

## FORMULATION AND EVALUATION OF GUAVA LEAF EXTRACT GUMMIES FOR MOUTH ULCER TREATMENT

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### Abstract

Herbal mouth ulcer gummies are a convenient and potentially effective way to address oral discomfort. Typically infused with soothing ingredients guava leaves these gummies aim to reduce inflammation and promote healing in the mouth. However, while they may provide temporary relief severe or persistent mouth ulcer should be evaluated by a healthcare professional. Guava leaves are known for their anti-inflammatory and antimicrobial properties. Incorporating guava leaves extract into gummies provides a convenient and palatable way to experience the benefits of this traditional remedies

### Introduction

As a human being we all are constantly waging a war against disease, mother nature has gifted us with various powerful weapons to fight against the diseases we are suffering from mouth ulcers are yellowish or white depression with red margination in the mucus lining of the mouth cavity, characterized by inflammation and pain.[1] Mouth ulcers are also known as canker sores or aphthous ulcer. The product formulated is claimed to show its mechanism of action by various routes. Traditional herbal medicine research has been heavily funded by China, India, Nigeria, the United States of America (USA), and world health organisation. The present research deals with a gel formulation using ethanol extract of guava leaves for the treatment of mouth ulcers. Guava is known for its medicinal properties. It is rich in antioxidants, vitamin C, fibre and many other nutrients. The leaves are high in limonene about 42.1% and caryophyllene about 21.3%. Guava leaves contain many volatile compounds. Guava's antibacterial properties may also aid oral health. There are many different types of ulcers, including vaginal, oesophageal, and mouth ulcers. These are painful round or oval sores that usually appear on the inside of the cheeks or lips and inside the mouth. Currently available topical application for the treatment of mouth ulcers is a gel or cream base that must be applied to the body, which is painful and often impossible to easily access the ulcer. This semi-solid layer lasts longer in the wound compared to a liquid film, which improves the treatment in terms of a longer duration of action. In addition, unlike semi-solid dosage forms, the formulation spreads spontaneously and thus does not require force applied to the wound, ultimately improving patient compliance.[2]

You can get mouth ulcers on your:

- Gums
- Tongue.
- Roof of mouth (palate).
- Inner cheeks.

- Inner lips.

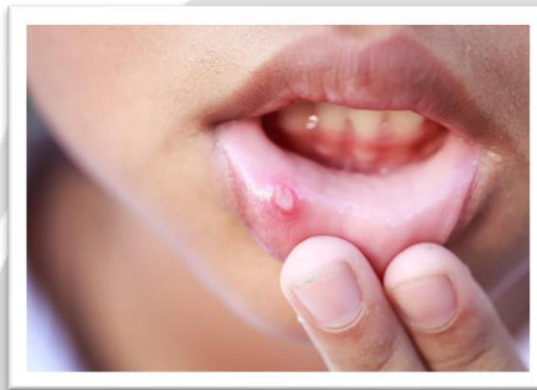
These sores are often painful and can make eating, drinking and speaking uncomfortable.

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.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**

### **2. Major ulcers**

It occurs 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**

### **3.Herpetiform ulcers**

It occurs 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**

#### 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 other serious conditions such as chronic infections.



**Figure 4 Ulcerative condition**

#### Infections:

Bacterial, viral or fungal infections may cause mouth ulcers.

**Foods and Drinks:**

Mouth ulcers may be triggered by acids in certain foods, including oranges, lemons, pineapples, strawberries, tomatoes, and others.

**Toothpaste or Oral Rinses:**

Pastes or rinses that contain sodium lauryl sulphate may contribute to the appearance of mouth ulcers.

**Vitamin Deficiencies:**

A deficiency of vitamins such as B-12, iron, folate or zinc could also be a cause of mouth ulcers.

**Quitting Smoking:**

Immediately after quitting smoking, you may get mouth ulcers. This is usually temporary. These are some of the causes for mouth ulcer and they can be treated accordingly by changing the habit and using herbal remedies

**Herbal Remedies for Mouth Ulcer**

As from the ancient era photogenic agents are used by for the prevention and treatment of mouth ulcer. Some of the botanical compounds with anti-ulcer activity include flavonoids (i.e., quercetin, naringin, silymarin, anthocyanosises, sophoradin derivatives) saponins (i.e., from Panaxjaponicus and Kochia scoparia), tannins (i.e., from Lin deraeumbellatae), gums and mucilage's (i.e., gum guar and myrrh). Among herbal drugs, liquorice, aloe gel and capsicum (chilli) can be used extensively having major effect in treatment of mouth ulcer. Ethnos medical systems employ several plant extracts for the treatment of ulcer.[3]

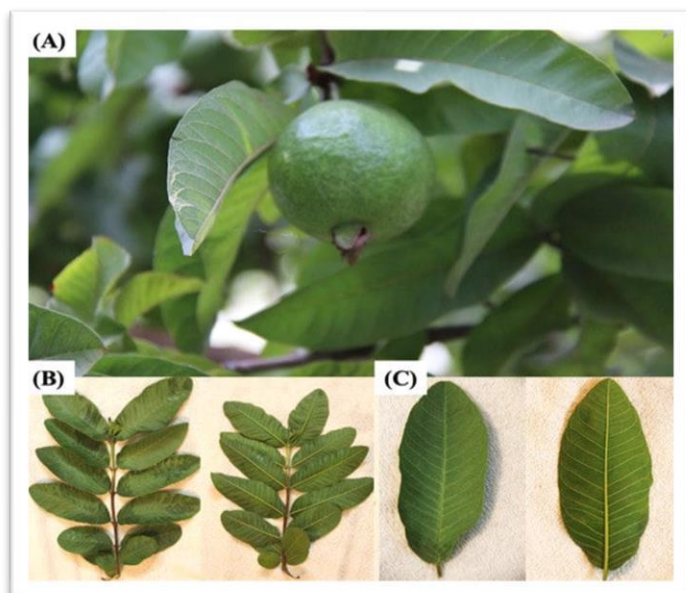
**Some of the Herbs that can be used as anti-ulcer drugs:**

1. Harra (*Terminalia chebulic*) [4,5,6] chewed after dinner cures mouth ulcers.
2. Basil leaves (*Ocimum sanctum*) [7] and Tomato juice (*Lycopersicum esculentum*) are taken for mouth ulcers.
3. Powder of nirgund (*Vitex negundo*) and Musli (*Chlorophytum borivilicum*) is prepared and can be taken four times a day for mouth ulcers
4. Mulberry (*Morus Alba*) juice is given to infants for this ailment.
5. Akar kara (*Spilanthes calva*) flower is chewed in mouth ulcers. It gives strength to the teeth.
6. Ash of burnt fruit bark of the water melon is also given.
7. Solanum and ginger oil are also used for mouth ulcer.

**Mouth ulcer treatment:**

One of the properties of mouth ulcers are that they can heal within 2 weeks without treatment but medicine and treatment may provide relief [8]. Treatment can numb the pain, protect the ulcer from further damage or decrease the chances of a bacterial infection; some medicines may speed up the healing if used early enough. Paste treatments, gel treatments, mouth washes, liquid paint treatments, neutralizing acid and numbing of the pain, pain killers, corticosteroids [9].

**Guava leaf**



#### Scientific classification:

- 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

The origin of guava is Psidium Guajava. It belongs to the Myrtaceae family.

#### Shape:

Guava fruits are usually 4 to 12 centimeters (1.6 to 4.7 inches) long and round or oval in shape, depending

on the species. They have a clear and precise smell, reminiscent of lemon peel, but less sharp. The skin can be hard, often bitter, or soft and sweet. The skin can vary in thickness and is usually green before growth, but can turn yellow, maroon or green as it matures. The pulp inside can be sweet or sour, white (“white” guava) to dark red (“red” guava). The number and hardness of the seeds in the average weight varies from variety to variety.

**Plant part used:** leaves

**Chemical composition:** Guava leaves contain carotenoids and polyphenols such as (+)- gallo catechin and

leucoanthocyanins. Red- orange guavas contain more polyphenols and carotenoids than yellowishgreen guavas, as some of these phytochemicals are responsible for skin color and texture. Guava leaves contain essential oils such as isopropyl alcohol, menthol,  $\alpha$ -pinene, terphenyl acetate, limonene,  $\beta$ -pinene, caryophyllene,  $\beta$ -bisabolene and Oleanolic acid.

**Use:**

Due to its high pectin content, guava is widely used to make desserts, preserves, jellies, jams and marmalades (such as Brazilian goiabada and Colombian and Venezuelan bocadillo) and as a bright jam. Red guava can be used as a base in the preparation of savories such as sauce, especially in place of tomatoes to reduce acidity. The drink can be made from the leaves and juice of guava leaves, which is called “tea” and is considered medicine. Guava (*Psidium guajava*): For the herbal treatment of a number of oral conditions, including toothaches, sore throats, inflamed gums, and ulcers, *Psidium guajava* has been Utilised.

**CONSTITUENTS: -**

Flavonoids are phytochemicals found in many plants, fruits, vegetables and leaves and can be used in medicinal chemistry. Flavonoids have many medicinal properties, including antibacterial, antifungal, antiviral and antibacterial properties. They also have neuroprotective and cardioprotective effects. These activities depend on the type of flavonoid, its (possible) mode of action and bioavailability. These cost- effective medicinal ingredients have important biological functions and their anti-inflammatory effects have been demonstrated. Recent studies have focused on their isolation, synthesis of analogues using various techniques and animal models, and their effects on human health. Thousands of flavonoids have been successfully isolated, and this number is constantly increasing. Therefore, we tried to summarize isolated flavonoids with important functions to better understand their effects on human health. Flavonoids are abundant and widespread throughout the plant kingdom. They are made from three parts acetate and one part phenylpropane (via the shikimate pathway). More than 2000 species are known; About 500 of these exist in the free form (aglycones) and the remainder are O- or C-glycosides. Flavonoid glycosides are mostly water soluble. There are three main types of carbon 3 based on its oxidation state. These are flavones, flavonols and flavonones. The properties of isoflavonoids are discussed in the Phytoestrogens section. Terpinene and pinene are found in aqueous extracts of the leaves and have antimicrobial activity

**Ingredients of gummy base :-**

#### 1. CORN STARCH

a) **Synonyms** - Amylum

b) **Biological Source** -

Starch consists of polysaccharide granules obtained from the grains of maize *Zea mays Linn* belonging to the family Gramineae.

c) **Geographical Source** -

Most of the tropical, as well as, subtropical countries prepare starch, commercially

d) **Chemical composition:**

Starch consists chemically two different polysaccharides Amylose (beta-amylose) & (alpha-amylose) in the proportion of 1:2. Amylose, is water soluble and amylopectin is water insoluble, but swells in water and is

responsible for the gelatinising property of the starch. Amylose gives blue colour with iodine, while amylopectin yields bluish, black colouration.

**e) Uses -**

Starch is well known as a gel agent. Starch is used as a nutritive, demulcent, protective, and as an absorbent. Starch is used in the preparation of dusting talcum powder for application over the skin. It is used as antidote in iodine poisoning, as a disintegrating agent in pills and tablets, and as dilute in dry extracts of crude drugs. It is a diagnostic aid in the identification of crude drugs. Glycerine of starch is used as an emollient and as a base for suppositories. Starch is also a material for the commercial manufacturer of liquid glucose, dextrose, and dextrin. Starch is industrially used for the sizing of paper and cloth.[10]



**Fig no. 5 - Corn Starch Powder**

## 2. SUCROSE

**a) Synonyms - Sugar, saccharose, table sugar, white sugar.**

**b) biological source: -**

Sucrose is majorly derived from sugarcane (*Saccharum officinarum* Linn) of the family Poaceae & beet sugar (*Beta vulgaris*)

**c)Geographical source: -**

Sugar cane was first recorded in Asia around 8000 bc and it is postulated that it originated as a native plant from Melanesia. Sugar cane now accounts for over 75% of the world's production of sugar. The major cane\_sugar producers are Brazil, India, China, Thailand, Mexico and Australia accounting for over 60% of the total cane sugar produced in 2003/4 and nearly 50% of the world's total production of sugar.

**c) Chemical composition: -**

Sucrose ( $\alpha$ -d-glucopyranosyl- $\beta$ -d-fructofuranoside) is a disaccharide with the general molecular formula  $C_{12}H_{22}O_{11}$ , and it has a molecular weight of 342.30.

**d) Uses: -**

It is mainly used as a sweetening agent. Caramellisation of sucrose can help in increasing the firmness of the formulation hence it is also used as a firming agent.[11]



Fig no. 6 - Sucrose

### 3. GROUNDNUT OIL

**a) Synonyms**- Peanut oil or Arachis oil

**b) Biological Source**-

The peanut is an annual herbaceous plant and are derived from the *Arachis hypogaea* plant which belong to the family Fabaceae, also known as Leguminosae, and commonly known as the legume, bean, or pea family.

**c) Geographical Source**-

This plant is native to South America, but can also be found in Mexico, Central America, Peru, Argentina, Nigeria, Australia, India, and Gambia. The kernels of the *Arachis hypogaea* plant are roasted and used to make vegetable oil, and the oil itself makes up 45–52% of the kernel content.

**d) Chemical composition**-

Its major component fatty acids are oleic acid (46.8% as olein), linoleic acid (33.4% as linoleic), and palmitic acid (10.0% as palmitin). The oil also contains some stearic acid, arachidic acid, behenic acid, lignoceric acid and other fatty acids

**e) Uses**-

Groundnut oil can be used for cooking because it has a smoking point of around 232 degrees Celsius, making it suitable for grilling, deep frying, and sauteing. Cold-pressed groundnut oil has a mild nutty flavour and aroma.

Groundnut oil is also a natural antioxidant that can help boost the immune system. It contains vitamin E, which can help maintain healthy skin, and phytochemicals, which can reduce inflammation.

Groundnut oil have been used as raw material for it's natural based plasticizer quality.





**Fig no. 7 - Groundnut oil**

#### 4. HONEY

**a) Synonyms:** -Madhu, Madh, Mel

**b) Biological Source:** -

Honey is a viscid and sweet secretion stored in the honey comb by various species of bees, such as *Apis mellifera*, *Apis dorsata*, *Apis florea*, *Apis indica* and other species of *Apis*, belonging to family Apidae.

**c) Geographical Source:** -

Honey is available in abundance in Africa, India, Jamaica, Australia, California, Chili, Great Britain and New Zealand.

**d) Chemical composition:** -

The average composition of honey is moisture 14–24%, dextrose 23–36%, levulose (Fructose) 30– 47%, sucrose 0.4–6%, dextrin and gums 0–7%. Besides, it is found to contain small amounts of essential oil, beeswax, pollen grains, formic acid, acetic acid, succinic acid, maltose.

**e) Uses:** -

Honey shows mild laxative, bactericidal, sedative, antiseptic and alkaline characters.

It is used for cold, cough, fever, sore eye and throat, tongue and duodenal ulcers, liver disorders, constipation, diarrhoea, kidney and other urinary disorders, pulmonary tuberculosis, marasmus, rickets, scurvy and insomnia.

Honey is an important ingredient of certain lotions, cosmetics, soaps, creams, balms, toilet waters and inhalations.

**It is applied as a remedy on open wounds after surgery. It prevents infection and promotes healing.**



Fig no. 8- Honey

Ingredients	%Composition	Role
Corn Starch	15	Gelling Agent
Groundnut Oil	5	Plasticizer
Sucrose	15	Firming Agent
Honey	15	Sweetener
Sodium Benzoate	0.5	Preservative

Sr.no	Ingredients	% Composition	Quality taken for Each 5gm gummy	Role of ingredients
1	Guava leaf extract	5%	250mg	Active ingredients
2	Corn starch	15%	750mg	Gelling agent

3	Sucrose	15%	750mg	Firming agent
4	Honey	15%	0.75ml	Sweetner
5	Groundnut oil	5%	0.25ml	Plasticizer
6	Sodium benzoate	0.5%	25mg	Preservative
7	Chocolate essence	2%	0.1ml	Flavouring agent
8	Water	q.s.	q.s.	Solvent

#### Procedure-

1. Take 15% Sucrose in a beaker and add 1 ml water to it. Heat the sucrose solution while stirring continuously with a stirrer until it becomes a little sticky.
2. In another beaker, take 15% Corn Starch and add 5 ml water and stir the starch solution until all the lumps disperse.
3. Add the Starch solution to the heating sucrose solution and stir well on flame until the starch starts to form translucent jelly like semi-solid.
4. To this sucrose and starch mixture add honey, groundnut oil and the extract solution which was prepared by dissolving 5% of extract in 1-2 ml methanol and stir well.
5. To this semi-solid mass add sodium benzoate and stir until it forms jelly like consistency.
6. Pour this mixture in a Mold and let it cool down and become firm for about 4-5 hours.[12]

#### To perform evaluation tests of herbal anti-mouth ulcer gummies-

Different evaluation test were performed on final formulation of gummies which are as follows:

#### 1. Physical evaluation:

Physical parameters such as color, appearance, Odor and taste was evaluated for the prepared formulation.

- **Color** - The color of the formulation was evaluated against white background.
- **Odor** - The Odor of the gummies was checked by mixing the gummy in water and taking the smell.
- **Taste** - The taste of the gummy was evaluated by placing a slice of gummy in the mouth.
- **Visual examination** - The shape of the gummies was checked visually and size of the gummies was checked using a scale.



Fig no. 9- Final Product with extract

## 2. Determination of weight variation:

Each of the 20 gummies were weighed individually, the average weight was calculated and the individual gummies weights were compared to it. If no more than two gummies fall outside the allowed % range as shown in table and no gummies deviates by more than twice the allowed range, the gummies pass the test.

## 3. Determination of pH:

1% solution of gummy sample was prepared by dissolving it in boiling water. The solution was allowed to cool at room temperature. pH was checked of the solution using pH meter.

## 4. Dispersion time:

Gummy was placed in 100 ml of distilled water along with a magnetic stirrer and was placed on rotator.

The dispersion time was noted.

## PLAN OF WORK

### 1. To carry out extraction of *Psidium guajava* leaf: -

Fresh leaves of *P.guajava* were collected from their natural habitat in and around the campus from SPCOP garden. Leaves were also collected from.

### 2. To formulate herbal anti-mouth ulcer gummies using extract of *Psidium guajava* leaf-

- A. Preliminary phytochemical screening** of *Psidium guajava* leaf extract was done which included test for alkaloids, tannins, saponins, carbohydrates, terpenoids, steroids, flavonoids, glycosides, amino acid and protein. Procedures for Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) was also carried out.
- B. Anti-Microbial Activity** of *Psidium guajava* leaf extract was determined by cup plate method.
- C. Analytical method development**
- D. Anti-Inflammatory Activity** of *Psidium guajava* leaf extract was determined by in-vitro antiinflammatory egg albumin denaturation assay method.
- E. Preparation of Blank batches of Gummies:** Blank batches of gummies were prepared using corn starch, sucrose, honey and groundnut oil in different ratios.

**F. Incorporation of extract in gummies:** Extract of *Psidium guajava* leaf was dissolved in hydro-alcoholic solvent in the ratio 1:1 and then added in the gummy base ingredients.

### **3. To perform evaluation tests of herbal anti-mouth ulcer gummies-**

Evaluation tests such as Physical appearance, pH test, Weight variation, Dispersion time and Friability test were performed on the final formulation.

### **4. Primary market research on herbal anti-mouth ulcer gummies by survey-**

Primary market research by carrying out survey to check the acceptance, scope and likability of the herbal anti-mouth ulcer gummies

#### **1. To carry out extraction of *Psidium guajava* leaf**

##### **a. Collection of plant material:**

Fresh leaves were collected from their natural habitat in and around the campus from SPCOP medicinal garden.

##### **b. Surface sterilization of herbs:**

The leaves were separated from other parts of the stem and washed thoroughly with tap water to obtain leaves free of dust and soil. Then the leaves were rinsed with distilled water to remove the remaining dirt. **drying:**

Guava leaves were dried in the sunlight till it completely loses its moisture and becomes dry. It takes around 8 to 10 days for the guava leaves to dry completely.



Fig no. 10 - Dried guava leaves.

##### **c. Preparation of coarse powder of guava leaves:**

Coarse powder of guava leaves was prepared by grinding the leaves in a mixer for less than one minute.

The coarse powder was then stored in an air tight container and used for further studies.



Fig no. 11 and 12 - Coarse powder of guava leaves

**d. Soxhlet extraction of guava leaves using hydroalcoholic solvent:**

- i. 50 gm of coarse powder was weighed subjected to hot continuous extraction using Soxhlet apparatus for 3-4 days (14 hours) with hydroalcoholic solvent (distilled water: ethanol-1:1).
- ii. About 300ml of hydroalcoholic solvent was used in the extraction process.



Fig no. 13- Soxhlet apparatus

**Collection of the semi-solid extract:**

After the extraction process was completed, an empty crucible was weighed and the liquid extract was then transferred to the empty crucible.

The crucible was then placed on a hot plate and the solvent was allowed to evaporate until a semi-solid extract was obtained.

The above step was repeated for several times and after evaporation the crucible was weighed.

The semi-solid extract was then stored in glass bottles at room temperature and kept away from sunlight.

**Calculation of total yield of extract:**

Weight of dried extract = weight of liquid extract with porcelain dish  
weight of empty porcelain dish.



Fig no. 14- Semi solid extract

**2. To formulate herbal anti-ulcer gummies using the extract of *Psidium guajava* leaves.**

**A. Preliminary phytochemical screening of *Psidium guajava* leaf extract:****1. Test for Alkaloid:****I) Dragendorff's test:**

2-3 ml of the filtrate was added to 0.5 ml of dragendorff's reagent. Formation of orange brown precipitate indicated the presence of alkaloids.

**2. Test for tannins and phenolic compounds: I) Lead acetate test:**

To the filtrate 5ml of 10% lead acetate solution was added. Formation of white precipitation indicates the presence of tannin

**II) Ferric chloride test:**

5% ferric chloride solution was added drop by drop in 2-3ml in guava extract filtrate.

A blue-green or black coloration indicated the presence of phenols and tannins.

**3. Test for Saponins: I) Foam test:**

Extract was placed in a test tube and shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

**4. Test for carbohydrates:****I) Molisch's test:**

To 2-3 ml aqueous extract, add few drops of alcoholic alpha-naphthol solution. Shake and add conc. H<sub>2</sub>SO<sub>4</sub> from sides of the test tube. Violet ring is formed at the junction of two liquids indicates the presence of carbohydrate.

**5. Test for steroids:****I) Salkowski test:**

To 2 ml of extract, add 2 ml chloroform and 2 ml conc. H<sub>2</sub>SO<sub>4</sub>. Shake well. Chloroform layer appears red and acid layer shows greenish yellow fluorescence indicates presence of steroids.

**6. Test for flavonoids: I) Alkaline reagent test:**

Two to three drops of sodium hydroxide were added to 2 mL of extract. Initially, a deep yellow colour appeared but it gradually became colourless by adding few drops of dilute HCL, indicating that flavonoids were present.

**7. Test for Cardiac Glycosides:****I) Keller-killiani test:**

To 2 ml extract, add glacial acetic acid, one drop 5% FeCl<sub>3</sub> and conc. H<sub>2</sub>SO<sub>4</sub>. Reddish brown colour appears at junction of the two liquid layers and upper layer appears bluish green indicates presence of cardiac glycosides.

**8. Test for amino acids:****9. I) Millions test:**

2 ml of millions reagent added in 2 ml of filtrate. The test tubes are then kept in the water bath for about 2 minutes if red coloured is not observed immediately. Red colour not formed indicates absence of amino acids.

## 10 Test for proteins:

### I) Biuret test:

1ml of plant extract was taken in a test tube followed by 4% NaOH and 1% CuSO<sub>4</sub>.

Violet pink colour not formed indicated the absence of proteins.

### B. Anti-Microbial Activity of *Psidium guajava* leaf extract by cup plate method:

Antimicrobial activities of different extracts were evaluated by Cup Plate Method. It is one of the official methods in IP, where the test samples diffuse from the cup through an agar layer in a petri plate to such an extent that the growth of added microorganism is restricted entirely to a circular area of zone around the cavity containing the solution of an antibiotic substance. The antimicrobial activity is expressed as zone diameter in millimetres, which is measured by a scale.

### I. Selection of organism

The most prevalent cause of mouth ulcers due to infection is caused by *Candida albicans*. *Candida albicans* is a symbiotic fungal specie that commonly colonizes human mucosal surfaces. Our literature search has shown that *C. albicans* enhances the adherence and facilitates the growth of other micro-organisms as well in the buccal environment. In this research, antimicrobial activity of guava leaf extract of different concentrations against *C. albicans* (laboratory sub-cultured) using Cup Plate Method.[13]

### II. Sub-Culturing

Sub-culturing is a procedure of transferring of microorganism into fresh nutritive medium from its stock culture. Sub-culturing is done to maintain culture in its active form (prolonging life and/or increase the number of cells) for varied applications. Sub-culture

*C. albicans* is done in the SDA (Sabouraud Dextrose Agar)

#### Procedure for sub-culturing *C. albicans* in slants:

1. In a sterilized conical flask weigh and add SDA (Sabouraud Dextrose Agar), to it add distilled water as per the quantity requirement.
2. Autoclave the SDA solution at 121°C for 15 minutes or in a pressure cooker up to 3 whistles after adding approximately 500 ml to the bottom of the pressure cooker.
3. Once autoclaved, let it become lukewarm. Prepare an aseptic area before proceeding.
4. Pour this SDA solution into sterilized test tubes and plug them with cotton. Keep them slant for 1-2 hours until the solution solidifies.
5. Sterilize an inoculation loop above a flame until it's red hot, then let it cool in the air.
6. Open the cotton plug of the tube containing the stock culture and rotate the neck of the tube over a flame.
7. Insert the loop into the tube and pick up the culture.
8. Close the tube's mouth with the plug.
9. Put the inoculating loop on the bottom of the slant and move it back and forth up the tube.
10. Replace the cap and sterilize the loop.
11. Place the tube in a dry and away from sunlight place at room temperature. Colonies are seen after about 48-72 hours.



#### 4. Primary market research on herbal anti-mouth ulcer gummies by survey: -

Primary market research by carrying out survey to check the acceptance, scope and likability of the herbal anti-mouth ulcer gummies.

In this research a survey form was generated with the following questions-

1. What do you understand by the term "mouth ulcer"?
2. How many ulcers are there during each episode/ time?
3. How often do you experience mouth ulcers
4. How long do your mouth ulcers typically last?
5. What do you think are the common causes of mouth ulcers?
6. Which of the common symptoms do you encounter during each episode?
7. How do you treat your mouth ulcers?
8. What type of medications do you use for mouth ulcers?
9. Are you familiar with using gummies as a treatment or relief for mouth ulcers?
10. Would you like to use gummies as a treatment for mouth ulcers?
12. What factors would influence your decision to use gummies as a treatment for mouth ulcers?

Based on the answers received to these questions a brief knowledge of what can be the target population, segmentation, acceptance of the product, scope in the market, likability, etc. factors can be determined.

## RESULTS

Extraction of *Psidium guajava* leaves by Soxhlet extraction method using hydro alcohol as solvent

Sr.no	Category	<i>Psidium guajava</i> leaves
1	Weight of the powdered crude drug A	50 gm
2	Weight of empty porcelain dish B	80 gm
3	Weight of porcelain dish with dried extract C	88.6gm
4	Total yield	8.6gm

Physical evaluation of extract

Physical appearance	<i>Psidium guajava</i>
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Consistency	Sticky
Colour	Dark brown

Phytochemical screening of *Psidium guajava* leaf

SR.NO	TESTS	<i>Psidium guajava</i> leaf extract
1	Alkaloids	+
2	Tannins and phenolic compounds	++
3	saponins	+
4	carbohydrates	+
5	steroids	+
6	flavonoids	+
7	Cardiac glycosides	+
8	Amino acids	-
9	protein	-

Phytochemical screening of *Psidium guajava* leaf

SR.NO	Solution (mg/ml)	Zone of inhibition (mm)
1	control	0
2	Drug (clotrimazole)	27.09
3	50	18.77
4	75	6.66
5	100	11.88
6	150	11.55
7	200	13.66
8	250	14

**Quality control tests of the final formulation****- Results of evaluation tests of final formulation**

SR.NO	EVALUTION PARAMETERS	INFERENCES
1	PHYSICAL APPEARANCE a) Colour b) Odour c) Taste	a) Brown b) chocolatey c) sweet chocolate flavour
2	Visual examination a) shape b) size	a) flower, heart b) length-1.3cm c) breadth-2.5cm
3	Weight variation	Complies the standard 7% deviation ra
4	Determination of PH	6.65
5	Dispersion time	11 min

**Fig no. 15- Final formulation.**

## SUMMARY

various herbal products such as tablets are available for the treatment of mouth ulcers in the market but gummies as a treatment choice for mouth ulcers is yet to be introduced in the market. Our product includes herbal Psidium guajava leaves extracts with anti-ulcers activity due to incorporation of herbal extract it reduces the chances of the harm to the oral cavity as well as to the gastro intestinal tract the anti-ulcers activity of the extract as well as the product is stated in the performing anti-microbial and antiinflammatory assay after obtaining the desired consistency of the gummies along with its firmness appropriate batch was selects for the formulation the formulation also includes honey which an anti-septic, prevents infections and promotes healing specially in disorders related to mouth and throat groundnut oil im our formulation adds to and anti-oxidants activity for our product herbal anti-ulcers gummies were successfully prepared by using extract of Psidium guajava leaves

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