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"Formulation And Evaluation Of Polyherbal Lozenges For Cold And Flu Defense"

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ABSTRACT

This study presents the formulation and evaluation of polyherbal lozenges designed to provide defense against cold and flu symptoms. Cold and flu are common viral infections affecting millions worldwide, leading to discomfort, productivity loss, and, in severe cases, complications. Herbal remedies have been historically used to alleviate symptoms associated with these infections due to their antiviral, anti-inflammatory, and immune-boosting properties. In this research, a combination of potent herbs known for their efficacy in combating cold and flu symptoms, such as Echinacea, elderberry, ginger, and licorice, were selected and incorporated into lozenges. The formulation process involved optimizing ingredient proportions to ensure maximum efficacy and palatability. The developed lozenges were subjected to various physicochemical and pharmacotechnical evaluations to assess their quality, including uniformity of weight, hardness, disintegration time, and drug release profile. Furthermore, the antimicrobial activity of the lozenges against common cold and flu viruses was investigated through in vitro assays. The results demonstrated that the polyherbal lozenges exhibited desirable physicochemical properties and demonstrated significant antiviral activity against the tested pathogens. Moreover, sensory evaluation studies indicated high acceptability among potential users. Overall, the findings suggest that these polyherbal lozenges have the potential to serve as an effective and convenient prophylactic or therapeutic option for individuals seeking natural remedies for cold and flu defense. Further clinical trials are warranted to validate their efficacy and safety in real-world settings

Key Word: Lozenges, Herbal plant, Phytochemical Screening, Anti-microbial activity etc.

I. INTRODUCTION:

➢ LOZENGES

Lozenges are dosage forms made of solid materials that are meant to dissolve or disintegrate gradually in the mouth. They have one or more active components in them. For a pleasing taste, they are seasoned and sweetened. Although its main purpose is topical, it may also contain substances with systemic effects. A lozenge is a solid concoction made of sugar and gum, the latter of which gives the lozenge firmness and cohesion and allows for the medication to release gradually. It is used to treat the throat and mouth in order to administer cough or digestive medications gradually. Anesthetics, demulcents, and antiseptics can all be found in lozenges.

➢ TYPES OF LOZENGES –

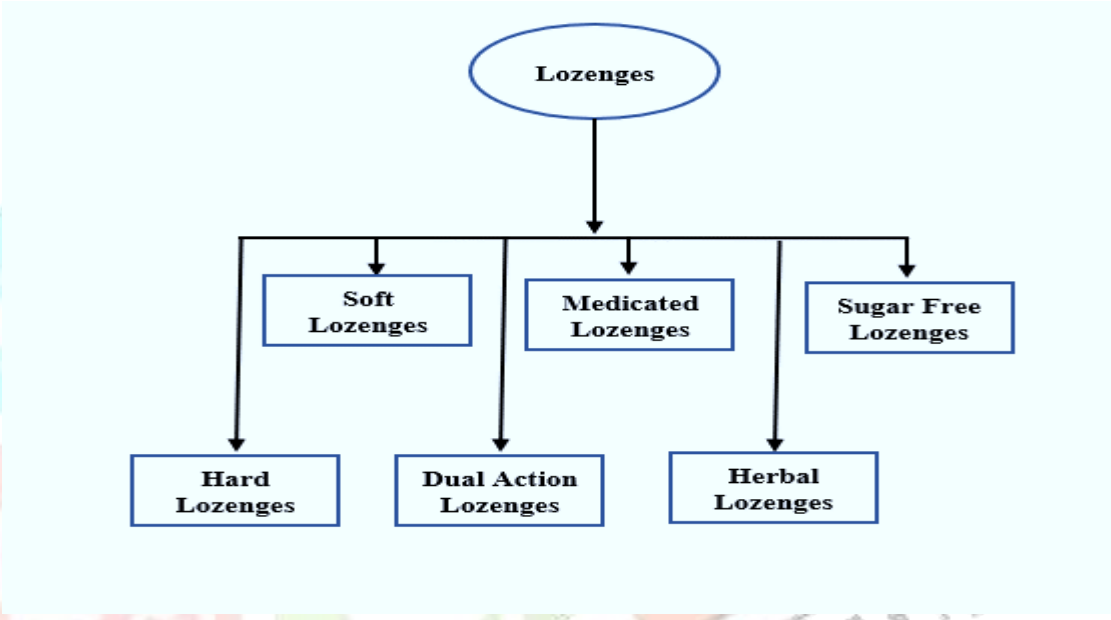

Figure1: Types of Lozenges

➢ HERBAL LOZEGES:

Herbal lozenges are medicated tablets designed to dissolve slowly in the mouth, releasing herbal extracts and other natural ingredients that provide therapeutic benefits. These lozenges are commonly used to soothe sore throats, alleviate coughs, and provide relief from various respiratory symptoms.

II. EXPERIMENTAL:

1. PLANTS: The standard raw herbal plant (Turmeric, Honey, Guduchi, Clove, Ginger) are procurement from local market.

2. METHODS:

➢ **CHARACTERIZATION OF HERBAL PLANTS:**

All plant materials and raw materials were characterized by their physical and morphological characters.

➢ **PREPARATION OF DECOCTION:**

1. Cloves Decoction:

The Cloves was taken wash it. These are place to boil with water until the extract is obtained. Now filter the extract.

2. Turmeric Decoction

The Turmeric rhizomes was taken and make pieces of these rhizomes. These pieces are place to boil with water until the yellow colour extract is obtained. Now filter out the extract.

3. Ginger Decoction

The Ginger rhizomes was taken and make pieces of these rhizomes. These pieces were place to boil with water until the extract is obtained. Now filter out the extract.

4. Guduchi (Giloy) Decoction

The stem of Guduchi was taken and makes pieces of this stem. These pieces were place to boil with water until the extract was obtained. Then filter out the extract.

➢ **DETERMINATION OF ASH VALUES:**

Crude drug incineration results in an ash residue having inorganic material.

a) Total Ash:

- 3 gm of each dried herbal plant powder was weight and take in to Silica Crucible.
- It was heated with burner, using flame about 2 cm high, heated till vapors almost cease to be evolved strongly until all the carbon is burnt off,
- The product was cooled in desiccator.
- Ash was weight and calculated the percentage of total ash.

b) Acid Insoluble Ash:

- The ash was boiled for 5 min with 25 ml of HCl.
- Insoluble matter was filtered by using ash less filter paper and washed with hot water.

- Ignited and weight the residue.
- Percentage of acid insoluble ash was calculated with reference of the air-dried herbal plant.

c) Water Insoluble Ash:

- 2gm of ash was boiled with 25 ml of water for 5 min.
- It was filter with ashless filter paper and insoluble matter.
- It was placed again to muffler furnace to get ash.
- The total wight and percentage was calculated of insoluble ash.

➢ DETERMINATION EXTRACTIVE VALUE:

5 gm of dried coarsely powdered of Clove, turmeric and ginger rhizomes, and guduchiwas taken separately. Macerated it with 100 ml of water and ethanol for 24 hours in close 250 ml conical flask. During first 6 hours shake this all flask frequently and allow it to stand for 18 hours. After 18 hours filter it.

Empty Petry disk should weight first. Taken 25 ml of filtrate to evaporated in Petry disk under oven at 105^0C. After drying of Petry disk weight it again. Now solvent soluble extractive percentage was calculated with reference to air dried drug.

➢ PHYTOCHEMICAL SCREENING:

It was done to determine the secondary plant constituents. The decoction of Cloves, Turmeric rhizomes, Ginger rhizome and Guduchi was taken. The test has performed on each decoction separately in test tube as follows.

Table 1: Phytochemical Screening of plant extract

PHYTOCHEMICAL INVESTIGATION	TEST	PROCEDURE	OBSEVATION
Alkaloids Test	**Dragendorff's Test**	Take 1ml of extract solution and one drop of Dragendorff's reagent (potassium bismuth iodide solution).	Shows reddish-brown precipitate in presence of alkaloid.
	Hager's Test	0.1ml of solution and 1 drop of Hager's reagent.	Shows Yellow precipitate in presence of alkaloid.
	Mayers Test	0.1ml of solution and 1 drop of Mayer reagent.	Shows Cream precipitate in presence of alkaloid.

	Wagner's Test	Take 0.1ml of extract solution and 1 drop of Wagner's reagent.	Shows Orange precipitate in presence of alkaloid.
Glycoside	**Raymond's Test**	The test arrangement takes on a violet tone when treated with dinitrobenzene in a hot methanolic antacid.	Give Voilet colour.
	Legal's Test	The extract was given a blood-red appearance after being treated with pyridine and then alkaline sodium nitroprusside solution.	Blood red colour appearance
Carbohydrates	**Anthrone Test**	Combine 0.5 ml hydroalcoholic acid and plant extract with 2 mL anthrone reagent solution.	Green or Blue colour appear
	Benedict's Test	Benedict's reagent was taken and added test solution (extract). Mixed it well. It was heated on boiling water bath for 5 min.	Shows green, red or yellow colour in presence of decreasing sugar.
	Fehling's Test:	It was carried out by mixing 1 ml of Fehling's solution A and B in equal parts with 2 ml of hydroalcoholic extract and heating for a few minutes.	The production of a crimson colour.
	Molisch test	Take the extract and added ά napthol and conc. H_2SO_4.	Purple colour if produced indicates +ve test.
Flavonoids	**Shinoda's Test**	Take extract with 5ml ethanol and few drops of concentrated HCl Added 0.5g magnesium turnings.	Shows orange pink red to purple colour in presence of flavonoids.
Steroids:	**Salkowski Tests**	Chloroform solution of the extract then add concentrated sulphuric acid with shaking and kept for some time.	Shows red colour in presence of steroids.
	Lieberman Burchard Tests	Take chloroform solution of the extract with few drops of acetic	Show reddish colour at the junction of 2

		anhydride and 1 ml of concentrated H_2SO_4.	layers form in presence of steroids.
Phenolic And Tannins Test	**Ferric chloride Test**	Take extract and add Ferric chloride sol.	Shows green or black colour in presence of Phenolic and tannins.
	Lead Acetate Test	Take extract and add Lead acetate solution	Shows precipitate in presence of phenolic compound and tannins.
	Phenazone test	In 5 ml of aqueous extract 0.5gm of sodium acid phosphate was added and warmed and then filtered. Then added 2% Phenazone solution.	Bulky precipitate was formed, which was often coloured.

➢ FORMULATION OF LOZENGES:

The lozenges formulation was prepared by using specific ratio of herbal extract and other excipients were used.

Table 2: Formulation composition of herbal lozenges

S. No.	Ingredient	Quantity (%)
1.	Sucrose	52.00
2.	Liquid Glucose	32.00
3.	Water	20.00
4.	Herbal Extract 3.00 gm of each of the herbal extract	15.00
5.	Citric acid	0.1
6.	Gum Acacia	0.9

➢ **EVALUATION TEST FOR LOZENGES:**

1. ORGANOLEPTIC CHARACTERISTICS:

It involves colour, odour, taste determination. The effectiveness and safety of produced herbal sweets was must be determined by quality evaluation. The formulation assesses using physicochemical and phytochemical comparisons to the standard criteria. Sensory assessment was also carried out and characterized as a field of study that aimed to elicit, quantify, examine, and interpret responses to those food and material features as viewed through the senses of sight, smell, taste, touch.

2. **PHYSICOCHEMICAL EVALUATION OF HERBAL LOZENGES:** Molisch Test, Fehling's Test, Mayers Test, Hanger Reagent Test, Wagner Test, Salkowski Test, Sulphuric Acid Test, Ferric Chloride Test was performed on the herbal lozenges. Result is shown on table 6.11

3. WEIGHT VARIATION TEST:

A suitable, previously calibrated balance was used to precisely weigh ten lozenges, and the average weight of each was noted. By adding together, the weight of all 10 of five batch lozenges and dividing it by 10, the average weight was determined.

4. HARDNESS TEST:

A specific level of firmness or hardness is necessary for solid formulations, whether they be lozenges, to withstand mechanical shocks from manufacturing, packaging, and shipping handling. Using the Monsanto tablet hardness tester is another way to measure hardness; with this test, when lozenges is sandwiched between two anvils, the anvils are forced together with such crushing power that the candy just breaks is captured. Zero reading is taken before the experiment begins. Ten lozenges of five batch were tested for hardness in Kg/cm2, and the average toughness was recorded.

Average Hardness = Total Hardness of Lozenges/ Numbers of Lozenges

5. TESTING FOR FRIABILITY:

Friability is a different indicator of a lozenge's durability. The Roche friabilator, a plastic circular chamber that rotates at 25 rpm and drops at 25 °C. The disintegration machine was turned on, and the amount of time needed to break up all ten lozenges of five batch was noted, and the mean time was determined.

- Ten prepared lozenges was selected randomly and weight (W_o)
- Placed the lozenges in friabilator drum, switch on the apparatus and timer was adjusted at 4 min and the speed at 25 rpm.
- At the end of operation, remove the tablet from the friabilator , dedust and reweigh (W_f).
- Friability was expressed as percentage loss in weight-

$$\% \text{ Friability} = (W_o - W_f / W_o) \times 100$$

W_o = Initial Weight, W_f = Final Weight

6. PH MEASUREMENT:

A 1% W/V solution of lozenges was made by dissolving 1 g of lozenges in 100 ml of distilled water, and the pH of the solution was recorded. The alkalinity or acidity of a product is expressed by using a pH meter, a scale from 1.0 to 14.0.

7. EVALUATION OF MICROBIAL ANALYSIS:

Determination of bacteria helps in analysis of sample quality after the production and storage practices. This is done by cup plate method and total plate count method (serialdilution method).

(a) Serial dilution method:

Procedure

- Mixed the bacterial suspension by rolling the test tubes between the palms of hands to ensure even dispersion of cells in the cultures.
- By using sterile pipette, aseptically transfer 1ml from the bacterial suspension to first flask containing 99ml saline solution.
- Discard the pipette in the beaker of disinfectant. The bacterial suspension has been diluted 100 times (10^{-2}). Mixed the contents of the first flask and transfer 1ml suspension to the second flask (containing 99ml saline) with a sterile pipette.
- Thisoriginal culture was diluted (10^{-4}).
- Mixed the contents of the second flask and transfer 1ml suspension to third flask containing 99ml sterile solution with a sterile pipette.
- Finally, in the third flask bacterial suspension is diluted to 10^{-6}.
- Add approximately 15 to 20ml nutrient agar medium into three large size test tubes, sterilize by autoclave at 121^0C for 15 minutes and cooled at 45^{o}C.
- Mixed all the dilutions and transfer 1ml from each dilution to large size test tubes.
- Mixed the bacterial suspension by rolling the test tubes between the palms of hands to ensure even dispersion of culture in the medium.
- Immediately pour the media of three test tubes into 3 sterile petri plates to solidify.
- Incubate these plates in an inverted position for 24 to 48 hours at 37^{o}C.

(b) Cup plate method

Procedure

- Each petri dish was filled to a depth of 4-5 mm with a nutrient agar medium that was previously inoculated with suitable inoculums of suitable test organism, and then allowed to solidify.
- The petri dish was specially selected with flat bottom and were placed on level surface so as to ensure that the layer of medium is in uniform thickness.
- The petri dishes were sterilized at 160-170°C in hot air oven for 30 mins before use.
- Small sterile borer of uniform size was placed approximately at 10 cm height, having an internal diameter of approximately 6-8 mm and made of aluminium (or) stainless steel.
- Each plate was divided in to five equal portions along the diameter. To each portion one cylindrical cavity was made in medium with the help of sterile borer. Five cavities for test compounds were made. The petri dishes were incubated at 37°C for 18 hours. Diameter of the zone of inhibition (ZOI) was measured and the average diameter for each sample was calculated.

➢ **SHELF-LIFE STUDY:**

Shelf-life study was started from the 2nd day of making the product. Herbal Lozenges was stored under the refrigerated condition for 4 weeks in its packaging materials. The product was observed at frequent intervals for any change in appropriate color, odour, texture, taste and moisture.

III. RESULT:

➢ **DETERMINATION OF ASH VALUES:**

Ash value of all herbal plant was determined by their value of total ash, insoluble in acid, insoluble water, which is shown on table 3

Table 3: Determination of Ash Value of herbal plant

Determination of Ash Value of herbal plant				
S. No.	**Herbal Plant**	**Total Ash Value (%)**	**Insoluble acid value (%)**	**Soluble in water value (%)**
1.	Clove	3.2	0.75	2.3
2.	Turmeric	5.3	0.25	3.1
3.	Ginger	4.8	0.58	4.1
4.	Guduchi	9.2	0.27	6.3

➢ DETERMINATION EXTRACTIVE VALUE:

The % water soluble extractive value of herbal plant was determined by calculating their weight of residue and weight of herbal drug table show the extractive value of herbal plants.

Table 4: Extractive value of Herbal Plants

S. No.	Solvent	Herbal Plant	Extract Colour	Extractive value (%) (w/w)
1.	Water	Clove	Dark Brown	9.5
2.	Water	Turmeric	Yellow	21.6
3.	Water	Ginger	Pale Yellow	10
4.	Water	Guduchi	Greenish-Brown	16.5

➢ PHYTOCHEMICAL SCREENING:

1. Test for Alkaloids:

The test of alkaloids for all herbal extract were performed and satisfactory result shown in table 5.

Table 5: Test for alkaloids of herbal plants

S. No.	Alkaloid Test	Turmeric	Clove	Ginger	Guduchi	Honey
1	Dragendorff's test	+	+	++	+	-
2	Hager's test	+	-	++	+	-
3	Mayers test	+	+	++	+	-
4	Wagner's test	+	+	+	+	-

2. Test for Glycoside:

The test for glycoside of all herbal plants was performed and result are shown in table 6.

Table 6: Test for glycoside of herbal plants

S. No.	Glycoside Test	Turmeric	Clove	Ginger	Guduchi	Honey
1	Raymond's test	+	+	++	+	-
2	Legal's test	+	-	++	+	-
3	Bromine water test	+	+	++	+	-

3. Test for Carbohydrate :

The test for carbohydrate of all herbal plant including honey was performed and results shown in table 7.

Table 7: Test for carbohydrates of herbal plants

S. No.	Carbohydrate Test	Turmeric	Clove	Ginger	Guduchi	Honey
1	Anthrone test	+	-	+	+	++
2	Benedict's test	+	-	+	+	++
3	Fehling's Test	+	+	+	+	++
4	Molisch test	+	-	+	+	++

4. Test for Flavonoids:

The test for flavonoid of all herbal plant including honey was performed and result shown in table 8.

Table 8: Test for flavonoids of herbal plants

S. No.	Flavonoid Test	Turmeric	Clove	Ginger	Guduchi	Honey
1	Shinoda's test	+	+	+	+	+

5. Test for steroids:

The test for steroids of all herbal plant extract was performed and result shown in table 9.

Table 9: Test for steroids of herbal plant

S. No.	Flavonoid Test	Turmeric	Clove	Ginger	Guduchi	Honey
1	Salkowski Tests	+	-	+	+	+
2	Lieberman Burchard tests	+	+	+	+	+

6. Phenolic and Tannin test:

The phenolic compounds and tannin test for all herbal plant was performed and results shown in table 10.

Table 10: Test for phenolic and tannin of herbal plants

S. No.	Flavonoid Test	Turmeric	Clove	Ginger	Guduchi	Honey
1	Lead acetate test	+	+	+	+	+
2	Ferric chloride test	+	+	+	+	+
3	Phenazone test	+	+	+	+	+

➢ FORMULATION OF LOZENGES:

The lozenges formulation was prepared by using specific ratio of herbal extract and other excipients were used. In which total weight of prepared lozenges was 3gm per lozenges. The formulation table shown in table 11.

Table 11: Formulation composition of herbal lozenges

S. No.	Ingredient	Quantity (%)	Quantity (Weight) for single Lozenges	Quantity for 100 Lozenges
1.	Sucrose	52.00	1.56 gm	156 gm
2.	Liquid Glucose	32.00	0.96 ml	96 ml
3.	Water	20.00	0.6 ml	60 ml
4.	Herbal Extract 3.00 gm of each of the herbal extract	15.00	0.45 gm	45 gm
5.	Citric acid	0.1	0.03gm	3 gm
6.	Gum Acacia	0.9	0.27 gm	27 gm
	Total		3 gm	300 gm

Figure 2: formulation of polyherbal lozenges

➢ EVALUATION TEST FOR HERBAL LOZENGES:

1. ORGANOLEPTIC CHARACTERISTICS:

The organoleptic evaluation test for prepared herbal lozenges was performed and result are shown in table 12.

Table 12: Organoleptic Character of herbal lozenges

Organoleptic Characters	Observation
Colour	Brownish- black
Odour	Pleasant
Taste	Sweet-Pungent
Self-life study (4 weeks)	Stable

2. PHYSICOCHEMICAL EVALUATION OF HERBAL LOZENGES:

The physiochemical evaluation of herbal lozenges was determined and result are shown in table 13.

Table 13. Phytochemicals evaluation of herbal lozenges

S. No.	Phytoconstituents	Test	Result
1.	Alkaloids	Mayers test	+
		Wagner's test	+
2.	Glycoside	Raymond's test	+
		Legal's test	-
3.	Carbohydrate	Fehling's Test	+
		Molisch test	+
4.	Flavonoid	Shinoda's test	+
5.	Steroid	Salkowski Tests	+
		Lieberman Burchard tests	+
6.	Tannin	Lead acetate test	+

Ferric chloride test	+

3. WEIGHT VARIATION TEST:

The weight variation of prepared herbal lozenges was determined for all batch which was formulated and result are shown in table 14.

4. HARDNESS TEST:

The evaluation of herbal lozenges by hardness test of all five batch was performed and result are shown in table 14.

5. TESTING FOR FRIABILITY:

The evaluation of herbal lozenges by friabilty test of all five bacth was performed and result are shown in table 14.

Table 14: Evaluation of herbal lozenges by weight variation, hardness test and friability test

Batch No.	Weight Variation Test (gm)	Hardness Test (kg/cm^2)	Friability Test
B1	3.1	6.8	2.9
B2	3.0	6.5	2.9
B3	2.9	7.2	2.7
B4	2.8	6.9	2.7
B5	3.2	7.1	3.0

6. PH MEASUREMENT:

The pH of 1% w/v solution of herbal lozenges in distilled water was determined by using pH meter, the results are shown in the table 6.13

Table 15: pH Measurement of prepared herbal lozenges

Batch No.	pH Measurement
B1	4.4
B2	4.7
B3	4.5
B4	4.5
B5	4.6

➢ EVALUATION OF MICROBIAL ANALYSIS:

(a) Serial Dilution Method:

The different dilution was prepared for microbial analysis of prepared lozenges by serial dilution method which are shown in figure 3.

Figure 3: Microbial analysis by Serial dilution

(b) Cup Plate Method:

The nutrient agar media was prepared by using different dilution of herbal lozenges for microbial analysis of prepared herbal lozenges by cup plate method and the microbial colony was countered after 2-3 days and the result of microbial analysis by serial dilution and cup plate method are shown in table 16.

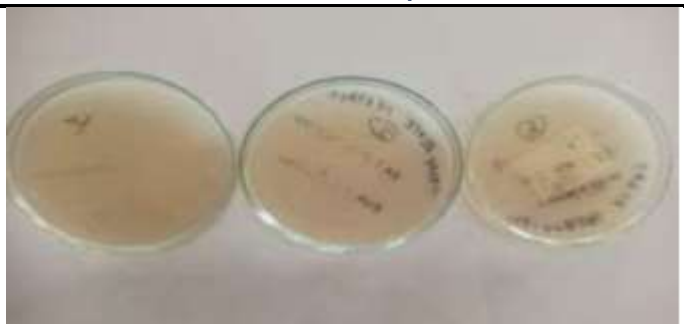

Figure 4: Microbial analysis by cup plate method

Table 16: Microbial analysis of herbal lozenges

Days	Dilution Used	Colony Count (cfu/ml)
Day 2	10^{-2}	2
	10^{-4}	3
	10^{-6}	0
Day 3	10^{-2}	0
	10^{-4}	0
	10^{-6}	0

➢ **SHELF-LIFE STUDY:**

The self-life of prepared herbal lozenges was determined and the observations are as follow:

Table 17: Self life study of prepared lozenges

Day	Observation
Day1	No change in color, odour, texture and taste
Day2	No change in color, odour, texture and taste

Day3	No change in color, odour, texture and taste
Day4	No change in color, odour, texture and taste

IV. CONCLUSION:

The preparation of lozenges based on herbal plants was the subject of this investigation. In terms of overall product attributes, the herbal lozenges formulations made with Turmeric, Clove, Ginger, Guduchi and Honey outperformed the others. Before formulate herbal lozenges the ash value and extractive value has been determined. The formulation was prepared based on sucrose and glucose and evaluation of prepared lozenges was evaluated by different physiochemical investigation, weight variation hardness testing and friability testing of prepared herbal lozenges. The microbial analysis of lozenges was done by serial dilution and cup plate method which give satisfactory result.

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