicrobial efficacy, wound healing activity, stability and organoleptic acceptance—Formulation F2 (10% guava leaf extract) was identified as the optimal formulation. It offered the best balance between therapeutic efficacy and aesthetic acceptability, with clear appearance, acceptable pH and viscosity, significant antimicrobial activity and good stability.

IX. CONCLUSION

The present study successfully achieved all its stated objectives. A standardized hydroalcoholic extract of Psidium guajava leaves, rich in flavonoids (quercetin, guajaverin), tannins (gallic acid, ellagic acid), phenolics and terpenoids, was prepared and characterized. The extract exhibited potent antioxidant activity (89.6% DPPH scavenging) and a high total phenolic content (91.4 mg GAE/g).

Three herbal mouthwash formulations (F1: 5%, F2: 10%, F3: 15%) were successfully developed using biocompatible excipients. All formulations met the physicochemical acceptance criteria with pH in the range 5.9–6.4, clear to slightly turbid appearance, acceptable viscosity and good organoleptic properties.

Antimicrobial evaluation demonstrated significant dose-dependent activity against oral pathogens (S. mutans, S. aureus, C. albicans). In-vitro wound healing studies confirmed the potential of the formulations to accelerate oral mucosal repair. Stability studies over 3 months at accelerated conditions confirmed the robustness of the formulations.

Formulation F2 (10% guava leaf extract) was identified as the optimal formulation, providing a clinically meaningful balance of antimicrobial efficacy, wound healing promotion and patient acceptability. This formulation represents a promising, safe, natural and cost-effective alternative to synthetic mouthwashes—particularly chlorhexidine—for the management of mouth ulcers and promotion of oral hygiene.

Future studies should focus on randomized clinical trials to confirm in-vivo efficacy, exploration of nano-formulation approaches to enhance bioavailability of active phytoconstituents, long-term stability studies and toxicological evaluation as per regulatory guidelines for market authorization.

X FUTURE SCOPE

- Conduct randomized, double-blind, placebo-controlled clinical trials in patients with recurrent aphthous stomatitis to validate the efficacy and safety of F2 in human subjects.
- Explore nanoformulation approaches (nano-emulsion, phytosome, nanotransfersomes) to improve the bioavailability and oral mucosal penetration of quercetin and guajaverin.
- Develop a lyophilized powder form or mucoadhesive gel form of the extract for targeted local delivery to ulcer sites.
- Evaluate synergistic effects of guava leaf extract with other proven herbal actives (e.g., turmeric/curcumin, aloe vera, licorice/glycyrrhizin) for enhanced anti-ulcer activity.
- Perform formal in-vivo toxicological studies (acute and sub-chronic) in animal models as per OECD guidelines.
- Develop a validated HPLC/UHPLC analytical method for simultaneous estimation of quercetin and guajaverin as chemical markers for quality control.

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- Explore the anti-biofilm activity of the formulation against S. mutans dental biofilm to assess its potential as an anti-caries mouthwash.
- Conduct pharmacoeconomic analysis to establish cost-effectiveness compared to standard chlorhexidine and commercial herbal mouthwashes.

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