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Research

PREPARATION AND EVALUATION OF ANTIDIABETIC LOZENGES FROM GYMNEMA SYLVESTRE

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Abstract

Gymnema sylvestre has the ability to lower glucose levels without causing hypoglycemia. The leaves of G. sylvestre are widely used in Indian proprietary medicines for the treatment of diabetes and as a diuretic, and in most of the country's herbal drug markets, the leaves are sold alongside the plant's aerial parts under the name Gurmarbuti. The leaves of G. sylvestre contain oleanane- and dammarene-class triterpene saponins. Oleanane saponins consist of gymnemic acids and gymnemasaponins, whereas dammarene saponins consist of gymnemasides. The leaves of G. sylvestre have been found to induce hypoglycemia in laboratory animals and to be useful in herbal medicine for the treatment of diabetes in adults. Gymnema leaf extract has laxative, diuretic, and cough suppressant properties. Gymnema may be useful in both adult-onset (NIDDM) and juvenile-onset (IDDM) diabetes mellitus to facilitate insulin entry into cells.

Keywords: Gymnema sylvestre, Gurmarbuti, Oleanane saponins, dammarene saponins, gymnema.

INTRODUCTION

The perennial woody vine Gymnema /gurmar (Gymnema sylvestre) is used in Ayurveda medicine. There is evidence that gymnema is effective against both type 1 and type 2 diabetes. In this regard, it may increase insulin secretion by increasing the number of pancreatic -cells and enhancing cell function. It has also demonstrated additional incretin-mimetic activity. Not only does it appear to lower blood glucose, but it has also been shown to decrease sweetness perception and increase bitterness perception. [1]

Gymnema sylvestre has the ability to lower glucose levels without causing hypoglycemia. There have been decreases in FBG, HbA1c, and urinary glucose excretion, resulting in a decreased need for conventional medication. It is believed that Gymnema sylvestre slows glucose absorption in the small intestine, and through its restorative effect on -cells, the pancreas produces more insulin. [2]

Since ancient times, the naturopathic treatment of diseases has been extensively studied and is gaining momentum in the present day. There are approximately 45,000 plant species in the Indian flora, of which several thousand have pharmacological significance. Diabetes mellitus is a significant endocrine disorder affecting nearly 10% of the world's population and a major concern. In its severe form, the disease affects major body systems, resulting in multiorgan complications. Oral hypoglycemic agents, such as sulphonylureas and biguanides are the conventional treatment for diabetes, but their adverse side effects are a major drawback. Herbal remedies are gaining popularity due to their superior efficacy and safety compared to commercially available drugs, as well as their greater efficacy in treating health issues. [3-5]

Gymnema sylvestre R.Br. (Asclepiadaceae)

Latin name: Gymnema sylvestre R.Br. (Asclepiadaceae)
English Name: Destroyer of sugar, Periploca of woods
Sanskrit/Indian name: Madhunashini, Gurmar, Meshashringi, Vishani, Merasingi
Parts used: Leaves, stems, roots

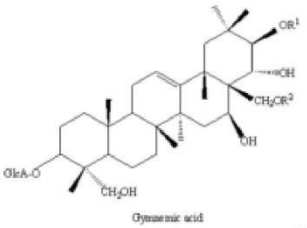
Taxonomic classification:

Kingdom: Plantae -Plant
Subkingdom: Tracheobionta – Vascular plants
Superdivision: Spermatophyta – Seed plants
Division: Magnoliophyta – Flowering plants
Class: Magnoliopsida – Dicotyledons
Subclass: Asteridae
Order: Gentianales
Family: Asclepiadaceae – Milkweed family
Genus: Gymnema R.Br. – gymnema
Species: Gymnema sylvestre (Retz.) Schult.

Antidiabetic plants are of great interest to the ethnobotanical community because they are known to contain valuable medicinal properties in various plant parts and a number of them have demonstrated hypoglycemic and anthyperglycemic activity to varying degrees.

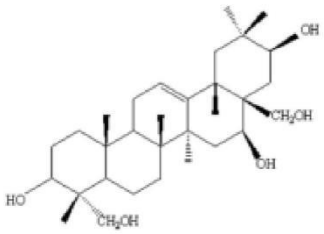
Triterpene saponins belonging to the oleanane and dammarene classes are present in Gymnema sylvestre leaves. Gymnemic acids and gymnemasaponins are oleanane saponins Gymnimasaponins include two aglycone saponins, such as gymnemagenis and gymnestrogenin. Dammarene saponins include gymnemasides (I-VII) (Sinsheimer et al., 1970; Foster et al., 2002; Khramov et al., 2008; Potawale et al., 2008). These secondary metabolites all possess anti-diabetic properties. In addition, the leaves contain resins, albumin, chlorophyll, carbohydrates, tartaric acid, formic acid, butyric acid, anthraquinone derivatives, inositol alkaloids, organic acid (5.5%), parabin, and calcium oxalate (7.3%), lignin (4.8%), cellulose (22%) and other substances with anti-diabetic activity (Dateo et al., 1973).

The triterpenoid glycoside gymnemic acid (Yoshikawa et al., 1989; Manohar et al., 2009) is not a pure compound; rather, it has a unique structure consisting of numerous types of compounds with a similar molecular formula. The aglycone portion is centrally located in this molecule, which contains a sugar moiety (such as glucuronic acid) or is conjugated with multiple ester groups. Gymnemic acid A consists of forms A1, A2, A3, and A4. [10-12] the aglycone fragment (gymnemagenin) is a hexahydroxytriterpene D-glucoronide. Another anti-sweet compound, Gymnemasaponin III contains twenty-three hydroxy longispinogenin as its aglycone moiety (Yoshikwa et al., 1989; Tiwari et al., 2014). [13-14]



Gymnemic acid

Gymnemic acid types	R^1	R^2
Gymnemic acid 1	Tigloyl	Ac
Gymnemic acid II	2-methylbutyroyl	Ac
Gymnemic acid III	2-methylbutyroyl	H
Gymnemic acid IV	Tigloyl	H



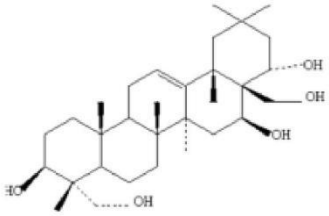


Figure No. 01. Gymnemic Acid. Figure No.02. Gymnemastrogenin Figure No.03: Gymnemanol

Pharmacognostical Studies

The leaves of G. sylvestre are widely used in Indian proprietary medicines for the treatment of diabetes and as a diuretic, and in most of the country's herbal drug markets, the leaves are sold alongside the plant's aerial parts under the name Gurmarbuti [13]. The following describes the macroscopic and microscopic characteristics of the aerial parts:

1.1. Large-Scale Characteristics

G. sylvestre has green-colored leaves and a hairy, light-brown stem. The leaf is 2-6 cm long and 1-4 cm wide. The leaves are simple, petiolate, base rounded to cordate, margin entire, opposite with acute apex, reticulately veined, and both surfaces pubescent. The aroma is distinctive, and the taste is slightly bitter and astringent. It also possesses the remarkable property of temporarily paralyzing the taste for sweet substances [13-14].

1.2. Microscopical Attributes

1.2.1. Petiole

The transverse section of the petiole resembles a horseshoe. The epidermis is single-layered, thick-walled, and barrel-shaped. It is covered with uniseriate, multicellular, nonglandular trichomes. The cortex is composed of collenchyma, and there are three amphicribal vascular bundles. Phloem parenchyma is made up of sieve tubes, companion cells, and phloem parenchyma. There are vessels, tracheids, and tracheidal fibres in the xylem. The starch grains are polygonal, simple or compound, and arranged in two or more groups. The calcium oxalate rosette crystals are more prevalent in the centre. [15-18]

1.2.2. Lamina

Lamina epidermal cells have a square shape with a convex outer wall and a thin cuticle. When viewed transversely, the epidermal cell surface is interrupted by uniseriate, multicellular trichomes with 2 to 5 cells that are abundant on both surfaces. Just below the adaxial epidermis, a single layer of closely packed palisade cells is present. Vascular bundles are amphicribal, and the mesophyll has a thickness of 3-5 cells [15-16].

1.2.3. Stem

The transverse section of the stem has a circular cross-section. The epidermis is cylindrical and has thick walls. Trichomes are multicellular, uniseriate, 185-485 microns in length, and 9-25 microns in width. The cork has three to five layers, and the cortical cells are elongated and collenchymatous. Well-developed phloem contains large sieve plates, companion cells, and phloem parenchyma. The xylem consists of a continuous cylinder traversed by thin medullary rays. [15-17] the endodermis is prominent and the pericycle is broad.

1.2.4. Powder

The powdered substance has a slight yellowish-green hue, a bitter flavour, and a pleasant aroma. Under the microscope, it reveals thick-walled, uniseriate, multicellular trichomes, anomocytic stomata, idioblast with rosette crystals of calcium oxalate, starch grains, remnants of collenchymatous and parenchymatous cells; vessels, tracheids, tracheidal fibres, bast fibres, and sieve plates [15-18].

1.3. Phytochemistry [19-29]

The leaves of G. sylvestre contain oleanane- and dammarene-class triterpene saponins. Oleanane saponins consist of gymnemic acids and gymnemasaponins, whereas dammarene saponins consist of gymnemasides [18-20]. Additionally, the leaves contain resins, albumin, chlorophyll, carbohydrates, tartaric acid, formic acid, butyric acid, anthraquinone derivatives, inositole alkaloids, organic acid (5.5%), parabin, calcium oxalate (7.3%), lignin (4.8%), and cellulose (22%).

The gymnemic acids contain a number of acylated (tiglolyl, methylbutyroyl, etc.) derivatives of deacylgymnemic acid (DAGA), which is a 3-O—glucouronide of gymnemagenin (3, 16, 21, 22, 23, 28-hexhydroxyolean-12-ene). Individual gymnemic acids (saponins) consist of gymnemic acids I through VII, gymnemosides A through F, and gymnemasaponins. G. sylvester has been reported to contain gymnemic acids, (+) quercitol, lupeol, (-) amyrin, stigma sterol, etc. In the aerial parts of G. sylvester, a new flavonol glycoside, kaempferol 3-O-beta-D-glucopyranosyl-(1—>4)-alpha-L- rhamnopyranosyl-(1—>6)-beta-D-galactopyranoside, has been identified [22-25]. beta-O-benzoylsitakisogenin 3-O-beta-D-glucopyranosyl (1-->3)-beta-D-glucuronopyranoside, the potassium salt of longiospinogenin 3-O-beta-D-glucopyranosyl (1-->3)-beta-D-glucuronopyranoside, and beta-O-benzoylsitakisogenin 3- -beta-D-glucopyranoside and 29-hydroxylongispinogenin 3-O-beta-D-glucopyranosyl (1-->3) potassium salt -beta-D-glucopyranoside and sodium salt of alternoside II were isolated from a G. sylvester leaf ethanol extract [26]. Four new triterpenoid saponins, gymnemasins A, B, C, and D, were identified as 3-O-[beta-D-glucopyranosyl (1—>3)-beta-D-glucopyranosyl]-beta-D-glucopyranosyl. -22-O-tiglyol-gymnemanol, 3-O-[beta-D-glucopyranosyl (1—>3)-beta-D-glucuro - nopyranosyl] - gymnemanol, 3-O-beta-D-glucuronopyranosyl-22-O-tigloyl-gymnemanol and 3- O-beta-D-glucopyranosyl-gymnemanol respectively. The newly discovered aglycone gymnemanol was identified as 3 beta-16 beta-22 alpha-23-28-penthydroxyolean-12-ene. Gymnestrogenin is a newly discovered pentahydroxytriterpene isolated from the leaves of G. sylvestre [28, 29].

1.4. Action mechanism of G. sylvestre (Gymnemic Acid)

The leaves of G. sylvestre have been found to induce hypoglycemia in laboratory animals and to be useful in herbal medicine for the treatment of diabetes in adults. When plant leaf extract is administered to a diabetic patient, the pancreas is stimulated, resulting in an increase in insulin secretion. It has also been discovered that these compounds increase cholesterol excretion in the faeces [30-32]. There are several possible mechanisms by which the G. sylvestre leaf extract or (Gymnemic acid) exerts its hypoglycemic acid effects, including: 1) It promotes islet cell regeneration, 2) It increases insulin secretion, and 3) It inhibits glucose absorption from the intestine. Increases the activities of enzymes responsible for glucose utilization by insulin-dependent pathways, including an increase in phosphorylase activity and a decrease in gluconeogenic enzymes and sorbitol dehydrogenase [30].

According to recent reports [33], formulations of gymnemic acid have also proven effective against obesity. This is a result of gymnemic acids' ability to delay glucose absorption in the blood. Similar to glucose molecules, gymnemic acid molecules have a similar atomic arrangement. These molecules occupy the receptor sites on the taste buds, preventing their activation by sugar molecules in the food and thereby reducing the desire for sweets. Similarly, gymnemic acid molecules occupy receptor sites in the absorptive outer layers of the intestine, preventing the absorption of sugar molecules by the intestine and resulting in a low blood sugar level [34].

The leaves of G. sylvestre have been found to cause hypoglycemia in laboratory animals and are used in herbal medicine to treat type 2 diabetes in adults (NIDDM). When Gymnema leaf extract is administered to a diabetic patient, the pancreas is stimulated, resulting in an increase in insulin secretion [33]. These compounds have also been found to increase faecal cholesterol excretion [34], but additional research is required to establish their clinical significance in treating hypercholesterolemia (high serum cholesterol). Additionally, Gymnema leaf

extract has laxative, diuretic, and cough suppressant properties. These other actions would be considered adverse reactions when Gymnema is used to treat diabetes by lowering blood glucose levels.

It has been discovered that Gymnema leaf extract, specifically the peptide 'Gurmarin,' inhibits the ability of the taste buds on the tongue to detect sweet and bitter flavours. Gymnemic acid has a comparable impact. It is believed that by inhibiting the sweet taste sensation, those who take it will reduce their consumption of sweet foods, which may contribute to its hypoglycemic effect [35].

There are several possible mechanisms by which the leaves of G. sylvestre, particularly the Gymnemic acids, exert their hypoglycemic effects. 1) It increases insulin secretion, 2) It promotes islet cell regeneration, 3) It increases glucose utilization by increasing the activities of enzymes responsible for glucose utilization by insulin-dependent pathways, an increase in phosphorylase activity, and a decrease in gluconeogenic enzymes and sorbitol dehydrogenase, and 4) It inhibits glucose absorption from the intestine.

It is believed that the gymnemic acid components inhibit the absorption of glucose in the small intestine, although the precise mechanism is unknown. It might involve multiple mechanisms [35].

The inability of insulin to enter cells through the insulin receptor is one of the mechanisms responsible for adult-onset diabetes mellitus. Gymnema may overcome this resistance, but additional research is required to confirm its efficacy and determine if the effect is clinically significant. If this effect is confirmed, Gymnema may be useful in both adult-onset (NIDDM) and juvenile-onset (IDDM) diabetes mellitus to facilitate insulin entry into cells. In the case of IDDM, insulin is administered intravenously and is not produced by the pancreas [36].

1.5. Anti-Diabetic Properties

Due to the presence of triterpene saponins referred to as gymnemic acids, gymnemasaponins, and gurmarin, the herb's sweet inactivation property can be explained. The hypoglycemic effect of G. sylvester on rats treated with beryllium nitrate and streptozotocin was confirmed by experiments. In diabetic rats treated with G. sylvester, C. auriculata, E. jam- bolanum, and S. reticulata, there was a slight increase in body weight and protein, as well as a significant reduction in fasting blood glucose; these effects were comparable to those observed in mice treated with insulin and glibenclamide. Kang et al. [58] used ethanolic extracts to investigate the antioxidant activity of Gymnema leaf extract and the function of antioxidants in diabetic rats.

Several antioxidant assays, including thiobarbituric acid (TBA) assay with slight modifications, using egg yolk lecithin or 2-deoxyribose (associated with lipid peroxidation), superoxide dismutase- (SOD-) like activity assay, and 2,2 -Azinobis (3-ethylbenzothiazole-6-sulfonic acid) (ABTS) assay (involved in Additional LC/MS analysis revealed the presence of anti- hyperglycemic compounds such as gymnemagenin and gymnemic acids in G. sylvester extract, and the level of lipid peroxidation was reduced by 31.7% in serum, 9.9% in liver, and 9.0% in kidney in diabetic rats fed the ethanolic extract. Transaminases involved in gluconeogenesis and ketogenesis in diabetes, such as glutamate pyruvate transaminase (GPT) in serum and glutathione peroxidase in cytosolic liver, returned to normal levels in diabetic rats following administration of ethanolic leaf extract [60].

The crude saponin fraction and five triterpene glycosides (Gymnemic acids I–IV and gymnemasaponin V) isolated from the methanolic extract of the leaves were reported to have an antihyperglycemic effect [61]. Compared to glibenclamide, intravenous administration of gymnemic acid (3.4/13.4mg/kg) decreased blood glucose levels by 14.0–60.0% within 6 hours. It has been reported that gymnemic acid IV increased plasma insulin levels in STZ-diabetic mice at a concentration of 13.4 mg/kg, but had no effect on -glucosidase activity in brush border membrane vesicles of the small intestine in normal rats.

Similarly, the anti-diabetic and hypolipidemic potential of dried powdered G. sylvestre leaves was investigated in an experiment. Non-diabetic and alloxan-diabetic rats were given G. sylvestre leaf extract to determine its effect. The Gymnema leaf extract had no effect on the alleviated glycemia caused by a balanced meal or by the administration of glucose or amylose, but it increased serum lipid level after SOC treatment. The subacute and chronic treatment of nondiabetic and alloxan diabetic rats with Gymnema extract had no effect on food and water intake, body weight gain, and blood glucose and lipid levels. Before the herbal formulation can be used to treat diabetes and hyperlipidemia, however, it must first receive clinical approval and scientific validation [62]. The studies concluded that the herb possesses an anti-diabetic effect and sugar-inactivating properties.

The water-soluble and petroleum ether (40–60°C) extract was found to be highly effective at preventing and treating arthritis. Furthermore, triterpenoids, steroids, and saponin glycosides were thought to be responsible for the most potent antiarthritic activity of the leaves [59]. Different extracts were suspended in 1% Tween 80, Diclofenac sodium was administered orally once per day for 21 days, and the effect was monitored. On induction with an adjuvant, it was observed that rats developed swelling in multiple joints, cellular inflammation, bone destruction, and reshaping.

The petroleum ether extract-treated group demonstrated a significant reduction in paw odema, possibly as a result of inhibiting the response of inflammatory cells or blocking the release of pain- and disability-causing mediators such as cytokines (IL-Ib and TNF-a), GM-CSF, interferons, and PGDF [64]. The other possible mechanism of action in chronic arthritic models [63] suggests protection against the release of joint cartilage and bone destruction. Multiple studies employing polar solvents in extract preparations by researchers demonstrated the leaf extract's antiarthritic potential.

Treatment for Dental Cavities Dental caries is an infection of the tooth caused by gram-positive cariogenic bacteria [65] such as S. aureus, S. mitis, and S. mutans, and fungus-like Candida albicans, which attaches to the tooth surface via the release of extracellular polysaccharides from sucrose and metabolises sugar to organic acid primarily lactic acid, resulting in demineralization of the tooth enamel [66].

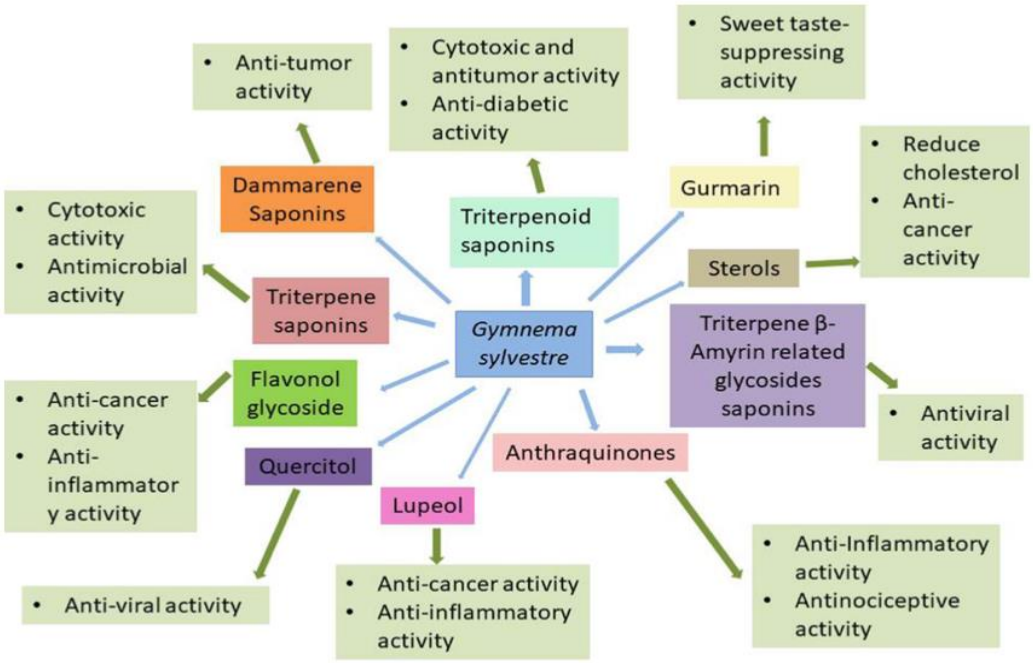


Figure. No. 04. Pharmacological activities of the constituents of Gymnema sylvestre

1.6. Introduction to Lozenges

Lozenges are flavoured dosage forms intended to be sucked and held in the mouth or pharynx, typically containing one or more medications in a sugary base [87-88]. The purpose of lozenges is to alleviate oropharyngeal symptoms, which are commonly caused by local infections, as well as for systemic effect, provided the drug is well absorbed through the buccal linings or when swallowed [9].Lozenges are used for patients who are unable to swallow solid oral dosage forms, as well as for medications designed to be slowly released to maintain a constant level of drug in the oral cavity or to bathe the throat tissues with a solution of the drug. Analgesics, anaesthetics, antimicrobials, antiseptics, antitussives, aromatics, astringents, corticosteroids, decongestants, and demulcents are frequently incorporated into lozenges. This is by no means an exhaustive list,

as numerous other medications may be suitable for delivery via lozenge. In addition, both single- and multi-ingredient lozenges can be compounded, depending on the needs of the individual patient [90].

ADVANTAGES:

It can be administered to patients with swallowing difficulties. It is simple to administer to geriatric and pediatric populations. It extends the duration of medication in the oral cavity to produce a specific effect. Basic absorption of medication is possible through the buccal cavity. Sugars and flavours can be used to mask the taste of medication. It can decrease dosing frequency. It can increase bioavailability. No disintegration. Not necessitating water for consumption. Reduced production time. Reduced production costs. If a dose is no longer necessary, the lozenge can be withdrawn.

1.7. Manufacturing Process of Lozenges

The candy base for chewy or caramel-based medicated lozenges is cooked at 95-125°C and transferred to a planetary or sigma cutting edge blender. It is permitted for mass to cool to 120°C. This is followed by the growth of whipping specialist below 105°C. The drugs are then added between 95 and 105 degrees Celsius. Shading is dispersed in humectant and added to the aforementioned mass at a temperature greater than 90OC. The cultivation of precious stones and flavour are then added below 85°C, followed by the addition of grease option above 80°C. Rope-forming is then used to shape confections.

Tablet compressible Lozenges:

1.7.1. Direct compression - Ingredients can be thoroughly mixed and compacted directly. [93]

In wet granulation, sugar is mechanically pulverized into a fine powder (40-80 mesh). Medicament is added to the mass, which becomes a mixed mass. The mixture is granulated with sugar or corn syrup and sieved through a 2-eighths-mesh screen. This is followed by drying and processing to a size of 10 to 30 mesh. Then, flavour and lubricant are added prior to compression.

Soft lozenges may be hand-rolled and subsequently cut into pieces, or the warm mass may be poured into a plastic mould. If Poly Ethylene Glycol (PEG) is utilised, the mould cavity should be overfilled, as PEG contracts as it cools. This is unnecessary given that chocolate does not shrink.

The candy base is prepared by dissolving the required quantity of sugar in one-third measure of water in a candy base cooker. This is continued until the temperature reaches 110°C. The corn syrup is added and the mixture is cooked until the temperature reaches 145-156°C. The mass of candy is removed from the cooker and transferred to a lubricated transfer container mounted on a weight check scale, where the mass's weight is measured. This is followed by the addition of colour using pastes, solutions, or colour cubes. The mass is then transferred to a stainless steel, water-jacketed table for blending, and flavouring, medication, and ground rescue are added.

While the mass is cooling, it is either poured into moulds or woven into a mesh, which is then sliced to the desired length. The got lozenges are wrapped in foil.

1.8. Excipients for Preparation of Lozenges

Sucrose, a disaccharide composed of glucose and fructose, is derived from sugarcane or sugar beets. Accessibility and geological considerations dictate whether sugar beets or natural sweeteners are selected.

Sucrose and sucrose derivatives are utilized in cured capsules due to their importance as independent sugars, their dissolvability properties, and their capacity as a "dryer" to reduce the mass of the sweet via crystallization.

Corn syrup: Corn syrup is used in all desserts to prevent the crystallization of sucrose and dextrose, which can lead to disintegration.

The combination of corn syrup, sucrose, and dextrose in the proper proportions permits the formation of a shapeless glass and produces a confection with an alluring appearance. In the preparation of medicated candies, the thickness, density, dextrose equivalent, hygroscopicity, sugar crystallization, consistency, edge freezing point depression, and osmotic pressure of corn syrup are crucial. Sucrose crystallization is observed in various food and pharmaceutical applications.

In sucrose crystallization, the average rate-limiting steps are dispersion of the sucrose from the mass answer for precious stone surface and combination of the sucrose atom into the grid structure.

Numerous factors, including temperature, supersaturation, and disturbance, and pollutants, can affect the development rate.

Binders: Generally, binders are used for compressed tablets that are intended to hold the particles of mass as discrete granules, such as acacia, corn syrup, sugar syrup, gelatin, polyvinylpyrrolidone, tragacanth and methylcellulose, HPMC, etc.

Lubricants, such as magnesium stearate, calcium stearate, stearic acid, and PEG, are used to prevent candies from sticking to teeth and to improve the flow of the final troche mixture.

Coloring agents: Coloring agents are added to medicated lozenges for product identification, aesthetics, and to mask physical degradation. Colorants are primarily used to give pharmaceutical dosage forms a distinct appearance. Dye and other organic colorants may be degraded by heat or light through oxidation, hydrolysis, photo oxidation, etc. Prior to selection, their compatibility with the drug, excipients, and process conditions must be determined. [94]

Flavoring agents: Flavor is a multisensory experience involving taste, touch, smell, sight, and sound. A variety of organic chemicals, including hydrocarbons, alcohols, aldehydes, ketones, acids, esters, and lactones, make up flavors. Low volatility and low molecular weight, typically less than 400 Daltons, account for a variety of sensorial sensations attributed to flavors. Since hard candy lozenges are hygroscopic, the water content may increase and bacterial growth may occur if they

are not packaged properly. Some sucrose would dissolve in the presence of water; the resulting highly concentrated sucrose solution would be bacteriostatic and would not support bacterial growth [95].

1.9. Analysis of Prepared Lozenges. Quality Control Checks

1.9.1 Physical and chemical examination

The lozenges' hardness is determined using a Pfizer or Monsanto hardness tester. The hardness of lozenges determines their resistance to shipping or breakage under conditions of storage, transportation, and handling prior to use.

Diameter and thickness:

A Vernier calliper is used to determine the lozenges' diameter and thickness.

Elasticity Friabilator

Roche is used to determine the friability of lozenges. The apparatus is rotated at 25 revolutions per minute for four minutes. Initial lozenge weights are determined and the lozenges are placed in a friabilizer. The lozenges were dedusted and reweighed after the revolution. The observed value cannot exceed 1%.

The formula for calculating friability is % friability = (1- Wt. / W) 100.

Where W= Initial lollipop weight Wt. = the weight of lozenges following the revolution.

Twenty lozenges were selected at random and individually weighed with an electronic balance to determine weight variation.

The mean and standard deviation of 20 tablets were calculated, or the initial weight was compared to the calculated mean.

Studies of Drug-Excipient Interactions:

Fourier Transform Infrared analysis, or FTIR, is utilized to study Drug-Excipient interactions.

The USP Disintegration apparatus is used to determine the disintegration time of lozenges during the 5.1.6 Disintegration test. The disintegration time is measured in phosphate buffer at pH 6.8 or in artificial saliva at 37oC. In- vitro drug dissolution study. The rate of drug dissolution in the lozenges determines the rate of drug absorption. The relationship between rate of dissolution and bioavailability and lozenge efficacy is direct. This research is conducted with USP II Dissolution apparatus (paddle type). The dissolution study was conducted in 900 ml of buffer pH 6.4 or artificial saliva using the USP II paddle method at 100 revolutions per minute. At 5-minute intervals, samples were withdrawn and immediately replaced with an equal volume of fresh buffer or artificial saliva before spectrophotometric analysis. Maintain a temperature of 37OC 2OC between dissolution studies.

The drug content is determined by crushing and dissolving an appropriate number of lozenges in a suitable solvent and measuring the absorbance of the solution spectrophotometrically.

As the manufacturing of the candy base commences, the following parameters are examined: Delivery mechanisms for corn syrup and sugar; temperature, steam pressure, cooking speed, and vacuum for precookers; temperature, steam pressure, cooking speed, and vacuum for candy base cookers [94 - 95].

The moisture content of lozenges is determined using gravimetric, Karl Fisher titration, and Azeotropic distillation techniques. In the gravimetric method, a 1g sample is weighed and placed in a 60-70°C vacuum oven for 12-16 hours.

Subtracting the final weight from the initial weight, the difference in moisture content is calculated. Karl Fischer titration requires calculating a sample to contain 10-250 mg of water in a titration flask, which is then titrated with Karl Fischer reagent. In azeotropic distillation, 10- 12 gm of crushed candy is added to a 500 ml flask containing 150-200 ml of toluene. Connecting a flask to a reflux condenser and refluxing it for 1-2 hours. The amount of water collected indicates the amount of water in the sample [95]

Microbial Test for Lozenges:

A microbial test for lozenges is conducted to check for bacterial, mould, or spore contamination in raw materials, cooling tunnels, finished products, machinery, environmental conditions, and storage drums. Testing for microorganisms in a laboratory should include multiple counts, such as total plate, total coliform, yeast and mould, E.coli, Staphylococcus, and Salmonella. Stability Evaluation

Lozenge stability testing is conducted under the following conditions:

1-2 months at 60°C 3 to 6 months at 45°C 9 to 12 months at 37°C 36 to 60 months between 25 and 40°C.The final packs of lozenges undergo stability testing under the following conditions: 25°C at 80% RH for 6-12 months; 37°C at 80% RH for 3 months; and 25°C at 70% RH for 6-12 months [96].For preparation of Herbal lozenge using Gymnema sylvestre, we will be needing a lozenges that release the drug in at least 15 minutes as it is to be used for diabetic patients, a fast release lozenge can result in faster action giving time to patient to settle and masking the taste of regular oral solid tablets.

MATERIAL & METHODS

1. **Extraction of Gymnema sylvestre:**
    a. Step 1: Extraction with Petroleum ether:

100 grams of dry leaf powder was packed into a clean soxhlet extraction unit. One liter of petroleum ether (60-80oC) was added and extracted for 3-6 hours till all the components are soluble in petroleum. Petroleum extract is collected and distilled in a distillation unit. Then a net weight of 25gm of petroleum ether extracts was obtained. Petroleum ether extraction was used for defatting dried leaf power.

b. Step 2: Extraction with 90% Methanol:
The plant dry powder material was then extracted with 90% methanol. 90% methanol was added and the extraction was carried out for 24-36 hours till the total methanol soluble extract was obtained. The methanol soluble extract was distilled and finally 150gm of the thick paste were obtained.

c. Step 3: Isolation of pure Gymnemic acid from Methanol Extract:
150gm dry leaf powder paste of methanol soluble extract was dissolved in 1% of KOH solution on continuously stirring for 45min to 1 hour. The solution was then filtered through filter paper to separate the undissolved particles. Diluted HCl acid was added slowly under constant stirring, during which the gymnemic acids were precipitated. Precipitated solution was filtered under suction and precipitate was dried. The pure gymnemic acid was obtained.

2. Preformulation studies:

a. Phytoconstituent Analysis:
Phenolic test: A pinch of gymnemic acid was taken into a clean test tube and dissolved 2ml of methanol. Then a few drops of 1% alcoholic ferric chloride were added.
Steroid test: A pinch of Gymnemic acid was added to a solution of 2ml CHCl3 and 1ml of acetic anhydride. A few drops of Conc. H2SO4 were added from the Sides of the Tubes.
Glycoside test: A pinch of Gymnemic acid was taken in a dried test tube and Dissolved in 2ml of methanol. 1ml of alpha naptholalcoholic solution was added from the sides of the test tube.

b. Calibration curve of Gymnemic acid:
Calibration curve for Gymnemic acid was constructed by dissolving it in Phosphate Buffer of pH 6.8 and series of dilution were made and absorbance was Measured at 210 nm.

3. Preparation of Lozenges:

4. Lozenges were prepared by wet granulation method. Accurately weighed amount of all the ingredients were added geometrically in mortar and add sufficient quantity of water to prepare to a damp mass. Pass the damp mass through sieve no. 16 and collect the wet granules on paper and shed dry it for 24 hours. Later, sieve the granules using sieve no. 85 to remove fine powder. Punch the tablet using Tablet compression machine.

5. Evaluations:

a. Organoleptic properties:
The organoleptic tests were carried out for colour, odour and taste of lozenges.

b. Moisture Content:
For Karl Fischer titration, 20ml of dehydrated methanol was added to the titration vessel and was titrated to the electrometric end point using Karl Fischer reagent. Prescribed amount of substance was weighed accurately and quickly transferred to the titration vessel. Stirred for one minute and titrated again to the electrometric end point using KF reagent. Now, lozenges were crushed in pestle mortar, weighed 4-5 times and then added in the KF reagent to attain a moisture percent value [2].

c. Hardness:
The Pfizer hardness tester was used to measure the lozenges hardness in terms of kg/cm2. Hardness of lozenge is the measurement of force which is applied across the diameter of the lozenge in order to break it as chipping or breakage during storage and handling always depends on the hardness [3].

d. Thickness:

The thickness of lozenges was measured by Vernier calliper and it is a significant feature in reproducing appearance. The average thickness for lozenges was measured in mm and presented with standard deviation [3].

e. Friability:

It is a measure of mechanical strength of tablets and was determined using Roche Friabilator.

Ten lozenges were preweighed and then they were placed in the Friabilator. The lozenges were

Then rotated at 25 rpm for 4 minutes (100 rotations) and then the lozenges were re-weighed [3].

Loss in the weight of lozenges is the measure of friability and is expressed as:

% Friability = [(W1 – W2) / W1] × 100

Where W1 = Initial weight of 10 lozenges

W2 = Weight of the 10 lozenges after testing

f. Disintegration time:

Disintegration time is defined as the time interval required for complete disappearance of a tablet or its particles from the tester net. Disintegration test apparatus was used to determine the disintegration time using phosphate buffer, pH 6.8 at 37 ºC [4].

g. % Drug Content:

5 mg lozenge was dissolved in 100 ml of phosphate buffer of pH 6.8 and absorbance was measured at 210 nm using UV visible spectrophotometer. % Drug Content was determined by using following formula;

% Assay = Sample Absorbance / Standard Absorbance X 100

h. In-vitro dissolution study:

USP apparatus II (paddle type) was used for the study. Accurately weighed formulations of lozenges were placed in 900 ml phosphate buffer of pH 6.8. The temperature was kept up at 37°C and mixed at a speed of 50 rpm. At 5 min time interval, a 5 ml aliquot of the sample was withdrawn and the volume was replaced with an equal measure of plain buffer kept at 37˚C. The obtained samples were filtered through 0.45 µm filter and measured at 210 nm using UV visible spectrophotometer. [5]

i. Stability Study:

The lozenges of optimized batch F3 were stored at 40˚C and 75% Relative humidity for 1 month and evaluated for all the physicochemical parameters like Colour, Odour, Taste, Moisture, Thickness, Hardness, Disintegration time, Friability and % Drug Content.

RESULTS AND DISCUSSION

5.1. Extraction of Gymnema sylvestre using petroleum ether and methanol in two steps gave significant results. For initial trials the powdered leaf was mixed with purified water and boiled until the concentration became half of original volume. 25 grams of dried leaf powder was taken in a beaker and 200 ml of purified water was added. Heating was initiated and the mixture was boiled unitl the mixture became of only 100 ml. This mixture was later filtered using a whatman filter paper and clear filtrate was analyzed on UV Spectrophotometer. Method of analysis is specified in material and method section. There was very small absorbance observed when extraction was done with purified water. The concentration of extract was compared to reference standard of Gymnemic acid and found that only 0.12% of the acid was extracted. Therefore, based on this finding, the extraction process was changed.

Based on structure of Gymnemic acid, petroleum ether was selected for initial extraction process. As direct boiling and filtration was not accurate choice, a distillation apparatus i.e. soxhlet apparatus was used. 100 grams of powder leaf was added to round bottom flask and 1000 ml of Petroleum Ether was added and boiling was initiated. It was made sure that boiling was done at $60^{o}C$ – $80^{o}C$. The boiling was continued for 3 – 6 hours. After 3, 4, 5 and 6 hours samples were collected using a syringe and analyzed on UV spectrophotometer. No significant amount of gymnemic acid was seen at any time point. The net 25 ml of petroleum ether concentrated with gymnemic acid was then taken for processing for step 2. The process of heating gymnema with petroleum ether causes defatting of dried leaf powder.

In step 2, the concentration solution of petroleum ether was mixed with 90% methanol (90% methanol and 10% water). This mixture was soaked for 36 hours. All the methanol soluble extracts were dissolved in methanol. This methanol extract was separated and kept for drying untill a thick paste was obtained. As gymnemic acid was soluble in methanol, the paste was processed for step3.

In step 3, 150 grams of paste collected in step 2, i.e. methanol soluble extract was dissolved in 1% potassium hydroxide solution. The mixture was stirred continuously for 45 minutes. This solution was then filtered through whatman filter paper to separate the undissolved particles. To precipitate the gymnemic acid for the mixture, dilute HCL was added and the precipitated acid was suction filtered and collected. The concentration was determined using a UV calibration curve as below:

Table No.01. UV Calibration table

Conc. (ug/ml)	Absorbance
0	0
5	0.195
10	0.41
15	0.595
20	0.803
25	0.975

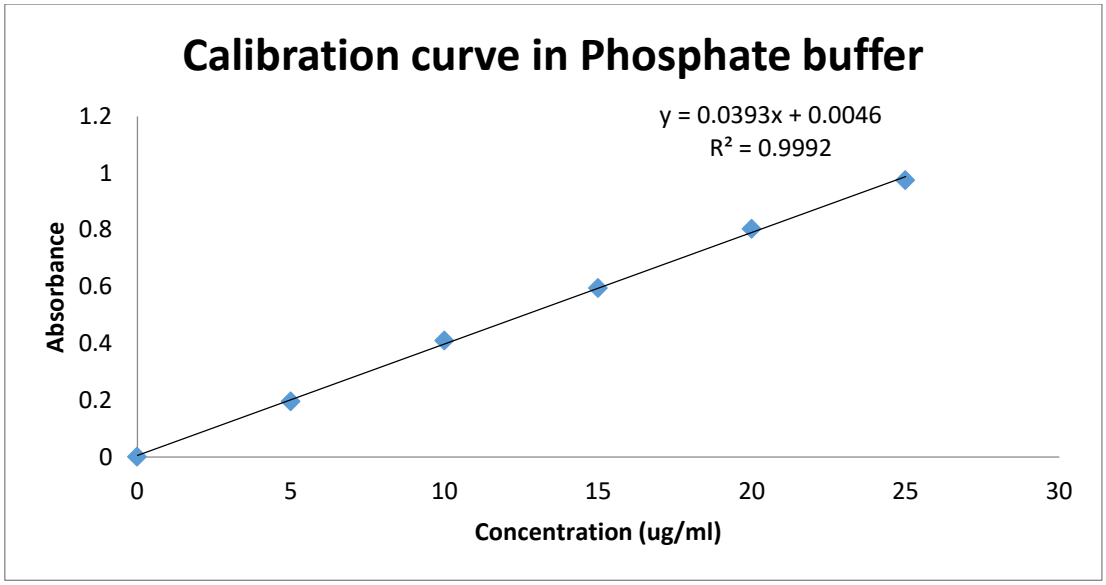


Figure No. 06 Gymnemic acid calibration curve.

The theoretical yield for gymnemic acid extracted form 100 grams of dried powder leaf was at 41.2%. I.e. almost 41.2 grams of gymnemic acid was extracted using this method. As the calibration curve has a R^2 of 0.999, the actual yield calculated here was accurate. The collected gymnemic acid was stored at room temperature away for light in dry place.

Initial evaluations of the gymnemic acid extract were done as below:

5.2. Preformulation Studies:

5.2.1. Phytoconstituents analysis:

Test for Phenolic constituents, Steroid and glycoside was done and positive results were observed. Implying presence of Phenolic, Steroid and Glycoside constituents in the final extract. Confirming the presence of Gymnemic acid in the extract. This was a secondary identification test.

As the final extract was ready for further use, the formulation development for Lozenges was initiated.

5.3. Formulation development.

As the intention of making these lozenges was for Gymnema sylvestre anti-diabetic properties, therefore, sugar or sugar related compounds were discarded. But to mask the taste of bitterness of the extract, some sweetener was required. Instead of artificial sweetener, we decided to use stevia powder (Only rebaudoside A - 99% extract) in the formulation. Stevia has been known as natural sweetener and is nearly 300 times sweeter than regular sugar.

The method of preparation of lozenges was by wet granulations. 100 grams of extract was taken and all ingredients like water and binder were mixed to make a thick paste of this extract. The damp mass was then passed through sieve no. 16 and collected wet granules were dried for 24 hours. After drying it was passed though sieve no. 85 to remove fin powder. The lozenges were prepared by tablet compression machine. As it was necessary to evaluate the granules and the final lozenges prepared.

Below is the formulation table using different polymer/gums and other excipients .

Table No.02. Formulation development

Formulation	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
Gymnemic acid (mg)	100	100	100	100	100	100	100	100	100
Stevia (mg)	10	10	10	10	10	10	10	10	10
Mannitol (mg)	100	200	300	-	-	-	200	300	400
Acacia (mg)	300	200	100	-	-	-	-	-	-
Xanthan Gum (mg)	-	-	-	200	300	400	-	-	-
Microcrystalline Cellulose (mg)	50	50	50	50	50	50	50	50	50
Lactose (mg)	440	440	440	640	540	440	640	540	440
Flavor (mg)	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.	q. s.
Total (mg)	1000	1000	1000	1000	1000	1000	1000	1000	1000

Each of the formulation listed in above table was compressed and lozenges or tablet was compresses and evaluation for is properties.

Table No.03. Organoleptic properties.

Batches	Colour	Taste	Odour
F1	Greenish Yellow	Sweet	Agreeable
F2	Greenish Yellow	Sweet	Agreeable
F3	Greenish Yellow	Sweet	Agreeable
F4	Greenish Yellow	Sweet	Agreeable
F5	Greenish Yellow	Sweet	Agreeable
F6	Greenish Yellow	Sweet	Agreeable
F7	Greenish Yellow	Sweet	Agreeable
F8	Greenish Yellow	Sweet	Agreeable
F9	Greenish Yellow	Sweet	Agreeable

All the formulation had similar properties. The lozenges were in good shape and size. The color kept was greenish as to have a natural look effect. Taste and odor was sweet and agreeable.

Table No. 04 Moisture Content of prototypes lozenges.

Batches	Moisture content (%)
F1	0.83 ± 0.03
F2	0.61 ± 0.04
F3	0.78 ± 0.02
F4	0.91 ± 0.07
F5	0.67 ± 0.06
F6	0.88 ± 0.01
F7	0.74 ± 0.02
F8	0.85 ± 0.05
F9	0.63 ± 0.07

The moisture content was in the range 0.61 to 0.91% implying very low water concentration. This was done by Karl-Fischer's method.
The three point test i.e. hardness, friability and thickness was studied and reported as below:

Table No. 05. Hardness, Thickness and Friability of Prototypes Lozenges.

Batches	Hardness (kg/cm²)	Thickness (mm)	Friability (%)
F1	10.4 ± 0.2	7.23 ± 0.06	2.43 ± 0.03
F2	9.2 ± 0.4	6.21 ± 0.02	3.71 ± 0.01
F3	7.8 ± 0.7	5.48 ± 0.02	0.98 ± 0.03
F4	8.5 ± 0.4	6.49 ± 0.04	1.21 ± 0.04
F5	11.3 ± 0.5	7.26 ± 0.03	1.54 ± 0.04
F6	9.7 ± 0.1	7.62 ± 0.05	2.95 ± 0.07
F7	8.3 ± 0.3	6.32 ± 0.06	3.02 ± 0.04
F8	11.2 ± 0.4	5.91 ± 0.08	1.16 ± 0.03
F9	7.6 ± 0.5	5.82 ± 0.09	1.85 ± 0.02

All the prototypes had pretty much same thickness. Some variations were observed in the hardness and friability. Prototype F3 and F9 had the least hardness compared to prototype F5 and F8 which have high hardness.

It can be logically incurred that more the hardness more time it will take to disintegration in mouth and vice versa.

Table No.06 Disintegration studies

Batches	Disintegration Time (min.)
F1	17 ± 0.5
F2	20 ± 0.1
F3	14 ± 0.4
F4	19 ± 0.2
F5	22 ± 0.6
F6	27 ± 0.6
F7	24 ± 0.4
F8	29 ± 0.7
F9	25 ± 0.2

Lozenge prototype F3 had the fastest disintegration time i.e. at 14 minutes all the solid was disintegrated. Formulation F9 when had similar hardness to that of F3 disintegrated at 5 minutes. This is due to the difference of polymer/ gum. F9 has microcrystalline cellulose causing delay in disintegration.

The drug content study was done for each prototype to know the amount of drug added during the time of making the formulation.

Table No 07. Drug content study:

Batches	**% Drug Content**
F1	98.75
F2	97.24
F3	99.48
F4	95.36
F5	97.63
F6	97.42
F7	96.29
F8	98.28
F9	99.37

All the lozenges prototype should drug content of 96.29% to 99.48%. Implying no degradation of gymnemic acid in the lozenges prepared during the granulation and compression time.
As the final product is lozenge, dissolution studies were performed for each prototype to understand the drug release profile. The data is shown below:

Table No.08 Dissolution studies. (All the data for F1 to F9 columns are in %)

Time (min.)	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	22.06	12.35	17.61	11.36	15.30	19.18	14.88	11.09	13.22
10	46.94	41.42	52.38	39.24	29.78	29.67	25.37	27.93	27.05
15	81.25	65.50	94.07	67.25	52.12	48.36	53.59	42.15	49.27
20	95.38	81.49	99.72	83.49	79.65	71.76	75.34	67.27	72.51

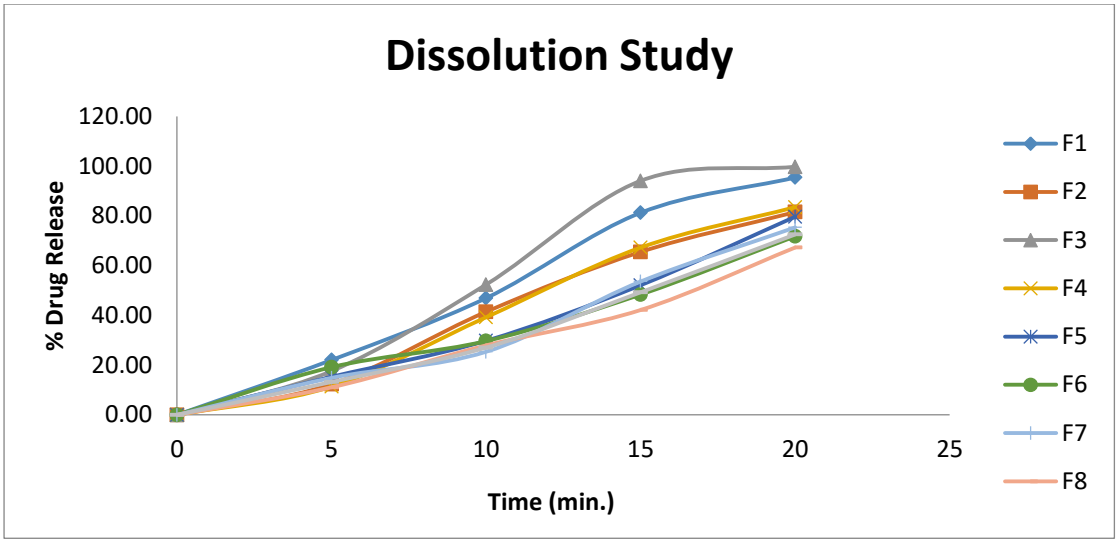


Figure No.07. Dissolution studies graph.

It can be observed from the graph and table above, that formulation F3 had the highest release at 15 minutes with % drug release of 94.07% compared to other prototypes this prototype has the fasted drug release.
Based on the Drug release profile and hardness and disintegration data, formulation F3 was taken as the lead prototype and selected for stability studies.
Stability studies were carried out for 1 month and all the parameters were studied. Evaluation data is shown below:

Table No.09 Stability data for Prototype F3

Batch F3	T0	1 month
Colour	Greenish Yellow	Greenish Yellow
Taste	Sweet	Sweet
Odour	Agreeable	Agreeable
Moisture content (%)	0.78 ± 0.02	0.70 ± 0.04
Hardness (kg/cm^2)	7.8 ± 0.7	7.9 ± 0.5
Thickness (mm)	5.48 ± 0.02	5.42 ± 0.04
Disintegration Time (min.)	14 ± 0.4	13.5 ± 0.2
Friability (%)	0.98 ± 0.03	0.99 ± 0.07
% Drug Content	99.48	99.45

After 1 month of stability, there is no significant change in the properties of Prototype F3 and it can be said that the formulation made to Lozenge is stable and ready for commercial application.

CONCLUSION

Lozenges was prepared using stevia as natural sweetener for Gymnema sylvestre extract. The extract process was of three steps using petroleum ether, 90% methanol and finally precipitation and filtration. A percentage yield of 41.2 was recorded using this extract technique. The lozenges were prepared using Acacia, xanthan gum and microcrystalline cellulose. Different formulations were prepared and evaluated for different physiochemical properties of which Formulation F3 should a drug release of 99.72% after 20 minutes. It also had fastest disintegration time compared to other formulations. As the lozenge was formulated for diabetic patients, the faster the drug release and disintegration, higher is the patient compliance.

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