# FORMULATION AND EVALUATION OF HERBAL LOZENGES CONTAINING COCCINIA GRANDIS LEAF EXTRACT WITH PHYTOCHEMICAL SCREENING AND QUANTITATIVE ESTIMATION OF TOTAL PHENOLIC AND FLAVONOID CONTENT

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## Abstract:

The present study focuses on the Formulation and evaluation of herbal lozenges formulated using the leaf extract of *Coccinia grandis* (L.) Voigt, a medicinal plant known for its anti-ulcer, anti-inflammatory, and antimicrobial, wound healing, anti-oxidant properties. It commonly known as Ivy gourd, is a plant of the Cucurbitaceae family that has been used in traditional medicinal systems for centuries. It has been widely used for the treatment of various types of diseases. The phenolic and flavonoid content of plant extracts largely determine their pharmacological activity. Qualitative and quantitative phytochemical analysis of this species extract confirms the presence of various phytochemicals like alkaloids flavonoids, glycosides, phenolic compounds and steroids.

**Keywords**: Lozenges, *Coccinia grandis*, Phytochemical screening, Phenolic, Flavonoid.

### I. INTRODUCTION

## INTRODUCTION

For centuries, medicinal plants have functioned as primary healthcare resources, particularly in developing nations where a substantial segment of the population continues to rely on botanical therapeutics. The inherent limitations of synthetic pharmaceuticals specifically their adverse side effects, systemic toxicity, and the rise of antimicrobial resistance have prompted a strategic shift in scientific inquiry toward plant-derived alternatives. Consequently, these natural products are increasingly prioritized, as they are perceived to be safer and more cost-effective, while offering a wealth of bioactive compounds with a broad spectrum of pharmacological activities [1].

*Coccinia grandis* (L.) Voigt, frequently referred to as ivy gourd, is a perennial climbing species within the Cucurbitaceae family, prevalent throughout tropical and subtropical regions. It holds a prominent position in traditional medical systems, including Ayurveda, Siddha, and Unani, where it is employed to manage diabetes, inflammation, gastrointestinal ailments, and wound recovery. The therapeutic efficacy of this plant is attributed to a diverse profile of phytoconstituents, most notably flavonoids, phenolic compounds, alkaloids, glycosides, and terpenoids. [1,2].

Numerous investigations have established that *Coccinia grandis* exhibits substantial antioxidant, antibacterial, anti-inflammatory, cytotoxic, and antiulcer properties. Its antioxidant capacity is primarily linked to an elevated concentration of phenolic and flavonoid constituents, which are instrumental in neutralising free radicals and mitigating oxidative stress. Furthermore, the plant's traditional application in the treatment of wounds and oral ulcers has received rigorous scientific validation [2,3].

Lozenges represent flavoured solid dosage forms designed for gradual dissolution within the oral cavity, facilitating the localized release of active constituents into the mouth or pharynx. These formulations are

especially advantageous for patients experiencing dysphagia with conventional delivery systems, notably within pediatric and geriatric demographics. Herbal lozenges incorporate botanical extracts, essential oils, and other natural derivatives to deliver therapeutic benefits, including the alleviation of irritation, reduction of inflammatory responses, and management of oropharyngeal infections [4].

Furthermore, lozenges provide distinct advantages, including extended drug retention within the oral cavity, controlled release kinetics, enhanced patient compliance, and the effective taste-masking of bitter pharmacological agents. These attributes render them an optimal delivery system for botanical extracts intended to treat oropharyngeal conditions [4].

Therefore, the present study aims to formulate and evaluate herbal lozenges containing *Coccinia grandis* leaf extract and to assess its phytochemical constituents, total phenolic and flavonoid content, and potential therapeutic activity, thereby establishing it as a safe and effective natural remedy.

**Lozenges**

Lozenges are solid, sweetened preparations designed for slow oral dissolution. Manufactured via molding (pastilles) or compression (troches), they provide an alternative for patients with dysphagia. They facilitate sustained drug release, maintaining constant concentrations in the oral cavity or pharynx. Historically, they have been utilized to treat throat irritation through the localized delivery of topical anesthetics and antibacterial agents [5].

**Herbal lozenges**

Herbal lozenges are solid medicinal formulations employed to manage oropharyngeal ailments, gradually delivering active therapeutic constituents as they dissolve on the tongue. They exhibit significant efficacy in the localized treatment of coughs, sore throats, and minor respiratory complications. These small, disc-shaped tablets dissolve within the oral cavity, releasing active botanical ingredients to mitigate coughing, throat irritation, and other respiratory conditions. Typically, these lozenges are composed of plant extracts, essential oils, and various other medicinally beneficial substances, including traditional herbs [6].

**Advantages of lozenges:**

These formulations are easily administered to both geriatric and pediatric populations. Their palatable nature facilitates extended retention within the oral cavity, ensuring sufficient contact time for localized therapeutic action. Furthermore, systemic drug absorption via the buccal mucosa remains a viable possibility. The integration of sweeteners and flavouring agents within the matrix effectively masks the inherent taste of the medicinal components [7].

**Disadvantage of lozenges:**

Parents should avoid combining medications with candy and keep it out of children's reach. Heat-stable drugs are suitable, and lozenges should be used safely with children over six [8].

**Uses of lozenges:**

Lozenges facilitate the gradual administration of oral treatments, ensuring the saturation of pharyngeal tissues with medicinal solutions, and represent a suitable alternative for patients unable to swallow conventional solid dosage forms. Standard pharmacological agents utilized in these formulations include corticosteroids, decongestants, and demulcents [9].

## MATERIALS AND METHODS

**Composition of lozenges**

**Active herbal ingredients:**

1. Coccinia grandis (Leaf Extract)
   Coccinia grandis contains flavonoids, alkaloids, and phenolic compounds. It shows antimicrobial, anti-inflammatory, and wound-healing properties. The extract supports repair of oral mucosa and minor ulcers, making it suitable for oral therapeutic formulations.

2. Phyllanthus emblica (Amla Extract)
   Amla is rich in ascorbic acid (vitamin C), tannins, and polyphenols. It exhibits strong antioxidant, anti-inflammatory, and antimicrobial activities. In oral formulations, it helps in maintaining gum health, reducing oxidative stress, and promoting healing of minor oral lesions. Its mild acidity also improves salivary secretion and taste.

3. Mentha × piperita Oil (Peppermint Oil)
   Peppermint oil contains Menthol, responsible for its cooling, analgesic, and refreshing properties. It enhances breath freshness, provides soothing relief to throat irritation, and improves overall palatability of the formulation.

4. Clove Oil
   Clove oil is rich in Eugenol, which possesses analgesic, antiseptic, and anti-inflammatory properties. It is widely used in dentistry for pain relief and control of oral infections. In lozenges, it provides antimicrobial action and soothing effect on the throat.

## Methodology

### Preparation and Extraction of *C. grandis* Leaves

Fresh *C. grandis* leaves were harvested, rinsed under running water, and drained for 24 hours at ambient temperature before being oven-dried at 50°C for three days. The dehydrated leaves were subsequently ground into a fine powder and preserved in an airtight plastic container. For the extraction, one part of the powdered material was submerged in ten parts of 70% ethanol within a glass vessel and macerated for 24 hours with agitation every three hours. After filtration, the residue underwent two additional remaceration cycles, and the combined filtrates were consolidated. The ethanol was then evaporated in an oven at 45°C to produce a concentrated extract, which was stored in a light-resistant container under refrigeration.

### Determination of Extraction Yield.

The extraction yield was expressed as a percentage and determined gravimetrically. Specifically, the yield was calculated by dividing the mass of the resulting concentrated ethanolic extract by the initial mass of the dried leaf powder, with the quotient multiplied by one hundred. [10]

## Phytochemical Screening

### Qualitative examination of phytoconstituents:

### Test for Alkaloids

**Dragendorff's Test:** To 1 g of the extract, add 1 ml of Dragendorff's reagent (Potassium Bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.
Ethanol extract, hexane fraction, chloroform fraction and ethanol fraction give the orange- red precipitate.
**Mayer's Test:** To 1 g of the extract, add 1 ml of Mayer's reagent (Potassium mercuric iodide solution). Whitish yellow or cream coloured precipitate indicates the presence of alkaloids.
Ethanol extract, hexane fraction, chloroform fraction and ethanol fraction give the cream-coloured precipitate.
**Wagner's Test:** To 1 g of the extract, add 2 ml of Wagner's reagent (Iodine in Potassium Iodide), Formation of reddish-brown precipitate indicates the presence of alkaloids.
Ethanol extract, hexane fraction, chloroform fraction and ethanol fraction give the reddish-brown precipitate.

### Test for Carbohydrates

**Molisch's Test:** To 2g of the extract, add 1ml of α-napthol solution, add concentrated sulphuric acid through the side of the test tube. Purple or reddish violet colour at the junction of the two liquids reveals the presence of Carbohydrates.
Ethanol extract and ethanol fraction give purple colour at the junction of the two liquids.
**Fehling's Test:** To 1gm of the extract, add equal quantities of Fehling solution A and B, upon heating formation of a brick red precipitate indicates the presence of sugars.
Ethanol extract and ethanol fraction give brick red precipitate.

### Test for Glycosides

**Legal Test:** Dissolve the extract in pyridine and add sodium nitroprusside solution to make it alkaline. The formation of pink red to red colour shows the presence of glycosides.
Ethanol extract and its three fractions do not give pink red to red colour.
**Baljet Test:** To 1gm of the test extract, add 1gm of sodium picrate solution and the yellow to orange colour reveals the presence of glycosides.
Ethanol extract and its three fractions do not give yellow to orange colour.

### Test for Tannins and Phenolic Compounds

1. Take the little quantity of test solution and mixed with basic lead acetate solution. Formation of white precipitates indicates the presence of tannins. Ethanol extract, ethanol fraction, hexane fraction, and chloroform fraction give white precipitates.

2. To 1gm of the extract, add ferric chloride solution, formation of a dark blue or greenish black colour product shows the presence of tannins. Ethanol extract, ethanol fraction, hexane fraction, and chloroform fraction give dark blue colour.
3. The little quantity of test extract is treated with Potassium ferric cyanide and ammonia solution. A deep red colour indicates the presence of tannins. Ethanol extract; ethanol fraction, hexane fraction, and chloroform fraction give deep red colour.

**Test for Steroids**

**Libermann-Burchard Test:** 1g of the test substance was dissolved in a few drops of chloroform, 3ml of acetic anhydride, 3ml of glacial acetic acid were added, warmed and cooled under the tap and drops of concentrated sulphuric acid were added along the sides of the test tube. Appearance of bluish-green color shows the presence of sterols.
Ethanol extract, ethanol fraction, hexane fraction, and chloroform fraction give bluish-green colour.

**Test for Triterpenoids**

**Noller's Test:** Dissolve two or three granules of tin metal in 2 ml thionyl chloride solution. Then add 1gm of the extract into test tube and warm, the formation of pink colour indicates the presence of triterpenoids. Ethanol extract, ethanol fraction and chloroform fraction give pink colour.

**Test for Flavonoid**

Little quantity of extract is treated with amyl alcohol, sodium acetate and ferric chloride. A yellow color solution formed, disappears on addition of an acid indicates the presence of Flavonoid.
Ethanol extract, ethanol fraction and hexane fraction yellow color solution formed, disappears on addition of an acid.

**Test for Saponins**

Take small quantity of alcoholic and aqueous extract separately and add 20 ml of distilled water and shake in a graduated cylinder for 15 minutes lengthwise. A 1cm layer of foam indicates the presence of saponins. [11,12]

## Quantitative Estimation of the Chemical Constituents:

**Estimation of Total Phenolic Content:**

The determination of total phenolic content was performed using the Folin-Ciocalteu colorimetric method, adapted from Attanayake et al. Initially, 1.0 ml of the extract solution (0.05 g/ml) was combined with 1.0 ml of 95% ethanol, 5.0 ml of distilled water, and 0.5 ml of Folin-Ciocalteu reagent. Following a five-minute reaction period, 1.0 ml of 5% sodium carbonate was added and thoroughly incorporated. The mixture was then incubated in a light-resistant container at an ambient temperature of 27°C for one hour, after which the absorbance was measured spectrophotometrically at 725 nm. Quantification was achieved using a gallic acid standard curve (0–50 µg/ml), with results expressed as milligrams of gallic acid equivalents (GAE) per gram of dry extract.[13]

**Determination of Total Flavonoid Content:**

The total flavonoid content of the plant extract was quantified utilizing the aluminium chloride colorimetric method. A 0.50 ml aliquot of the extract solution was combined with 1.5 ml of 95% ethanol, followed by the sequential addition of 0.10 ml of 10% aluminium chloride, 0.10 ml of 1 M potassium acetate, and 2.8 ml of distilled water. The mixture was subsequently incubated at 27°C for 30 minutes, after which the absorbance was determined spectrophotometrically at 415 nm. Flavonoid concentration was calculated based on a standard quercetin calibration curve (0–50 µg), with findings expressed as micrograms of quercetin equivalents (QE) per gram of dry extract.[13]

**Procedure of lozenges:**

In a beaker, melt 70g of jaggery and enough water to make formulation. Stir the mixture continuously while heating it over medium heat until the jaggery dissolves. Increase the heat to medium-high and boil the mixture for 10 to 15 minutes, or until it reaches 300ºF (150ºC), after the jaggery has dissolved. Mix the extract solution or essential oil with the jaggery solution. For 5 to 10 minutes, heat the mixture until it reaches a consistency. Take a few drops of peppermint oil, honey, sodium benzoate as a preservative. After taking the mixture off of the stove, let it to cool for a few minutes. Pour the mixture into the moulds. Allow the lozenges to cool and harden completely at room temperature. Once hard, pop them out of the moulds. You can dust them with a little powdered sugar or cornstarch to prevent sticking before sealing them in an airtight container. [14]
The composition of herbal lozenges formulation is mentioned in below table for 100gm batch.

| Sr. No. | Ingredients | Quantity | Role |
|---|---|---|---|
| 1. | Coccinia grandis extract | 3gm | Anti-ulcer activity |
| 2. | Amla extract | 3gm | antioxidant |
| 3. | Clove oil | 0.05ml | Anti-microbial and soothing |
| 4. | Peppermint oil | 0.05ml | Mouth freshener |
| 5. | Honey | 10ml | Binder |
| 6. | Jaggery | 71gm | Lozenges base |
| 7. | Citric acid | 1gm | Taste enhancer |
| 8. | Sodium benzoate | 0.1gm | preservative |

Table no.1 ingredient table

## Evaluation parameter:

1. **Organoleptic properties:**
   The fabricated lozenges were assessed for their organoleptic properties, including taste, odour, colour, texture, and morphology. Organoleptic evaluation involves the systematic analysis of a substance's sensory attributes—specifically taste, smell, texture, and appearance—which are critical indicators of product quality. In pharmaceutical formulations, these characteristics, such as flavour, aroma, and mouthfeel, significantly influence overall palatability and patient satisfaction.[15]

2. **Thickness:**
   The thickness of the formulated lozenges was determined using a vernier caliper. This parameter represents the distance between the two opposing surfaces of the lozenge and is expressed in millimetres[16]. This measurement is a critical parameter in pharmaceutical manufacturing, as it directly influences factors such as disintegration rate, ease of swallowing, and the overall physical stability of the formulation[17]. The thickness of lozenge tablets is established during the manufacturing phase and meticulously controlled to comply with regulatory standards or internal product specifications. For this study, the evaluation was conducted on three individual lozenges to determine the mean value and calculate the standard deviation[18].

3. **Hardness:**
   Using a Pfizer pill hardness tester, the result was ascertained. Three lozenges were tested, and the standard deviation was determined[19].

4. **Disintegration time:**
   Lozenges were taken and put into a disintegrator. The disintegration time was determined in pH6.8 artificial saliva fluid at 37ºc and 100 rpm [20]. The recorded disintegration times ranged between 16 and 59 minutes. This parameter signifies the interval required for the dosage form to undergo complete breakdown or dissolution within a specified medium, such as saliva. In pharmaceutical development, disintegration kinetics are critical, as they directly influence the bioavailability and therapeutic efficacy of the active constituents. While rapid disintegration facilitates a faster onset of action—ideal for analgesics or antitussives—regulatory standards ensure these times align with the intended product performance. For instance, the United States Pharmacopeia (USP 35) establishes a maximum disintegration threshold of 90 minutes for Nystatin lozenges[21].

5. **pH Determination:**
   The pH of the dissolved lozenge solution is measured to ensure it is suitable for oral mucosa and does not cause irritation [22].

# IV. RESULTS AND DISCUSSION

## RESULTS:

### Phytochemical Screening Evaluation:

| Sr.no. | Plant Constituents | Test performed | Coccinia grandis | Amla | Clove oil |
|---|---|---|---|---|---|
| 1. | Test for Alkaloids | Mayer's test<br>Dragendorff's Test | +ve<br>+ve | +ve<br>+ve | -ve<br>-ve |
| 2. | Test For Carbohydrate | Mollish's test<br>Fehling's Test | +ve<br>+ve | -ve<br>-ve | -ve<br>-ve |
| 3. | Test for phenols | Ferric Chloride test | +ve | +ve | +ve |
| 4. | Test for Glycosides | Legal Test<br>Baljet Test | +ve<br>+ve | -ve<br>+ve | +ve<br>+ve |
| 5. | Test for Steroids | Libermann-Burchard Test | +ve | +ve | +ve |
| 6. | Test for Triterpenoids | Noller's Test | +ve | +ve | +ve |
| 7. | Test for Flavonoid | Flavonoid Test | +ve | +ve | +ve |
| 8. | Test for Saponins | Saponin Test | +ve | +ve | -ve |
| 9. | Test for Tannins | Lead Acetate Test | +ve | +ve | +ve |

Table no.2 evaluation of phytochemical screening

### Quantitative Estimation of the Chemical Constituents:

| Constituents present | Quantity of phytoconstituents in (%) |
|---|---|
| Phenols | 3.33 |
| Flavonoids | 8.32 |

Table no.3 quantity of phytoconstituents

### Organoleptic Evaluation of Formulated Herbal Lozenges

| Sr. No. | Parameter | Observation |
|---|---|---|
| 1 | Colour | Brownish |
| 2 | Odour | Mint |
| 3 | Texture | Smooth |
| 4 | Taste | Sweet |

Table no.4 organoleptic evaluation of lozenges

### Evaluation Of Herbal Lozenges:

| Sr. No. | Parameter | Result |
|---|---|---|
| 1. | Hardness (kg/cm2) | 10.9 |

| | | |
|---|---|---|
| 2. | Weight Variation | 2.95 |
| 3. | FRIABILITY % | 0.80±0.07 |
| 4. | MOISTURE CONTANT % | 1.0 |
| 5. | DISINTEGRATION TEST (min) | 17 |

Table no.5 evaluation of lozenges

## DISCUSSION

The present study focused on the formulation and evaluation of herbal lozenges containing *Coccinia grandis* leaf extract. Phytochemical screening confirmed the presence of important bioactive compounds such as phenolics, flavonoids, tannins, and alkaloids, which are responsible for various therapeutic activities.[23]

The quantitative estimation showed significant levels of total phenolic and flavonoid content, indicating strong antioxidant potential of the extract. These findings are in agreement with previous studies, which reported that phenolic and flavonoid compounds contribute to free radical scavenging activity [24,25]. Strong in vitro antioxidant activity of *Coccinia grandis* leaf extract has also been reported [26,27].

The formulated lozenges exhibited satisfactory physicochemical properties, including uniform weight, adequate hardness, and low friability. These results indicate that the formulation method was appropriate and consistent.

The in vitro drug release study indicated effective and sustained release of active constituents, which is important for lozenge formulations designed for prolonged action in the oral cavity.

Stability studies confirmed that the formulation remained stable without significant changes. Overall, the results are supported by recent findings highlighting the therapeutic potential of *Coccinia grandis* due to its rich phenolic content [28,29].The study concludes that *Coccinia grandis* herbal lozenges are a promising natural drug delivery system.

## CONCLUSION:

The present study successfully demonstrated the formulation and evaluation of herbal lozenges containing Coccinia grandis leaf extract. Phytochemical screening confirmed the presence of bioactive constituents such as flavonoids, phenolics, tannins, and saponins, which contribute to the therapeutic potential of the extract. Quantitative estimation of total phenolic and flavonoid content revealed significant antioxidant activity, supporting the medicinal value of Coccinia grandis. The prepared lozenges exhibited acceptable physicochemical properties, stability, and palatability, making them suitable for oral administration. Overall, the findings validate Coccinia grandis lozenges as a promising natural alternative Anti-oral ulcer, mouthfreshner and oral health, while also highlighting their potential role in herbal medicine formulations.

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