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RESEARCH ARTICLE

Pharmacokinetic Profile of Polyherbal Tablets Comprising Extracts of Antidiabetic Medicinal Plants.

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ABSTRACT

Diabetes is a chronic metabolic disease that affects millions of individuals across the world and has a significant impact on human existence. Diabetes mellitus is a well-known endocrine condition that is becoming increasingly prevalent in India. In this study, using suitable animal models, researchers evaluated an extract produced from Gymnema Silvestre leaves, Momordica charantia fruit juice extract, and Synzigium cumini seeds extract for pharmacological activities such as antihyperglycemic and antidiabetic activity. Following that, nanoparticles were created and characterised utilising a variety of techniques. Following that, a polyherbal tablet was developed and tested in a variety of pre and post compression conditions. Finally, the pharmacokinetic characteristics of a made-from-scratch polyherbal tablet was compared to a commercially available formulation.

KEYWORDS:
Diabetes, Polyherbal Tablets, Gymnema Silvestre, Momordica charantia, Synzigium cumini.

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1. INTRODUCTION

Diabetes is a chronic metabolic disease that affects millions of individuals across the world and has a significant impact on human existence. Diabetes mellitus is a well-known endocrine condition that is becoming increasingly prevalent in India. It's possible that lifestyle and hereditary factors are to blame. Diabetic monocytes generate more superoxide anion as a result of these variables. Diabetes is a key risk factor in patients with early atherosclerosis and oxidative stress. Diabetes is a condition characterised by inadequate insulin production or increased insulin resistance. Chemical medicines are commonly employed to treat this distressing illness, however herbal diabetes therapies have shown to be effective in individuals with insulin-dependent and non-insulin-dependent diabetes, diabetic retinopathy, diabetic peripheral neuropathy, and other conditions. Diabetes is rapidly affecting the global population, particularly type 2 diabetes, which affects 90-95 percent of the population and is caused by impaired insulin production or consumption, according to the World Health Organization, which estimates that it will affect 300 million or more people by 2025. Many oral hypoglycemic medications, such as biguanides, glinides, and sulfonylureas, are used to control diabetes today, but many have negative side effects, thus research is mostly focused on finding safer antidiabetic drugs. [1-14] Herbal remedies have been a key source of medications for the prevention and treatment of illnesses, including diabetes mellitus, for millennia. There are about 200 plant species that have hypoglycemic characteristics. Diabetic treatment

frequently presents challenges in terms of medicine selection, dosage, and undesirable side effects. As a result, novel medicines with the fewest side effects but the greatest efficacy are constantly sought. Nanotechnology has offered fresh promise for the development of different medicines to treat a variety of illnesses, including diabetes, in recent years. Because nano-structured systems could potentiate the action of plant extracts, promote sustained release of active constituents, reduce the required dose, have low toxicity, reduce side effects, and improve activity, the strategy of applying nanotechnology to plant extracts has been widely cited in the literature. Furthermore, nano-encapsulated plant extracts have been employed in numerous investigations to improve the effectiveness of their biological activity. As a result, a recent subject centred on the usage of nanoparticles in the development of an anti-diabetic herbal medicine treatment. [15-18]

In this study, using suitable animal models, researchers evaluated an extract produced from Gymnema Silvestre leaves, Momordica charantia fruit juice extract, and Synzigium cumini seeds extract for pharmacological activities such as antihyperglycemic and antidiabetic activity. Following that, nanoparticles were created and characterised utilising a variety of techniques. Following that, a polyherbal tablet was developed and tested in a variety of pre and post compression conditions. Finally, the pharmacokinetic characteristics of a made-from-scratch polyherbal tablet was compared to a commercially available formulation. [19-22]

2. MATERIALS AND METHODS

Plant Profile

1. Gymnema Silvestre

The plant is native to central and western India, tropical Africa and Australia.

Synonyms-

Sanskrit: Meshashringi, madhunashini,

Hindi: Gur-mar, merasingi,

Marathi: Kavali, kalikardori, vakundi,

Plant description

G. sylvestre (Asclepiadaceae), a vulnerable species is a slow growing, perennial, medicinal woody climber found in central and peninsular India. It is a potent antidiabetic plant and used in folk, ayurvedic and homeopathic systems of medicine. It is also used in the treatment of asthma, eye complaints, inflammations, family planning and snakebite. In addition, it possesses antimicrobial, antihypercholesterolemic, hepatoprotective and sweet suppressing activities. It also acts as feeding deterrents to caterpillar, Prodenia eridania; prevent dental caries caused by Streptococcus mutans and in skin cosmetics. G. sylvestre is a large, more or less pubescent, woody climber. It is occasionally cultivated as medicinal plant. Leaves are opposite, usually elliptic or ovate (1.25–2.0 inch × 0.5–1.25 inch). Flowers are small, yellow, in umbellate cymes. Follicles are terete, lanceolate, upto 3 inches in length. [23-25]

2. Momordica charantia

Common Name: balsampear

Family: Cucurbitaceae

Native Range: Tropical Africa, tropical Asia

Plant Description: Yellow flowers and green to yellow to red fruits. M. charantia is widely distributed throughout tropical and subtropical regions on all continents. It appears to be native to the African and Australian continents, but its actual origin has been obscured by its spread as a food crop. Currently it can be found cultivated and naturalized in North, Central and South America, the West Indies and on several islands in the Pacific Ocean.

Rich, humusy, well-drained soils in full sun. Loves high heat. Best grown on a support structure such as a fence or trellis. Although it may be allowed to sprawl along the ground, its ornamental display of attractive foliage, flowers and fruit will be lost. Plants are generally grown like cucumbers. Start seed indoors (best in peat pots which decompose in the soil because seedlings dislike being transplanted) about 4 weeks prior to last spring frost date. Plant seedlings outside about the same time as tomatoes are typically planted. Plants will die in fall at the time of first fall frost.

Momordica charantia, commonly called bitter-melon or ampalaya, is a vigorous, tendril-bearing, frost tender, annual vine of the cucumber family that will grow rapidly to 12-20' long in a single growing season. It is native to tropical and sub-tropical parts of Asia and Africa. It was introduced into Hawaii where it has naturalized on several of the islands.

Rounded dark green leaves (1-4" diameter) have 3-7 deep palmate lobes with sharply toothed margins. Gourd-like yellow flowers (1" diameter) with 5 spreading petals bloom from the upper leaf axils in summer. Flowers are followed by cylindrical, torpedo-shaped, warty fruits (4-8" long) with wrinkled surfaces that ripen from green to yellow to orange at which point they split into three curling segments revealing the inner seed surrounded by showy scarlet pulpy arils. Ripe fruits are ornamentally attractive but malodorous. Young fruits (green or early yellow colored) are a popular vegetable consumed in SE Asia, India, China and Japan. Red mature fruit and seeds are toxic and should not be eaten. Leafy shoot tips are often used as salad greens.

Uses

The plant has many medicinal uses, including use as an anthelminthic, purgative and pain reliever, and to treat haemorrhoids, internal parasites and rashes. A seed extract has the capacity to inactivate certain cancerous tumours and may have anti-leukemic activity; it also helps ameliorate the

effects of diabetes mellitus. Its pharmacological properties have been recently investigated by Zafar and Neerja (1991). Leaves of the plant are brewed in hot water to create a tea to treat malaria and diabetes. The leaves are allowed to steep in hot water before being strained thoroughly so that only the remaining liquid is used for the tea.

3. Synzigium cumini

Common Name- Jambolan, Java Plum, Malabar Plum, Jambu

Family- Myrtaceae

Habitat- Most tropical and subtropical forest habitats in India, ranging from evergreen broadleaved to deciduous and coniferous, from wet to fairly dry areas, near the coast and even in swamps.

Plant Description- Syzygium cumini, Jambolan or otherwise known as Java Plum, is a medium-sized tropical and evergreen tree, about 10-30 m in height. The leaves are smooth, opposite, shiny, leathery and oval. The flowers are pink or nearly white. The fruits are oval, green to black when ripe, with dark purple flesh. it contains a large seed. The seeds and fruits are used in the treatment of diabetes. Seeds and bark are used against dysentery. Bark juice is used for treating wounds and enlargement of the spleen. Bark infusion is used to treat irregular menstruation, diarrhea, dysentery, children's thrush, etc. Fruits are used in the treatment of colic and diarrhea. Leaf infusion is used for diarrhea and diabetes. Fruits can be eaten raw or processed into desserts. It is juicy, purple, and olive-shaped. Jambolan also functions as a hedge in some areas and is interplanted with crops as a shade tree. The bark is a source of tannins and brown dye used in coloring and preserving fish nets. The branches are uses to whiten teeth. The wood is used in exterior joinery and carpentry, construction, boat building, plywood, agricultural implements, furniture, etc. Jambolan can tolerate waterlogged conditions and can withstand strong winds.

Uses

Both the seeds and the fruit are diuretic and have important carminative and astringent properties. The seeds also reduce blood sugar levels and are useful in the treatment of diabetes. The seeds and bark are well known in the Far East for the treatment of dysentery and in the control of hyperglycaemia and glycosuria in diabetic patients. The juice of the bark is considered good for treating wounds and enlargement of the spleen. The bark is astringent. An infusion is used to treat irregular menstruation, diarrhoea, dysentery, children's thrush etc. The bark is used as a gargle to strengthen gums, treat mouth ulcers etc. The ripe fruit is astringent and is used as an effective treatment for diabetes. Fruits are used as a relief for colic and to treat diarrhoea. An infusion of the leaves is used in the treatment of diabetes and diarrhoea. The wood yields a sulphate pulp that has medicinal uses. The roots are sometimes used as a treatment for epilepsy.

PLANT MATERIAL

1. Collections and Drying

Fruit of Momordica charantia, leaves of Gymnema sylvestre, stems and seeds of Synzigium cumini were collected from from nursery and local market, Nanded, Maharashtra, India, in the month of March-May.

2. Authentication

The plants, Gymnema Silvestre, Momordica charantia and Synzigium cumini was authenticated by C.R. Jadhav, Botanist, Botanical Survey of India, Pune by comparing morphological features. The herbarium of the plant specimen was deposited at Botanical Survey of India, Pune; with the Voucher specimen number 01-03 (Ref. No.BSI/WRC/IDEN. CER. /CRC/2016/H3 Dated 21/10/2016).

PHARMACOGNOSTIC STUDY

For all plants Gymnema Silvestre, Momordica charantia and Synzigium cumini pharmacognostic study were carried as per below procedure and methods.

1. Macroscopy

Organoleptic characters, extra feature and macroscopical details for all parts of plants were carried out. (Khandelwal, 2003)

2. Microscopy

Microscopical study was done as per the method described by Khandelwal, (2005). Transverse section of stem and leaf was taken, stained with phloroglucinol: Hydrochloric acid (1:1) and observed under microscope at 10X, 45X.

EVALUATION OF PHYSICAL CONSTANTS [26-28]

- **Determination of foreign organic matter**

  5 gm of air dried coarsely powdered drug was spreaded in a thin layer. The sample was inspected with the unaided eye or with the use of 6X lens. The foreign organic matter was separated manually as completely as possible. Sample was weighed and percentage of foreign organic matter was determined from the weight of the drug taken (Indian Pharmacopoeia., 1996).

- **Determination of moisture content**

  Accurately weighed glass-stopper, shallow weighing bottle, was dried. 2gm of sample was transferred to the bottle and covered, the weight was taken, and sample was distributed evenly and poured to a depth not exceeding 10 mm. Then loaded bottle was kept in an oven and was removed. The sample was dried to constant weight. After drying it was collected to room temperature in a desiccator. Weighed and the loss on drying was calculated in terms of percent w/w (Indian

Pharmacopoeia., 1996).

- **Ash value**

  Ash value is used to determine quality and purity of crude drug. Ash value contains inorganic radicals like phosphates carbonates and silicates of sodium, potassium, magnesium, calcium etc. sometimes inorganic variables like calcium oxalate, silica, carbonate content of the crude drug affects 'total ash value'. Such variables are then removed by treating with acid and then acid insoluble ash value is determined (Khandelwal, 2005).

- **Determination of Total ash**

  Accurately weighed 2gm of air dried crude drug was taken in a tared silica dish and incinerated at a temperature not exceeding 450°C until free from carbon, cooled and weight was taken. The percentage of ash was calculated with reference to the air-dried drug (Indian Pharmacopoeia., 1996).

- **Determination of Water- soluble ash**

  The ash was obtained as per method described above and boiled for 5 minutes with 25 ml of water, filtered and collected the insoluble matter on an ash less filter paper, washed with hot water and ignited for 15 minutes at a temperature not exceeding 450°C and weight was taken. Subtracted the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water-soluble ash. The percentage of water –soluble ash was calculated with reference to the air-dried drug (Indian Pharmacopoeia., 1996).

- **Determination of Acid -insoluble ash**

  The ash was obtained as per method described above and boiled for 5 minutes with 25 ml of 2M hydrochloric acid, filtered and collected the insoluble matter on an ash less filter paper, washed with hot water and ignited cooled in a desiccator and weighed. The percentage of acid –insoluble ash was calculated with reference to the air-dried drug. (Indian Pharmacopoeia., 1996).

- **Extractive values**

  Different extractive values like alcohol soluble extractive, water soluble extractive values were performed by standard method (Indian Pharmacopoeia, 1996).

- **Determination of water-soluble extractive value**

  Five gm of air dried coarsely powdered drug was macerated with 100 ml of chloroform water in a closed flask for 24 hours, and it was shaken frequently during first 6 hours and allowed to stand for 18 hours. Then it was filtered, 25 ml of the filtrate was evaporated in a flat shallow dish, and dried at 1050c and weighed. Percentage of water-soluble extractive value was calculated with reference to air-dried drugs. (Indian Pharmacopoeia, 1996)

- **Determination of Alcohol-soluble extractive value**

  Five gm of air-dried coarsely powdered drug was macerated with 100 ml of ethanol of specified strength in a closed flask for 24 hours, and it was shaken frequently during first 6 hours and allows standing for 18 hours. Then it was filtered, during filtration precaution was taken against loss of ethanol, 25 ml of the filtrate was evaporated in a flat shallow dish, and dried at 1050c and weighed. Percentage of ethanol soluble extractive value was calculated with reference to air-dried drugs. (Indian Pharmacopoeia, 1996)

EXTRACTION

PLANT EXTRACTS

Gymnema Silvestre -The leaves of Gymnema sylvestre was collected and dried in the shade. Then the dried material is pulverized in grinder.

Processing of Plant materials- Gymnema sylvestre leaves 1.5 kg of cleaned dried leaves powdered passed through 40 mesh and stored in a closed vessel for further use.

Method- Continuous hot Soxhlet extraction.

Solvents

The extraction was carried out with 85% ethanol. The extraction was carried out in Soxhlet extractor till all the constituents were extracted. The completion of extraction was indicated by taking sample of siphon tube on TLC plate and placing it in iodine chamber. Absence of colored spot-on plate indicated complete extraction. After completion of extraction, solvent was distilled off and concentrated extract was air-dried. The extract was stored in airtight container. Finally, the new dried powdered material was refluxed for about 3 hours with distilled water to obtain ethanolic extract.

Preparation of ethanolic extract from leaves of Gymnema sylvestre-

Extract was prepared according to Farzana et al., 2010 with slight modification. 1.5 kg of dry plant leaf material was packed in a Soxhlet thimble and extracted continuously with 85 % ethanol until the material is completely exhausted. The final product is a dark green amorphous powder after the evaporation of solvent. The collected solutions were filtered through Whatman No-1 filter paper. The solvent was evaporated under reduced pressure at 90 °C by Rotary evaporator and the green gummy extract was stored at -20 °C in a freeze until used for further analysis.

2. Momordica charantia- Fruit extract

Momordica charantia fruits (5 Kg) were purchased from the

local market. Dried fruit of Momordica charantia underwent a reflux extraction process using 15 L water (dH2O) between 75 and 80°C for 3 hours. Subsequently, the extract was filtered and solidified in the −80°C refrigerator for 24 hrs. It was then freeze-dried for 3–5 days until the powder of aqueous extract was formed.

3. Synzigium cumini- Seed extract

The powdered sample were percolated by using Soxhlet apparatus successively with the organic solvent such as methanol (70% w/v) respectively. The extracts were taken and kept for further studies.

Preliminary Phytochemical Screening for ethanolic Extract: (Khandelwal, 2005)

1) Test for Carbohydrates

a) Molisch test (General test)

Two ml of extract solution was added with few drops of 15 % ethanolic alpha naphthol solution in a test tube and 2ml of concentrated sulphuric acid was added carefully along the side of the test tube. The formation of reddish violet ring at the junction of two layers indicates the presence of carbohydrates.

2) Test for Proteins

i) Biuret test

The extract was treated with 1 ml of 10 percent sodium hydroxide solution and heated. A drop of 0.7 percent copper sulphate solution was added to the above mixture. The formation of purple violet color indicates the presence of proteins.

ii) Millon's test

The extract was treated with 2 ml of Millon's reagent. Formation of white precipitate indicates the presence of proteins and amino acids.

3) Test for Amino acids

Ninhydrin test

The extract was treated with Ninhydrin reagent at pH range of 4-8 and boiled. Formation of purple color indicates the presence of amino acids.

4) Test for Steroids

i) Salkowski test

One ml of concentrated sulphuric acid was added to 10 mg of extract dissolved in 1 ml of chloroform. A reddish brown color exhibited by chloroform layer and green fluorescence by the acid layer suggests the presence of steroids.

ii) Liebermann-burchard test

10 mg extract was dissolved in 1 ml of chloroform and 1 ml of acetic anhydride was added following the addition of 2 ml of concentrated sulphuric acid from the side of the test tube. Formation of reddish violet color at the junction indicates the presence of steroids.

iii) Liebermann's test

To 2 ml of the residue a few ml of acetic anhydride was added and gentle heated. The content of the test tube was cooled and 2 ml of concentrated sulphuric acid was added from the side of the test tube. Development of blue color gave the evidence for presence of steroids.

5) Test for Terpenoids

One ml of extract added with one ml of Vanillin sulfuric acid. Development of violet color gave the evidence for presence of Terpenoids.

6) Test for Saponins

Foam formation test

One ml solution of the extract was diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. The development of stable foam indicates the presence of Saponins.

IN-VIVO PHARMACOLOGIAL STUDY

The water-soluble (W-S) and water-insoluble (W-INS) fractions of a methanolic extract of Gymnema sylvestre were separated after being agitated in distilled water at room temperature. In total ethanolic extract, the yields of W-S and W-INS were 60 percent and 40 percent, respectively. These fractions were applied in the same proportion as they were obtained. These fractions were made using the method described by Alam et al (2005). The water-soluble fraction and water insoluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg and 80 mg/kg, respectively) were triturated with 1% carboxy methyl cellulose (CMC) solution in 0.9 percent w/v normal saline and given to male Wistar albino rats by oral route for the experimental study.

Animals

Swiss Albino mice of either sex weighing 20-30 g were selected from animal house of SGRS College of Pharmacy, Saswad. The animals were maintained in house at room temperature, humidity and light. They were feed with standard pellet feed and water ad libitum. Protocol was approved by the Institutional Animal Ethics Committee (IAEC), SGRS College of Pharmacy, Saswad, No. IAEC/SGRS/2021/5.

Alloxan-induced diabetes in Wistar rats

Acute Toxicity Studies

Organization for Economic co-operation and Development (OECD) regulates guideline for oral acute toxicity study.

Methods for Acute Toxicity Study

Nine adult albino rats were divided into three groups, each with three individuals. All of the animals were fasting for the night. The extracts of all three plants were dissolved in 1% CMC and given orally at dosages of 300, 1000, and 2000 mg/kg body weight, respectively. The animals were monitored for 2 hours and then for another 4 hours for any signs of death. Gross behaviour, pupil size, general motor activity, convulsion, water intake, faecal output, writhing, response to tail pinching, sedation, and any other toxic signs were monitored for 72 hours, after which the animals were maintained under observation for another 14 days.

Induction of Type-II Diabetes in Rats

Two weeks before to the start of the trial, the animals were acclimatised. All animals except those in group I (Normal control) were given a 10% fructose solution instead of drinking water for three weeks, after which a single dosage of 40 mg/kg of Alloxan was administered i.p. into fasting rats. In 0.1M citrate phosphate buffer, alloxan was newly synthesised (pH 6.3). After 48 hours, blood glucose levels were measured, and animals with blood glucose levels more than 250 mg/dl were separated into the following groups. Animal classification:

Group-I : Normal non-diabetic rats.

Group-II : Diabetic control rats.

Group-III : Diabetic rats fed with extract of Gymnema Silvestre (250 mg/kg body wt.)

Group-IV : Diabetic rats fed with extract of f Momordica charantia (250 mg/kg body wt.)

Group-V: Diabetic rats fed with extract of Synzigium cumini (250 mg/kg body wt.)

Group-VI : Diabetic rats fed with metformin

Preparation of nanoparticle

1 mM AgNO3 solution preparation

One milimolar (mM) solution of AgNO3 was produced by dissolving (0.17 mg/ml) it in 500 ml distilled water (DW) and storing it in an amber-colored container in a cold, dry location.

In an Erlenmeyer flask, 500 mL, 5-10mL of Gymnema Silvestre leaf extract, Momordica charantia fruit extract, and Synzigium cumini seed extract (NPF1-NPF6) Was added into 90-95ml of aqueous solution of 1mM silver nitrate the mixture was exposed to a range of controlled temp for 24 hr. The solution was then kept in dark for further analysis collected and stored for 40c for further use.

The reaction mixture was stirred at 200 rpm with a magnetic stirrer until the colour of the solution changed from yellow to dark brown, indicating the production of AgNPs. To get clear supernatant, the decreased solution was centrifuged at 5000 rpm for 30 minutes. To get pure nanoparticles, the supernatant was removed and the particles recovered were centrifuged with water many times. Using Gymnema Silvestre leaf extract, Momordica charantia fruit extract, and Synzigium cumini seed extract, several silver nanoparticle formulations (NPF1-NPF6) were created. The powders (nanoparticles) were lyophilized and kept at 40 degrees Celsius until needed.

Table 1: Formula of different formulations of leaf extract of Gymnema Silvestre leaf, fruit extract of Momordica charantia and seed extract of Synzigium cumini extract loaded chitosan nanoparticles

S. No.	Name of ingredients	NPF1	NPF2	NPF3	NPF4	NPF5	NPF6
1	Gymnema Silvestre leaf extract	5mL	10mL	-	-	-	-
2	Momordica charantia fruit extract	-	-	5mL	10mL	-	-
3	Synzigium cumini seed extract	-	-	-	-	5mL	10mL
4	AgNO3	95ml	90ml	95ml	90ml	95ml	90ml

All values are in % Characterizations of Nanoparticles

1. Zeta potential study

A zeta sizer was used to examine the zeta potential (surface charge) of silver nanoparticles. The produced nanoparticle formulations (NPF1-NPF6) were diluted with water (0.1ml) and put in an electrophoretic cell with a 15.5 V/cm electrical field to evaluate their zeta potential. Each sample was measured in three different ways.

2. Scanning electron microscopy

Scanning electron microscopy was used to examine the nanoparticle's morphology. In a first stage, 100l of silver nanoparticle formulations (NPF1-NPF6) were applied to a 10mm glass slide and dried overnight at room temperature in a vacuum desiccator till SEM examination was done. Nanoparticles were mounted on appropriate support and coated with gold using a gold sputter module in a higher vacuum evaporator for analysis. At a voltage of 15kv, observations were made at various magnifications.

3. Drug encapsulation efficiency

The ultra-centrifugation technique was used to assess the

drug encapsulation effectiveness of silver nanoparticle formulations (NPF1-NPF6). Using ultracentrifugation at 10,000 rpm for 30 minutes, AgNO3 containing the silver nanoparticle was separated from the silver nanoparticle. The pellets were re-dissolved in distilled water, and the supernatant was scanned with a UV-visible spectrophotometer in this parameter (for Gymnema Silvestre, 486nm, Momordica charantia, 440nm, and Synzigium cumini, 277.5nm).

The drug encapsulation efficiency was determined by using the relation in this equation.

% Drug encapsulation efficiency= experimental drug contentx100/Theoretical drug content

--Formula 1

4. Production yield of nanoparticles

The yield of nanoparticles were determined by comparing the whole of nanoparticle formed against the combined weight of the copolymer and drug.

% Yield calculation = Amount of drug X100 / Amount of drug +polymer

--Formula 2

5. In-vitro release study of drug release

The in-vitro release of produced silver nanoparticles in phosphate buffer saline (PBS) (PH 7.4) at 370C was studied. Silver nanoparticles were dialyzed for 12 hours against 50 ml of PBS with continuous shaking in a dialysis bag. Aliquots were removed on a regular basis. The deleted sample volume in PBS is replaced with a new volume of PBS. The quantity of medication released was measured by using a UV-visible spectrophotometer to measure absorbance (for Gymnema Silvestre, 486nm, Momordica charantia, 440nm, and Synzigium cumini, 277.5nm).

6. Transmission electron microscopy

TEM is a type of microscopy in which a beam of electrons is sent through an ultra-thin object and interacts with it as it passes. The interaction of electrons passing through the specimen creates an image, which is amplified and focussed onto an imaging device, such as a fluorescent screen, a layer of photographic film, or a sensor to be detected. Because of the short de Broglie wavelength of electrons, transmission electron microscopes can image at a far greater resolution than light microscopes. In a variety of scientific areas, including physical and biological sciences, TEM is a common method of analysis. It's used in cancer research, virology, materials science, pollution, nanotechnology, and semiconductor research, among other fields.

Transmission electron microscopy (TEM) was used to characterise the morphology of AuNPs (JEOL-JEM 2100, 1.4 Angstrom Unit, Tokyo, Japan). Drops of diluted AgNP solutions were air-dried on carbon sheets supported by copper grids to create the samples. Under the microscope, TEM images were seen at 120 kV.

7. Stability of Nanoparticle

The stability of the produced nanoparticles was evaluated by keeping the optimum formulation in a stability chamber for eight weeks (4°C, RT-25°C) for three months. The particle size, zeta potential, entrapment efficiency and physical appearance were determined at different time intervals of one, two and three months. (According to ICH Q1A).

Preparation of Polyherbal tablet

Formulation

The prepared lyophilized powder blends of optimized formulations (F2, F4 and F6) i.e. 1:1 is taken to formulate the tablet and to carryout the pre formulation study.

Pre-formulation studies

The following Pre-formulation studies were performed:

Organoleptic studies

In this study the organoleptic features like colour, odour and physical appearance were observed and recorded.

Angle of repose

Angle of repose is an important parameter to study the Flow property analysis of any powdered formulation with respect to their frictional forces. It was measured by taking reading of height of pile and radius of pile. Mathematically it was calculated by level of the pile (H) divided by radius of the pile (R).

Angle of repose (Tan θ) = height of the pile (H)/ radius of the pile(R)

Loss on drying

Weighing bottle was dried a in an oven at 105ºC and weight (w1) was taken. 3 g of the powder was to be found in it. It was dried at temperature of 100-1050C in oven approximately for 3 hours. Drug was then allowed to cool in desiccators. And weigh it again (w2).

% Loss on drying (LOD) = {(w1- w2) / w1} x100

Total ash

The W1 that was weight of crucible obtained by weighing of crucible after heating for 30 minutes followed by cool in desiccators. The sample powdered drug is weight in crucible (3 gm). The crucibles gross weight was noted with the contents (w2). The specimen was spread evenly and dried at 100-105ºC for 1 hour. Ignited to a constant mass. Allowing, the crucible to cool in desiccators after ignition. The weight (w3) of crucible was determined after cooling in desiccators. The calculation of total ash was done in % w/w by the following formula.

Total ash in % w/w= {(w3-w1)/ (w2-w1)} X 100

Where, w1-Mass of the empty crucible in grams

w2- Mass of the crucible + sample in grams

w3- Mass of the crucible + Residue obtained in grams

Bulk density

Bulk density was determined by measuring the amount of sample required to fill 3/4th volume of a 10ml. capacity graduated measuring cylinder via a funnel and measuring the volume occupied and weighed. The following formula was used to determine bulk density.

Bulk density (Db) = Mass of powder (M) / bulk volume (Vb)

Tapped density

The following formula was used to determine tapped density.

Tapped density (Dt) = mass of powder (M) /tapped volume (Vt)

Tapped density was determined by tapping the graduated 10ml. measuring cylinder 100 times from a height of about 1.5 inch.

% Compressibility

% Compressibility determined by formula given below

Tapped density – Bulk density/ tapped density. Then multiplied value obtained by100

Hausner ratio

Hausner ratio can be obtained by formula given below.

Hausner ratio = Tapped density / Bulk density

Formulation of tablets

Extract powders of nanoparticles from Gymnema Silvestre, Momordica charantia, and Synzigium cumini (F2, F4, and F6) were used in the current investigation. Individual powdered extracts of Gymnema Silvestre, Momordica charantia, and Synzigium cumini were used to make six formulations. Then, using a mixture of the various extracts, a formulation was created. PHF is the acronym for a combination formulation (Poly herbal formulation).

The following is the structure of a formula.

Table 2. Formulation containing extracts of leaf of Gymnema Silvestre, fruit extract of Momordica charantia and seed extract of Synzigium cumini.

Sr. No.	Ingredient	F1	F2	F3	F4	F5	F6	F7 (Poly)
1	Powder of Gymnema Silvestre	100	75	-	-	-	-	50
2	Powder of Momordica charantia	-	-	100	75	-	-	50
3	Powder of Synzigium cumini	-	-	-	-	100	75	50
4	Lactose	90	115	90	115	90	115	40
5	Talc	5	5	5	5	5	5	5
6	Starch	35	30	35	30	35	30	35
7	Microcrystalline Cellulose-101	15	20	15	20	15	20	15
8	Sodium benzoate	5	5	5	5	5	5	5

Quantity of per tablet (mg) Total Weight – 250mg

Granules preparation by wet granulation process

Take Starch and measured it. It converts into granulating liquid with water and applied the sodium benzoate as a preservative to it. The starch emulsion was very well cooked in a water bath unless translucent semi-solid mass was created. The weighed excipient quantities were thoroughly mixed with extract adding gradually the cooked starch until the powder was a moist mass. This humid mass was moved across sieve number 20 and dried for 3 hours in an oven at a temperature of 60oC until the granules were adequately dried. Then the dried granules are moved via sieve number 20 and lubricated. The dried powder extract and other components were evenly mixed and the wet granulation process was used to produce granules. In an 8-station tablet press machine with 500 mg die cavity, the lubricated granules were pressed into tablets.

Evaluation of Tablets

The various physical parameters were used to evaluate the tablets.

Organoleptic properties

Size (thickness), shape, colour, taste was determined.

Weight Variation Test

For determination of weight variation take 20 tablets. Weight these 20 tablets and their average was determined. Compare the average of tablet weight with single tablet.

Hardness

The tablet strength was represented as the capacity of the tensile (Kg/cm2). The tablet crush load, in which the pressure force needed to break a tablet in half. A tablet

hardness tester (Monsanto hardness tester) was used to calculate this.

Friability

Friability testing was carried out to evaluate friction and shocks effect. This can often lead to chipping, caping, and breaking of tablets. For this reason, Roche friabilator was used. This machine puts many tablets under the cumulative impact of abrasion. The tablet was shaken in a plastic chamber which rotates with speed of 25 RPM. The tablets were dropped from height of 6 inch per rotation. The tablets were again weight after dusting. The loss of weight of tablets should not more than 1 %.

Disintegration Test

Electrolab disintegration test apparatus was used for the test. One tablet was placed in each of the six tubes of the basket and the apparatus was maintained at 37 ± 0.50°C of the immersion liquid. The time required for complete disintegration of tablet was noted. All disintegrated particles should pass from 10# sieve screen of apparatus.

Stability Study (Accelerated)

For 6 months, accelerated stability testing was carried out according to the ICH stability testing recommendations. Temperature and relative humidity are maintained in accordance with the standards.

The research was carried out with the use of a Photo Stability Chamber from Make-Labtop India.

Pharmacokinetic Profile of formulated tablet by comparing with marketed formulation.

Dissolution study

The dissolution profile (F1-F7) was determined in 900 ml of simulated gastric liquid (0.1 N HCl) at a stirring speed of 100 rpm using the USP dissolution device II. Different aliquot samples were taken at 5, 10, 15, 30, 45 and 60 min respectively with simulated substitute fluid of the same quantity. The quantity of medication released was measured by using a UV visible spectrophotometer to measure absorbance (for Gymnema Silvestre, 486nm, Momordica charantia, 440nm, and Synzigium cumini, 277.5nm). Similarly, a dissolving test was performed on a commercial pill (diabohills-550mg). The disintegration profiles of each were compared.

Animal groups:

Anti-diabetic study

The In vivo study was conducted in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, Government of India) guidelines and approved by the Institutional Animal Ethics Committee (IAEC), SGRS College of Pharmacy, Saswad, No. IAEC/SGRS/2021/5.

Alloxan-induced diabetes in rabbits

Acute Toxicity Studies

The Organization for Economic Co-operation and Development (OECD) oversees oral acute toxicity research guidelines.

White albino rabbits weighing 1.5-2.0 kg were ynthesi in the experiment. The rabbits were bought from local vendors and housed in an animal home at the SGRS College of Pharmacy, Saswad. The cage was kept at a constant temperature of 25°C.

Methods for Acute Toxicity Study

Nine adult albino rabbits were divided into three groups, each with three individuals. All of the animals were fasting for the night. The formulation tablets were dissolved in 1% CMC and given orally at dosages of 300, 1000, and 2000 mg/kg body weight, respectively. The animals were monitored for 2 hours and then for another 4 hours for any signs of death. Gross behaviour, pupil size, general motor activity, convulsion, water intake, faecal output, writhing, response to tail pinching, sedation, and any other toxic signs were monitored for 72 hours, after which the animals were maintained under observation for another 14 days.

Induction of Type-II Diabetes in Rabbits

Two weeks before to the start of the trial, the animals were ynthesized . All animals except rats in group I (Normal control) were given a 10% fructose solution instead of water for three weeks, after which a single dosage of 40 mg/kg of Alloxan was administered intraperitoneally into fasting rabbits. In 0.1M citrate phosphate buffer, alloxan was newly ynthesized (pH 6.3). After 48 hours, blood glucose levels were measured, and animals with blood glucose levels more than 250 mg/dl were separated into the following groups. Animal classification:

Table 11: Animal groups for in vivo study. (Validation batch)

Groups	Treatment	No. of Rabbits
Group 1st	Control	4
Group 2nd	Formulation F1	4
Group 3rd	Formulation F2	4
Group 4th	Formulation F3	4
Group 5th	Formulation F4	4
Group 6th	Formulation F5	4

Group 7th	Formulation F6	4
Group 8th	Formulation F7-Polyherbal	4

Following the induction of diabetes, all batches F1-F7 tablets were delivered by stomach intubation. The rabbit was restrained during the administration of the tablet and the collection of the blood sample. Blood samples were taken at specified intervals of 0, 5, 10, 15, 30, 45, 60 minutes from marginal ear veins. Heparinized tubes were used to retain the extracted blood sample. Following centrifugation of blood at 3500 rpm for 5 minutes at 4 °C, plasma samples were collected and stored frozen until analysis. Blood glucose levels were measured in the samples.

3. RESULTS AND DISCUSSION

For all plants Gymnema Silvestre, Momordica charantia and Synzigium cumini pharmacognostic study were carried and results obtained were discussed below,

Macroscopical Examination

All the three plants were morphologically examined for their colour, odour, taste, texture, size, shape. They were morphologically found to be identical with reference material.

Determination of foreign organic matter

Foreign organic matter of extract of Gymnema Silvestre, Momordica charantia and Synzigium cumini was found to be 0.5% w/w, 0.6% w/w and 0.5% w/w when observed under 6X lens.

Determination of moisture content

Moisture content of extract of Gymnema Silvestre, Momordica charantia and Synzigium cumini was found to be within limit.

Table 3: Moisture content of extract of Gymnema Silvestre, Momordica charantia and Synzigium cumini.

Time (hrs.)	Gymnema Silvestre, (%w/w) -Extract 1	Momordica charantia (%w/w) -Extract 2	Synzigium cumini (%w/w) - Extract 3
0	0.000	0.000	0.000
01	0.200	0.210	0.198
02	0.210	0.212	0.201
03	0.220	0.213	0.209
04	0.220	0.213	0.209

Determination of Ash value

Ash value of respected parts of each plant was done. The total ash, acid insoluble ash, and water-soluble ash were determined and mentioned below,

Table 4: The total ash, acid insoluble ash, and water-soluble ash

Sr. No.	Evaluation Parameters	Value (%w/w)- Extract 1	Value (%w/w)- Extract 2	Value (%w/w)- Extract 3
1.	Total ash value	4 %	5 %	3 %
2	Acid insoluble ash value	0.7 %	0.8 %	0.6 %
3	Water soluble ash value	0.2%	0.3%	0.3%

Determination of Extractive values

Extractive value of respected parts of each plant was done. The water soluble and Alcohol soluble extractive values were determined and mentioned below,

Table 5: Extractive values

Sr. No	Extractive values	Extractive value (%w/w)- Extract 1	Extractive value (%w/w)- Extract 2	Extractive value (%w/w)- Extract 3
1	Methanol soluble extractive values	13	5.7	11
2	Water soluble extractive values	5.8	12	5.6

In the case of extracts 1 and 3, the methanol-soluble extractive value was found to be larger than the extractive value, indicating that the chemicals present in the leaves are highly soluble in alcohol. The fact that extract 2 has a lower extractive value suggests that the chemical in fruit extract is

more soluble in water than in any other solvent. This might help us isolate the most active components from the plant.

Preliminary Phytochemical Screening

The presence of steroids, terpenoids, flavones, and saponins has been confirmed in preliminary phytochemical screening of Gymnema Silvestre, Momordica charantia, and Synzigium cumini. The role of various constituencies was screened in all extracts.

The results of this preliminary phytochemical study are summarised in the table below.

Table 6: Preliminary Phytochemical Screening of Various Extracts of Gymnema Silvestre, Momordica charantia and Synzigium cumini.

Extracts	**Aqueous Extract**	**Ethanol Extract**	**Methanol Extract**
Tests for carbohydrates	-	-	-
Molish Test	+	-	-
Fehling Test	-	+	+
Benedict Test	-	-	-
Test for Monosaccharide	-	-	-
Barfoed's Test	-	-	-
Test for Non-reducing polysaccharides	-	-	-
Iodine Test	-	-	-
Test for Proteins	-	+	-
Biuret test	+	-	-
Millions test	-	-	-
Tests for Steroids			
Salkowaski reaction	-	+	+
Libermann Burchard reaction	-	+	+
Libermann reaction	-	-	-
Tests for Terpenoids	+	+	+
Test for Saponin			
Foam test	+	+	+
Tests for Flavonoids			
Shinoda test	+	+	+
Lead acetate Test	-	-	-
Sod-hydroxide Test	-	+	+
Test for Tannins & Phenolic compounds			
FeCl3	+	+	+
Lead acetate	+	+	+

+ Indicates presence of phytoconstituents, - Indicates absence of phytoconstituents

TLC- Characterization of extract of Gymnema Silvestre, fruit extract of Momordica charantia and seed extract of Synzigium cumini

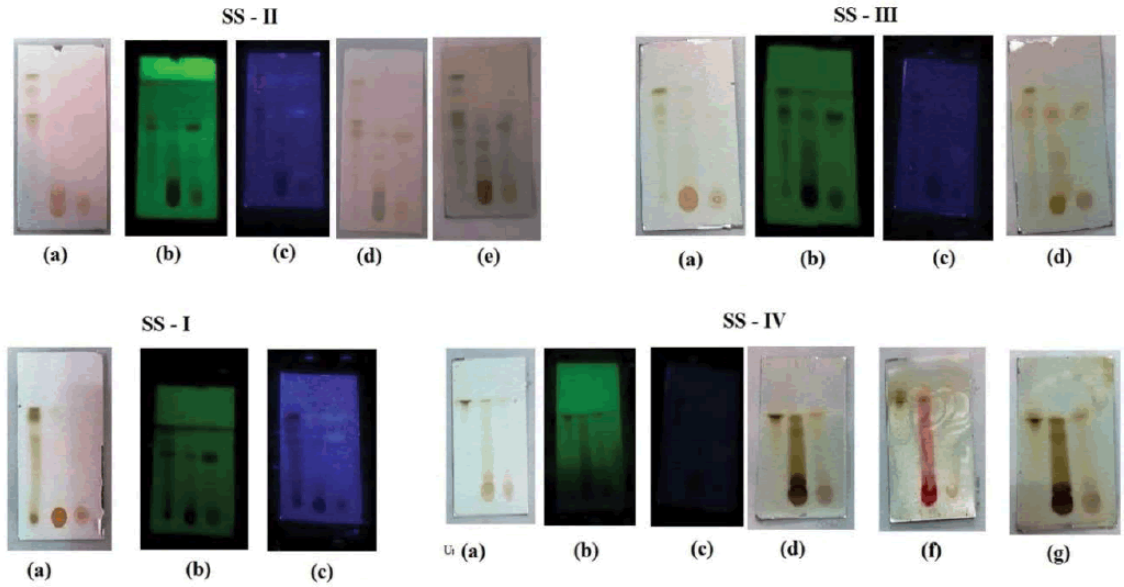


Fig.1: TLC Analysis of all plant extracts in various solvent system

SS- Solvent System; a-under visible light; b- under UV short wave length; c- under UV long wave length; d- under NH3 sparay; e- under Folin-Ciocalteu spray, f-under vanillin HCl spray; g-under potassium hydroxide in ethanol spary.

IN VIVO PHARMACOLOGICAL STUDY

In-vivo study was carried out with extract of Gymnema Silvestre, Momordica charantia and Synzigium cumini.

Acute Toxicity

The acute oral toxicity in mice indicated that extract of Gymnema Silvestre, Momordica charantia and Synzigium was nontoxic at 200-400mg /kg body weight.

Table 7: Acute toxicity profile

Groups	No. of animals in group	Dose (mg/kg)	Results
Extract 1	9	300	No toxic sign
Extract 2	9	300	No toxic sign
Extract 3	9	300	No toxic sign

Hypoglycemic study

Effect of Extract 1-Extract 3 on Blood Glucose levels in Diabetic rats

The obtained extract of Gymnema Silvestre, Momordica charantia and Synzigium cumini after administration in rats were studied for antidiabetic activity for 1 month. The blood samples were collected at the defined intervals and checked for blood glucose levels.

Table 8: Blood Glucose levels in Diabetic rats

Groups	0 week	1 week	2 week	4 week
Non diabetic Control	124	124.5	124	124
Diabetic control	288	299	301	318
Extract 1	293	229	202	184
Extract 2	292	211	180	169
Extract 3	295	198	179	167
Standard	296	201	178	162

All values are given as mean ± SEM, N = 6. **P < 0.01 compared to diabetic control animals at specified interval.

The extracts of Gymnema Silvestre, Momordica charantia, and Synzigium cumini have potential anti-diabetic action, according to blood glucose levels. When compared to the control group, the alloxan induced diabetic group's mean blood glucose levels were substantially (p0.01) lower for extract 1, extract 2, and extract 3.

Initially, there is no substantial drop in blood glucose levels after the "0" week, but after the 1st week, there is a considerable decrease in blood glucose levels, i.e., 30%, which progresses to a 45 percent fall in blood glucose by the end of the 4th week.

Characterizations of Nanoparticles

The prepared nanoparticles of extract of Gymnema Silvestre, Momordica charantia and Synzigium cumini were subjected for different evaluations parameters.

1. Particle size determination by Zeta sizer

The particle size of nanoparticles (F1-F6) made from extracts of Gymnema Silvestre, Momordica charantia, and Synzigium cumini was determined. The nanoparticles' particle size was determined to be between a range of 1-100nm.

Table 9: Particle size and zeta potential of extract of Gymnema Silvestre, Momordica charantia and Synzigium cumini.

Sr. No.	Sample	Nanoparticle Size (nm)	Zeta Potential (mV)
1	Extract of Gymnema Silvestre-F1	43.1 ± 12	-28.45
2	Extract of Gymnema Silvestre-F2	42.5 ± 12	-29.12
3	Extract of Momordica charantia-F3	43.3 ± 12	-50.25
4	Extract of Momordica charantia-F4	41.8 ± 12	-51.58
5	Extract of Synzigium cumini-F5	39.1 ± 18	-30.12
6	Extract of Synzigium cumini-F6	38.6 ± 18	-29.08

Values are shown as the mean ± standard deviation; n=5.

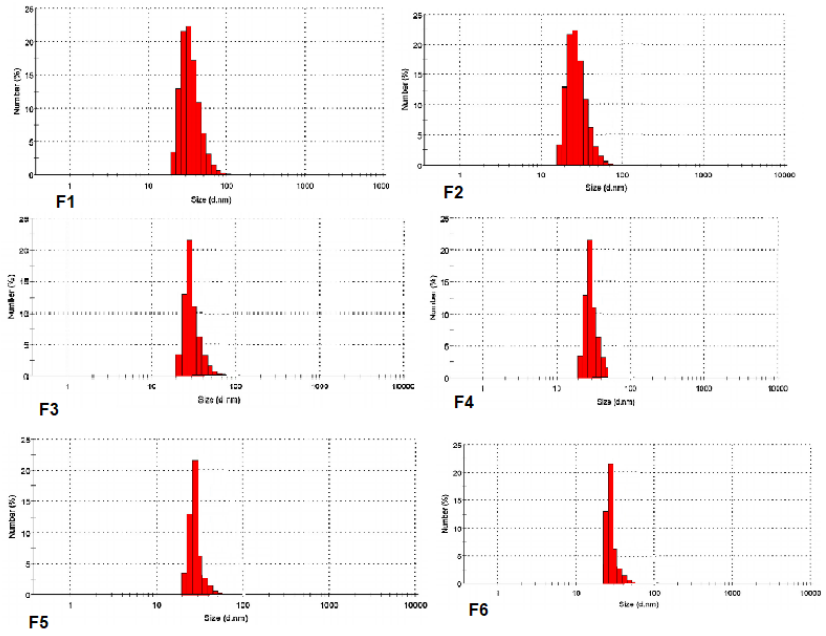
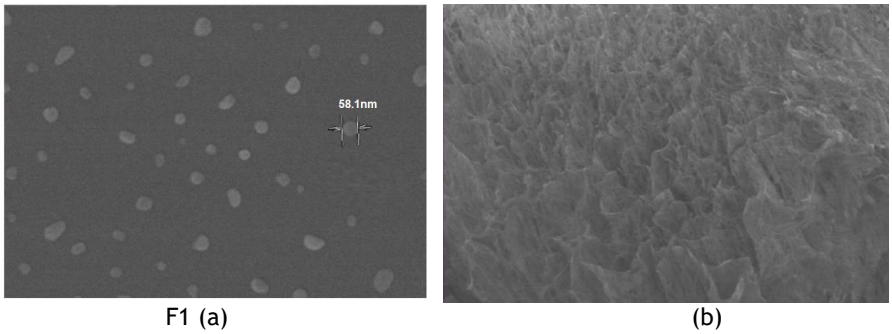


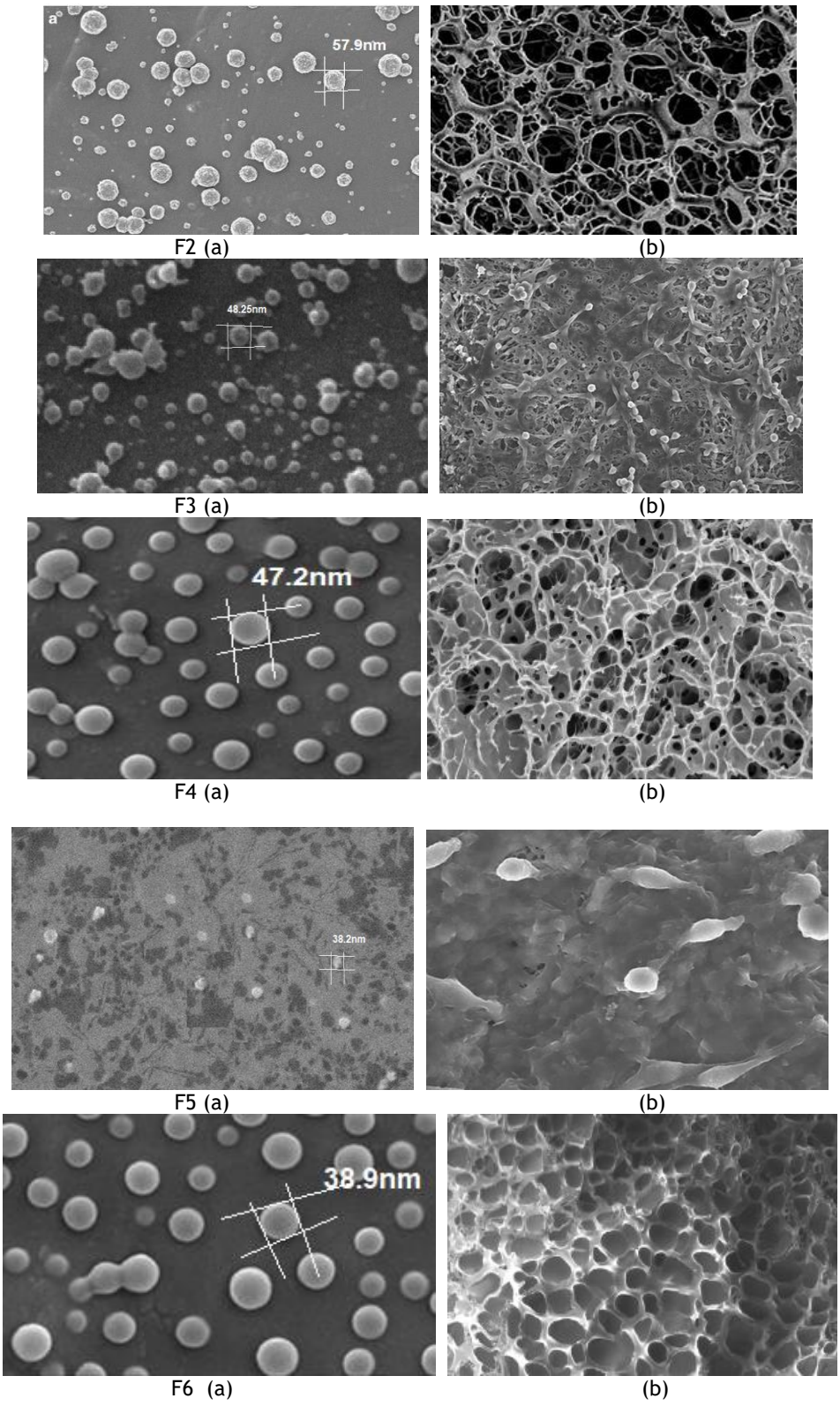
Fig.2: Results of Particle Density Index of extracts derived extract of Gymnema Silvestre loaded nanoparticles (F1-F6).

2. Scanning electron microscopy

The majority of the nanoparticles were spherical (Figure F1-F6). The size of the nanoparticles obtained by extract of Gymnema Silvestre was approximately 58nm, which matches the results obtained by light scattering for the nanoparticles obtained by extract of Gymnema Silvestre. Nanoparticles derived from Momordica charantia extract are approximately 47.2nm, whereas those obtained from Synzigium cumini extract are around 38.5nm. The dispersion of nanoparticles was freeze-dried, resulting in sponge-like structures. SEM was used to determine the morphology of the sponge.



F1 (a) (b)



F2 (a) (b)
F3 (a) (b)
F4 (a) (b)
F5 (a) (b)
F6 (a) (b)

Fig.3: Scanning electron micrograph of nanoparticles (F1-F6) obtained by extract of Gymnema Silvestre (a) and freeze-dried Gymnema Silvestre nanoparticles (b).

3. Drug encapsulation efficiency

The entrapment efficiency of Gymnema Silvestre extract, Momordica charantia extract, and Synzigium cumini loaded silver nanoparticle was observed in the range of '50 percent to 80 percent' for 'F1 to F6' formulation, indicating higher drug encapsulation efficiency.

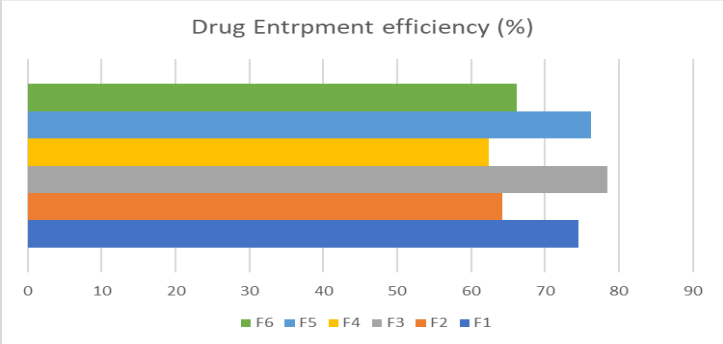


Fig.4: Graphical representation of entrapment efficiency

4. Production yield of nanoparticles

It was discovered that as the concentration of polymer increased, the yield decreased. This was due to the fact that increased polymeric concentration made the solution viscous, making it difficult to pour and causing it to cling to the beaker's wall. However, in this situation, we utilised a comparable concentration of polymer, resulting in a consistent yield of Gymnema Silvestre, Momordica charantia, and Synzigium cumini loaded silver nanoparticle extract.

Table 10: Production yield of all formulation (F1-F6)

Formulation	Production yield (%)
F1	72.11
F2	64.11
F3	73.26
F4	63.25
F5	71.02
F6	61.89

5. In-vitro release study of drug release

Gymnema Silvestre, Momordica charantia, and Synzigium cumini loaded silver nanoparticles of all formulations were studied in vitro for drug release. For formulations F1-F6, the maximal drug release was found to be about 91-93 percent.

Silver nanoparticles of all formulations were studied in vitro for drug release. The maximal drug release indicated that formulation F1 released 93.10 percent of the medication, 92.88 percent from formulation F3, and 91.15 percent from formulation F5. The drug release was determined to be 88.76, 87.23, and 89.12 for formulations F2, F4, and F6. It was discovered that as the polymer concentration was increased, drug release from the nanoparticle reduced. Because the drug release rate from nanoparticles decreases as the hardness of the nanoparticle increases, F1 formulation demonstrated prolonged drug release from nanoparticles in compared to others.

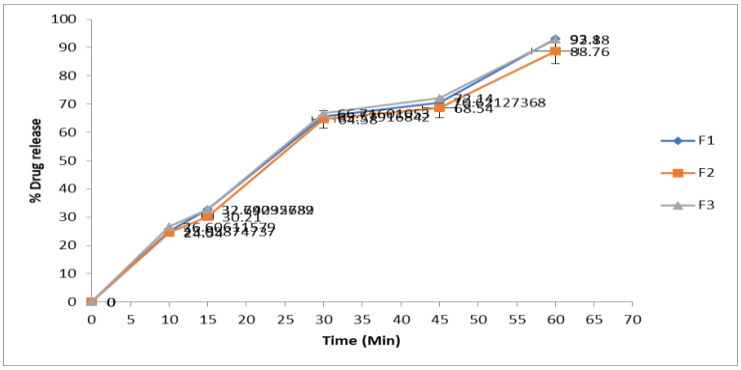


Fig.5: %Cumulative drug release (F1-F3)

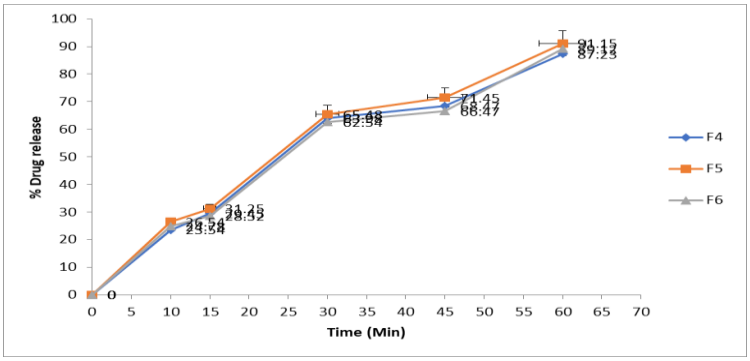


Fig.6: %Cumulative drug release (F4-F6)

6. Transmission electron microscopy

Below is a TEM micrograph of all nanoparticles (F1-F6) samples. The particle size ranges from 30 to 60 nanometers, with a 45-nanometer average. The particles have a spherical form. The same sample was also subjected to electron diffraction examination to ensure that no other forms of metal oxide were present.

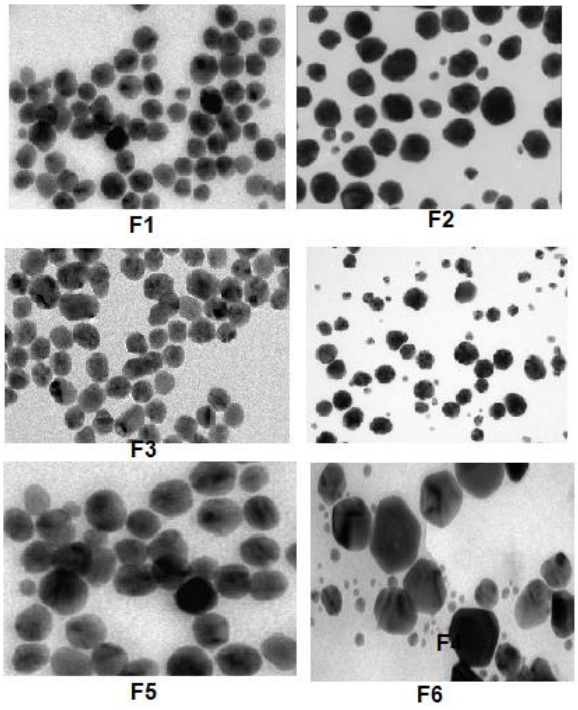


Fig.7: TEM of formulation F1-F6.

7. Stability of Nanoparticle

After 8 weeks, the stability of the silver nanoparticle was evaluated by analysing its absorption spectra. There were no significant changes over the storage period, and the silver nanoparticles did not agglomerate, indicating that they were more stable.

The pattern of change in entrapment efficiency, particle size and zeta potential were same for prepared nanoparticles. There is slight increase in the size of the nanoparticles (1.5%) was observed after three months of storage at 4°C. The entrapment efficiency of nanoparticles was decreased by about 1-2%, whereas zeta potential was found to be decreased by 4%. The changes observed during the storage are negligible.

Evaluation of Polyherbal tablet

Preformulation study

The prepared lyophilized powder blends of all formulations and polyherbal formulation (F1-F7) taken to formulate the tablet and to carry out the pre formulation study.

Organoleptic studies

Powder of blend was found to be off-white.

Extract-excipient compatibility study

IR spectroscopy-

By observing at the FTIR spectra of pure extract for three plants namely Gymnema Silvestre, Momordica charantia and Synzigium cumini and a physical combination of extract and

excipients. There were no significant changes in the primary peaks of the medication. This shows that the medication was not incompatible with the polymers and excipients utilized in the formulation. Generally, all the parameters fell within the acceptable range as indicated in the USP.

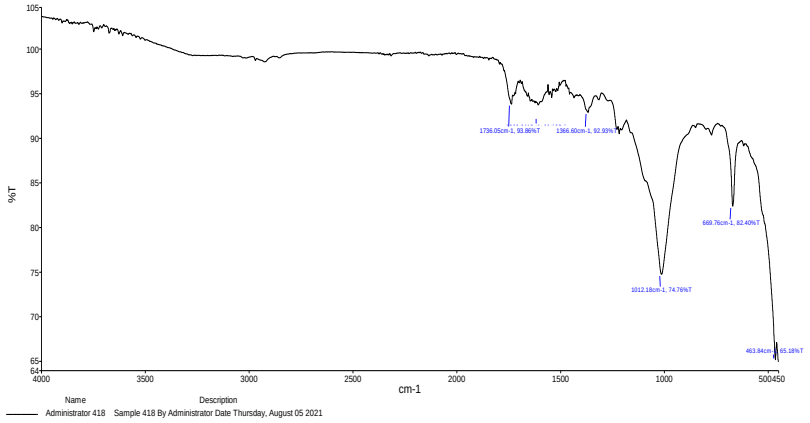


Fig.8: IR spectra of physical mixture.

Table 11: Pre-compression parameters of formulation

Sr. No.	Parameter	F1	F2	F3	F4	F5	F6	F7 (Poly)
1	Angle of repose	32	30	31	31	30	32	31
2	Loss on drying (%)	12.5	10.9	11.02	10.7	12.1	13.0	12.5
3	Ash value (%)	4.12	4.85	2.89	3.58	4.10	3.12	3.65
4	Bulk density (%)	0.66	0.69	0.67	0.66	0.67	0.68	0.69
5	Tapped density (%)	0.87	0.88	0.89	0.91	0.87	0.88	0.88
6	% Compressibility	24.13	21.59	24.71	27.47	22.98	22.72	21.59
7	Hausner ratio (%)	1.31	1.27	1.32	1.37	1.29	1.29	1.27

Evaluation of Tablets (Post compression parameters)

Organoleptic properties

All batches (F1-F7) were assessed for organoleptic properties like color, odor, and taste and found to be acceptable in all aspect.

General appearance: The formulated tablets were assessed for its general appearance and observations were made for shape, colour and texture.

a. Shape- Round

b. Colour- off white

c. Texture- smooth

Table 12: Post compression evaluation parameters.

Sr. No.	Parameter	F1	F2	F3	F4	F5	F6	F7 (Poly)
1	Hardness (kg/cm2)	5.5±0.57	5.3±0.58	5.5±0.89	5.3±0.68	5.4±0.98	5.4±0.2	5.4±0.56
2	Friability	0.12±0.02	0.10±0.05	0.14±0.03	0.13±0.08	0.10±0.03	0.14±0.08	0.10±0.05
3	Weight variation	±4.55	±4.89	±4.90	±4.51	±4.23	±4.56	±4.71
4	Disintegration time	24.15	25.45	23.12	25.41	24.20	23.12	24.12

Stability Study (Accelerated)

For Formulations F1 through F7, an accelerated stability study was conducted in accordance with ICH stability recommendations. Hardness and cumulative percent drug release were among the characteristics examined. The look, texture, and colour of prepared tablets from batches F1 through F7 did not vary. Hardness and cumulative percent drug release did not vary much. During the investigation, prepared tablets from batches F1 to F16 were determined to be stable.

Pharmacokinetic Profile of formulated tablets by comparing with marketed formulation.

For 01 hours, in-vitro drug release studies of formulations (F1-F7) were conducted. It was discovered that the drug release was slow and substantial for all formulations F1-F6. When the dissolution rate of the formulation nanoparticles containing tablet was compared to the dissolution rate of the marketed tablet, F7, it was discovered that the formulation

nanoparticles including tablet for all plant extracts containing exhibits a promising and competitive drug release. The drug release was determined to be non-fiction and to fit the korsemeyer-peppas paradigm. As a result, the pharmacokinetic profile of manufactured tablets is compared to commercial formulations, achieving the study's goal.

Formulation F1 includes a 0:50 ratio of silver, resulting in a faster release than Formulation F2, which has a 1:1 ratio of silver.

For F1 through F2, the percent medication release increases as the silver concentration in the pill drops. At 60 minutes, the formulation F1 exhibited a percent release of 88.31, whereas the formulation F2 showed a percent release of 81.33. The percent release graphs for formulations F1-F2 containing Gymnema Silvestre extract are shown below. (Table 39, Figure 48-55)

Characterization of Release Kinetics

There are two types of release patterns: those that release medication at a slow zero or first order rate and those that deliver an initial fast dosage followed by slow zero or first order sustained component release. Controlled release systems are designed to keep medication concentrations in the blood or target tissues at a desired level for as long as feasible.

The release kinetics of formed batches F1 through F7 were studied, and the release exponent n, which describes the drug's release mechanism, was determined. Batches F1 through F7 were found to fit Peppas' model for drug release, which is based on non-Fickian transport.

Table 13: Characterization of Release Kinetics F1-F7

Formulation Code	Time in Min	R2 Square Value					n Value	Best fit model
		Zero order	1st order	Matrix	Peppas	Hix. Crow.		
F1	60	0.682	0.676	0.022	0.960	0.029	0.6067	Peppas
F2	60	0.059	-	0.230	0.991	0.082	0.5955	Peppas
F3	60	0.319	-	0.354	0.842	0.582	0.6610	Peppas
F4	60	0.033	-	0.305	0.725	0.244	0.6715	Peppas
F5	60	0.203	-	0.939	0.829	0.440	0.6569	Peppas
F6	60	0.023	-	0.150	0.738	0.150	0.6437	Peppas
F7	60	0.988	-	0.016	0.851	0.846	0.6634	Peppas

Anti-diabetic study

1. Acute Toxicity

The acute oral toxicity in rabbits indicated that tablets formulated by preparing extract of Gymnema Silvestre, Momordica charantia and Synzigium was nontoxic at 200-400mg/kg body weight.

Table 14: Acute toxicity profile of tablets

Formulation/Group	No. of animals in group	Dose (mg/kg)	Results
F1	3	200	No toxic sign
F2	3	200	No toxic sign
F3	3	200	No toxic sign
F4	3	200	No toxic sign
F5	3	200	No toxic sign
F6	3	200	No toxic sign
F7-Polyherbal	3	200	No toxic sign

Hypoglycemic study

Effect of formulated tablet F1-F7 on Blood Glucose levels in Diabetic rats

The formulated tablet F1-F7 containing extract of Gymnema Silvestre, Momordica charantia and Synzigium cumini after administration in rabbits were studied for antidiabetic activity for 1 month.

Table 15: Blood Glucose levels in Diabetic rabbits

Groups	0 week	1 week	2 week	4 week
Non diabetic Control	124	124.5	124	124
Diabetic control	288	297	302	317
F1	293	226	198	171
F2	291	212	184	161
F3	294	191	178	168
F4	288	227	206	171
F5	293	196	177	165
F6	294	196	179	163
F7	291	211	181	160
Marketed Tablet	296	215	184	160

All values are given as mean ± SEM, N = 6. **P < 0.01 compared to diabetic control animals at specified interval.

The blood glucose levels revealed that the manufactured pill F1-F7 comprising Gymnema Silvestre extract, Momordica charantia extract, and Synzigium cumini extract had potential antidiabetic action. When compared to the marketed standard, the alloxan induced diabetic group's mean blood glucose levels were substantially (p0.01) lower for formulation F1-F7.

Initially, there is no substantial drop in blood glucose level after the "0" week, but after the 1st week, there is a considerable decrease in blood glucose level, i.e., 35 percent, which progresses to a 50 percent fall in blood glucose by the end of the 4th week.

4. CONCLUSION

In a number of medical therapies, herbal medicines have been advocated for the treatment of a variety of diseases. The data imply that the poly herbal pill's anti-diabetic efficacy may be attributable to non-specific mechanisms. More study is needed, however, to identify the plant's exact mechanism(s), active principles, and safety profile as a medical hypoglycemic therapy. Overall, the use of prepared pills in the future is both cost-effective and non-harmful. The polyherbal pills are non-hazardous plant-based alternative treatments that might be studied further because they do not have the main side effects associated with manufactured medications. According to long-term toxicity testing, the produced tablet requires more investigation to determine the basic mechanism of action. A crucial criterion also considers the stability and formulations of crude medications. The purpose of this research was to see how effectively the standardized antidiabetic activity in herbal medications functioned. According to the outcomes of this investigation, the herbal formulation met all of the government's requirements. The results of this study clearly show that there is no uniformity in the preparation of formulations, and that there is a great deal of commercial adulteration or replacement, due to the diverse geographical locations in which plants grow, as well as the problem of different vernacular names known to these plants. According to the general health science concept, it may be useful in selecting an acceptable formulation in clinical practice and, as a consequence, effective rational therapy.

5. CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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