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Development and Characterization of Glycyrrhizin Loaded Herbal Lozenges for Mouth Ulcer Treatment

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Abstract

Abrus precatorius Linn. A member of the Fabaceae family, this plant has numerous benefits. The seeds, roots, and leaves are used medicinally. The roots and leaves include astringent, pleasant, emetic, diuretic, and anthelmintic properties. The oral gel dosage form is extensively used in the treatment of mouth ulcers, but it has numerous disadvantages, including blistering, burning, itching, and skin irritation, as well as the fact that it can be swallowed and dissolve in the mouth. To address the aforementioned issue, herbal lozenges were made from Abrus precatorius L. leaves. Microscopy (powder research), morphology, extraction (maceration method), acidic isolation, and thin-layer chromatography were employed to prepare glycyrrhizin acid extract. Then hard lozenges were made and evaluated using a variety of procedures, including average weight and weight fluctuation, friability, hardness testing, disintegration, and moisture content measurement. The leaves contained fibers, trichomes, and xylems. The powder properties of Abrus precatorius leaves revealed the presence of glycyrrhizin. Glycyrrhizin was extracted from a water extract of leaves and identified using chemical testing and Thin Layer Chromatography. Then, lozenges were made in five batches. The first three batches had been consolidated, therefore only the fourth and fifth batches were evaluated. According to observations and data, the fourth batch of lozenges outperformed batch-5 in all tests. So, batch-4 lozenges are preferable. So it is concluded that the lozenge composition can outperform other formulations.

Keywords: *Abrus precatorius* L., glycyrrhizin, mouthulcer, Herbal Lozenges, Herbal Formulation, Thin layer chromatography

Introduction

Since ancient times, people have found and utilized therapeutic plants, often known as medicinal herbs, in traditional medical practices[1]. Many plants with therapeutic promise have not yet been subjected to rigorous scientific analysis to determine their phytochemical composition and, if any, pharmacological effects, hence defining their efficacy and safety[2]. The roots and leaves of *Abrus precatorius* L. have astringent, pleasant, emetic, diuretic and anthelmintic properties[3]. Oral gel dosage forms are frequently used to treat mouth ulcers, but they have a number of drawbacks, including the potential for skin irritation, blistering, burning, and dissolving in the mouth. Additionally, you should wait 30 minutes after applying oral gel to a mouth ulcer before eating or drinking anything.

To solve the aforementioned issue, herbal lozenges made from the leaves of the *Abrus precatorius* plant are prepared; they have no negative impact on human health. Medicinal uses of *Abrus precatorius*: Eye complaints like purulent conjunctivitis, Epithelioma, Ulcers, Inflammation that spreads to the face and neck, Granular ophthalmia, Keratitis, Angina pectoris, Myocardial infarction, Valvular insufficiency, Cellulitis, Gangrene, Gastritis, Hypertension, Nephritis, Brain tumor, Cardiomyopathies, Epilepsy, Septicemia shock, Tetanus, Purpura, Typhoid, Dysentery, Snake Bite, Purgative, Emetic, Tonic, Aphrodisiac[4].

The mucous membrane of an oral cavity can become inflamed and become a mouth ulcer, also known as a mouth ulcer or a mucosal ulcer[5]. They are uncomfortable, round, or oval sores that typically develop in the mouth on the inner part of the cheeks or lips. Even though mouth ulcers normally have no important underlying causes, they can occur in connection with an extensive variety of diseases and via a wide range of mechanisms[6]. The most frequent causes of mouth ulcers include poor oral healthcare, infections, anxiety, indigestion, mechanical damage, allergies to foods, hormonal imbalances, skin conditions, and a lack of nutrients like iron insufficiency and vitamin shortages, especially B12 and C deficiency[7]. The three primary types of oral ulcers are: Minor ulcers[8]: These have a diameter of 2 to 8 mm and often go away in 10 to 2 weeks. Major ulcers: Larger, deeper, and frequently with an elevated or uneven border, major ulcers are more severe. In addition to leaving a mouth scar, this kind of ulcer can take many weeks to heal[9]. Herpeti-form ulcers: They are composed of a number of tiny, pinhead-sized lesions that are grouped together[10]. Ulcerative Conditions: Mouth ulcers are quite prevalent and are typically brought on by trauma, such as from poorly fitting dentures, broken teeth, or fillings. To rule out cancer or other

serious illnesses including chronic infections, patients with ulcers that have been present for longer than three weeks should undergo a biopsy or other testing[11].

Lozenges are flavored pharmaceutical dosage forms that are meant to be sucked and retained in the mouth or pharynx. They typically include one or more medications in a sweetened foundation[12]. If the medication is well absorbed through the buccal linings or when it is ingested, lozenges may also have a systemic effect in addition to relieving or pharyngeal symptoms, which are frequently brought on by local illnesses[13]. Lozenges commonly contain drugs like astringents, antitussives, aromatics, antimicrobials, analgesics, antiseptics, anesthetics, corticosteroids, demulcents, and decongestants. However, there are a lot more medications that can be taken as lozenges, so this list is not exhaustive. Additionally, depending on the needs of the specific patient, combining can be done for both a single- and multi-ingredient lozenges[14]. Lozenges can be divided into several different types based on various factors, such as the site of action: i. Local effect Ex. Antiseptics, Decongestants ii. Systemic effect Ex. Vitamins, Nicotine, according to texture and composition- i. Chewy or caramel-based medicated lozenges ii. Compressed tablet lozenges iii. Soft lozenges iv. Hard Lozenges[15].

Although oral gel dosage forms are frequently employed in the treatment of mouth ulcers, there are a number of drawbacks to this gel dosage type like blistering, burning, itching, irritation of skin and main disadvantage is sometimes people swallow and dissolve in mouth this type of gel and its harmful for the body and after oral gel application in on mouth ulcer, people should not eat or drink anything for 30 min. So, to overcome the above problems here Herbal lozenges can be prepared from plant *Abrus precatorius* leaves and it have no side effects on the human body.

Materials and Methods

Materials: The plant material of *Abrus precatorius* L. was obtained from locally. Phluoroglucinol, Ammonia, ethanol, ethyl acetate and silica gel G were procured from Piyush chemicals, Ahmedabad, Gujarat, India. Hydrochloric acid and methanol was obtained from Advent Chembio Pvt. Ltd., Mumbai, Maharashtra, India. Ethanol was procured from Shree Madhi Vibhag Khand Udhyog Sahakari Mandli Ltd., Surat, Gujarat, India. Ammonia solution was obtained from High Purity Lab. Chemicals Pvt. Ltd., Mumbai, Maharashtra, India. Citric acid, sucrose and Hydroxypropyl Methyl Cellulose were procured from S D Fine-Chem Ltd. Mumbai, Maharashtra, India. Corn starch was procured from Roquette India Private Limited, Viramgam, Gujarat, India.

Authentication: The plant was authenticated by Dr. A.M Patel, Botanist, Principle of J&J collage of Science, Nadiad, and herbarium specimen (DDU|FOP|21-22|E-01) was deposited at the department of Pharmacognosy, faculty of pharmacy, Dharmsinh Desai University, Nadiad.

Identification: The seeds were black and crimson. The leaves were pinnate, glabrous, and arranged in pairs with several leaflets. The plant produced clusters of tiny racemes of orange-pink flowers.

Collection: *Abrus precatorius* L. plants were collected by plucking method from nearer villages from Nadiad.

Morphology: Morphology of aerial parts of *Abrus precatorius* L. can be done. Their physical appearance, texture, color odor can be determined by morphology[16].

Microscopy: Microscopy of the dried leaves can be done by powder study.

Powder study: The dry leaves of the plant, which were finely powdered, looked at under a microscope, and analyzed, are known as *Abrus precatorius* L. Take a watch glass, add a small amount of powder, phluoroglucinol, HCl, and water, then form a solution with the brush and a small amount of the prepared solution on a slide and a cover slip that has been coated with glycerin to view under a microscope[17].

Extraction of glycyrrhizin from plant leaves: In this maceration process was used. In order to make a coarse leaf powder, the dry leaves of *Abrus precatorius* L. were first removed and combined. Leaf powder, which had been pounded into a coarse powder, was put into a 1000 ml beaker. After that, the mixture received a suitable volume of water and was cooked for an entire hour. Transfer the remedy to a beaker after an hour. To reach room temperature, the solution that had been heated was cooled. The sample solution was then given methanol, and it was then covered with aluminium foil and left for 24 hours. On the next day, recovered it and drained it using filter paper. Following that, the resulting filtrate was put into a porcelain dish then allowed to evaporate in a hot water bath[18].

Isolation of the glycyrrhizin of the plant: The beaker was filled with coarse leaf powder, which was then given enough water, boiled, and stirred. Decant the liquid supernatant after that. The filtrate from the remaining residue was collected. The pH was then brought down to 2.8 by adding acid (HCl), after which glycyrrhizin precipitated out. Precipitates were filtered and collected after that. Precipitates that require to be acid-free are washed in cold water. Then the precipitate was poured onto the porcelain dish and gently heated to evaporate the water. Then a brown, glossy mass of glycyrrhizin appeared[19].

Thin layer chromatography (TLC) of the Glycyrrhizin: The TLC plate and chamber were taken apart and cleaned. After adding some water, thicken the slurry by adding more silica gel G to the mortar. After that, a thick layer of silica gel G slurry was applied to the plate of TLC (stationary phase), and the plate was put in a hot air oven at 80°C for 30 minutes to activate it. Then, a TLC plate was prepared with the mobile phase to be used for the TLC chamber. Water (2.25), ethanol (6.25), ethyl

acetate (16.25), and ammonia (0.25), for the glycyrrhizin mobile phase. Set up the TLC plate's extract place after that, and then immediately start the mobile phase[20].

Method of preparation for Lozenges: Weigh accurate amount of sugar and add sugar to water, and stir to dissolve. Then add maize starch and citric acid, followed by polymer (HPMC 15cps). Then mix the drug into the solution and add water to make up. Properly dissolve the above substance. Produce a thick solution. Fill the mold with this solution. After that, let it stand for 24 hours to solidify[21].

Evaluation of Lozenges: Average weight and Weight variation test: Weighing 20 lozenges was done. The average weight was then determined. Following that, the average weight of all the lozenges was compared. Confirmed whether or not it came within the acceptable limits. The weight variation was then calculated[22].

$$\text{Average weight}=\frac{\text{Weight of 20 lozenges}}{20}$$

$$\%\ \text{weight variation} = \frac{\text{Initial weight}-\text{Average weight}}{\text{Average weight}} * 100$$

Friability test: A friabilitor was used to evaluate each batch's 20 lozenges for friability. For 4 minutes at a 25 rpm speed. After de-dusting and reweighing the lozenges, the equation was used to calculate the percentage loss in weight[22].

$$\%\ \text{Friability} = \frac{(\textit{Initial weight.} - \textit{weight after friability})}{\textit{Initial weight}} * \mathbf{100}$$

Hardness Test: Three tablets of each formulation were examined by a Monsanto hardness tester to determine the crushing strength of the diametrically opposed compounds. The values of mean and SD were calculated[22].

In vitro mouth Dissolving Time: Each batch formulation's mouth-dissolving time was calculated using USP disintegration equipment. Lozenges were inserted in each tube of the apparatus, and the time it took for them to completely dissolve was recorded using a 100ml phosphate buffer solution with a pH of 6.8 at 37°C. Three duplicates of this test were run. The standard deviation of the average lozenge dissolving time was determined[22].

Moisture content analysis: In a mortar, the sample was weighed and crushed. Following this, a gm of the material was weighed and dried for 24 hours in a desiccator. The sample is weighed 24 hours later. By deducting the end weight from the initial weight of the lozenges, the moisture content may be calculated[22].

Table-1: Composition of lozenges.

S. No.	Ingredient	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5
1.	Sugar	30%	40%	50%	50%	50%
2.	Citric acid	3%	3%	3%	3%	3%
3.	Corn Starch	2.5%	2.5%	3.5%	2.5%	2.0%
4.	HPMC 15cps	2.5%	3.5%	3.5%	3.5%	3.5%
5.	Glycyrrhizin	5%	5%	5%	5%	5%
6.	Flavoring and sweetening Agent	0.5%	0.5%	0.5%	0.5%	0.5%
7.	Water	q. s	q. s	q. s	q. s	q. s

Table-2: Limits of % weight variation.

The average weight of tablet (According to IP/BP)	Limit	The average weight of tablet (According to USP)
80 mg or less	± 10%	130 mg or less
More than 80 mg or less than 250 mg	± 7.5%	130 mg to 324 mg
250 mg or more	± 5%	More than 324 mg

Results and Discussion

Morphology of plant: Stem: The stems are smaller than those of other plants because it is a creeper. When supported by other plants, this attractive, twining, woody vine can grow as high as 10 to 20 feet.

Leaves: They are compounded with tiny oblong leaflets, alternate, and have a feathery texture. The leaves are light green in color, abruptly pinnate, and have individual leaves that are about 1cm long and total leaflets that range in length from 5 to 10 cm. Each leaflet has 10–20 ligulate, oblong leaves that are hairy underneath. The leaves have a sweeter flavor.

Flowers*:* The flower clusters are 1-3 inches long and have a more compact structure. There are red, purple, pink, and even white versions of them. They have a long calyx that is smooth and slightly silky, long pink or white hair that is curled and rounded, deformed monadelphous stamens.

Seeds: Fully developed seeds are found in pods, or as they are sometimes known, are encased in a pod with a light brown hue. Pods are typically 2 to 3cm long. A pod contains 3-5 seeds, each of which is 5–6 mm long and has a smooth surface. The three colors that make up seeds are red, white, and black. Since the seeds are oblong-round in shape, they must resemble ladybird insects. They frequently have an edge marked in black and are a brilliant red color. They are very poisonous.

Roots: The colors they frequently take on range from light brown to dark yellowish. They have a sweeter flavor and a more hairy exterior.

Microscopy of Leaves powder: i. Trichomes: Elongated tubular out growth of an epidermal cell is termed as trichome. ii. Xylem: Xylem tissue comprises a network of dead, hollow elongated conduits such as tracheids or vessels. iii. Fibers: Fibers are elongated thick walled cells with pointed ends, cell walls of which may consist of cellulose and may or may not contain lignin.

Extraction: The extraction was done by Hot Extraction Method. The liquid Extract of *Abrus precatorius* L. leaves was dark brown in color.

Isolation of Glycyrrhizin: The isolated Glycyrrhizin was glossy and black crystalline powder.

Thin Layer Chromatography method for the Glycyrrhizin: i. Spraying Reagent: Vanillin, ii. Mobile phase for glycyrrhizin: Ethyl acetate (16.25): Ethanol (6.25): Water (2.25): Ammonia (0.25),

$$R_f = \frac{\text{Distance travelled by Solute}}{\text{Distance travelled by solvent}}$$

$$R_f \text{ Value} = \frac{4.4}{7.5} = 0.51$$

Preparation of Lozenges: The lozenges were made in five batches. The evaluation was conducted on Batches 4 and 5 because the first three batches failed to solidify and took the form of soft gels.

Evaluation of Lozenges: Here the average weight and weight variation, friability, hardness, In-vitro mouth dissolution time (disintegration time) and moisture content tests of Batch-4 and Batch-5 of prepared lozenges are shown in the Table-3.

According to the aforementioned results, Batch-4 passed the average weight and weight variations test, but Batch-5 failed in accordance with IP, USP, and BP limitations. In this case, friability, as determined by IP, BP, and USP, is not greater than 1%; it passed in batch 4 but failed in Batch-5. Batch-4 has passed the IP, USP, and BP hardness tests, whereas batch 5 failed since the hardness limit is not greater than 10. Here, in vitro mouth dissolving times (also known as disintegration times) for Batches 4, 5, and the bath batch are passed according to IP, USP, and BP. And at last, batch-4's moisture content analysis was successful since it was within the limit of 1.0%, whereas batch 5's analysis failed because it was above the limit of 1.5%.

Table-3: Evaluation of lozenges.

Sr. No	Test	Batch 4	Batch 5
1	Average Weight and weight Variation Test	2.04%	8.7%
2	Friability Test	0.5%	9.0%
3	Hardness Test	10	20
4	Invitro Mouth dissolution time	24	22
5	Moisture Content Analysis	1%	2%

Conclusion

Identification, collection, morphology, microscopy and extraction from plant leaves, chemical tests, isolation of glycyrrhizin, TLC of glycyrrhizin, preparation of lozenges, and parameter evaluations are all carried out here. The batch-4 of lozenges passed all tests than Batch-5, which can be concluded from observation and results. Therefore, Batch-4 lozenges are preferable than Batch-1 to Batch-3, and Batch-5 in terms of preparation. Based on the aforementioned results, batch 4's average weight and weight variation test passed, while batch 5 failed in accordance with IP, USP, and BP limits. Here, friability is less than 1%, passed in batch 4, and failed in batch 5, according to IP, BP, and USP. Batch-4 has passed the IP, USP, and BP hardness tests, while batch 5 failed because the hardness limit is not greater than 10. Here, in vitro mouth dissolving times (also known as disintegration times) for batches 4, 5, and the both batches are passed according to IP, USP, and BP. Finally, batch 4's moisture content analysis was successful because it was below the limit of 1.0%, while batch 5's analysis was unsuccessful because it was above the limit of 1.5%. Thus, lozenges were administered more effectively than other dosage forms.

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