



PREPARATION AND CHARACTERISATION OF HERBAL LOZENGES CONTAINING SOLANUM NIGRUM LEAF EXTRACT FOR THE MANAGEMENT OF MOUTH ULCERS

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ABSTRACT :

Oral ulceration is one of the commonest complaints met with in dental and general practice, and most of the preparations available for it give only short symptomatic relief. The present work was taken up to formulate herbal lozenges containing an ethanolic leaf extract of *Solanum nigrum* and to evaluate them for local use in oral ulcers. Leaves were collected locally and authenticated at the Siddha Central Research Institute, Chennai. The dried leaf powder was extracted with ethanol in a Soxhlet apparatus for about six hours, and 100 g of powder yielded roughly 12.5 g of semisolid extract. Preliminary phytochemical screening of the extract showed alkaloids, carbohydrates, cardiac glycosides, flavonoids, phenols, tannins, saponins, terpenoids and steroids, while amino acids were not detected. Three batches of lozenges (F1, F2 and F3) were prepared by the moulding method using 0.5 g, 1.0 g and 1.5 g of extract respectively, with sucrose, liquid glucose, gelatin, citric acid and peppermint oil kept constant across the batches. The lozenges were evaluated for organoleptic characters, weight variation, hardness, pH, drug content and in-vitro drug release. All three batches were smooth, intact and acceptable in taste, with pH between 6.22 and 6.23 and hardness between 4.5 and 4.6 kg/cm². Release at 30 minutes was 92.4 per cent for F1, 93.0 per cent for F2 and 90.1 per cent for F3. F2 was accordingly taken as the optimised formulation.

1. INTRODUCTION

1.1. Oral ulcer

An oral ulcer is a break in the lining of the mouth in which the covering epithelium is lost and the connective tissue beneath it is laid bare. This is what separates a true ulcer from an erosion, where only part of the epithelial thickness is lost. The lesion is usually round or oval, carries a yellowish white fibrinous slough over its floor, and is ringed by a red inflammatory halo. Ulcers may be single or multiple and may run an acute or a chronic course. Some arise on their own and others appear as one feature of a systemic illness. Because the oral mucosa is exposed continuously to chewing forces, to a mixed microbial flora and to chemical irritants in food, ulceration of the mouth is among the complaints seen most often in dental and general practice.



Figure: 1 Mouth ulcer

1.2. Epidemiology

Recurrent aphthous stomatitis is the commonest of these lesions. Reported prevalence varies a good deal between studies, with figures between about 5 and 25 per cent depending on the diagnostic criteria used, the age of the group studied and the region surveyed. The peak occurrence falls in the second and third decades of life, though the condition is not restricted to any age. A few surveys have reported higher rates among students and among people in the higher socioeconomic groups, and a lower rate among tobacco users, which has been attributed to the keratinising effect of tobacco on the mucosa. Traumatic ulcers, being the result of mechanical, thermal or chemical injury, occur in almost everyone at some time and follow no particular age or demographic pattern. Taken with the discomfort they cause, this frequency explains why oral ulcers account for so large a share of self-medication and over the counter drug use.

1.3. Causes and risk factors

The cause is seldom single, and for recurrent aphthous stomatitis it remains incompletely settled. Local trauma from cheek or tongue biting, sharp or fractured teeth, ill-fitting dentures and orthodontic appliances, or burns from hot and caustic substances, accounts for a large share of cases. Deficiency of iron, folic acid, vitamin B12 and zinc has been linked with more frequent aphthous episodes. An altered local cell mediated immune response with disturbed cytokine production, particularly of tumour necrosis factor alpha and the interleukins, is believed to lie behind aphthous ulceration itself. A positive family history raises both the likelihood and the severity of the recurrent form.

Patients frequently report hormonal swings during the menstrual cycle, emotional stress and anxiety, and sensitivity to foods such as chocolate, coffee, citrus fruit and gluten containing products as triggers for an episode. Streptococcal species and herpes simplex virus have been implicated, although their exact role is still argued. Ulceration may also appear as part of Crohn's disease, ulcerative colitis, coeliac disease, Behcet's disease and HIV infection, or follow the use of non-steroidal anti-inflammatory drugs, nicorandil and certain cytotoxic agents.

1.4. Clinical features

The size, number and distribution of the lesions differ with type and cause, but the presenting picture is fairly consistent. The ulcer is well defined, round to oval, and depressed at the centre. Its floor is yellow, grey or white, representing fibrin clot and necrotic tissue, and the surrounding mucosa is red from local vasodilatation. Pain or burning is often out of proportion to the size of the lesion and is made worse by spicy, acidic or hot food and by contact during speech, chewing and swallowing. The lesion is tender on palpation. In the recurrent forms a tingling or burning sensation may precede the visible ulcer by 24 to 48 hours. Minor ulcers heal without a scar, whereas major aphthous ulcers, being deeper, often leave one.

1.5. Pathophysiology

Aphthous ulceration begins with a pre-ulcerative stage in which lymphocytes and mononuclear cells infiltrate the epithelium. Vacuolation of the epithelium follows, and small micro-ulcerations form and run together into a lesion large enough to be seen. Local production of tumour necrosis factor alpha, interleukin-2 and interferon gamma destroys keratinocytes and holds up re-epithelialisation. Cell mediated cytotoxicity directed at the oral epithelial cells, possibly set off by cross reactive antigens or by local trauma, keeps the process going. Once the barrier is breached the connective tissue below it meets the oral flora and the mechanical and chemical irritation of saliva and food, and the inflammatory response is sustained. Healing then passes through the usual phases of haemostasis, inflammation, proliferation and remodelling, but the mouth is wet, heavily colonised and constantly in motion, so repair here is slower and less straightforward than at a skin site.

1.6. Types of oral ulcers

Minor aphthous ulcers make up the bulk of recurrent aphthous stomatitis. They measure less than 10 mm, are shallow and round or oval, and carry a clear red margin. They favour the non-keratinised mobile mucosa of the lips and cheeks, the floor of the mouth and the underside of the tongue, and heal on their own in 7 to 14 days without scarring.

Major aphthous ulcers, also called Sutton's disease or periaadenitis mucosa necrotica recurrens, are larger and deeper, often above 10 mm, and may take several weeks to a few months to heal. Scarring is common. The soft palate, tonsillar pillars and lips are the usual sites, and the pain and disturbance of function are correspondingly greater.

Herpetiform ulcers are the least common variant. The name refers to their resemblance to herpetic lesions and not to any role of herpes simplex virus. They appear as multiple pinpoint ulcers, sometimes dozens at a time, which may run together into larger irregular areas. Adult women are affected more often, and healing is quicker than in the major form although episodes tend to recur.

Traumatic ulcers follow direct mechanical, thermal, chemical or factitious injury. Unlike the aphthous types they do not recur at the same site once the irritant is removed, and they heal quickly thereafter.

Several other conditions present with oral ulceration and need to be kept in mind, though they lie outside the scope of this study. These include herpetic gingivostomatitis, hand-foot-and-mouth disease, oral lichen planus, pemphigus vulgaris, mucous membrane pemphigoid, mucositis following chemotherapy or radiotherapy, and oral squamous cell carcinoma presenting as a non-healing ulcer. Separating these from a simple aphthous or traumatic ulcer matters clinically, since management differs and a malignant ulcer calls for urgent referral.

1.7. Conventional management and its limitations

Topical corticosteroids remain the mainstay of symptomatic treatment. Triamcinolone acetonide in an adhesive dental paste, fluocinonide and clobetasol propionate gels suppress the local inflammatory and immune response and so reduce pain, redness and the length of the episode. They work best when applied in the prodromal or early ulcerative phase, and prolonged or careless use predisposes to oral candidiasis.

Antiseptic rinses, chlorhexidine gluconate above all, are advised alongside to hold down secondary bacterial colonisation of the ulcer floor. Hydrogen peroxide and povidone iodine rinses are used in the same way. They do not speed up epithelial repair directly, but they lower the risk of secondary infection and may shorten the symptomatic period.

For pain relief, benzydamine hydrochloride rinse, lidocaine gel or spray and choline salicylate gel act locally, while paracetamol or a systemic non-steroidal anti-inflammatory drug is used when discomfort is severe. Some of the latter have themselves been reported to cause mucosal ulceration, so they need care. Antibiotics have no place in the routine treatment of simple aphthous ulcers, which are not primarily infective, although topical tetracycline rinses have been used for their anti-collagenase and anti-inflammatory effect and systemic antibiotics are justified when secondary infection is evident.

The drawbacks of these agents are well known. Corticosteroids carry the risk of fungal infection, mucosal atrophy and, with heavy use, systemic absorption. Chlorhexidine stains teeth and tongue, alters taste and irritates the mucosa on long use. Topical anaesthetics relieve pain briefly and do nothing for healing. Beyond this, most conventional preparations are washed away quickly by saliva and by the movement of the tongue and cheeks, so their contact time at the ulcer is short. These shortcomings, set against a condition that recurs over years, are the reason interest has continued in plant-based alternatives that can be used repeatedly with less concern.

1.8. Herbal drugs in oral healthcare

Herbal medicine, or phytotherapy, is the use of plants and plant derived preparations to prevent or treat disease. The World Health Organization includes under this head herbs, herbal materials, herbal preparations and finished herbal products containing parts of plants as the active ingredients. Such preparations have carried the traditional systems of Ayurveda, Siddha, Unani and Chinese medicine for centuries and still supply a large part of primary healthcare in the developing world. Plant derived compounds have also been a major source of leads for modern drug development.

Their appeal in the mouth rests on a few practical points. A single extract often carries several actions at once, commonly anti-inflammatory, antioxidant and antimicrobial together, which suits a condition as mixed in origin as oral ulceration. Used sensibly they have a reasonable safety record, which matters when treatment has to be repeated over years and where prolonged corticosteroid use would not be acceptable. Patients also tend to accept them readily. Plants have long been used in oral care, from chewing sticks and herbal rinses to modern toothpastes, mouthwashes, gels and lozenges carrying standardised extracts.

1.9. Plant profile: *Solanum nigrum*

Solanum nigrum, known as black nightshade and as manathakkali in Tamil, is an annual or short-lived herb of warm regions. The leaves are ovate with entire or wavy margins, the flowers are white and the berries turn black on ripening. Leaves and berries have been used for a long time in the treatment of inflammation, fever, wounds and stomach complaints. The taxonomical position of the plant is given in Table 1.

S. No	Category	Classification
1	Kingdom	Plantae
2	Family	Solanaceae
3	Genus	<i>Solanum</i>
4	Species	<i>S. nigrum</i>
5	Common name	Black nightshade
6	Tamil name	Manathakkali

Table: 1 Taxonomical classification of *Solanum nigrum*

The plant carries a wide range of constituents, and these are held responsible for the activities reported from it. The steroidal glycoalkaloids and saponins, chiefly solasonine and solamargine, are regarded as the principal bioactive group in the genus. Quercetin and its glycosides, along with kaempferol and rutin, account for much of the antioxidant activity, and the leaves also contain phenolic acids, tannins, beta-sitosterol, stigmasterol, ascorbic acid and further alkaloids of the solanine type. The exact profile shifts with the part of the plant used, its maturity, the place it was grown and the method of extraction.

Extracts of the leaves and fruits have been examined for anti-inflammatory activity, which is put down largely to the flavonoid and glycoalkaloid content and to their effect on inflammatory mediators. Free radical scavenging activity is well reported and follows from the phenolic and flavonoid content. Activity against a range of bacteria and fungi has been shown in several studies, which is relevant in a cavity as heavily colonised as the mouth. Most

pertinent here, the plant has been used traditionally and tested experimentally for wound healing, with effects reported on wound contraction, epithelialisation time and the tensile strength of healing tissue. A direct effect on fibroblast proliferation and collagen synthesis has been suggested alongside the anti-inflammatory and antioxidant actions.

Healing of an oral ulcer depends on inflammation settling, on secondary colonisation being kept in check, on oxidative injury being limited and, on the epithelium, repairing itself. An extract active across all four is well matched to the problem. That, together with the long traditional use of the plant, its ready availability and its safety record, is why *Solanum nigrum* leaf extract was chosen as the active ingredient in this work.



Figure: 2 *Solanum nigrum* plant

1.10. Anatomy of the oral cavity

The oral cavity is the entrance to the digestive tract. It is bounded in front by the lips, behind by the oropharyngeal isthmus, at the sides by the cheeks, above by the hard and soft palate, and below by the floor of the mouth and the tongue. It is lined throughout by oral mucosa and holds the teeth, the gingiva and the openings of the major and minor salivary glands. Site matters when a preparation is meant to act inside the mouth, because the nature of the covering mucosa and the local conditions govern how long the dosage form stays put and how the drug is released.

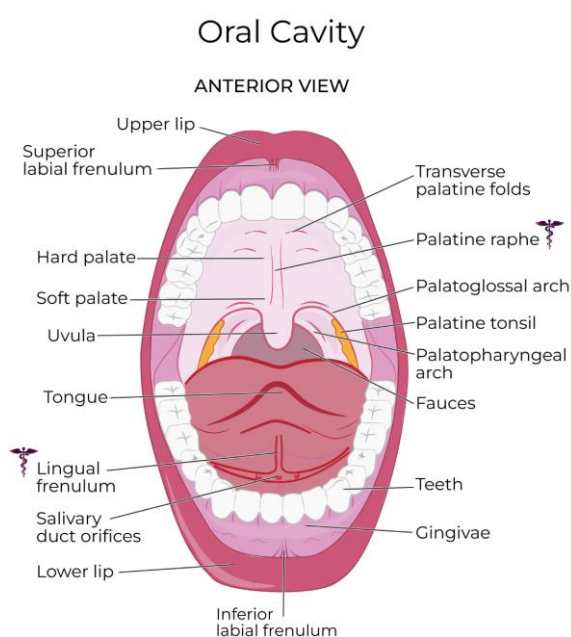


Figure: 3 Oral cavity

The mucosa itself falls into three kinds. Masticatory mucosa is keratinised and covers the gingiva and hard palate. Lining mucosa is non-keratinised and more elastic and covers the cheeks, lips, ventral tongue, floor of the mouth and soft palate. Specialised mucosa on the dorsum of the tongue bears the papillae and taste buds. The non-keratinised lining mucosa is thinner and more permeable, which makes it both more liable to ulcerate and more amenable to local absorption. These are precisely the sites at which aphthous ulcers appear most often. The buccal mucosa in particular is highly vascular and relatively permeable, which is why it has attracted attention for local and even systemic delivery.

Saliva is secreted by the parotid, submandibular and sublingual glands and by numerous minor glands spread through the mucosa. It lubricates the lining, starts carbohydrate digestion, buffers oral pH, contributes lysozyme and immunoglobulin A, and carries dissolved substances to the taste buds. From a

formulation point of view its continuous flow is the main obstacle, since it dilutes and washes away anything applied locally and cuts down residence time at the site of action. This is the central consideration in designing a lozenge meant to release its contents slowly in the mouth.

1.11. Lozenges

Lozenges, also called troches or pastilles, are solid single dose preparations meant to dissolve or disintegrate slowly in the mouth. The active ingredient is released gradually as the unit dissolves, which keeps it in contact with the oral or pharyngeal mucosa for a prolonged period. In some cases absorption through the mucosa into the circulation follows, but for the present formulation the intended action is local, at the ulcer itself. Early lozenges were sugar and gum preparations used to carry soothing substances to the throat, and confectionery technique was gradually adapted to pharmaceutical use, so that what began as hard sugar candy is now a precisely dosed dosage form carrying local anaesthetics, antiseptics, decongestants and, increasingly, herbal actives.

The advantages of the form are straightforward. The unit stays in the mouth while it dissolves, which gives the sustained local contact that a topical rinse or gel cannot. No water is needed, so it can be taken at any time. It is non-invasive and is generally accepted by children and by elderly patients who find tablets and capsules difficult. Unpleasant taste can be covered with flavouring and sweetening agents, and as a solid it is more stable than a mouthwash or gel.

There are limitations to set against this. The patient has to let the lozenge dissolve rather than chew or swallow it, so the effect depends on cooperation. Lozenges are unsuitable for very young children because of the risk of choking. A conventional sugar base is a concern for diabetic patients and for caries risk, which is why sugar free bases are used where those matters. Dissolution time varies with salivary flow and with the way the patient uses it, and the dose that can be carried in a single unit is small compared with other oral forms.

Lozenges are classified by the way they are made and by what they contain. Hard candy lozenges are prepared by fusion, with sugar and other carbohydrates melted, combined with the active and cast into moulds; the result is glassy and dissolves slowly. Soft lozenges use a gelatin or similar base and give a chewable or slowly dissolving consistency. Chewable lozenges are meant to be chewed and release the active faster. Compressed lozenges are made like conventional tablets, by compressing a powder blend, and disintegrate more quickly than fusion moulded units. Moulded lozenges are made by pouring a semi-solid or liquid mass into moulds and allowing it to set. Medicated lozenges are those carrying a defined pharmacologically active ingredient, and herbal lozenges are the subset in which that ingredient is a plant extract rather than a synthetic substance.

A well-made lozenge should be uniform in weight and in drug content across a batch, hard enough to survive handling, packaging and transport without chipping or crumbling yet still able to dissolve properly in the mouth, and smooth and free from cracks, pits and air bubbles. Since it remains in the mouth for some minutes, taste has to be acceptable. Dissolution should be gradual, over several minutes, and the formulation should hold its appearance, texture, hardness and drug content through its shelf life.

1.12. Choice of preparation method

Two methods were considered. In the moulding method the active is combined with a base such as gelatin or sugar syrup to give a semi-solid mass, which is poured or pressed into moulds and allowed to cool, wet or dry. In the compression method the active is blended with diluents, binders, sweeteners, flavours and lubricants and the blend is compressed on a tablet press. Compression is efficient at scale and gives faster disintegration, but it requires the active and the excipients to flow and compress acceptably.

The moulding method was selected here. *Solanum nigrum* leaf extract carries flavonoids and glycoalkaloids that can be degraded by heat, and the lower processing temperature of moulding was considered necessary to keep them intact. Moulding was also simpler and more reproducible at the laboratory scale on which this work was carried out.

2. AIM AND OBJECTIVES

The aim of the study was to formulate and characterise herbal lozenges of *Solanum nigrum* leaf extract for the possible management of oral ulcers. The objectives were to authenticate the plant material, to prepare and evaluate the leaf extract, to formulate three lozenge batches designated F1, F2 and F3 using suitable excipients, and to evaluate their physicochemical and in-vitro release characteristics so that an optimised formulation could be selected on the basis of the results obtained.

3. MATERIALS AND METHODS

3.1. Collection and authentication of plant material

Leaves of *Solanum nigrum* were collected locally. The species was identified and authenticated by the Siddha Central Research Institute (Central Council for Research in Siddha, Ministry of AYUSH, Government of India), Anna Government Hospital Campus, Arumbakkam, Chennai 600106.

3.2. Materials and equipment

The materials used and their sources are listed in Table 2, and the equipment used with the respective manufacturers in Table 3.

S. No	Equipment	Manufacturer
1	Soxhlet extraction apparatus	Labus
3	UV-Visible spectrophotometer	Hitachi U-3900
4	Digital pH meter	Ohaus
5	Hardness tester	Polylab India
6	Digital weighing balance	Polylab India
7	Heating Mandle	WKM

Table 2: Equipment and their manufacturers

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Table 3: Equipment Used

3.3. Excipient profile

The ingredients used in the formulation and the function of each are given in Table 4.

S. No	Ingredient	Use
1	Solanum nigrum	Wound healing and anti-inflammatory
2	Sucrose	Lozenge base
3	Gelatin	Binding agent
4	Citric acid	Preservative and taste enhancer
5	Peppermint oil	Flavouring and cooling agent
6	Liquid glucose syrup	Prevent crystallization
7	Distilled water	Vehicle

Table 4: Excipients Used and Their Function

3.4. Extraction procedure

The leaves were washed, shade dried and powdered. One hundred grams of the dried leaf powder was weighed accurately and packed into a Soxhlet extraction thimble. Five hundred millilitres of ethanol was placed in the round bottom flask as the extraction solvent. The apparatus was assembled and heated gently so that the solvent evaporated, condensed and passed repeatedly through the powdered material. Extraction was continued for about six hours, until the siphoning solvent ran comparatively clear. The extract was filtered through suitable filter paper to remove insoluble plant matter and then

concentrated by controlled temperature evaporation. About 12.5 g of semisolid extract was obtained and used for the preformulation studies and for preparing the lozenges.

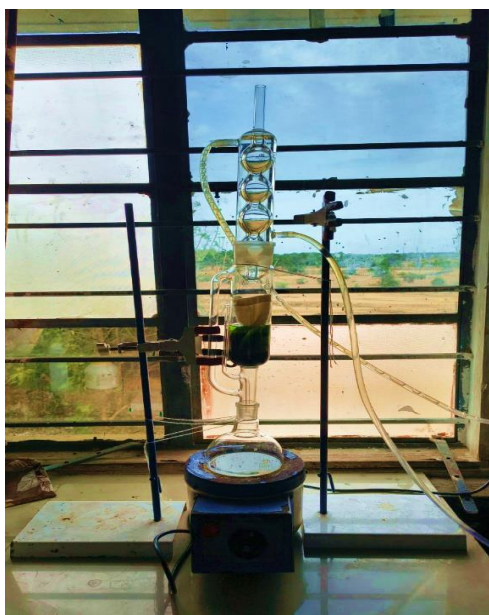


Figure: 4 Soxhlet extraction

3.5. Preparation of the lozenges

The lozenges were prepared by the moulding method, in which the medicated mass is made by mixing the required ingredients, transferred to suitable moulds and allowed to cool and set into the desired shape and size. The same procedure was followed for all three batches and only the quantity of extract was varied.

Gelatin, 0.5 g, was weighed and allowed to swell in a small quantity of distilled water for about ten minutes, then warmed gently until a uniform solution was obtained. Separately, 14 g of sucrose was taken in a beaker with the required quantity of distilled water and heated gently with constant stirring until it had dissolved completely. Liquid glucose syrup, 3 g, was added slowly to this solution and mixed thoroughly. The mixture was heated further with gentle stirring until a concentrated, uniform lozenge mass was obtained.

The mass was cooled slightly and the gelatin solution was added and mixed in. The *Solanum nigrum* leaf extract was then added and mixed until uniform, using 0.5 g for F1, 1.0 g for F2 and 1.5 g for F3. Citric acid, 0.1 g, was mixed in next, followed by 0.2 mL of peppermint oil. The warm mass was transferred to a 2 g mould and the cavities were filled evenly. The moulded lozenges were left to cool and set at room temperature, removed carefully from the mould and stored in a well closed container until evaluation. The composition of the three batches is given in Table 5.

S. No	Ingredients	F1	F2	F3
1	<i>Solanum nigrum</i> extract	0.50 g	1 g	1.5 g
2	Sucrose	14 g	14 g	14 g
3	Gelatin	0.5 g	0.5 g	0.5 g
4	Citric acid	0.1 g	0.1 g	0.1 g
5	Peppermint oil	0.2 ml	0.2 ml	0.2 ml
6	Liquid glucose syrup	3 g	3 g	3 g
7	Distilled water	q.s.	q.s.	q.s.

Table 5: Composition of Herbal Lozenges

4. EVALUATION

4.1. Evaluation of the herbal extract

4.1.1. Preliminary phytochemical screening

The ethanolic extract of *Solanum nigrum* leaves was subjected to the standard chemical tests for alkaloids, flavonoids, phenolic compounds, carbohydrates, proteins, tannins, glycosides and terpenoids.

S. No	EXPERIMENT OBSERVATION INFERENCE	EXPERIMENT OBSERVATION INFERENCE	EXPERIMENT OBSERVATION INFERENCE
1	Test for carbohydrates To 2ml of plant extract, 1 ml of Molisch's reagent and few drops of concentrated sulphuric acid were added	Purple Colour formation	Indicates the presence of carbohydrates
2	Test for Tannins To 1ml of plant extract, 2ml of 5% ferric chloride was added	Formation of greenish black	Indicates the presence of Tannins
3	Test for Saponins . To 2ml of plant extract, 2ml of distilled water was added and shaken in a graduated cylinder for 15 minutes lengthwise	Formation of 1 cm layer of foam	Indicates the presence of saponins
4	Test for Flavonoids 5ml of dilute ammonia solution was added to a portion of the aqueous filtrate of plant extract followed by addition of concentrated sulphuric acid.	Appearance of yellow coloration	Indicates the presence of Flavonoids
5	Test of Alkaloids To 2ml of plant extract, 2ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added	Presence of green colour	Indicates the presence of alkaloids.
6	Test for Anthocyanin and Betacyanin To 2ml of plant extract, 1ml of 2N sodium hydroxide was added and heated for 5 minutes at 100°C	Formation of yellow colour	Indicates the presence of Betacyanin
7	Test for Glycosides To 2ml of plant extract, 3ml of chloroform and 10% ammonia solution was added	Pink colour formation	Indicates the presence of Glycosides
8	Test for Phenols To 1ml of the extract, 2ml of distilled water followed by few drops of 10% ferric chloride was added	Formation of green colour	Indicates the presence of phenols

Table 6: Preliminary Phytochemical Screening of *Solanum nigrum* Leaf Extract

4.2 EVALUATION OF HERBAL LOZENGES:

4.2.1 Organoleptic Evaluation

The prepared lozenges were evaluated visually for colour, appearance, odour, and taste.

4.2.2 Weight Variation Test

Twenty lozenges from each formulation were individually weighed, and the average weight along with the percentage deviation of individual lozenges from the average was calculated as per standard pharmacopeial limits.

4.2.3 Thickness and Diameter

The thickness and diameter of the lozenges were measured using a digital vernier caliper for ten randomly selected units from each batch.

4.2.4 Hardness Test

The hardness of the lozenges, indicating their resistance to chipping or breakage during handling and packaging, was determined using a hardness tester.

4.2.5 Measurement of pH

A weighed quantity of lozenge, dissolved/dispersed in a fixed volume of distilled water, was used to measure the pH using a calibrated digital pH meter, to ensure compatibility with the oral cavity.

4.2.6 Thickness and Diameter:

The thickness and diameter of the prepared herbal lozenges were determined using a digital vernier caliper. The measurements of individual lozenges were recorded in millimetres (mm), and the average thickness and diameter were calculated.

4.2.7 Drug Content

A specified number of lozenges were powdered, and an accurately weighed quantity equivalent to the extract content was dissolved in a suitable solvent, filtered, and analysed spectrophotometrically at the predetermined λ_{max} against the calibration curve to determine the percentage drug content.

4.2.8 In-vitro Drug Release Study

In-vitro release of the extract from the lozenges was studied using a Franz diffusion cell fitted with a cellophane membrane, with simulated saliva or phosphate buffer as the dissolution medium maintained at $37 \pm 0.5^\circ\text{C}$. Aliquots were withdrawn at predetermined time intervals, replaced with fresh medium, and analysed spectrophotometrically to determine the cumulative percentage drug release.

5. RESULTS AND DISCUSSION

5.1. Preliminary phytochemical screening

The observations recorded in the individual chemical tests and the inference drawn from each are set out in Table 6.

S · N O	NAME OF THE TEST	OBSERVATION	INFERENCE
TEST FOR ALKALOIDS			
A	Mayer's test	Cream- coloured precipitate is formed	Presence of alkaloids
B	Dangendroff's test	Orange-brown precipitate is formed	Presence of alkaloids
C	Wagner's test	Reddish-brown precipitate is formed	Presence of alkaloids
TEST FOR CARBOHYDRATES			
A	Molisch's test	Violet ring is formed	Presence of carbohydrates
B	Fehling's test	Reddish-brown precipitate is formed	Presence of carbohydrates
C	Benedict's test	Red/orange precipitate is formed	Presence of carbohydrates
TEST FOR GLYCOSIDES			
A	Keller-killani test	Bluish-green colour ring is observed	Presence of cardiac glycosides
TEST FOR FLAVONOIDS			
A	Shinoda's test	Pink/ red colour is developed	Presence of flavonoids
B	Alkali test	Yellow colour is developed	Presence of flavonoids
C	Lead acetate test	Yellow/white precipitate is formed	Presence of flavonoids
TEST FOR PHENOLS			
A	Ferric chloride test	Green colour is developed	Presence of phenolic compounds

B	Lead acetate test	Precipitate is formed	Presence of phenolic compounds
TEST FOR TANNINS			
A	Ferric chloride test	Green colour is developed	Presence of tannins
B	Vanillin-HCL test	Pink/ red colour is developed	Presence of tannins
TEST FOR SAPONINS			
A	Foam test	Persistent foam is formed	Presence of saponins
TEST FOR TERPENOIDS			
A	Salkowski's test	Reddish-brown colour is formed at the junction	Presence of terpenoids
TEST FOR PROTEINS/ AMINO ACIDS			
A	Ninhydrin test	No Purple colour is developed	Absence of amino acids

Table: 7 Phytochemical test for *Solanum nigrum*

Screening of the ethanolic leaf extract showed alkaloids, flavonoids, phenols, tannins, saponins, terpenoids, carbohydrates, steroids and cardiac glycosides. Amino acids were the only group not detected. The consolidated result is given in Table 8. The flavonoids, phenols and tannins are of most interest for the present purpose, since these are the groups usually credited with the antioxidant and anti-inflammatory activity reported from this plant, and tannins additionally have an astringent action at a raw mucosal surface.

S.NO	COMPOUNDS	RESULT (<i>Solanum nigrum</i>)
1	Carbohydrates	+
2	Alkaloids	+
3	Flavonoids	+
4	Steroids	+
5	Cardiac glycosides	+
6	Phenols	+
7	Tannins	+
8	Amino acids	–
9	Terpenoids	+
10	Saponins	+

Table: 8 Result for phytochemical test

5.2. Absorption maximum and calibration curve

The absorption spectrum of the extract was recorded between 200 and 400 nm. Maximum absorbance was obtained at 265 nm, and this wavelength was used for the estimations that followed. The calibration curve was constructed over 10 to 50 micrograms per mL. Absorbance readings are given in Table 8 and the plot in Figure 15.

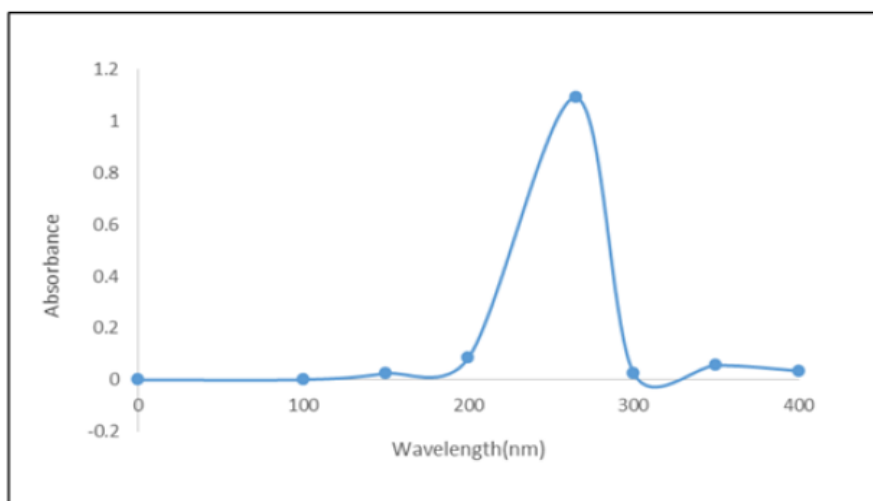


Figure: 5 Absorption spectrum of Solanum nigrum extract

Concentration (µg/mL)	Absorbance
0	0
10	0.304
20	0.608
30	0.912
40	1.216
50	1.520

Table: 9 Calibration curve data for Solanum nigrum extract

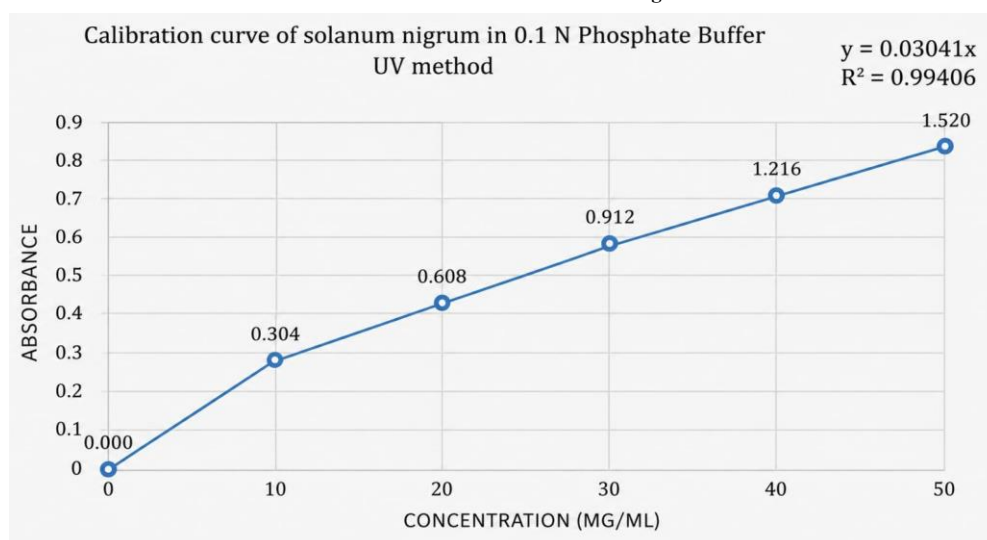


Figure: 6 Calibration curve of Solanum nigrum extract

Absorbance rose in direct proportion to concentration across the range examined, with a regression coefficient of 0.994 for the fitted line, so the method was taken as acceptable for estimating the extract in the formulations.

5.3. Organoleptic properties

All three batches gave smooth, uniform and intact moulded lozenges, sweet in taste with a mild peppermint odour. F1 and F2 were yellowish green. F3 was distinctly darker, a dark brown, which follows from the larger quantity of extract it carries. The finished lozenges are shown in Figure 16 and the detailed observations in Table 10.

Formulation	Colour	Appearance	Taste/Odour
F1	Yellowish green	Smooth, uniform, intact molded lozenge	Sweet with mild peppermint odour
F2	Yellowish green	Smooth, uniform, intact molded lozenge	Sweet with mild peppermint odour
F3	Dark brown	Smooth, uniform, intact molded lozenge	Sweet with mild peppermint odour

Table: 10 Result for organoleptic properties



Figure: 7 Formulated herbal lozenges

5.4. Weight variation

Average weights of the three batches are given in Table 10. The values were close to one another and to the capacity of the mould, which indicates that the mass filled the cavities evenly and that the moulding step was reproducible.

Formulation	Avg. Weight (mg)
F1	2.0 mg
F2	2.0mg
F3	2.1 mg

Table: 11 Result for weight variation test

HARDNESS TEST:

The hardness of the formulation was found to be

Formulation	Hardness (kg/cm ²)
F1	4.6
F2	4.5
F3	4.5

Table: 12 Result for Hardness test



Figure: 8 Hardness testing

PH MEASUREMENT

The pH of the formulation was found to be

Formulation	pH
F1	6.23
F2	6.22
F3	6.22

Table: 13 Result for pH



Figure: 9 pH of formulation 1

Figure: 10 pH of formulation 2

THICKNESS AND DIAMETER:

The thickness and diameter of the formulation was found to be

Table: 14 Result for Thickness and Diameter

Formulation	Thickness (mm)	Diameter (mm)
F1	9.39	20.06
F2	9.90	20.09
F3	9.90	20.09



Figure: 11 Thickness of the lozenges



Figure: 12 Diameter of the lozenges

DRUG CONTENT:

The drug content of the three formulations was found to be

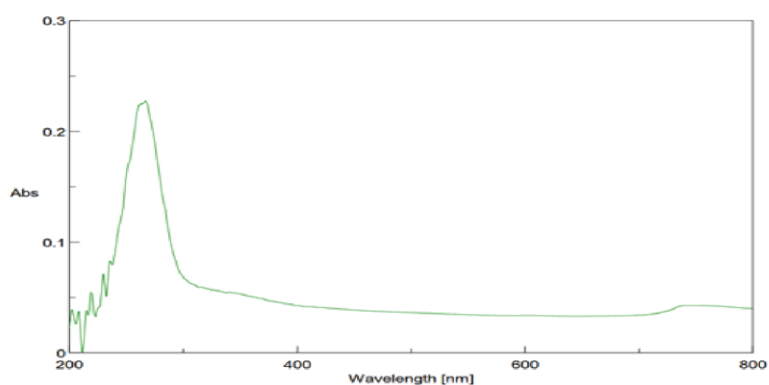


Figure: 13 Absorption spectrum of formulation 1

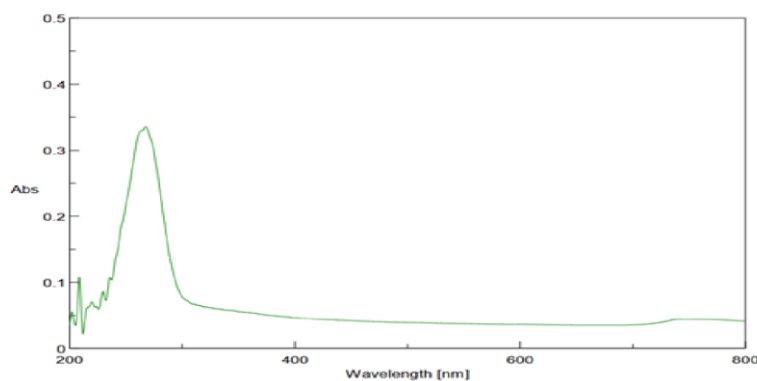


Figure: 14 Absorption spectrum of formulation 2

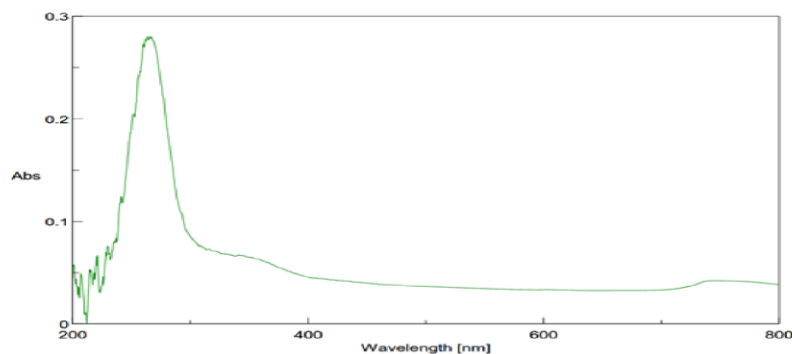


Figure: 15 Absorption spectrum of formulation 3

Parameter	F1 Spectrum	F2 Spectrum	F3 Spectrum	Interpretation
Instrument Used	JASCO UV-VIS V-730	JASCO UV-VIS V-730	JASCO UV-VIS V-730	Same instrument ensures consistent results.
Wavelength Range (nm)	200 – 800	200 – 800	200 – 800	Full UV-Visible range covered.
λ_{max} (Peak Absorbance Wavelength)	~270-310 nm	~280–320 nm	~300-350 nm	Indicates conjugated systems/aromatic compounds.
Absorbance Intensity (Peak)	~0.25	~0.35	~0.30	Suggests F2 has the highest concentration or a stronger chromophore
Data Interval (nm)	0.2 nm	0.2 nm	0.2 nm	High resolution for peak detection.
Scan Speed	400 nm/min	400 nm/min	400 nm/min	Standard UV-Vis analysis speed.
Possible Functional	π - π^* transitions (conjugated compounds,	π - π^* transitions (conjugated compounds,	π - π^* transitions (conjugated compounds,	Presence of bioactive compounds such as flavonoids or phenolics.

Table: 15 Interpretation table for drug content

- All three spectra (F1, F2, and F3) show peaks in the 250-400 nm range, which suggests the presence of bioactive compounds like flavonoids, phenolics, or alkaloids commonly found in plant extracts.
- F2 exhibits the highest absorbance (~0.35), followed by F3 (~0.30), and F1 (~0.25). This suggests that F2 has a higher concentration of the absorbing species or a slight structural variation.
- The shift in λ_{max} (peak absorbance wavelength) from F1 to F3 might indicate changes in molecular structure, solvent effects, or interactions with other compounds.
- Further analysis (e.g., FTIR, HPLC, Mass Spectrometry) is needed to confirm the exact nature of these compounds.

INVITRO DRUG RELEASE:

The drug release of the formulation was found to be

FORMULATION	DRUG RELEASE					
	ABSORBANCE					
	5 mins	10 mins	15 mins	20 mins	25 mins	30 mins
F1	18.80%	35.90%	53.20%	69.80%	82.50%	92.40%
F2	20.50%	38.60%	56.70%	73.40%	87.80%	93%
F3	17.20%	33.40%	49.50%	66.10%	78.20%	90.10%

Table: 16 Result for drug release



Figure:16 Diffusion cell F1 Figure:17 Diffusion cell F2 Figure:18 Diffusion cell F3

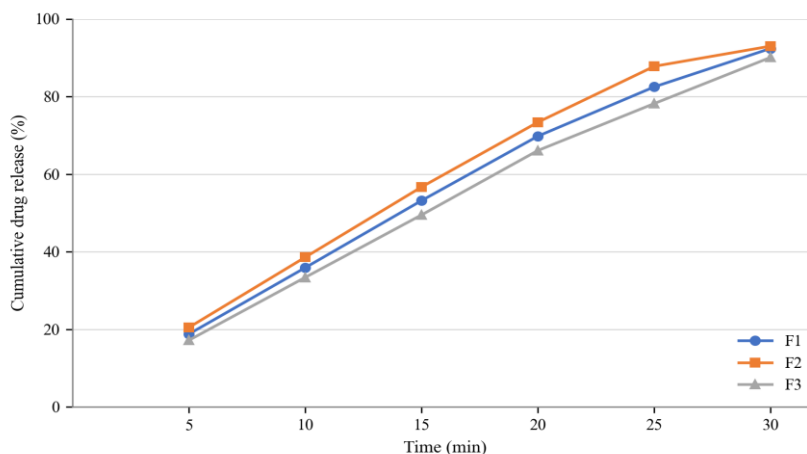


Figure:19 In-vitro drug diffusion profile

Release dose steadily with time in all three batches, with no burst effect at the early time points and no plateau before 30 minutes, which is the behaviour wanted from a lozenge meant to act locally over several minutes. F2 released 20.5 per cent at 5 minutes and 93.0 per cent at 30 minutes, and stayed ahead of the other two at every interval. F1 followed closely with 18.8 per cent at 5 minutes and 92.4 per cent at 30 minutes. F3 was the slowest throughout, giving 17.2 per cent at 5 minutes and 90.1 per cent at 30 minutes.

The order is worth noting, because F3 carries the largest quantity of extract and yet releases the least. The semisolid extract is itself viscous, and at 1.5 g per batch it appears to have made the moulded mass denser and slower to erode in the dissolution medium, so the extra drug loading was offset by slower release from the matrix. F2, at 1.0 g, seems to sit at the point where the loading is high without the matrix being affected in this way. The same is reflected in the spectral data, where F2 gave the highest peak absorbance of the three.

Taking the results together, all three batches were acceptable on appearance, weight, hardness and pH, and the differences between them lay mainly in the release behaviour and drug content. On those grounds F2 was selected as the optimised formulation.

SUMMARY

- The study was undertaken to develop and evaluate herbal lozenges containing *Solanum nigrum* leaf extract, with peppermint, for the management of oral ulcers.
- The lozenges were prepared by the moulding method using three different concentrations of the leaf extract, designated F1, F2 and F3.
- The batches were evaluated for organoleptic characters, weight variation, hardness, pH, drug content and in-vitro drug release.
- All three gave acceptable physical characteristics and progressive release over 30 minutes. F2 gave the highest release at 93.0 per cent, followed by F1 at 92.4 per cent and F3 at 90.1 per cent.
- F2 was taken as the optimised formulation on the basis of its overall evaluation and release behaviour.
- The lozenge developed here may serve as a herbal oral dosage form for the management of oral ulcers, subject to further study.

CONCLUSION

Three herbal lozenge formulations containing *Solanum nigrum* leaf extract with peppermint were prepared successfully by the moulding method. The batches were evaluated for appearance, weight variation, hardness, pH, drug content and in-vitro drug release, and all three were found acceptable as a moulded lozenge dosage form. The extract incorporated into them showed progressive release through the study period, which supports the use of this form for local delivery in the mouth. Of the three, F2 performed better overall, particularly on drug release, and was selected as the optimised formulation. The lozenge developed in this work may therefore offer a herbal dosage form for the management of oral ulcers, although further studies are needed to establish its efficacy and its stability on storage.

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There is no conflict of interest.

Author contribution:

Madhan. M, Mathimugaselvan. K, Mohamed zahiruddin. S, Nandhini. S had principal responsibility for the conception, design of the study and drafting of manuscript. Dr. G. Mariyappan was responsible for the revision of the manuscript for important intellectual content, Dr. J. Karthi and Dr. S. Swarnalatha contributed towards reviewing of the literature. All authors agreed on the final version of manuscript.

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