FORMULATION AND EVALUATION OF AQUEOUS GEL OF POWDERED GUAVA LEAVES FOR MOUTH ULCER

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ABSTRACT
The main objective behind this formulation of herbal Guava Leaves gel was to overcome the pain & discomfort by the mouth ulcers. As we know there are different types of mouth ulcers that cause inflammation & pain. The most common oral ulcers are Local trauma & Aphthous Stomatitis. Now a day’s a lots of herbal medicines are important stay of primary healthcare because of good response & highly efficient treatment with less amount of side effects. The formulated gel contains main ingredients is Guava Leaves Powder & Carbopol 934 as gelling agent & Propylene glycol as co-solvent. The other ingredients as Haldi & Lavang act as antiseptic & antifungal agents. The formulated gel was evaluated for different parameters like physicochemical parameters (pH, viscosity, spreadability, etc.), zone of inhibition, etc. The formulated gel was made transparent homogeneous mixture with pH ranges between 6-6.5. This herbal gel was stable at room temperature which is protected from any of microorganisms & thus it is safer for use for mouth ulcers.

KEYWORDS: Oral ulcer, Guava leaves, Haldi, Lavang, Carbopol 934 & Gel.

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.

They are painful round or oval sores that form in the mouth, mainly on the inside of the cheeks or lips.
Mouth ulcers, also known as aphthous ulcers, can be painful while eating, drinking or brushing teeth.

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.[1]

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.[2]

Types of Mouth Ulcer

On the basis of ulcer size and number
1. Minor ulcers: These are around 2-8mm in diameter and they usually clear up in 10 days to 2 weeks.
2. Major ulcers: These are bigger and deeper, often with a raised or irregular border.
3. Herpetiform ulcers: This type of ulcer is a cluster of dozens of smaller sores about the size of pinheads order.
4. Ulcerative Conditions: Mouth ulcers are very common and are mainly due to trauma such as from ill-fitting dentures, fractured teeth, or fillings.[1]

Factors responsible for the mouth ulcers
- Toothpastes and mouthwashes that contain sodium lauryl sulfate
- Emotional stress / Psychic stress
- Hormonal changes
- Nutritional deficiencies
- Mechanical trauma
- Viral infections
- Allergies and sensitivities
- Genetics
- Infectious agents (both bacterial and viral)
- Medical conditions.[1]

Gel

Gels are typically semi-solid formulations having a liquid phase that has been thickened
with other components. Uses of topical gel preparations are for skin application or percutaneous penetration of medicament or local action to certain mucosal surfaces.[2]

The Commercially available gels containing synthetic and semisynthetic active agents which have several disadvantages like staining on the teeth, irritation, and burning sensation only because presence of high degree of alcohol content and some organic compounds.[2]

The present investigation deals with use of herbal powdered Guava Leaves in the treatment of mouth ulcer in pharmaceutical gel.

![Image of Guava Gel](image-url)

**Plant Profile**

**Guava**

**Biological source:** Guava is botanically termed as *Psidium guajava*, belonging to the family *myrtaceae*.

![Image of Guava Plant](image-url)

**Macroscopic Identification**

**Habitat:** Medium sized tree with thin smooth, patchy, peeling whitish brown bark, but under
high moisture conditions, grows to 6-9 m in height.

**Plant part used:** Leaves, roots, fruits.

**Root:** Tap, branched.

**Stem:** Erect, aerial, branched, woody, cylindrical, solid, white or brown.[3]

**Leaves:** Simple, alternate, short petiolate, exstipulate, aromatic, gland dotted.

The leaves are 10-12 cm in length, 5-7 cm in width. They have a green color & leathery texture.

The lamina is green, simple with acute apex, entire margin, and symmetric-asymmetric base.

The upper & lower surface is pubescent.[3]

**Chemical constituents:** Guava leaves contain both carotenoids and polyphenols like (+)-gallocatechin and leucocyanidin. As some of these phytochemicals produce the fruit skin and flesh color, guavas that are red-orange tend to have more polyphenol and carotenoid content than yellow-green ones.[1]

The guava fruit contains vitamin A, C, iron, phosphorus and calcium. It has more vitamin C than the orange.

The fruit contains saponin, oleanolic acid, lyxopyranoside, guaijavarin, quercetin and flavonoids.

Ascorbic acid and citric acid are the major ingredients of guava that play important role in anti-mutagenic activity.[4]

The chemical structures of quercetin and ascorbic acid have been shown in Fig.

![Chemical structures of Quercetin & Ascorbic acid.](image)

**Uses:** Gastrointestinal, anti-spasmodic, for treatment of rheumatism, Guavas are extensively used to make candies, preserves, jellies, jams. Red guavas can be used as the base of salted products such as sauces, substituting for tomatoes, especially to minimize acidity.

Used as Antioxidant, Antibacterial activity, Anti-inflammatory activity, anticancer
activity.[5]

The main traditional uses of guava leaves in the main producer countries
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 and as anti-bacterial in Nigeria. For skin and wound applications, poultice is externally used in Mexico, Brazil, Philippines, and Nigeria. In addition, chewing stick is used for oral care in Nigeria.[6]

Figure 1: Main traditional uses of Guava leaves in principle producer countries.

Medicinal Importance of Guava
Guava contains high content of organic and inorganic compounds like secondary metabolites e.g. antioxidant, polyphenols, antiviral compounds and anti-inflammatory compounds. Guava has a lot of compounds which have anti-cancerous activities. It has a higher number of vitamins and minerals. Phenol compounds like flavonoids also find an important place in the
guava.\textsuperscript{[4]}

**Antimicrobial activity**
Guava has a high antimicrobial activity. Guava leaf’s extract doses can reduce the amount of cough due to its anti-cough activity. Guava leaves have high antibacterial activity in extracts that can inhibit the growth of S. aureus. Plant leaf and bark methanolic extracts of \textit{P. guajava} have high antimicrobial activity.\textsuperscript{[4]}

**Anti-inflammatory activity**
Extract of guava in ethyl acetate can stop the germ infection and thymus production. It can act as anti-viral agent.
Guava can alter the homo oxygenase-1 protein’s work. And due to this reason, it can be used as anti-inflammatory agent for skin.
Extract of guava in ethanol inhibit the lipopolysaccharide from manufacturing of nitric oxide. It suppresses the expression of E2. In this way it works as anti-inflammatory agent.\textsuperscript{[4]}

**Antioxidant activity**
Antioxidants are molecules which retard the oxidation process. The oxidation reactions may produce free radicals which damage the cells by starting various chain reactions. Free radicals which damage the cells cause cancer and many other diseases. Antioxidants terminate the free radicals and stop the chain reactions.

Examples of antioxidants include \textit{beta-carotene}, \textit{lycopene}, \textit{vitamins C, E,} and \textit{A} and other substances.

Guava contains high amount of antioxidants and anti-providing nutrients.

Extracts of guava in water and organic solvents have a large quantity of antioxidants which can stop the oxidation reaction.

Guava is highly rich in antioxidants which are helpful in decreasing the incidences of degenerative diseases such as \textit{brain dysfunction, inflammation, heart disease, cancer, arteriosclerosis} and \textit{arthritis.}\textsuperscript{[4]}

**AIM AND OBJECTIVES**
The Aim of this work is:
“Formulation & Evaluation of Aqueous Gel of Powdered Guava Leaves for Mouth Ulcer.”

The treatment of mouth ulcers like conditions with the use of traditional medicinal plants is an essential aspect in the field of medicinal science.

In present study, our particular interest was plant vitamins obtained from *Psidium guajava* used to treat mouth ulcer.

For ex.: Minor & Major ulcers, Herpetiform ulcers & Ulcerative conditions.

**Carbopol 934** is used as gelling agent in the present study also possesses some pharmaceutical activities.

The basic **Aim** of this work is **to evaluate the formulation parameter & ulcer healing activity of carbopol 934 & the active ingredients in the form of gel.**

In order to achieve this goal, the following major **Objectives** are as:

- **To procure & characterize the material.**
- **To prepare the gel of powdered Guava leaves with carbopol 934 as gelling agent.**
- **To evaluate & optimize the formulation.**
- **To carry out the stability study of the formulation.**

**PLAN OF WORK**

- Literature
- Formulation of Herbal Gel
- Physicochemical Parameters
  1. Determination of physical appearance
  2. Determination of microscopic characters
  3. Determination of pH
  4. Determination of Viscosity
  5. Determination of Zone Inhibition

**MATERIALS AND METHODS**

**Collection of Plant Materials:** The fresh plant materials of *Psidium guajava, Aloe vera, and acacia leaf* were collected & fresh plant leaves were washed under distilled water and shade drying was carried out.\[^2\]
Preparation of herbal Gel\textsuperscript{[5]}

Carbopol 934 dispersed into distilled water.

\[ \downarrow \]

5 ml distilled water + methyl paraben and propyl paraben.

\[ \downarrow \]

Heated on water bath.

\[ \downarrow \]

After cooling propylene glycol was added.

\[ \downarrow \]

Further varying concentration of \textit{Psidium guajava} powder was mixed to the above mixture.

\[ \downarrow \]

Volume was made up to 20 ml with distilled water.

\[ \downarrow \]

At last full mixed ingredients were mixed to Carbopol 934 gel properly.

\[ \downarrow \]

With continuous stirring triethanolamine was added drop wise to adjust pH (6.8-7).

Preparation of herbal Gel

Specified amount of Carbopol 934 was dispersed in required amount of distilled water with continuous stirring. 5 ml of distilled water was taken and required quantity of methyl paraben and propyl paraben were dissolved by heating on water bath after cooling propylene glycol was added. Further varying concentration of \textit{Psidium guajava} powder was mixed to the above mixture and volume was made up to 20 ml with distilled water. Finally full mixed ingredients were mixed properly to the Carbopol 934 gel with continuous stirring and triethanolamine was added drop wise to the formulation for adjustment of required pH (6.8-7).\textsuperscript{[9]}

The composition of herbal gel prepared from the powdered guava leaves coded as G1, G2, and G3 & tabulated as below:-

Table 1:

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Ingredients</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Guava Leaves</td>
<td>2gm</td>
<td>3.32gm</td>
<td>10gm</td>
</tr>
<tr>
<td>2</td>
<td>Propylene glycol</td>
<td>2gm</td>
<td>3.32gm</td>
<td>10gm</td>
</tr>
<tr>
<td>3</td>
<td>Carbopol 934</td>
<td>2.5gm</td>
<td>6.65gm</td>
<td>12.5gm</td>
</tr>
<tr>
<td>4</td>
<td>Haldi</td>
<td>2gm</td>
<td>3.32gm</td>
<td>10gm</td>
</tr>
<tr>
<td>5</td>
<td>Lavang</td>
<td>2gm</td>
<td>3.32gm</td>
<td>10gm</td>
</tr>
<tr>
<td>6</td>
<td>Methyl paraben</td>
<td>0.0015gm</td>
<td>0.005gm</td>
<td>0.0075gm</td>
</tr>
<tr>
<td>7</td>
<td>Propyl paraben</td>
<td>0.01gm</td>
<td>0.033gm</td>
<td>0.05gm</td>
</tr>
<tr>
<td>8</td>
<td>Triethanolamine</td>
<td>q.s.</td>
<td>q.s.</td>
<td>q.s.</td>
</tr>
<tr>
<td>9</td>
<td>Distilled water</td>
<td>Up to 25ml</td>
<td>Up to 50ml</td>
<td>Up to 125ml</td>
</tr>
</tbody>
</table>
Evaluation of Herbal Gel

Physical appearance (macroscopic analysis): The prepared gel formulation containing Guava Leaves were inspected visually for their color, homogeneity, consistency & phase separation.[7]

Microscopic analysis: The microscopic study by the Optic Microscope with magnification of 10 & 40 for uniformity, gel texture & bubbles.[8]

Clarity of gel: The clarity of all the three batches was determined by visual inspection.[9]

Homogeneity: All developed gel formulations were tested for homogeneity by visual inspection after the gels have been set in to the container. They were tested for their presence and appearance of any aggregates.[9]

Centrifugation test: Each of the chosen formulations were separately centrifuged in a test tube of 10cm long & 1cm width for 5, 15, 30 & 60 minutes with 2000 rpm & then studied for sedimentation & gel stability.[8]

Stability study: Stability studies were done with open and close container. Here, by subjecting the product to room temperature for 1 month.[9]

pH determination: The pH of developed gel formulations was determined using digital pH meter. 1gm of gel was dissolved in 100 ml distilled water and kept aside for 2 hours. The measurement of pH of each formulation was done in triplicate and average values are calculated.[7]

Viscosity: Viscosity was determined by using Brookfield viscometer (DV-III programmable Rheometer). Formulated gels were tested for their rheological behaviors at 250C. The measurement was made over range of speed from 10rpm to 100rpm with 30seconds between 2 successive speeds and then in a reverse orders.[5]

Spreadability test: Spreadability was determined by the apparatus which consists of a wooden block, which was provided by a pulley at one end. By this method spreadability was measured on the basis on slip and drag characteristics of gels. An excess of gel (about 2 gm) under study was placed on this ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with
the hook. A one kg weighted was placed on the top of the two slides for 5 min. to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped off from the edges. The top plate was then subjected to pull of 80 gm. With the help of string attached to the hook and the time (in sec.) required by the top slide to cover a distance of 7.5 cm be noted. A shorter interval indicates better spreadability.[7]

Spreadability was calculated using the following formula:

\[ S = \frac{M \times L}{T} \]

Where,

- \( S \) = Spreadability,
- \( M \) = Weight in the pan (tied to upper slide),
- \( L \) = Length moved by the slide, \( T \) = Time (in sec.).

**Gel strength:** Gel strength was determined by the time in seconds required by the weight to penetrate in the gel. A Sample amount of 5 gm of each of the optimize batches was taken and 3.5 gm weight was placed on the surface of gel. The time in seconds required by the weight to penetrate 0.5 cm in the gel.[2]

**Determination of zone inhibition:** The antifungal activity of all developed batches of formulation and without drug containing gel formulation i.e. blank formulation were carried out by *Cup-plate method* in comparison with marketed antifungal formulation (Zolef cream). There are one bacteria culture used were Aspargilious aureus. The antifungal test was performed using the agar well diffusion Prepared nutrient brought and poured in to sterile petri plates and kept for drying and cooling. After that each bacterial culture were spread by micron wire loop. A sterile cork borer 6 mm diameter was used to drill holes 4 mm deep. Then 0.5 gm of gel from each batches add in to this holes. Plates were then incubated at 27°C for 48 hrs. The zone of inhibition (diameter in mm) developed, if any, was then measured for the particular compound with each fungal strength.[5]
RESULTS AND DISCUSSION

Table 2: In vitro evaluation parameters.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical appearance</td>
<td>Greenish yellow</td>
<td>Greenish yellow</td>
<td>Greenish yellow</td>
</tr>
<tr>
<td>Ph</td>
<td>6.4±0.5</td>
<td>6.3±0.7</td>
<td>6.5±0.5</td>
</tr>
<tr>
<td>Homogeneity</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
</tr>
<tr>
<td>Viscosity</td>
<td>3.111 ± 0.004</td>
<td>3.017 ± 0.049</td>
<td>2.219 ± 0.012</td>
</tr>
<tr>
<td>Spreadability</td>
<td>4.30 ± 0.1</td>
<td>5.70 ± 0.15</td>
<td>5.86 ± 0.57</td>
</tr>
<tr>
<td>Gel strength</td>
<td>42±0.75</td>
<td>36±0.07</td>
<td>27±0.5</td>
</tr>
</tbody>
</table>

From the above mention results in table, it is clearly shows that all the prepared gel formulations have good appearance. The pH of all gel formulations was found in suitable range. The all gel formulations have good homogeneity. The rheological behavior (viscosity) was studied with ranging between 2.219 to 3.11 Pa.S. which indicate that formulated gel is neither too thick & nor too thin. The study of spreadability shows that with increasing viscosity of formulation spreadability decreases & vice-versa. The gel strength of all batches was found in suitable range.

Table 3: In vitro evaluation parameters: Stability study.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Open container (1 month)</th>
<th>Closed container (1 month)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>Not stable</td>
<td>Stable</td>
</tr>
<tr>
<td>G2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

One month stability study was done with open and close container and it’s showed that open container containing gel was not stable and close container gel was stable. Formulated gel containing open container when expose to ambient room temperature then syneresis was observed it means the contraction of gel by separating out of liquid. Syneresis it means the form of instability in aqueous gels.

Table 4: In vitro zone inhibition study.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>Marketed preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspargillus-aureus (mm)</td>
<td>19±0.5</td>
<td>22±0.4</td>
<td>20±0.6</td>
<td>25±0.4</td>
</tr>
</tbody>
</table>

All the three batches of developed formulation showed antifungal activity against Aspargillus aureus as it is the main microorganism responsible for mouth ulcer and formulation it can also use to treat mouth ulcer infection.
CONCLUSION

From the mentioned results, it was conclude that the prepared gel formulations are in good appearance with suitable pH range. All the batches have good homogeneity, proper gel strength & spreadability. The prepared formulations have viscosity ranging between 2.2 to 3.1 Pa.S. indicating that the gel is neither too thick nor too thin. The all formulated gels are found to be stable in closed container as compared to open container. The formulation showed the action against pathogenic anti-fungal activity.

Therefore, the study concluded that the natural remedies are more acceptable & they are safer with minimum side effects than synthetic preparations.

Thus, the data presented in this study, it was conclude that the formulated gel of powdered Guava Leaves possesses a significant therapeutically efficacious & have suitable vehicle for drug delivery.

Thus, the formulated gel of powdered Guava Leaves is suitable for treatment of mouth ulcer.

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REFERENCES
