

## Formulation and evaluation of mouthwash using guava leaves for aphthous ulcer treatment

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### Abstract

Mouth ulcers are small sores that form on your gums, lips, tongue, inner cheeks or roof of your mouth. Lots of different things can cause them, including minor injuries, hormonal changes, biting your cheeks or tongue, lack of sleep, eating acidic food, emotional stress and oral hygiene. Many mouth ulcers go away on their own. Others may require treatment. Generally, for prevention of Mouth ulcers/canker sores the different types of formulations are used, such as pastes, gels, tablets, paints and mouthwashes. *Psidium guajava* Linn. (guava) is used not only as food but also as folk medicine in subtropical areas around the world because of its pharmacologic activities. It is demonstrated that the pharmacological ability of this plant to show antioxidant, hepatoprotective, anti allergy, antimicrobial, antispasmodic, cardioactive, antidiabetic, anti inflammatory and antinociceptive activities. Guava leaves mouthwash was effective for aphthous ulcers in terms of reduction of symptoms of pain and faster reduction of ulcer size. Further formulating mouthwash which has efficiency to inhibit the growth of the microorganism. This study is an attempt to outline such natural substances, which may be used as effective mouthwashes.

**Keywords:** Aphthae; Aphthosis; Aphthous Stomatitis; Canker Sore

### 1. Introduction

Oral health is a key indicator of overall health, wellbeing and quality of life. WHO defines oral health as “a state of being free from chronic mouth and facial pain, oral and throat cancer, oral infection and sores, periodontal (gum) disease, tooth loss, and other disease and disorder that limits an individual capacity in biting, chewing, smiling, speaking and psychosocial wellbeing”. World Health Organization (2003).<sup>[15]</sup> Traditional herbal medicines are naturally occurring plant-derived substances that have been used in local or regional healing therapy procedures with little or no industrial processing to cure illness. In the field of medicine, traditional herbal remedies are gaining popularity. In the treatment of SARS, traditional Chinese herbal therapy played a key role. Traditional herbal medicine is used by 80% of Africans, and the global annual demand for these treatments is estimated to reach \$60 billion. Many traditional herbal medicine studies will be evaluated by the global health community. Traditional herbal medicine research has been heavily funded by China, India, Nigeria, the United States of America (USA), and the World Health Organization.<sup>[17]</sup> Phytogenic agents are traditionally used by herbalists and indigenous healers for the prevention and treatment of ulcer. Ethnomedicine, which refers to the study of traditional medical practice, is an integral part of the culture and the interpretation of health by indigenous populations in many parts of the world. For example, Indian Ayurveda and traditional Chinese medicine are among the most enduring folk medicines still practiced. These systems try to promote health and improve the quality of life, with therapies based on the use of indigenous drugs of natural origin. Given that plants have been widely used as herbal medicines, several approaches are now being carried out to discover new bioactive compounds<sup>[7]</sup>.

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The importance of mouth and teeth cleanliness has been recognized from the earliest days of civilization to the 21st century. Patients and oral health practitioners are faced with a multitude of mouthwash products containing many different active and inactive ingredients. Making informed decisions as to the suitability of a particular product for a particular patient can be a complex task. Although many popular herbal products have helped to control dental plaque and gingivitis, they have been used for a short time and only as an adjunct to other oral hygiene measures such as brushing and flossing. Various herbal products and their extracts such as Guava, Pomegranate, Neem, Liquorice, Tulsi, Green Tea, Cranberry, Grapefruit etc. have shown significant advantages over the chemical ones. Natural mouthwashes may offer significant advantages over the chemical ones. If such mouthwashes can be formulated which can be easily prepared and used safely by people at home using natural products, it may lead to improvement in the general dental health of the population<sup>[3]</sup>. Mouthwash is an aqueous solution which is most often used for its deodorant, refreshing and antiseptic properties or for control of plaque. It may contain alcohol, glycerin, synthetic sweetness, surface active agents, flavoring agents, coloring agents, etc. This can vary from breath fresheners to treatment of life threatening secondary infections such as oral mucositis in patients undergoing bone marrow transplant therapy. The use of mouthwashes requires a correct diagnosis of the oral condition and a thorough knowledge of the product to achieve effective treatment.<sup>[3]</sup>

Major side effects for some marketed brand are: Skin irritation, Allergic Skin Reaction, Redness of skin, Acneiform eruptions, Thyroid imbalances, Tooth/tongue staining, Increased tartar, Mouth/throat irritation, Dry mouth, Unusual or unpleasant taste in your mouth, Decreased taste sensation, Tongue swelling, Gingivitis.<sup>[3]</sup> To overcome all these side effects, Herbal Mouthwash can be formulated. Herbal mouthwash contains a natural ingredient called phytochemical that contains the desired anti-microbial and anti-inflammatory effect. *Psidium guajava* L. is a fruit-bearing tree commonly known as “guava”, which belongs to the family, Myrtaceae. The leaves and bark of guava trees have an extensive record of medicinal uses. Many researchers have demonstrated the presence of a wide variety of bioactive compounds in the leaf of *P. guajava* that are capable of demonstrating valuable effects on human well-being. Literature has not provided conclusive evidence regarding potential side-effects of guava leaf extracts when taken in a dose-dependent manner. In general, phytochemical studies of *P. guajava* have revealed the presence of bioactive substances like tannins, triterpenes, flavonoids, essential and fixed oils, saponin, carotenoids, lectins, vitamins (C&A), alkaloids, glycosides, and reducing-sugars, These flavonoids are known for its antibacterial effect along with quercetin which is responsible for an anti-oxidant effect, which maintains the effective functioning of the immune system.<sup>[3]</sup>

Medicinal plants can be used to find out effective alternatives to synthetic drugs (Jadhav, 2015). The use of the medicinal plant based medication is gradually becoming popular throughout the world. Nearly half of the world's twenty five bestselling pharmaceutical innovator agents are derived from natural products (Das, 2011). The use of medicinal plants as raw materials in the preparation of new drugs is ever increasing because of their potentials and the problem of drug resistance in microorganisms. Demand for medicinal plants is increasing in both developed and developing countries. Research on herbal medicinal plants is one of the leading areas of research globally (Dwivedi, 2012).

The anti-inflammatory effect at a cellular level has been explained by Jang et al. wherein the authors found that guava leaf extract significantly impeded the lipopolysaccharide (LPS)- induced production of nitric oxide and prostaglandin E2 in a dose-dependent manner. Considering its effect on local tissues, guava leaf extract has been used for aphthous ulcers displaying a marked improvement in pain and resolution of ulcer within 7 days. In view of literature references on valuable qualities of *P. guajava*, a preliminary investigation was carried out to check the efficacy of the leaf variants of *P. guajava* and based on these results, further research was carried out.

### 1.1. 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.

You can get mouth ulcers on your:

- Gums.
- Tongue.

- Roof of mouth (palate).
- Inner cheeks.
- Inner lips.

These sores are often painful and can make eating, drinking and speaking uncomfortable.

## 1.2. 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:

### 1.2.1. 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

### 1.2.2. 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

### 1.2.3. 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.



**Figure 3** Herpetiform ulcer

#### 1.2.4. 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/apthous ulcers

### 1.3. 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

#### 1.3.1. 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.
- 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.

#### 1.4. Anatomy of oral cavity

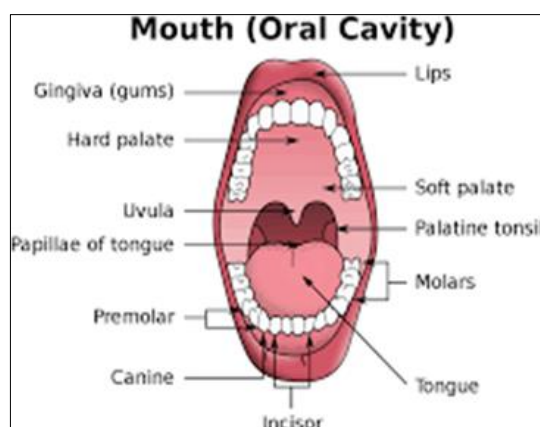


Figure 5 Oral cavity

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 posteriorly, 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

The oral cavity incorporates the lips, gingivae, retromolar trigone, teeth, hard sense 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.

#### 1.5. Need of herbal medicine

Herbal medicine has its origins in ancient cultures. It involves the medicinal use of plants to treat disease and enhance general health and wellbeing. Some herbs have potent (powerful) ingredients and should be taken with the same level of caution as pharmaceutical medications. In fact, many pharmaceutical medications are based on man-made versions

of naturally occurring compounds found in plants. For instance, the heart medicine digitalis was derived from the foxglove plant.

Herbal medicines contain active ingredients. The active ingredients of many herbal preparations are as yet unknown. Some pharmaceutical medications are based on a single active ingredient derived from a plant source. Practitioners of herbal medicine believe that an active ingredient can lose its impact or become less safe if used in isolation from the rest of the plant. For instance, salicylic acid is found in the plant meadowsweet and is used to make aspirin. Aspirin can cause the lining of the stomach to bleed, but meadowsweet naturally contains other compounds that prevent irritation from salicylic acid. According to herbal medicine practitioners, the effect of the whole plant is greater than its parts. Critics argue that the nature of herbal medicine makes it difficult to give a measured dose of an active ingredient. Herbal medicine aims to return the body to a state of natural balance so that it can heal itself. Different herbs act on different systems of the body.

Herbal formulations have nowadays undergone more thorough investigation for their potential in preventing and curing oral disease (Silva, 2012). Herbs have long been used traditionally for routine cleaning of teeth and dental disease and to treat various oral diseases (Deepa, 2011). Oral diseases like oral cancer, dental caries and periodontal diseases are among the most important oral health problems. There is a well-established link between the activities of microbial species that form part of the microbiota of the oral cavity and oral diseases. The big need for alternative treatment, products and prevention options for oral diseases that are safe, economical and effective comes from the rise in disease incidence particularly in developing countries, increased resistance by pathogenic bacteria to currently used chemotherapeutics and antibiotics opportunistic infections in immunocompromised individuals and financial that is economical considerations in developing countries. Moreover, allopathic medicine is too expensive and capital intensive for a developing country like India and has only limited success in the prevention and treatment of oral diseases and periodontal disease. Hence, the plant extracts used in traditional medicine and alternative products are considered as good alternatives to synthetic and organic medicine (Nagi, 2015)&(Jose, 2011). The present investigation deals with use of herbal Guava Leaves in treatment of mouth ulcer in Mouthwash.

#### 1.5.1. Guava leaves



**Figure 6** Guava leaves

Study of traditional medical practice is an integral part of the culture and the interpretation of health by indigenous populations in many parts of the world. For example, Indian Ayurveda and traditional Chinese medicine are among the most enduring folk medicines still practiced. These systems try to promote health and improve the quality of life, with therapies based on the use of indigenous drugs of natural origin. Given that plants have been widely used as herbal medicines, several approaches are now being carried out to discover new bioactive compounds.

Since ancient times, guava has been widely utilized and appreciated for its health benefits. Due to its climate adaptation, it can be grown in all tropical and subtropical locations. But it favors predominantly arid environments. The parts of the guava plant that are most frequently used include the leaves, roots, bark, and fruits. The major producers of this plant in India are Assam, Uttar Pradesh, Bihar, Maharashtra, Andhra Pradesh, and West Bengal. It has been utilized as food and as a vital component of human health since the dawn of time. Essential oil, flavonoids, carotenoids, polyphenolic substances, pentacyclic triterpenoids, esters, aldehydes, and other substances are among the active components found in guava. The treatment of diarrhea, dysentery, vomiting, gastroenteritis, and flatulence involves the

use of guava leaf extract . Guavas come in a variety of types, and depending on the species, the fruits range in size from 1.6 to 4.7 inches and are either round or oval in shape. Guava fruit has a high concentration of vitamins A and C, calcium, potassium, phosphorus, and dietary fibers, all of which help lower blood pressure, cholesterol, and triglycerides . Various portions of this plant contain a variety of medical qualities, from antibacterial activity to anticancer property, and are utilized not just as food but also as folk medicine . Studies show that Aqueous extracts of *Psidium guajava* are effective at reducing the development of harmful microorganisms . Guava can withstand droughts but need full sunlight, mild temperatures, and well spaced rainfall throughout the year to grow. This review may serve as a guide for future research on the domestication and development of the guava . The guava leaf's active ingredients are also thought to have immunostimulatory properties . Numerous studies have shown that the leaf, seed, and bark of *Psidium guajava* contain a wide range of bioactive substances that can have positive benefits on human health.

Guava (*Psidium guajava*) as a mouthwash for swollen gums and ulceration of the mouth and also for bleeding gums. Guava leaves have anti-inflammatory action and antibacterial ability that fights infections and kills germs and people consuming guava leaves at home will help curb toothaches. The juice from the guava leaves is also said to provide relief from oral ulcers.

Guava leaves contain vitamins A, C and antioxidants that can protect skin layers (Mishra et al., 2017). And flavonoids in guava leaves can be used as antibacterial and antimicrobial. The activity of flavonoids can reduce the number of bacteria and reduce infection so that it can reduce the production of exudate.

Help Reduce Menstruation Pain Guava leaves may relieve menstrual pain and discomfort, especially for women. Their soothing properties can ease cramps and make periods more manageable. Good for hair and skin: Guava leaves have antibacterial and antioxidant properties that can help to treat skin conditions like acne.

Depending upon the illness, the application of the remedy is either oral or topical. The Consumption of decoction, infusion, and boiled preparations is the most common way to overcome several disorders, such as rheumatism, diarrhea, diabetes mellitus, and cough in India, China, Pakistan, and Bangladesh, while in Southeast Asia the decoction is used as gargle for mouth ulcers. For skin and wound applications, poultice is externally used in Mexico, Brazil, Philippines, and Nigeria. In addition, chewing sticks are used for oral care in Nigeria. Currently, there is increasing interest in studying plants regarding their chemical components of bioactive compounds, their effects on several diseases, and their use for human health as functional foods and/or nutraceuticals. In recent years, guava leaves tea and some complementary guava products are available in several shops in Japan as well as on the Internet, because guava leaf phenolic compounds have been claimed to be food for specified health use (FOSHU), since they have beneficial health effects related to the modulation of blood-sugar level. In this present investigation we tried to find out the antifungal and antibacterial properties of guava leaves and the probable cause behind it.

#### 1.6. Plant profile:<sup>[13]</sup>

- **Habit:** A dwarf tree with its bark white, 4-angled branches that are thin peeling off young.
- **Roots:** Shallow root system. It has low drooping branches from bottom and suckers through the root's
- **Stem:** It has hairy younger stems which are generally four-angled in cross-section (quadrangular) and relatively large yellow fruit (2.5-10 cm long). The dominant compounds in the essential oil of the stem of *Psidium guajava* were  $\alpha$ -pinene, 1, 8-cineole, and  $\beta$ -bisabolol .
- **Leaves:** Leaves are Opposite, elliptic and acute in arrangement. *Psidium guajava*'s leaves have been used to isolate five chemicals, including one new pentacyclic triterpenoid called guajanoic acid and four previously identified ones ie sitosterol, ursolic acid, oleanolic acid, and ursolic acid uvaol.
- **Inflorescences:** Cymes or solitary flowers (1 to 3 flowers) generally come from the leaf axils.
- **Flower:** The flowers originate singly in the upper leaf forks (axils).
- **Calyx:** Splitting irregularly into 2- 4 lobes, whitish and hairy
- **Corolla:** Cream coloured with 5–7 petals and 150–185 stamens
- **Seed:** Guava contains 100 to 500 seeds in it
- **Branches:** Fruit is a berry, which consists of a chubby pericarp and fleshy pulp of seed cavity





Figure 7 Plant profile

### 1.7. Botanical description

The root system of *Psidium guajava* is typically superficial and very extensive, frequently extending well beyond the canopy. There are some deep roots, but there is no clear taproot. It is a large dicotyledonous shrub or small evergreen tree, typically 3–10 m high, with many branches, crooked stems, and bark that is light to reddish brown and thin, smooth, and constantly flaking. The fruit's surface is fleshy, while its interior is made of a seedy pulp<sup>[13]</sup>

#### 1.7.1. Scientific classification:<sup>[13]</sup>

- **Kingdom:** *Plantae - Plants*
- **Subkingdom:** *Tracheobionta Vascular plants*
- **Superdivision:** *Spermatophyta Seed plants*
- **Division:** *Magnoliophyta Flower plants*
- **Class:** *Magnoliopsida Dicotyledonous*
- **Subclass:** *Rosidae*
- **Order:** *Myrtales*
- **Family:** *Myrtaceae*
- **Subfamily:** *Myrtoideae*
- **Tribe:** *Myrtaceae*
- **Gender:** *Psidium*
- **Species:** *Psidium guajava*

**Table 1** Nutritional profile of guava leaves

SR.No	Compounds	Content composition
1]	Elements and ascorbic acid	
	Potassium	1.11%
	Phosphorus	0.23%
	Nitrogen	1.02%
	Ascorbic acid	142.55mg/100g
2]	Carbohydrates/phenols/Sulphates	
	Fucose	1.44%
	Rhamnase	3.88%

	Arabinose	22.6%
	Galactose	29.41
	Glucose	33.79%
	Mannose	0.59%
	Xylose	7.71%
	Phenol	15.28%
	Sulfate	18.58%
	Carbohydrate	48.13%
	Sulfate polysaccharide	66.71%
3]	Protein	

### 1.8. Chemical composition

Guava leaves are a great source of several macro and micronutrients that are good for health, as well as bioactive substances. They have 1717 mg of gallic acid equivalents (GAE)/g of total phenolic compounds, 103 mg of ascorbic acid, 3.64% ash, 0.62% fat, 18.53% protein, and a moisture content of 82.47%.<sup>[13]</sup>

### 1.9. Origin

*Psidium guajava* belongs to the Myrtaceae family, is the most important fruit of the genus in spite of it included about 150 species. It originated from Central and South America. Today, guavas are grown in Florida, southern California and different parts of the world.<sup>[13]</sup>

## 2. Material and methods

### 2.1. Materials

A mouthwash generally consists of water, ethanol, a humectant, a surfactant, flavor, color and an active agent.

**Table 1** Ingredients for Guava Mouthwash For Ulcer

INGREDIENTS	F1 (80ml)	F2 (100ml)	F3 (100ml)
Guava Leaves Powder	0.12%	0.15%	0.15%
Propylene Glycol	12ml	15ml	15ml
Chlorhexidine Gluconate	2%	2%	2%
Peppermint Oil	0.1%	0.1%	0.1%
Sodium Benzoate	0.2%	0.2%	0.2%
Tween-80	1%	1%	1%
Glycerol	12 gm	15 gm	15 gm
Ethanol	2%	2%	2%
Green colorant	q.s	q.s	q.s
Water	q.s	q.s	q.s

ACT AS

- Guava leaves powder(Antioxidant),
- Chlorhexidine Gluconate(antiseptic and disinfectant) ,
- , propylene glycol(Thickener/Sweetener), Peppermint oil (Essential oil/Flavouring Agent), Sodium Benzoate (Preservative),

- Tween-80(Surfactant), Glycerol (Humectant), Ethanol (Bactericide).

## 2.2. 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

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## 3. Evaluation parameter

### 3.1. Organoleptic properties

- **Color:** Greenish, Light green
- **Odor:** Pungent
- **Taste:** normal sweet, pungent, bitter

### 3.2. Physicochemical parameters

#### 3.2.1. pH determination

pH of mouthwashes was measured using a digital pH meter.

#### 3.2.2. Stability study

Stability studies were done with open and close containers. Here, by subjecting the product to room temperature for 1 month. The purpose of stability testing is to provide evidence how the quality of drug substance or drug product varies with time under the influence of variety of in environmental factor such as temperature, humidity and light to retest period for drug substance or a shelf life of drug.

#### 3.2.3. Antimicrobial activity of drug

The formulated mouthwash was inoculated in the plate of agar media by a zone of inhibition test a control was prepared. The plates were placed in the incubator and were incubated at 37<sup>o</sup>c for 24 hours. After the incubation period plates were taken out and checked for microbial growth by comparing it with the control.

#### 3.2.4. Palatability detection

Palatability is the property of being acceptable to the mouth. The mouthwashes were tested separately for that Criterion by three research members in a blind-style.

The test was done on scale of 5 levels:

5 = Really Good; 4 = Good; 3 = Not Sure; 2 = Bad; And 1 = Really Bad.

## 4. Result

### 4.1. Physical Parameters

**Table 1** Physical Parameters

	<b>F1</b>	<b>F2</b>	<b>F3</b>
COLOUR	Dark greenish	Light green	green
TASTE	bitter	unpleasant	Slightly bitter
ODOUR	minty	Fruity	pungent

### 4.2. pH determination

**Table 2** pH determination

<b>FORMULATION</b>	<b>pH</b>
F1	6.2
F2	5.8
F3	6.0

### 4.3. Stability study

The stability can be determined by , subjecting the product to room temperature for 1 month.(After 1 month)

**Table 3** Stability study

<b>Formulation</b>	<b>Color</b>	<b>Odour</b>	<b>Microbial growth</b>
F1	Dark green	Decay	Formation
F2	Light green	minty	No formation
F3	green	fruity	No formation

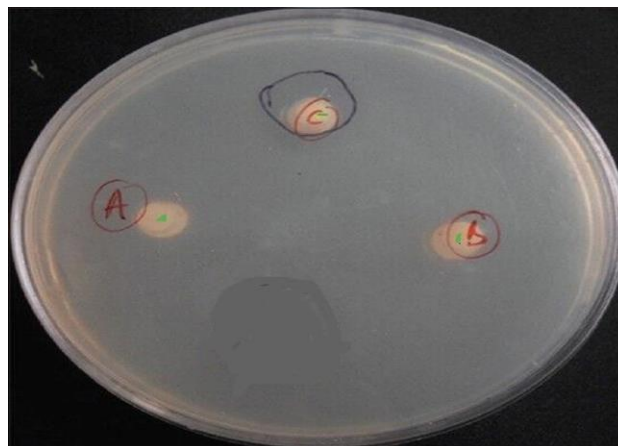
The study found that F1 was failed to resist in temperature , humidity, light and F2 has resist in all condition but it has less effective. The stability testing can evaluated that F3 has very good stability because it resist to all parameters of stability like temperature, humidity, light which is need for determination and is more reactive.

### 4.4. Antimicrobial activity of drug

The zone of inhibition can be used to measure the susceptibility of the bacteria to the antibiotic. Larger zones indicate decreased bacterial growth with greater antibiotic susceptibility.

**Table 4** No. of zone of inhibition

<b>Formulation</b>	<b>Zone of inhibition</b>
F1	0.10
F2	0.12
F3	0.14



**Figure 8** Agar plate zone of inhibition

F3 has greater antibiotic susceptibility than F1 AND F2 Because of their larger zone of inhibition.

#### 4.5. Palatability study of drug

For the given formulations of mouthwashes of

- F1=BAD
- F2= Good
- F3= Very Good

#### 4.6. Future perspective

Guava mouthwash is used to control microbial growth of organism which causes infection and pain in the ulcer site. for small children rather than prescribing the ulcer gel , guava mouthwash is effective solution on mouth ulcers for their used . Guava mouthwash gives cooling sensation after using which gives relief to mouth from burning sensation

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### 5. Conclusion

The data presented in this study, it was concluded that the developed Guava mouthwash possesses a significant, therapeutically efficacious, suitable vehicle for drug delivery at low cost but definitely with high potential. Guava leaves mouthwash is effective as an alternative treatment for aphthous ulcers, Among 3 Formulation, F3 are effective because in F1 formulation guava leave extract particles are visible due to poor filtration and there zone of inhibition was less and F2 Formulation has good filtration was done but there zone of inhibition was less than F3.SO, the final conclusion was F3 are more effective on aphthous ulcer.

Guava mouthwash reduces the bacteria that can irritate the site. The other ulcer formulations such as ointment and gel can burn the skin, irritate ulcers, numbness, peeling of skin and get redness on applied site, so for avoiding such circumstances Guava mouthwash can be very useful for handling the pain of the ulcers. it's just simple procedure, Take the mouthwash solution into mouth and gargle it for 30 seconds and spit it out of mouth. The antioxidant effect of guava leaves and Disinfectant or antiseptic effect of chlorhexidine can use to control the bacterial growth organisms. The Given formulation give the minimal side effect to the site of application and provide protection to the skin. generally, small childrens can't handle the pain of gels or ointments so it is very useful for them for handling the ulcers. It helps to improve oral hygiene with given formulation and it helps to reduce the bad odor from mouth. Guava mouthwash helps to reduce ulcers, give oral hygiene and protection to inner skin from irritation. Thus there is a need to create awareness among prescribers and public about the use of mouthwashes for ulcers and production of such natural ingredient mouthwashes rather than using of harmful chemicals and many other such studies should be encourage.

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