STANDARDIZATION QUALITY CONTROL AND DEVELOPMENT OF POLY HERBAL FORMULATION FOR THE MANAGEMENT OF TYPE 2 DIABETES MELLITUS

A dissertation submitted to

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CHENNAI - 600 032

In partial fulfillment of the requirements for the award of the degree of

MASTER OF PHARMACY

IN

PHARMACOGNOSY

Submitted by

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2.

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T.Sampath kumar,
Reg. No. 261720656.

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1. INTRODUCTION

HERBAL MEDICINE[1,2]

Herbal medicine have become the remedy for most of the diseases. In conjunction with a healthy diet and lifestyle they target specific health goals providing every cell the most appropriate and optimal nourishment. These herbal supplements do not have any harmful side effects that might disturb physical health unlike synthetics. For every synthetic drug present there is an alternative herbal drug. Man in his everlasting search for cure of serious illnesses, at last finds his way to our indigenous medicine.

Indigenous system of medicine which is also known as traditional or folk medicine encompasses of medical knowledge systems that have germinated over generations within various societies before the era of modern medicine. Indigenous medicines include Herbal, Ayurveda, Siddha medicine, Unani, ancient Iranian medicine, Islamic Medicine, Traditional Chinese medicine, Traditional Vietnamese medicine, Acupuncture, Muti, Ifa, Traditional African medicine, and other medicinal practices all over the world.

The World Health Organization (WHO) defines traditional medicine as:

"The health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral-based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illnesses or maintain well-being."

From available literature, the use of herbs dates back 5,000 years to the ancient Sumerians, who described well-established medicinal uses for plants. Nature always stands as a golden mark to exemplify the outstanding phenomena of symbiosis. Nowadays people are well versed with the potency and side effects of synthetic drugs. Hence, there is an increasing interest in the natural product remedies with a basic approach towards the nature. Natural products obtained from plant,

animal and minerals have been the backbone of the treatment of human diseases. At about 80 % of people in developing countries still hinge upon traditional medicine, based largely on species of plants and animals for their primary health care. Indigenous system of medicine is the need of the day.

IMPORTANCE[3-5]

Ayurveda is a traditional system of medicine using a wide range of modalities to create health and wellbeing. The main aspire of Ayurveda health care is to restore the physical mental and emotional balance in patients, thus improving the health, preventing disease and to treat any current illness. The number of patients looking for alternate and herbal therapy is growing exponentially. Thus the herbal medicines are now in great demand in the developing world for primary healthcare not only for its inexpensiveness but also for better cultural acceptability; better compatibility with the human body and minimal side effects.

Herbal medicine is still the mainstay of about 75 – 80% of the world population for primary healthcare mainly in the developing countries. However among the estimated 250,000 – 400,000 of plant species, only about 6% have been studied for biological activity, and about 15% have been investigated based on its phytochemicals. Therefore it seems necessary to evaluate the herbs properly.

The first reason for the use of herbals is that it is part of the culture and belief of some people for maintenance of health or to treat certain ailments. The second reason for the increased use of herbals is the relatively cheaper cost of herbal products and hence affordability to the lower income group. The third reason is that the public has the impression of herbals being natural and that anything natural is safe. There is also this notion that herbal products do not contain chemicals and only those chemicals found in modern medicines, are linked to toxicity, and hence are more harmful.

FORMULATION OF HERBAL PRODUCTS[6]

An herbal formula consists of a selective combination of individual herbal ingredients that are formulated for a specific ailment or group of disease-conditions. When herbs are combined together, they become more potent and effective within the body than single herb due to their activating or catalyzing influence upon one another. These combinations acts as powerful catalysts in order to activate over own individual healing energies (or vital force) which permeate the entire organism and reside in each and every cell in our bodies.

Advantages of Herbal Formulations:

There are a number of advantages associated with using herbal medicines as opposed to allopathic products. Examples include the following:

- **Reduced risk of side effects:** Most herbal medicines are well tolerated by the patient, with fewer unintended consequences than pharmaceutical drugs. Herbs typically have fewer side effects than traditional medicine, and may be safer to use over time.

WHO Guidelines for Standardization of Herbal Formulation[7,8]

Standardization is an important aspect for maintaining and assessing the quality and safety of the polyherbal formulation as these are combinations of more than one herb to attain the desire therapeutic effect. Standardization minimizes batch to batch variation, assures safety, efficacy, quality and acceptability of the polyherbal formulations. Standardisation involves:

- Quality control of crude drugs material, plant preparations and finished products.
- Stability assessment and shelf life.
- Safety assessment, documentation of safety based on experience or toxicological studies.
- Assessment of efficacy by ethnomedical informations and biological activity evaluations

TABLETS[9]

Tablets are defined as solid preparations intended for oral administration, each containing a single dose of one or more active ingredients. Tablets are prepared by compaction and contain drugs and formulation additives. They vary in shape and differ

greatly in size and weight, depending on amount of medicinal substance and the intended mode of administration. Tablet may be coated or uncoated, consist of one or more active substances with or without excipients. The excipients include substances such as diluents, binders, disintegrants, glidants and lubricants, substances capable of modifying the behavior of the preparation in digestive tract, colorants and flavoring substances. It is the most popular dosage form and 70% of the total medicines are dispensed in the form of tablet.

METHODS OF TABLET FORMULATION

Tablets are prepared by,

- Direct compaction
- Granulation Methods
    - Wet granulation
    - Dry granulation

ADVANTAGES

- They are dosage form and offer the greatest capabilities of all oral dosage forms for the greatest precision and least content variability.
- Low cost among all oral dosage forms.
- They are the easiest and cheapest to package and ship.
- Product identification requires no additional processing steps when employing embossed or monogrammed punch face.
- Provides greatest ease of swallowing with the least tendency for hang up above the stomach.
- They lend themselves to certain special release profile products E.g. enteric coated or delayed release profiles.
- Easy large scale production than other oral dosage forms.

- They have the best combined properties of chemical, mechanical and microbiological stability among all the oral dosage forms.
- The emergency supplies of the drug can be conveniently carried by the patient.

DISADVANTAGES

- Drugs with poor wetting, slow dissolution properties, intermediate to large dosages, optimum absorption high in the gastro intestinal tract or any combination of this features may be difficult or impossible to formulate and manufacture as tablet that will still provide adequate or full drug bioavailability.
- Bitter tasting drugs, drugs with an objectionable odour or drugs that are sensitive to oxygen or atmospheric moisture may require encapsulation or entrapment prior to compression, or the tablets may require coating. In such cases, the capsules may offer the best and lowest cost approach [3].

***Diabetes mellitus*[10-13]**

As per WHO, Diabetes Mellitus is defined as heterogenous metabolic disorder characterised by common feature of chronic hyperglycemia with disturbance of carbohydrate, protein and fat metabolism.

Types of diabetes

- **Type - I diabetes** (insulin dependent diabetes mellitus)
- **Type - II diabetes** (formerly, non-insulin dependent diabetes mellitus)
- **Gestational diabetes** (first recognition during pregnancy)
- **Diabetes due to other causes** (genetic defects or medication)

Type 1 diabetes (formerly known as insulin-dependent) in which the pancreas fails to produce the insulin which is essential for survival. This form develops most frequently in children and adolescents, but is being increasingly noted later in life.

Type 2 diabetes (formerly named non-insulin-dependent) which results from the body's inability to respond properly to the action of insulin produced by the pancreas. Type 2 diabetes is much more common and accounts for

around 90% of all diabetes cases worldwide. It occurs most frequently in adults, but is being noted increasingly in adolescents as well.

Certain genetic markers have been shown to increase the risk of developing Type 1 diabetes. Type 2 diabetes is strongly familial, but it is only recently that some genes have been consistently associated with increased risk for Type 2 diabetes in certain populations. Both types of diabetes are complex diseases caused by mutations in more than one gene, as well as by environmental factors.

GESTATIONAL DIABETES

Diabetes that's triggered by pregnancy is called gestational diabetes (pregnancy, to some degree, leads to insulin resistance). It is often diagnosed in middle or late pregnancy. Because high blood sugar levels in a mother are circulated through the placenta to the baby, gestational diabetes must be controlled to protect the baby's growth and development.

The rate of gestational diabetes is between 2% to 10% of pregnancies. Gestational diabetes usually resolves itself after pregnancy. Up to 10% of women with gestational diabetes develop type 2 diabetes. It can occur anywhere from a few weeks after delivery to months or years later.

Diabetes in pregnancy[gestational diabetes] may give rise to several adverse outcomes, including congenital malformations, increased birth weight and an elevated risk of perinatal mortality. Strict metabolic control may reduce these risks to the level of those of non-diabetic expectant mothers.

Symptoms

The symptoms of diabetes may be pronounced, subdued, or even absent.

- In Type 1 diabetes, the classic symptoms are excessive secretion of urine (polyuria), thirst (polydipsia), weight loss and tiredness.
- These symptoms may be less marked in Type 2 diabetes. In this form, it can also happen that no early symptoms appear and the disease is

only diagnosed several years after its onset, when complications are already present.

Epidemiology of diabetes

The incidence of diabetic is growing rapidly in United States and worldwide. Globally as of 2010, an estimated 285 million people had diabetes, with type II making up about 90% of the cases. In 2013, according to International Diabetes Federation an estimated 381 million people had diabetes, its prevalence is increasing rapidly.

PATHOPHYSIOLOGY

Pancreas

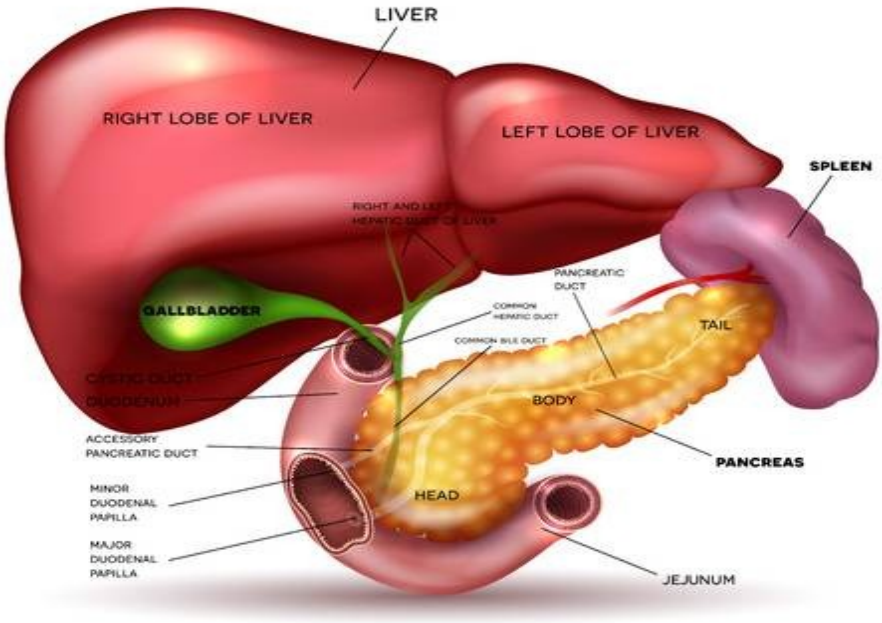

Figure :1 Human Pancreas

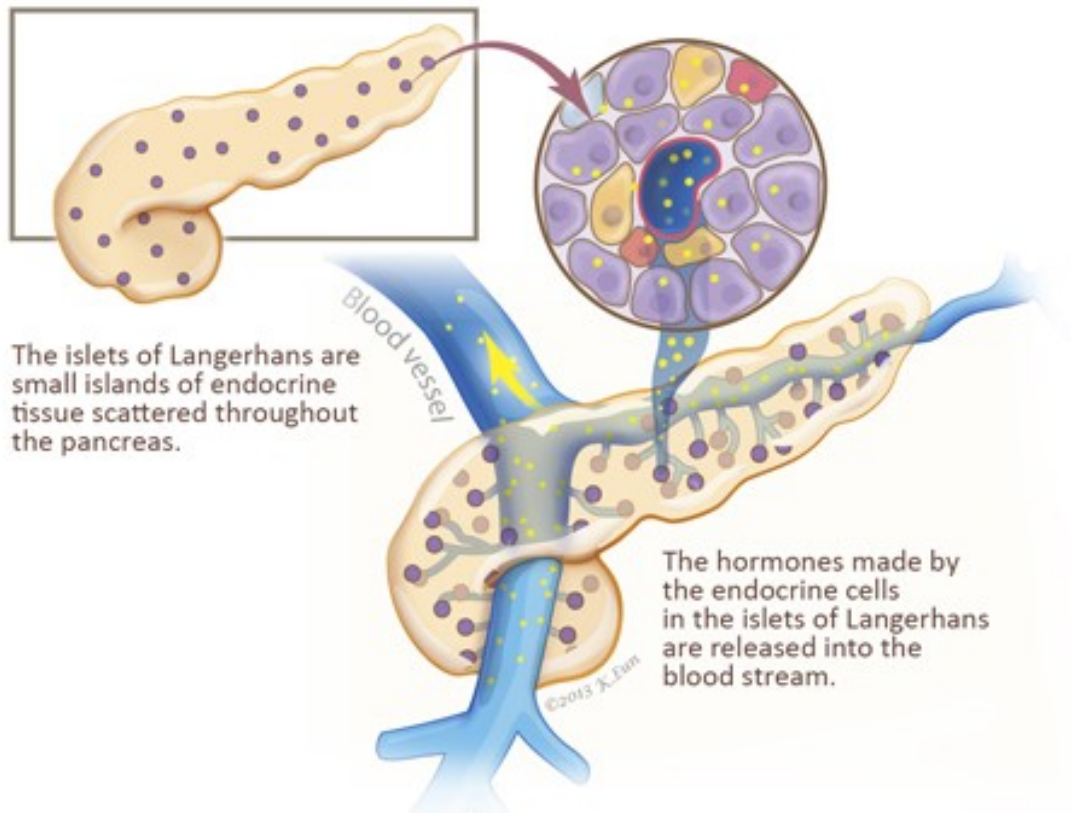

Figure: 2 Langerhans

The hormones play an important role in regulating the metabolic activities of the body, particularly the hameostasis of blood glucose. The pancreas is both an endocrine and exocrine gland, in which endocrine produces the peptide hormone insulin, glucagon and somatostatin and exocrine gland produces digestive enzymes The peptide hormones are secreted from cells located in the islet of langerhans

(β cells produce insulin, alpha cells produces glucagon and δ cells produce somatostatin).

Insulin

Insulin was discovered in 1921 by Banting and best who demonstrated the hypoglycaemic action of an extract of pancreas. In 1922 an extract

containing insulin was first used on a 14 year old boy suffering from severe diabetes mellitus with excellent response. Insulin was then purified in a few years.

Insulin Structure

Insulin is composed of two chains of amino acids named chain A (21 amino acids) and chain B (30 amino acids) that are linked together by two disulfide bridges. There is a 3rd disulfide bridge within the A chain that links the 6th and 11th residues of the A chain together.

In most species, the length and amino acid compositions of chains A and B are similar, and the positions of the three disulfide bonds are highly conserved. For this reason, pig insulin can be used to replace deficient human insulin levels in diabetes patients. Today, porcine insulin has largely been replaced by the mass production of human proinsulin by bacteria (recombinant insulin).

Insulin secretion

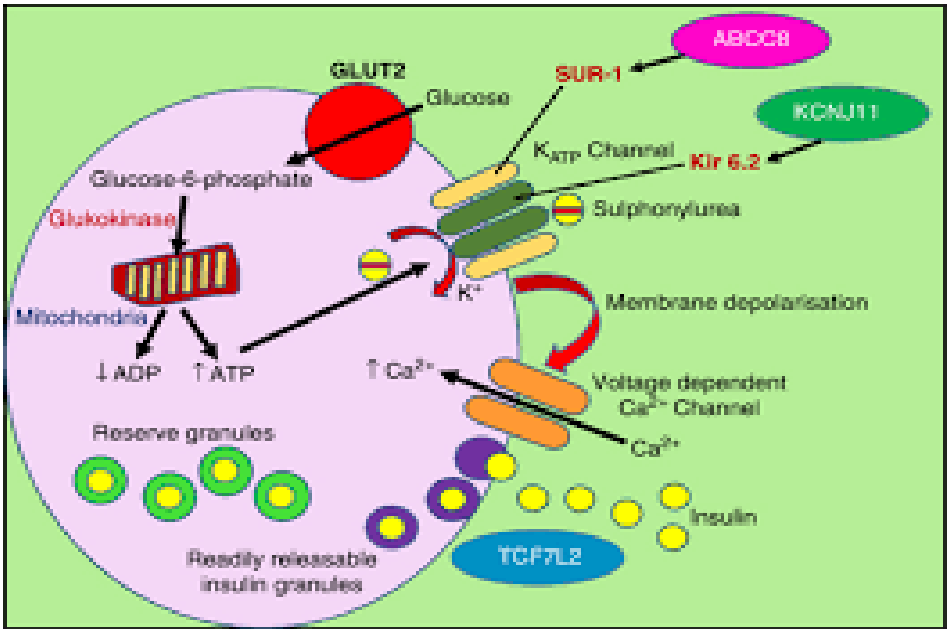

Figure:3 Secretion of Insulin in β cells

The insulin-making cells of the body are called beta cells, and they are found in the pancreas gland. These cells clump together to form the "islets of Langerhans", named for the German medical student who described them.

Rising levels of glucose inside the pancreatic beta cells trigger the release of insulin:

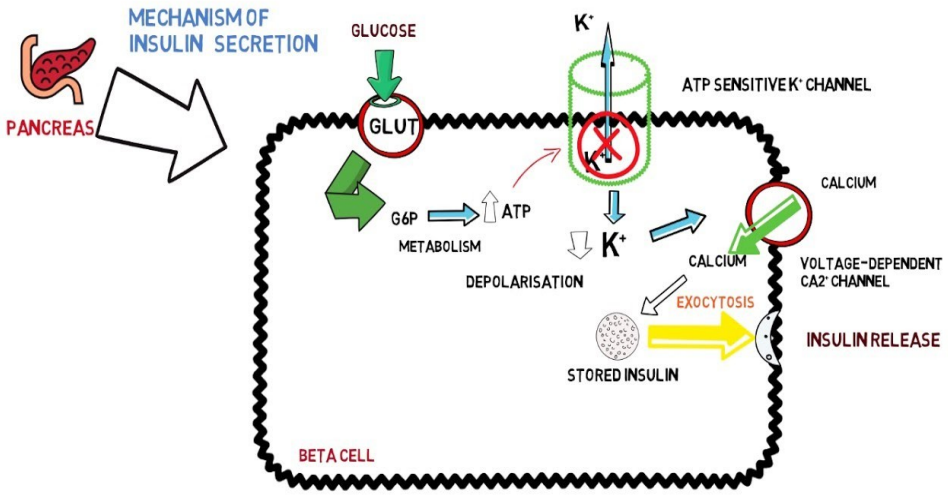

Figure 4 : Mechanism of Insulin secretion

1. Glucose is transported into the beta cell by type 2 glucose transporters (GLUT2). Once inside, the first step in glucose metabolism is the phosphorylation of glucose to produce glucose-6-phosphate. This step is catalyzed by glucokinase—it is the rate-limiting step in glycolysis, and it effectively traps glucose inside the cell.
2. As glucose metabolism proceeds, ATP is produced in the mitochondria.
3. The increase in the ATP:ADP ratio closes ATP-gated potassium channels in the beta cell membrane. Positively charged potassium ions (K^+) are now prevented from leaving the beta cell.
4. The rise in positive charge inside the beta cell causes depolarization.
5. Voltage-gated calcium channels open, allowing calcium ions (Ca^{2+}) to flood into the cell.
6. The increase in intracellular calcium concentration triggers the secretion of insulin via exocytosis.

There are two phases of insulin release in response to a rise in glucose. The first is an immediate release of insulin. This is attributable to the release of preformed insulin, which is stored in secretory granules. After a short delay, there is a second, more prolonged release of newly synthesized insulin.

Once released, insulin is active for a only a brief time before it is degraded by enzymes. Insulinase found in the liver and kidneys breaks down insulin circulating in the plasma, and as a result, insulin has a half-life of only about 6 minutes. This short duration of action allows rapid changes in the circulating levels of insulin.

Insulin Receptor

The net effect of insulin binding is to trigger a cascade of phosphorylation and dephosphorylation reactions. These actions are terminated by dephosphorylation of the insulin receptor.

Similar to the receptors for other polypeptide hormones, the receptor for insulin is embedded in the plasma membrane and is composed of a pair of alpha subunits and a pair of beta subunits. The alpha subunits are extracellular and contain the insulin-binding site. The beta subunits span the membrane and contain the enzyme tyrosine kinase. Kinases are a group of enzymes that phosphorylate proteins (the reverse reaction is catalyzed by a group of enzymes called phosphatases).

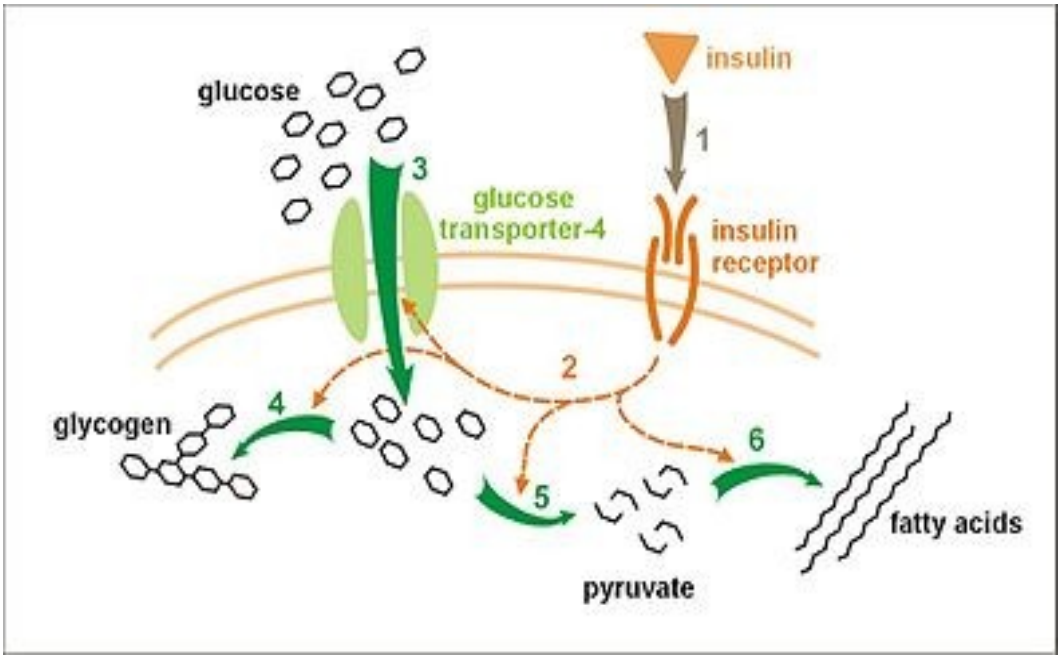

Figure: 5 The insulin receptor

The insulin receptor is a tyrosine kinase receptor and is composed of a pair of alpha subunits and a pair of beta subunits. Insulin binds to the alpha subunits and induces a conformational change that is transmitted to the beta subunits that autophosphorylate and initiate a cascade of phosphorylation and dephosphorylation reactions.

Insulin binding to the alpha subunits induces a conformational change that is transmitted to the beta subunits and causes them to phosphorylate themselves (autophosphorylation). A specific tyrosine of each beta subunit is phosphorylated along with other target proteins, such as insulin receptor substrate (IRS). As these and other proteins inside the cell are phosphorylated, this in turn alters their activity, bringing about the wide biological effects of insulin.

Insulin Action

The binding of insulin results in a wide range of actions that take place over different periods of time. Almost immediately, insulin promotes the uptake

of glucose into many tissues that express GLUT4 glucose transporters, such as skeletal muscle and fat. Insulin increases the the activity of these transporters and increases their numbers by stimulating their recruitment from an intracellular pool to the cell surface. Not all tissues require insulin for glucose uptake. Tissues such as liver cells, red blood cells, the gut mucosa, the kidneys, and cells of the nervous system use a glucose transporter that is not insulin dependent.

Over minutes to hours, insulin alters the activity of various enzymes as a result of changes in their phosphorylation status.

Over a period of days, insulin increases the amounts of many metabolic enzymes. These reflect an increase in gene transcription, mRNA, and enzyme synthesis.

After a Meal—the Role of Insulin

The rise in blood glucose following a meal is detected by the pancreatic beta cells, which respond by releasing insulin. Insulin increases the uptake and use of glucose by tissues such as skeletal muscle and fat cells. This rise in glucose also inhibits the release of glucagon, inhibiting the production of glucose from other sources, e.g., glycogen break down.

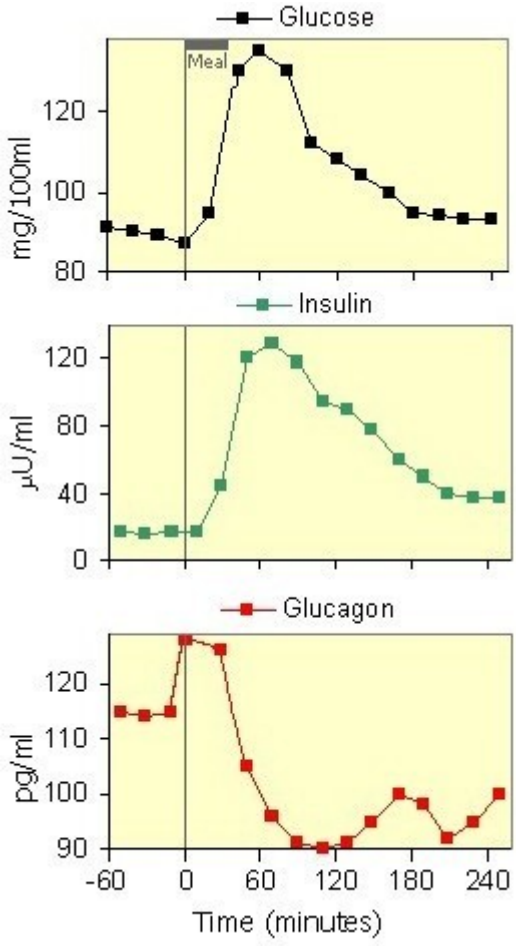

Figure: 6 Changes in key hormones after a meal

Changes in blood levels of glucose, insulin, and glucagon after a carbohyrate-rich meal (ingested at time 0 minutes

1. Use Glucose

Once inside the cell, some of the glucose is used immediately via glycolysis. This is a central pathway of carbohydrate metabolism because it occurs in all cells in the body, and because all sugars can be converted into glucose and enter this pathway. During the well-fed state, the high levels of insulin and low levels of glucagon

stimulate glycolysis, which releases energy and produces carbohydrate intermediates that can be used in other metabolic pathways.

2. Make Glycogen

Any glucose that is not used immediately is taken up by the liver and muscle where it can be converted into glycogen (glycogenesis). Insulin stimulates glycogenesis in the liver by:

- stimulating hepatic glycogen synthetase (the enzyme that catalyzes glycogen synthesis in the liver)
- inhibiting hepatic glycogen phosphorylase (the enzyme that catalyzes glycogen breakdown in the liver)
- inhibiting glucose synthesis from other sources (inhibits gluconeogenesis)

Insulin also encourages glycogen formation in muscle, but by a different method. Here it increases the number of glucose transporters (GLUT4) on the cell surface. This leads to a rapid uptake of glucose that is converted into muscle glycogen.

3. Make Fat

When glycogen stores are fully replenished, excess glucose is converted into fat in a process called lipogenesis. Glucose is converted into fatty acids that are stored as triglycerides (three fatty acid molecules attached to one glycerol molecule) for storage. Insulin promotes lipogenesis by:

- increasing the number of glucose transporters (GLUT4) expressed on the surface of the fat cell, causing a rapid uptake of glucose
- increasing lipoprotein lipase activity, which frees up more fatty acids for triglyceride synthesis

In addition to promoting fat synthesis, insulin also inhibits fat breakdown by inhibiting hormone-sensitive lipase (an enzyme that breaks down fat stores). As a result, there are lower levels of fatty acids in the blood stream.

Insulin also has an anabolic effect on protein metabolism. It stimulates the entry of amino acids into cells and stimulates protein production from amino acids.

Fasting—the Role of Glucagon

Fasting is defined as more than eight hours without food. The resulting fall in blood sugar levels inhibits insulin secretion and stimulates glucagon release. Glucagon opposes many actions of insulin. Most importantly, glucagon raises blood sugar levels by stimulating the mobilization of glycogen stores in the liver, providing a rapid burst of glucose. In 10–18 hours, the glycogen stores are depleted, and if fasting continues, glucagon continues to stimulate glucose production by favouring the hepatic uptake of amino acids, the carbon skeletons of which are used to make glucose.

In addition to low blood glucose levels, many other stimuli stimulate glucagon release including eating a protein-rich meal (the presence of amino acids in the stomach stimulates the release of both insulin and glucagon, glucagon prevents hypoglycemia that could result from unopposed insulin) and stress (the body anticipates an increased glucose demand in times of stress).

"Starvation in the Midst of Plenty"

Diabetes is often referred to as ‑starvation in the midst of plenty‖ because the intracellular levels of glucose are low, although the extracellular levels may be extremely high.As in starvation, type 1 diabetics use non-glucose sources of energy, such as fatty acids and ketone bodies, in their peripheral tissues. But in contrast to the starvation state, the production of ketone bodies can spiral out of control. Because the ketones are weak acids, they acidify the blood. The result is the metabolic state of diabetic ketoacidosis (DKA). Hyperglycemia and ketoacidosis are the hallmark of type 1 diabetes.

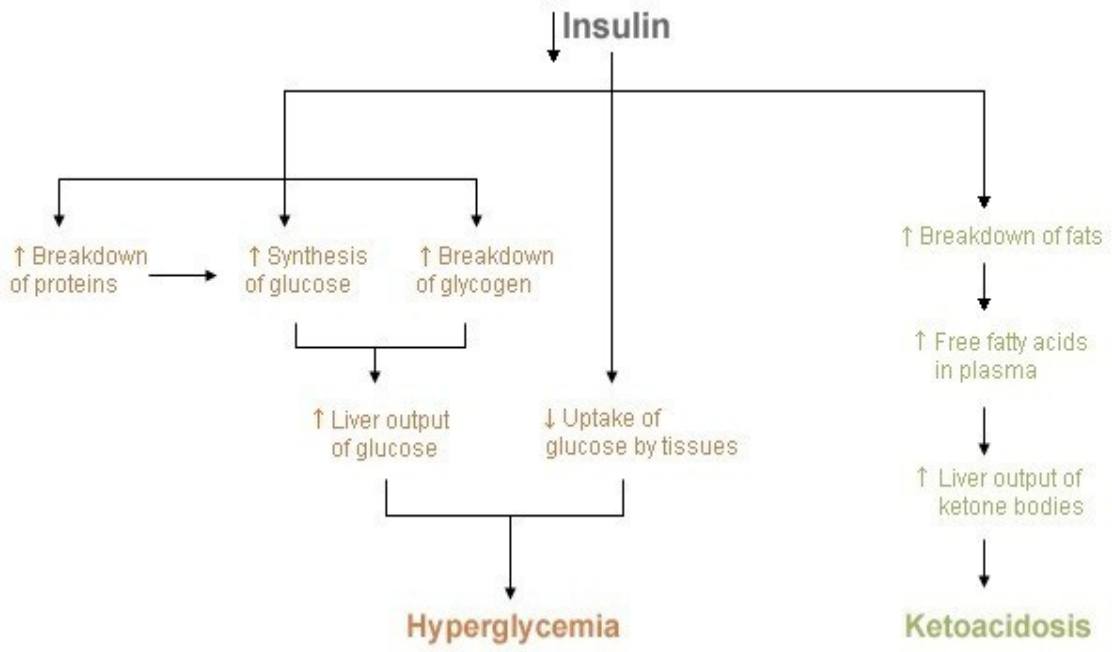

Figure: 7 Metabolic changes in Ketoacidosis

Hyperglycemia is caused by the increased production of glucose by the liver (driven by glucagon) and the decreased use of glucose of insulin by peripheral tissues (because of the lack of insulin

Hypertriglyceridemia is also seen in DKA. The liver combines triglycerol with protein to form very low density lipoprotein (VLDL). It then releases VLDL into the blood. In diabetics, the enzyme that normally degrades lipoproteins (lipoprotein lipase) is inhibited by the low level of insulin and the high level of glucagon. As a result, the levels of VLDL and chylomicrons (made from lipid from the diet) are high in DKA.

Prevalence

- Recently compiled data show that approximately 150 million people have diabetes mellitus worldwide, and that this number may well double by the year 2025. Much of this increase will occur in

developing countries and will be due to population growth, ageing, unhealthy diets, obesity and sedentary lifestyles.

- By 2025, while most people with diabetes in developed countries will be aged 65 years or more, in developing countries most will be in the 45-64 year age bracket and affected in their most productive years.

Diagnosis

- WHO has published recommendations on diagnostic values for blood glucose concentration. The diagnostic level of fasting blood glucose concentration was last modified in 1999.

Treatment

- The mainstay of non-pharmacological diabetes treatment is diet and physical activity.
- About 40% of diabetes sufferers require oral agents for satisfactory blood glucose control, and some 40% need insulin injections. This hormone was isolated by Frederic Banting and Charles Best in 1921 in Canada. It revolutionized the treatment of diabetes and prevention of its complications, transforming Type 1 diabetes from a fatal disease to one in which long-term survival became achievable.
- People with Type 1 diabetes are usually totally dependent on insulin injections for survival. Such people require daily administration of insulin. The majority of people suffering from diabetes have the Type 2 form. Although they do not depend on insulin for survival, about one third of sufferers needs insulin for reducing their blood glucose levels.

Complications associated with diabetes mellitus

- Diabetic retinopathy is a leading cause of blindness and visual disability. Diabetes mellitus is associated with damage to the small blood vessels in the retina, resulting in loss of vision. Findings, consistent from study to study, make it possible to suggest that, after 15 years of diabetes, approximately 2% of people become blind, while about 10% develop severe visual handicap. Loss of vision due

to certain types of glaucoma and cataract may also be more common in people with diabetes than in those without the disease.

- Good metabolic control can delay the onset and progression of diabetic retinopathy. Loss of vision and blindness in persons with diabetes can be prevented by early detection and treatment of vision-threatening retinopathy: regular eye examinations and timely intervention with laser treatment, or through surgery in cases of advanced retinopathy. There is evidence that, even in developed countries, a large proportion of those in need is not receiving such care due to lack of public and professional awareness, as well as an absence of treatment facilities. In developing countries, in many of which diabetes is now common, such care is inaccessible to the majority of the population.
- Diabetes is among the leading causes of kidney failure, but its frequency varies between populations and is also related to the severity and duration of the disease. Several measures to slow down the progress of renal damage have been identified. They include control of high blood glucose, control of high blood pressure, intervention with medication in the early stage of kidney damage, and restriction of dietary protein. Screening and early detection of diabetic kidney diseae are an important means of prevention.

- Diabetic neuropathy is probably the most common complication of diabetes. Studies suggest that up to 50% of people with diabetes are affected to some degree. Major risk factors of this condition are the level and duration of elevated blood glucose. Neuropathy can lead to sensory loss and damage to the limbs. It is also a major cause of impotence in diabetic men.
- Diabetic foot disease, due to changes in blood vessels and nerves, often leads to ulceration and subsequent limb amputation. It is one of the most costly complications of diabetes, especially in communities with inadequate footwear. It results from both vascular and neurological disease processes. Diabetes is the most common cause

of non-traumatic amputation of the lower limb, which may be prevented by regular inspection and good care of the foot.

Prevention

Large, population-based studies in China, Finland and USA have recently demonstrated the feasibility of preventing, or delaying, the onset of diabetes in overweight subjects with mild glucose intolerance (IGT). The studies suggest that even moderate reduction in weight and only half an hour of walking each day reduced the incidence of diabetes by more than one half.

Diabetes is a serious and costly disease which is becoming increasingly common, especially in developing countries and disadvantaged minorities. However, there are ways of preventing it and/or controlling its progress. Public and professional awareness of the risk factors for, and symptoms of diabetes are an important step towards its prevention and control.

HERBAL DRUGS FOR DIABETES MELLITUS[14-16]

In the Ayurvedic system of medicine, as mentioned in ancient Indian books like Charak Samhita, Mahdhav Nidan and Astang Sanghra, there are about 600 plants, which are stated to have antidiabetic property. Wide arrays of plant derived active principles representing numerous phytochemicals have demonstrated consistent hypoglycemic activity and their possible use in the treatment of diabetes mellitus.

Indian plants which are most effective and commonly studied in relation to diabetes are namely *Allium cepa, Allium sativum, Aloevera, Berberis aristata, Cajanus cajan, Coccinia indica, Caesalpinia bonducella, Cyperus rotundus, Ficus bengalenesis, Gymnema sylvestre, Momordica charantia, Ocimum sanctum, Pterocarpus marsupium, Swertia chirayita, Syzigium cumini Terminalia belerica, Terminalia chebula, Tinospora cordifolia, Trigonella foenum, Phyllanthus emblica, Annona squamosa* etc.

Herbal medicines have been used to cure diabetes as anti-diabetic regimens alone or in compound. Therefore, research is still on the nascent stage

to find more effective and safer hypoglycemic agents. For a long time, several medicinal plants have been used for the treatment of diabetes in the form of compound drugs. Moreover, after the reference made by researchers on diabetes mellitus, investigations on the hypoglycemic activity of compound drugs of medicinal plants have been more important.

Polyherbal formulations may enhance the pharmacological activity and reduce the concentrations of single herbs, thereby reducing adverse effects. Plant formulation and combined extracts of plants have been used as a drug rather than individual.

Exploring an effective drug either single or in combination against diabetes is challenging still. Hence we planned to develop antidiabetic polyherbal formulation in the form of tablet containing extracts of *Nigella sativa* (dried Seed),

Moringa oleifera (seed), *Linum usitatissimum* (seed), *Trigonella foenum* (seed) and *Cinnamum zeylanicum (bark*), *Macrotyloma uniflorum* (seed). Development of this preparation into a suitable drug delivery system in the form of tablet was sought to be of appropriate pharmacopoeial quality and would have similar release of the actives as that of the traditional dosage form.

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3. PLANT PROFILE

1. Nigella sativa[28]

Figure: 8 *Nigella sativa*

Synonym	:	Kalonji
Family	:	Ranunculaceae
Common name	:	black cumin, nigella
Parts used	:	seed

Vernacular names

Sanskrit	:	Upakuncika, Susavi
English	:	Small Fennel, Nigella seed
Hindi	:	Kalaunji, Mangaraila
Tamil	:	Karunjeerakam, Karunjiragam

Plant taxonomy

Scientific Classification

Kingdom	:	Plantae
Division	:	Magnoliophyta
Order	:	Ranunculales
Family	:	Ranunculaceae

Genus : Nigella

Species : sativa

Distribution : Punjab, Himachal Pradesh, Bihar and Assam.

Description : Seeds, flattened, oblong, angular, rugulose tubercular, small, funnel shaped, 0.2 cm. long and 0.1cm.wide, black, odour, slightly aromatic, taste, bitter.

Phytoconstituents : Fixed oils : arachidonic,eicosadienoic, linolenic, oleic acid, volatile oils, resin saponin, and tannin

Ethno medicinal uses : Asthma, Bronchitis, High blood pressure, inflammation, Rheumatoid arthritis.

Dose : 1-3 g of the drug in powder form.

2.Moringa oleifera[31]

Figure: 9 *Moringa oleifera*

Synonym	:	*Moringa pterygosperma*
Family	:	Moringaceae
Common name	:	Drum stick tree, horseradish tree
Parts used	:	seed

Vernacular names

Sanskrit	:	Sobhanjana,Aksiva, Mocaka
English	:	Drum stick tree
Hindi	:	Sahajana, Munga
Tamil	:	Muringai, Muringai Virai

Plant taxonomy

Scientific Classification

Kingdom	:	Plantae
Division	:	Tracheophyta

Order	:	Brassicales
Family	:	Moringaceae
Genus	:	*Moringa*
Species	:	*oleifera*

Distribution : found wild in sub-Himalayan tract, and also commonly cultivated all over the plains of the country

Description

Seeds hard, trigonous, having short wings; size 0.5 to 1.0 cm long and 0.3 to 0.5 cm wide; colour greyish-cream; odour, not characteristic; taste; slightly bitter.

Phytoconstituents

Edible oil called ben oil , polyphenols, The seeds contain high levels of vitamin C and moderate amounts of B vitamins and dietary minerals

Ethno medicinal uses :

Antioxidant, anti inflammatory, anti diabetic, reduce cholesterol.

Dose : 5-10 gm of the drug in powder form.

3.Linum usitatissimum [29]

Figure: 10 *Linum usitatissimum*

Synonym	:	Atasi, flax seed
Family	:	Linalaceae
Common name	:	Flax seed
Parts used	:	Seed

Vernacular names

Sanskrit	:	Uma, Ksuma
English	:	Linseed
Hindi	:	Alsi
Tamil	:	Ali, Virai

Plant taxonomy

Scientific Classification

Kingdom	:	Plantae
Division	:	Magnoliophyta
Order	:	Malpighiales

Family : Linaceae

Genus : Linum

Species : *Linum usitatissimum*

Distribution : extensively cultivated throughout the plains of India upto an altitude of 800 m.

Description

Seed small, brown, glossy with minutely pitted surface, about 4-6 mm long and 2-2.5 mm in maximum width, elongated-ovoid, flattened, rounded at one end and obliquely pointed at the other.

Phytoconstituents :

Fixed oil, mucilage and protein.

Ethno medicinal uses :

Used in the treatment of Diarrhea, anti diabetic, gastro intestinal infections.

Dose :

3-6 g of the drug in powder form.

4.Trigonella foenum [32]

Figure: 11 Trigonella foenum

Synonym	:	fenugreek seed
Family	:	Fabaceae
Common name	:	Methi
Parts used	:	seed

Vernacular names

Sanskrit	:	Methini
English	:	Fenugreek
Hindi	:	Methi
Tamil	:	Mendium, Ventaiyam

Plant taxonomy

Scientific Classification

Kingdom	:	Plantae
Division	:	Magnoliophyta
Order	:	Fabales
Family	:	Fabaceae
Genus	:	*Trigonella*
Species	:	*Trigonella foenum-graecum* L.
Distribution	:	Tamilnadu, kerala, Andhra,Orissa,U.P, Punjab, Himachal Pradesh, Bihar and Assam.

Description

Seed oblong, rhomboidal with deep furrow running obliquely from one side, dividing seed into a larger and smaller part, 0.2-0.5 cm long, 0.15-0.35 cm broad,

Phytoconstituents :

Alkaloid, Sapogenins and Mucilage

Ethno medicinal uses :

Arthritis, poor thyroid function, anti inflammatory action, anti diabetic.

Dose :

3-6 g. of the drug in powder form.

5.Cinnamum zeylanicum [30]

Figure: 12 *Cinnamum zeylanicum*

Synonym	:	Ceylon cinnamon, *Cinnamomum cassia*
Family	:	Lauraceae
Common name	:	Dalchini
Parts used	:	bark

Vernacular names

Sanskrit	:	Darusita
English	:	Cinnamon bark
Hindi	:	Dalchini
Tamil	:	Lavangapattai, Karuvapattai

Plant taxonomy

Scientific Classification

Kingdom	:	Plantae
Division	:	Tracheophyta
Order	:	Laurales
Family	:	Lauraceae
Genus	:	Cinnamomum
Species	:	*Cinnamomum cassia*

Distribution : cultivated on the Western Ghats and adjoining hills, bark collected during April-July and October-December.

Description

Bark pieces about 0.5 mm thick, brittle, occurs as single or double, closely packed compound quills, up to a metre or more in length and upto about 1 cm in diameter.

Phytoconstituents

Essential oil, tannin and mucilage

Ethno medicinal uses

Anti oxidants, anti inflammatory, anti diabetic effect, neurodegenerative diseases, anti cancer, anti bacterial .

Dose 1-3 g of the drug in powder form

6.Macrotyloma uniflorum [32]

Figure : 13 *Macrotyloma uniflorum*

Synonym	:	Dolichos biflorus
Family	:	Fabaceae
Common name	:	Horse gram, Kulthi bean
Parts used	:	seed

Vernacular names

Sanskrit	:	Kulathika, Sweta beeja, Kulattha
English	:	Madras gram, Horse gram, poor man's pulse
Hindi	:	Kulthi
Tamil	:	Kaanam, Kollu

Plant taxonomy

Scientific Classification

Kingdom	:	Plantae

Division	:	Tracheophyta
Order	:	Fabales
Family	:	Fabaceae
Genus	:	*Macrotyloma*
Species	:	*Macrotyloma uniflorum*

Distribution : Karnataka, Andhra Pradesh, Orissa, Tamil Nadu, Madhya Pradesh, West Bengal, Jharkhan, Uttaranchal and Himachal Pradesh

Description :

Trifoliate leaves, white coloured flowers, long linear pubescent pods with curved beak, flattened small seeds with light red, brown, grey, black or mottled testa with photo and thermo-sensitive nature.

Phytoconstituents

Phenolic acids namely 3,4 dihydroxybenzoic, 4 hydroxybenzoic, vanillic, caffeic, p – coumaric , ferulic, syringic and sinapic acids.

Ethno medicinal uses :

Anti diabetic, anti oxidant, anti inflammatory, antimicrobial, anti urolithiatic, antihistamine, anthelmintic, hemolytic.

4. AIM AND OBJECTIVE

AIM

The aim of the present work is to develop a polyherbal anti diabetic tablet from the selected plant material and evaluate the tablet.

OBJECTIVE

- To perform the raw material analysis
- To develop polyherbal tablets.
- Standardization quality control of developed tablets.
- To evaluate the antidiabetic activity by *in vitro* methods

5. PLAN OF WORK

I. Collection and Authentication

II. Processing of raw materials

III. Raw Materials Standardization

- Physico-chemical Evaluation.
  - Loss on Drying
  - Determination of Ash values
    - ✓ Total ash value
    - ✓ Acid insoluble ash value
    - ✓ Water soluble ash value
    - ✓ Sulphated ash value
  - Determination of Extractive values
    - ✓ Water soluble extractive value
    - ✓ Alcohol soluble extractive value
    - ✓ Ether soluble extractive value
- Quantitative Estimation of Heavy metals and Inorganic elements

IV. PHYTOCHEMICAL STUDIES

- Preliminary phytochemical screening of extract
- HPTLC - Finger print analysis

V. DEVELOPMENT OF FORMULATION

Pre formulation studies

Selection of excipients

Flow property measurement

- Bulk density

- Tapped density
- Compressibility index
- Hausner's ratio
- Angle of repose

Trial batches (1 to 9) (Selection of optimized batches)

Formulation of tablets

VI. STANDARDIZATION OF POLYHERBAL TABLETS

- Description pH
- Uniformity of weight
- Disintegration time
- Ash value
- Extractive value
- Quantitative estimation of Phytoconstituents
- Quantitative Estimation of Heavy metals

VII. PHARMACOLOGICAL STUDIES

- ✓ *In vitro* studies:

  α-glucosidase inhibitory assay.
- ✓ 3T3 *cell* line - DNS ASSAY

6. MATERIALS AND METHODS

MATERIALS

Polyherbal antidiabetic formulation consists of six herbs viz., *Nigella sativa* (seed), *Moringa oleifera* (seed), *Linum usitatissimum* (seed),*Trogonella foenum*(seed) , *Cinnamum zeylanicum* (bark) and *Macrotyloma uniflorum* (seed).

Table 1. MATERIALS SELECTED FOR FORMULATION

S.no	Name of the materials	Manufacturer /Supplier	Use in formulation
1.	*Nigella sativa*	Amsar PVT.LTD, Indore, Madhya Pradesh.	Active ingredient
2.	*Moringa oleifera*	Amsar PVT.LTD, Indore, Madhya Pradesh.	Active ingredient
3.	*Linum usitatissimum*	Amsar PVT.LTD, Indore, Madhya Pradesh.	Active ingredient
4.	*Trogonella foenum*	Amsar PVT.LTD, Indore, Madhya Pradesh.	Active ingredient
5.	*Cinnamum zeylanicum*	Amsar PVT.LTD, Indore, Madhya Pradesh.	Active ingredient
6.	*Macrotyloma uniflorum*	Amsar PVT.LTD, Indore, Madhya Pradesh.	Active ingredient
7.	Crospovidone	Sai Mirra Pharmaceuticals Pvt Ltd	Disintegrant
8.	Micro crystalline cellulose	Sai Mirra Pharmaceuticals Pvt Ltd	Diluent
9.	Coloidal Silicon dioxide	Sai Mirra Pharmaceuticals Pvt Ltd	Glidant
10	PVP (Polyvinylpyrrolidone)	Sai Mirra Pharmaceuticals Pvt Ltd	Binder
11	Magnesium stearate	Sai Mirra Pharmaceuticals Pvt Ltd	Lubricant

LOSS ON DRYING

The test for loss on drying determines both water and volatile matter in the crude drug. The loss on drying test is important when the herbal substances are known to be hygroscopic. An excess of water in herbal materials will encourage microbial growth, presence of fungi, insects and deterioration. In modern pharmaceutical technology, the water content provides information concerning the shelf life and the quality of the drugs. Loss on drying is the loss of mass expressed as % w/w. About 10 g of drug was weighed in a tarred flat weighing bottle previously dried and dried at 105^{o}C and cooled in a suitable dessicator and weighed. The drying was continued and weighed to a constant weight at one hour interval.

Loss on drying = Finalweight / Initialweight. X 100

DETERMINATION OF ASH VALUES

The ash content of crude drug is generally taken as the residue remaining after incineration. It usually represents the non-volatile inorganic salts like metallic salts and silica naturally occurring in the drug and adhering to it, but it may include inorganic matter added for the purpose of adulteration, contamination and substitution. This is important parameter for the evaluation of crude drugs. The ash value can be determined by three different methods like total ash, acid insoluble ash and water soluble ash. Sulphated ash is also ash value to find out the sulphated residue.

Total ash

Incinerated 2g of the powdered drug in a tared silica crucible at 450ºC in a muffle furnace until carbon completely ashes and ignited to constant weight, removed, cooled in a suitable dessicator for 30 minutes and weighed. Percentage of total ash content was calculated with reference to the air-dried drug.

% Total Ash = wt of residue /wt of sample X 100

Acid insoluble ash

Boiled the ash obtained in total ash for 5 minutes with 25 ml of dilute hydrochloric acid collected the insoluble matter in an ashless filter paper, washed with hot water and ignited at 450ºC to constant weight. Percentage of acid insoluble ash content was calculated with reference to the air-dried drug.

% Ash insoluble ash = wt of residue / wt of sample X 100

Water-soluble ash

The difference in weight between the total ash and the residue after treatment of the total ash with water.

Determination of Water-soluble ash

To the crucible containing the total ash, add 25 ml of water and boil for 5 minutes. Collect the insoluble matter in a sintered-glass crucible or on an ashless filter paper. Wash with hot water and ignite in a crucible for 15 minutes at a temperature not exceeding 450^0C. Subtract the weight of this residue in mg from the weight of total ash. Calculate the content of water-soluble ash in mg per g of air-dried material.

% water soluble ash = wt of residue/ wt of sample X 100

Sulphated ash

Heated a silica crucible to redness for 10 minutes, cooled in a dessicator and weighed. 1g of the substance was transferred into the crucible, ignited gently at first, until the substance is thoroughly charred. Cooled and moistened the residue with 1ml of sulphuric acid, heated gently until white fumes are no longer evolved and ignited at

800^{o}C until all black particles disappeares. Allowed the crucible to cool, added a few drops of sulphuric acid and ignited as before to a constant weight cooled and weighed.

% water sulphated ash = wt of residue/ wt of sample X 100

DETERMINATION OF EXTRACTIVE VALUES

The method determines the amount of active constituents in a given amount of crude drugs when extracted with the solvents. The extraction process of crude drug with a particular solvent yields a solution containing different phytoconstituents. The composition of these phytoconstituents provides the preliminary information on the quality of a particular drug sample.

Water soluble extractive value

Macerated 5 g of the air dried, coarsely powdered drug, with 100 ml of chloroform water in a closed flask for twenty-four hours, shaking frequently during six hours and allowed to stand for eighteen hours. Filtered and evaporated 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, dried at 105ºC to constant weight and weighed. The percentage of water-soluble extractive value with reference to the air-dried drug was calculated.

% water soluble extractive = wt of residue /wt of sample X 100

Alcohol soluble extractive value

Macerated 5 g of the air dried, coarsely powdered drug, with 100 ml of ethanol (95%) in a closed flask for twenty-four hours, shaking frequently during six hours and allowed to stand for eighteen hours. Filtered and evaporated 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, dried at 105ºC to constant weight and weighed. The percentage of water-soluble extractive value with reference to the air-

dried drug was calculated.

% Alcohol soluble extractive = wt of residue /wt of sample X 100

Ether soluble extractive

The type of ether soluble extractive values determined for evaluation of crude drugs are volatile and non volatile ether soluble extractives. The volatile ether soluble represents volatile oil content of the drug, while non volatile ether soluble represent resin, fixed oils or colouring matter present in drugs. The percentage of ether soluble extractive was calculated.

% Ether soluble extractive = wt of residue /wt of sample X 100

QUANTITATIVE ANALYSIS OF HEAVY METALS[33]

INSTRUMENTATION PARAMETERS:

Instrument name:

Inductive coupled plasma-Optical emission spectroscopy.

Instrument Model:

PE Optima 5300DV ICP-OES; Optical system Dual view-axial or radial.

Detector system:

Charge coupled detector, (UV-Visible detector which is maintaining at 40º C) to detect the intensity of the emission line.

Light source (Torch):

Positioned horizontally in the sample compartment along the central axis of the spectrometer optics. Changing from axial to radial viewing is a simple software command and is accomplished by computer control of a mirror located in the optical path. The torch assembly of this system comprises of two concentric quartz

tubes.

Standard alumina injector	:	2.00mm inner
diameter Spray Chamber	:	Scott type
Nebulizer	:	Cross flow gem tip

Preparation of sample by acid digestion method:

50mg of powder was treated with acid mixture of sulphuric acid: water in the ratio of 4:1in the Kjeldahl flask and heated continuously till the solution is colourless. The sample mixture was then transferred in a 25ml volumetric flask and made up to the volume with distilled water. Blank solution was prepared as above without sample.

The standards of Arsenic, Lead, Mercury and Cadmium were prepared as per the protocol and the calibration curve was developed for each of them.

Detection:

Samples were analyzed for the detection and quantification of the calcium, sulphate, borate, silver, aluminium, copper, potassium, chloride by Inductively Coupled Plasma Emission Spectrometry.

PHYTOCHEMICAL STUDIES [34, 35]

Herb is a biosynthetic laboratory, which contains chemical compounds such as carbohydrates, proteins and lipids that are utilized as food. It also contains secondary products like glycosides, alkaloids, flavonoids, tannins etc. The detection of these active principles in medicinal plants plays a strategic role in the phytochemical investigation of crude drugs and extracts and is very important in regard to their potential pharmacological effects. These tests facilitate the quantitative estimation and qualitative separation of pharmacologically active chemical compounds and subsequently may lead to the drug discovery and development.

All the plant raw materials were subjected to preliminary phytochemical

screening for the detection of various plant constituents.

PRELIMINARY PHYTOCHEMICAL SCREENING

Triterpenoids

Salkowski test - Powdered crude drug was treated with few drops of concentrated sulphuric acid, formation of yellow colour indicates the presence of triterpenoids.

Flavones

Shinoda test - To the powdered crude drug, few magnesium turnings and few drops of concentrated hydrochloric acid were added and boiled for five minutes formation of red coloration indicates the presence of flavones.

Alkaloids

Dragendorff's reagent - To the powdered crude drug, few drops of potassium bismuth iodide solution was added, reddish brown colour indicates the presence of alkaloids.

Carbohydrates

Molisch's test - In a test tube containing powdered drug, 2 ml of distilled water and 2 drops of freshly prepared 20% alcoholic solution of α- napthol were added. Mixed well and added 2ml of concentrated sulphuric acid along the sides of the test tube. Formation of red violet ring, which disappears on addition of excess alkali solution, indicates the presence of carbohydrates.

Glycosides

Extracted 200 mg of drug with 5 ml dilute sulphuric acid by warming on a water bath, filtered and neutralized the acid extract with 5% solution of sodium hydroxide. 1ml of Fehling's solution A and B were added until it became alkaline (test with pH paper) and heated on a water bath for 2 minutes. Formation of red precipitate was observed, which indicates the presence of glycosides.

Phenols

Ferric chloride test - Dissolved a small quantity of the drug with 2ml of distilled water, added a few drops 10% aqueous ferric chloride solution. A blue or green colour was produced, which indicates the presence of phenols.

Proteins (Biuret test)

To 1ml of ethanolic extract of the drug, 5 to 8 drops of copper sulphate solution (10%) was added. Formation of violet colour indicates the presence of proteins.

Resin

Dissolved a small quantity of the ethanolic extract of the drug with 5 -10 ml of acetic anhydride by gently heating the solution. Cooled and added 0.05 ml of concentrated sulphuric acid. A bright purplish red colour was seen, which rapidly changed to violet indicates the presence of resins.

Saponins

To 5 ml of an extract of the drug, a drop of sodium bicarbonate solution was added. Shake the mixture vigorously and left it for 3 minutes. Honey comb like froth developed indicates the presence of saponins.

Tannins

The powdered drug was mixed with basic lead acetate solution. Formation of white precipitate, which indicates the presence of Tannins.

Steroids

Libermann Burchard's test - The powdered drug was treated with few drops of acetic anhydride, boiled and cooled and added concentrated sulphuric acid from the side of the test tube. A brown ring was formed at the junction two layers and upper layer turns green indicates the presence of steroids.

HPTLC FINGERPRINT PROFILE

HPTLC is one of the advanced and versatile chromatographic technique which helps in the identification of compound and thereby authentication of purity of herbal drugs. It is very quick process. In addition to qualitative detection, HPTLC also provides semi-quantitative information on major active constituents of a drug thus enabling an assessment of drug quality.

HPTLC serves as a convenient tool for finding the distribution pattern of the phytoconstituents which is unique to each plant. The finger print obtained is suitable for monitoring the identity and purity of drugs and for detecting adulteration and substitution. HPTLC technique is helpful in order to check the identity, purity and standardize the quantity of active principles present in the herbal extracts.

Sample Preparation:

1mg of Polyherbal extract was dissolved in 1 ml of methanol.

Developing Solvent System:

A number of solvent systems were tried, for extract. The satisfactory resolution obtained for the phytochemical constituent alkaloid was in the solvent butanol-acetic acid-water (7:2.5:0.5).

Chromatography:

Chromatography was performed on silica gel 60 F254 TLC pre- coated plates using Hamilton syringe and CAMAG LINOMAT 5 instrument. 2 µl of standard solution and 2 µl of the test solution (extract) were loaded as 5 mm band length in the 4×10 glass plates, with the help of a CAMAG LIWOMAT 5 sample applicator at the distance of 10 mm from the edge of the plates.

Development of Chromatogram:

After the application of sample, the chromatogram was developed in Twin trough glass chamber 10x10 cm saturated with previously equilibrated mobile phase for 30 minutes. The chromatographic conditions were previously optimized to active the best resolution and peak shape.

Detection of Spots:

The air-dried plates were viewed in ultraviolet radiation to mid- day light.The chromatograms were scanned by densitometer at 366 nm. Quercetin was used as the reference standard. The plates were kept in Photo-documentation chamber (CAMAG SAVIT-GOLAY 7) and captured the images at White light, UV 254 nm. After scanning the plates the Peak table, Peak display and Peak densitogram were recorded. The retention factor (Rf) was calculated by the WinCats software.

DEVELOPMENT OF FORMULATION

The extracts., *Nigella sativa* (seed), *Moringa oleifera* (seed), *Linum usitatissimum* (seed),*Trogonella foenum*(seed) , *Cinnamum zeylanicum* (bark) and *Macrotyloma uniflorum* (seed) were subjected to freeze drying process.

The extracts were dried for a period of time according to their rate of drying . Diluents like, Microcrystalline cellulose, Magnesium stearate, Lactose,starch were dried.

All active ingredients were weighed according to the formula, mixed with MCC followed by diluents and glidant like Aerosil and magnesium stearate as lubricant as specified in formula were mixed well. The mixture was blended thoroughly for 30 minutes.

Then the powder was transferred to the polythene bags and labelled for further studies.

Table 2. PROPOSED STRENGTH OF FORMULATION

S.NO.	ACTIVE INGREDIENTS	STRENGTH (in mg)
1	*Nigella sativa*	62.5
2	*Moringa oleifera*	62.5
3	*Linum usitatissimum*	125
4	*Trogonella foenum*	62.5
5	*Cinnamum zeylanicum*	125
6	*Macrotyloma uniflorum*	62.5

PREFORMULATION STUDIES [36]

Prior to formulation, it is essential that fundamental physical and chemical properties of the drug molecule and other derived properties of the drug powder are determined. This information decides many of the subsequent events and approaches in formulation development. This first learning is known as Preformulation. It aims to optimize the process of turning a drug into a drug product. During preformulation the physiochemical properties of the drug candidate are determined.

Definition

Preformulation involves the application of biopharmaceutical principles to the physicochemical parameters of drug substance are characterized with the goal of designing optimum drug delivery system.

Before beginning the formal preformulation programs the manufacturing scientist must ensure the following factors:

- The amount of drug available
- The physicochemical properties of the drug already known.
- Therapeutic category and anticipated dose of compound.

- The nature of information, a formulation should have or would like to have.

SELECTION OF THE EXCIPIENTS[37]

The majority of materials filled in the capsules are formulated as powders that are typically mixtures of the active ingredients together with a combination of different types of excipients. Normally, there are three types of excipients used in tablet formulation i.e. diluents, glidants and lubricants.

Diluents:

Diluents/Fillers are added where the quantity of active ingredient is less (or) difficult to filling. Common tablet/capsule filler include Lactose, Dicalcium phosphate, Microcrystalline cellulose, etc.

Lubricants:

They reduce friction during the filling process. In addition, they aid in preventing adherence . Magnesium Stearate, Stearic acid, Hydrogenised vegetable oils and talc are commonly used lubricants.

Glidant:

It is used to improve flow of the powder materials by reducing the friction between the particles. The most effective glidants are the Colloidal silicon dioxide, Talc and Starch.

FLOW PROPERTY MEASUREMENTS[38,39]

The flow property of the blended powder is an important parameter to be measured since it affects the uniformity of dose. It was assessed by the following parameters.

- Bulk density
- Tapped density
- Compressibility index
- Hausner's ratio
- Angle of repose

Bulk density (ρb)

It is determined by measuring the volume of a known mass of powder sample that has been passed through a screen into a graduated cylinder or through a volume measuring apparatus into a cup. It is expressed in g/ml and is given by,

$$\rho b = M/Vo$$

Where, M - is the mass of powder

Vo- is the bulk volume of the powder.

The inter particle interactions that influence the bulking properties of a powder are also the interactions that interfere with powder flow, a comparison of the bulk and tapped densities can give a measure of the relative importance of these interactions in a given powder. Such a comparison is often used as an index of the ability of the powder to flow.

Tapped density (ρt)

It is achieved by mechanically tapping a measuring cylinder containing a powder sample. After observing the initial volume, the cylinder is mechanically tapped and volume readings are taken until little further volume change is observed.The mechanical tapping is achieved by raising the cylinder and allowing it to drop under its own weight at a specific distance.The tapped volume was measured by tapping the powder to constant volume. It is expressed in g/ml and is given

$$\rho t = M/Vt$$

Where, M - Mass of powder and Vt- Tapped volume of the powder

Compressibility index: (CI)

Compressibility is the ability of powder to decrease in volume under pressure. Compressibility is a measure that obtained from density determination. Weighed quantity of granules was transferred to 50 ml graduated cylinder, volume occupied by granules was noted down. Then cylinder was subjected to 500/ 750 and 1250 taps. The difference between two tabs should be less than 2%. The percentage Compressibility Index is calculated by using formula.

Hausner's Ratio

$$\mathbf{CI = Vo\text{-}Vi \times 100/Vo}$$

Where, Vo- Untapped density; Vi- Tapped density

It is measurement of frictional resistance of the granular material. The Ideal range should be 1.2 -1.5, it was determined by the ratio of tapped density and bulk density.

$$\mathbf{Hausner's\ Ratio = Vi / Vo}$$

Where, Vo -Untapped density, Vi -Tapped density

Angle of repose

The tangent of angle of repose is equal to the coefficient of friction between the particles. Hence the rougher and more irregular the surface of particles, the greater will be angle of repose. For determination of angle of repose (θ), the blends were poured through the walls of a funnel which was fixed at a position such that its lower tip was at a height of exactly 2.0 cm above a hard surface. The drug or the blends were poured till the time when upper tip of the pile surface touched the lower

tip of the funnel. Angle of repose was calculated using following equation.

$$\textbf{Tan } \theta = \textbf{h/r,}$$

$$\theta = \textbf{tan-1 (h/r)}$$

Where, θ - angle of repose, h- height in cm and r- radius in cm.

Based on the Angle of repose, Compressibility index and Hausner's ratio, the flow property of the granules can be characterized.

Table 3:Angle of Repose, Compressibility Index and Hausner's Ratio

Flow property	Angle repose	Compressibility Index	Hausner's ratio
Excellent	25-30	<10	1.00-1.11
Good	31-35	11-15	1.12-1.18
Fair	36-40	16-20	1.19-1.25
Passable	41-45	21-25	1.26-1.34
Poor	46-55	26-31	1.46-1.59
Very poor	56-65	32-37	1.46-1.59
Very very poor	>66	>38	>1.60

DEVELOPMENT OF FORMULATION -TRIAL BATCHES

Nine trial batches of tablets were formulated by varying the composition off the excipients proportions for excellent flow property.

Table 4. DEVELOPMENT OF FORMULATION

The blended powder of all nine trial batches were analysed for its flow characteristics like bulk density, tapped density, compressibility index, Hausner's ratio and Angle of repose.

MATERIALS	F1	F2	F3	F4	F5	F6	F7	F8	F9
Herbal extract	500	500	500	500	500	500	500	500	500
MCC	83	76	70	64	70	58	58	52	46
PVP	6	13	19	25	19	31	31	31	31
Crospovidone	19	19	19	19	19	19	19	25	31
Aerosil	6	6	6	6	6	6	6	6	6
Magnesium stearate	6	6	6	6	6	6	6	6	6
Total weight	620	620	620	620	620	620	620	620	620

From the above trial batches, the trial batch 9th trial was found to be the perfect batch and it was selected for the consideration of further studies.

Table :5 Final Batch

S.NO	MATERIALS	TRAIL9 (mg)
1	*Nigella sativa*	62.5
2	*Moringa oleifera*	62.5
3	*Linum usitatissimum*	125
4	*Trogonella foenum*	62.5
5	*Cinnamum zeylanicum*	125
6	*Macrotyloma uniflorum*	62.5
7	MCC(Micro crystalline cellulose)	46
8	PVP (poly vinyl pyrrolidone)	31
9	Aerosil	6
10	Crospovidone	31
11	Magnesium stearate	6

POST COMPRESSION STUDIES

PHYSICAL PARAMETERS

General appearance

The general appearance of the tablets from each formulation batch was observed. The general appearance parameters like shape, color, presence or absence of odour and taste were evaluated.

Uniformity of weight

Twenty tablets were selected at random and weighed individually. The average weight was also measured. The percentage deviation of tablets was calculated and compared with standard specifications.

Table 6.Uniformity of weight

S.NO	IP	LIMIT
1.	80mg	±10%
2.	80mg or less than 250mg	±7.5%
3.	250mg or more	±5%

Thickness and diameter

The thickness and diameter was measured to determine the uniformity of size and shape. Thickness and diameter of the tablets were measured using Vernier caliper.

Hardness

Hardness is defined as the force required for breaking a tablet at diametric compression test and it is termed as tablet crushing strength. Hardness of the prepared formulations was determined using Monsanto hardness tester. It was expressed in kg/cm^2

Friability

Friability of the prepared formulations was determined by using Roche Friabilator. Pre- weighed sample of tablets was placed in the friability tester, which was then operated for 100 revolutions. Tablets were de-dusted and reweighed.

The friability of the tablets was calculated using the formula mentioned below,

$$\text{Friability} = \frac{\text{Initial weight of the tablets} - \text{Final weight of the tablet}}{\text{Initial weight of the tablets}}$$

DISINTEGRATION TIME

This test is done to measure the time taken by the drug to disintegrate in the body. This is done to determine whether the tablet disintegrates within the prescribed time when placed in a liquid medium under the prescribed experimental conditions. One each tablet was added to each of the six tubes of the basket and a disc was added to each of the tube. The tubes were dipped in 0.1N HCl solution maintained at 37°C.

PHYSICOCHEMICAL PARAMETERS [43]

Physicochemical parameters like loss on drying, ash and extractive values were carried out as per pharmacopoeial procedures as discussed in earlier.

PHYTOCHEMICAL STUDIES

I. QUALITATIVE PHYTOCHEMICAL ANALYSIS were carried out to detect the presence of phytoconstituents in the polyherbal formulation.

II. QUANTITATIVE ESTIMATION OF TOTAL PHENOLIC, FLAVONOID AND TANNINS CONTENT [44,45,46]

ESTIMATION OF FLAVONOIDS

The total flavonoid content is usually determined spectrophotometrically using Ultra-violet spectroscopy.

Preparation of Standard Stock solution

Accurately weighed 25 mg of Quercetin standard transferred to 100 ml of volumetric flask and dissolved with dimethyl sulfoxide(DMSO). The serial dilution (12.5µg/ml to 200µg/ml) were made with dimethyl sulfoxide.

Preparation Of Test Solution

The Polyherbal formulation was weighed accurately equal to the weight of Standard Quercetin and transferred to 100 ml volumetric flask and the extract dissolved with dimethyl sulfoxide (DMSO). The serial dilution (12.5µg/ml to 2005µg/ml) were made with dimethyl sulfoxide.

PROCEDURE

From the prepared solution of standard and test solutions 2ml was withdrawn from each concentration to the test tube and added equal volume of 2% Aluminium Chloride solution to every single concentration. Incubate the solution about 10 minute at ambient temperature. After 10 minute, measure the absorbance spectrophotometrically at 435 nm with the standard and test sample solutions.

ESTIMATION OF PHENOLIC CONSTITUENTS

Total phenolic content of the extracts were determined using Folin – Ciocalteu's assay. 0.5ml extract solutions were mixed with 2.5ml of 10 fold diluted Folin Ciocalteu's reagent and 2.5ml of 7.5% sodium carbonate. After incubation at 40°c for 30 minutes, the absorbance of the reaction mixtures was measured at 765nm in a spectrophotometer. Three replicates were made for each test sample. Gallic acid was used as a standard and total phenolic content of the extract was expressed in mg of Gallic acid equivalents (mg GAE/g extract)

ESTIMATION OF TANNIN CONTENT

The tannin content was determined by using $FeCl_3$ and gelatin tests 0.1g of the extracts were transferred to a 100ml flask, 50ml of water was added and boiled for 30min. After filtration with cotton filter, filtrate was transferred to a 500ml flask and the volume was made up to the mark with distilled water. 0.5 ml aliquots were transferred to vials, 1ml of 1% $K_3Fe(CN)_6$and 1 ml of 1% $FeCl_3$ were added and the volume was made up to 10ml with distilled water. After 5 min absorbance was measured at 510nm against a reagent blank spectrophotometer and concentration of tannins in the test sample was

determined and expressed as mg equivalent of tannic acid per gram of sample.

Quantitative analysis of Heavy metal

The formulation was analyzed for its heavy metals as discussed earlier.

PHARMACOLOGICAL STUDIES

IN VITRO ANTI DIABETIC ACTIVITY

ALPHA GLUCOSIDASE[47]

Sample extraction

25 gram of powdered sample were taken and extracted with ethanol using soxhlet apparatus**.** The extract was collected condensed under reduced pressure in rotary vacuum evaporator and stored at 4^{o}C.

Determination of alpha – glucosidase inhibitory activity

Materials required

Phosphate buffer	:	50mM, pH 6.8
Sodium carbonate	:	(0.1M).
PNPG	:	1Mm
Sample	:	extract with range of concentrations 20-100 µg /ml
Alpha- glucosidase	:	1u /ml-SRL

Procedure:

Alpha-glucosidase inhibitory activity of extracts was carried out according to method of Bachhawat *et al 2011* with slight modification. Reaction mixture containing 50µl phosphate buffer, 10µl alpha-glucosidase and 20µl of varying concentrations of extracts was pre-incubated at 37^{o}C for 15 min. Then 20µl p-nitrophenyl-α-D-

Glucopyranoside (PNPG) was added as a substrate and incubated further at 37^{o}C for 30 min. The reaction was stopped by adding 50µl sodium carbonate .The yellow color produced was read at 405nm. Each experiment was performed along with appropriate blanks. Acarbose at various concentrations (20-100 µg/ml) was included as a standard. Negative control without extracts was set up in parallel. The result is expressed as percentage inhibition.

Calculation:

Inhibition (%) = Abs. Control – Abs. Sample / Abs. control X 100,

DNS ASSAY[48]

Cell line and culture:

3T3 *cell* line was obtained from NCCS, Pune. The cells were maintained in DMEM with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO_2 at 37 °C.

Subculture:

1. Take T 25 flask from incubator and check under microscope.
2. Check for Confluence of cells.
3. Remove the medium using a pipette.
4. Gently rinse the cells with DMEM without FBS 2 - 3 times.
5. Add 4 – 5 ml of TPVG (Trypsin, PBS, Versin and Glucose) over the cells.
6. Allow TPVG to act for 3-5 minutes
7. Discard the TPVG and add 5ml of 10% FBS DMEM
8. Break off the cell clusters by gently pipetting back and forth with pipette.
9. Count the cells in haemocytometer

10. Keep sterile Tissue culture flask properly labeled and corked ready.
11. Add 5ml of growth medium (DMEM with serum) to each of the Tissue Culture flask.
12. Add cell suspension to each of the Tissue Culture flask based on the cell count (1 lakh cell per ml of medium). Shake the bottle gently so as to allow uniform dispersion of cells.
13. Label on flask should indicate cell line, date of seeding, passage number.
14. Stopper the newly seeded flask tightly and incubate at 37^0C in 5% CO2 atmosphere.
15. Observe the cell growth every day.
16. If attains 60% confluence, follow procedure of MTT assay.
17. Collect the cells with 60% confluence from the TC flask by the means of adding TPVG and passing into a 15ml centrifuge tube.
18. Centrifuge and remove the supernatant.
19. To the cell pellet add 15ml of fresh 10%FBS, DMEM and resuspend the cells (Solution A: Cell + Medium).

CELL GROWTH WITH SAMPLE AND GLUCOSE

1. Take a 6 well plate and name it.
2. Take 5ml of Solution A (Cell + Medium) and add in 6 well plate
3. Add 1ml of sample (Sample at selected dosage) to 5ml of solution A in the 6 well plate.
4. Incubate for a day (24hrs).Sub samples were collected from the culture medium at 0^{th} hr, 5^{th} hr, 10^{th} hr, 15^{th} and 20^{th} hr.DNS assay was carried out.

GLUCOSE SAMPLE PREPARATION:

1. After 24hrs, take the 6-well plate from the incubator and suck the entire medium from the plate.
2. Take 2ml of 10XPBS and add to the 6-well plate for washing and suck out PBS and close the plate.
3. Take 150µl of 10%SDS, add to the plates and shake well.
4. Take a cell scraper and scrap the cells from the bottom of the plate and shift the cells into a corner of the plate.
5. Take 150µl, take the total content from the plates and pour in eppendorf tube and named it separately.
6. Allow to settle for 5 min.
7. The 6 eppendorf tubes were centrifuged at 14000 rpm for 15min at 25^0C.
8. Then take pipette and take supernatant and add to labeled eppendorfs.
9. Store in refrigerator 4^0C for further use.

DNS ASSAY – STANDARD CURVE:

DNS REAGENT:

1. Take 1g of DNS add 50ml of distilled water and dissolve it. Then, add 30g of sodium potassium tartarate tetrahydrate solution turns milky yellow color. Then add 20ml of 2N NaOH (1.6g of NaOH in 20ml of water) – turns to transparent orange yellow color. Make the final volume 100ml by adding distilled water. Take the solution in a reagent bottle and cover it with aluminum foil.

GLUCOSE STOCK:

450mg of glucose is weighed and made up to 100ml with distilled water (0.025m).

PROCDURE: For standard Graph

1. Take the required no. of test tubes and label as follows
2. Take 200, 400, 600, 800, 1000 µl of glucose stock in each test tube. Make up the volume of 2ml with distilled water.
3. Take 100µl of each sample supernatant in each test tube and make up a volume of 2ml with distilled water.
4. Take 2ml of distilled water as a blank.
5. Add 1ml of prepared DNS reagent to all the tubes.
6. Observe all the test tubes are of equal volume(3ml)
7. Cover the entire test tube top with aluminum foil.
8. Keep the test tubes with rack at 100^0C in a water bath for 5 min.
9. Observe for the colour change.
10. Take 1ml of the solution and observe OD at 540nm.
11. Draw graph with amount of glucose in mg (mg/ml) as X axis and OD at 540nm as Y axis
12. Measure absorbance of the samples and detect the unknown concentration from the standard.

7. RESULTS AND DISCUSSION

PHYSIO CHEMICAL EVALUATION

LOSS ON DRYING

Loss on drying for the raw materials were done. The results obtained and the standard values are given

Table: 7 Loss on drying

SNO	PLANT NAME	LOD(%w/w)
1.	*Nigella sativa*	2.91
2.	*Linum usitatissimum*	0.19
3.	*Moringa oleifera*	3.47
4.	*Trigonella foenum*	4.21
5.	*Cinnamum zeylanicum*	4.34
6.	*Macrotyloma uniflorum*	1.45

DETERMINATION OF ASH VALUES

Total ash content:- Total ash content of raw materials was determined , the values obtained and their acceptable limits defined are given in table.

Table: 8 Total ash value

SNO	PLANT NAME	TOTAL ASH VALUE (w/w%)
1.	*Nigella sativa*	4.82
2.	*Linum usitatissimum*	1.09
3.	*Moringa oleifera*	3.99
4.	*Trigonella foenum*	3.0
5.	*Cinnamum zeylanicum*	4.75
6.	*Macrotyloma uniflorum*	4.95

WATER SOLUBLE ASH

The total ash content, the water soluble ash content of individual raw materials was determined and results are enumerated in table.

Table: 9 water soluble ash value

S.NO	PLANT NAME	WATER SOLUBLE ASH(% w/w)
1	*Nigella sativa*	0.78±0.01
2	*Moringa oleifera*	2.31±0.02
3	*Linum usitatissimum*	1.76±0.37
4	*Trogonella foenum*	2.74±0.59
5	*Cinnamum zeylanicum*	5.73±0.21
6	*Macrotyloma uniflorum*	4.91±0.37

SULPHATED ASH

The total ash content, the Sulphated ash ash content of individual raw materials was determined and results are enumerated in table.

Table: 10 Sulphated ash value

S.NO	PLANT NAME	SULPHATED ASH (% w/w)
1	*Nigella sativa*	1.25±0.02
2	*Moringa oleifera*	0.12±0.02
3	*Linum usitatissimum*	1.5±0.12
4	*Trogonella foenum*	2.2±0.04
5	*Cinnamum zeylanicum*	2.8 ±0.12
6	*Macrotyloma uniflorum*	5.7±0.32

DETERMINATION OF EXTRACTIVE VALUES

Water Soluble Extractive Value

Water soluble Extractive values for the raw materials were determined and the results are tabulated in table.

Table: 11 Water soluble Extractive

S.NO	PLANT NAME	WATER SOLUBLE EXTRACTIVE (%w/w)
1	*Nigella sativa*	4.29±0.04
2	*Moringa oleifera*	1.94±0.17
3	*Linum usitatissimum*	4.63±0.20
4	*Trogonella foenum*	2.61±0.69
5	*Cinnamum zeylanicum*	3.54±0.15
6	*Macrotyloma uniflorum*	3.76±0.56

ALCOHOL SOLUBLE EXTRACTIVE VALUE

Alcohol soluble Extractive values for the raw materials were determined (90% ethanol) and the results are enumerated in table.

Table: 12 Alcohol soluble extractive

S.NO	PLANT NAME	ALCOHOL SOLUBLE EXTRACTIVE (%w/w)
1	*Nigella sativa*	4.82±0.17
2	*Moringa oleifera*	4.66±0.16
3	*Linum usitatissimum*	4.36±0.22
4	*Trogonella foenum*	4.88±0.28
5	*Cinnamum zeylanicum*	4.08±20
6	*Macrotyloma uniflorum*	2.13±66

ETHER SOLUBLE EXTRACTIVE VALUE

Ether soluble extractive values for the raw materials were determined and the results are enumerated in table.

Table : 13 Ether soluble extractive value

S. NO	PLANT NAME	ETHER SOLUBLE EXTRACTIVE(%w/w)
1	*Nigella sativa*	3.28±0.64
2	*Moringa oleifera*	5.35±0.42
3	*Linum usitatissimum*	2.68±0.24
4	*Trogonella foenum*	2.50±0.25
5	*Cinnamum zeylanicum*	9.25±0.24
6	*Macrotyloma uniflorum*	7.67±0.21

ANALYSIS OF HEAVY METAL

Estimation of heavy metals in the raw materials were carried out and the results were recorded and detailed in table.

Table: 14 Test for heavy metals

S.NO	PLANT NAME	OBSERVATION (in ppm)			
		Arsenic (NMT 5)	Lead (NMT 10)	Cadmium (NMT 0.3)	Mercury (NMT 0.5)
1	*Nigella sativa*	0.002	0.001	0.024	0.004
2	*Moringa oleifera*	0.004	0.003	0.005	0.002
3	*Linum usitatissimum*	0.002	0.004	0.001	0.001
4	*Trogonella foenum*	0.001	0.005	0.04	0.04
5	*Cinnamum zeylanicum*	0.005	0.002	0.002	0.004
6	*Macrotyloma uniflorum*	0.003	0.001	0.004	0.002

The estimation of heavy metals in the sample revealed heavy metals are within the prescribed limits. It is safe and does not cause any harm on consumption.

PHYTOCHEMICAL ANALYSIS

The chemical tests for various Phytoconstituents in the raw materials were carried out and the results were recorded and detailed in table.

Table: 15 Phytochemical analysis

Phyto-constituents	*Nigella sativa*	*L. usitatis -simum*	*M. oleifera*	*T.foenum*	*C. zeyla -nicum*	*M.uni -florum*
Phenolic compounds	-	+	+	+	+	+
Flavanoids	-	+	+	+	-	-
Tannins	-	-	+	+	+	+
Alkaloids	+	+	+	+	+	-
Steroids	-	-	+	-	+	+
Glycosides	+	+	+	+	-	-
Saponins	+	+	+	-	+	-
Proteins	+	+	+	+	-	+
Carbohydrates	+	+	+	+	-	+
Terpenoids	-	-	+	+	+	+

QUANTITATIVE ESTIMATION OF PHYTOCONSTITUENT

The Polyherbal formulation was found to contain various phytochemical constituents and hence it is desirable to quantify few of them in order to establish a standard to maintain its quality. Among them the estimation of total phenolics, Flavanoids, and Tannin content in the ethanolic extract were decided to be taken as parameters. Samples were drawn from three random samples of polyherbal capsules and the total Phenolics, Flavonoids, and Tannin content present in them were estimated.

TOTAL PHENOLIC CONTENT

Table:16 Total Phenolic content

S.NO	Concentration of standard solution(µg)	ABSORBANCE (765nm)
1	2	0.016
2	4	0.028
3	6	0.038
4	8	0.052
5	10	0.063
6	PHF	0.058

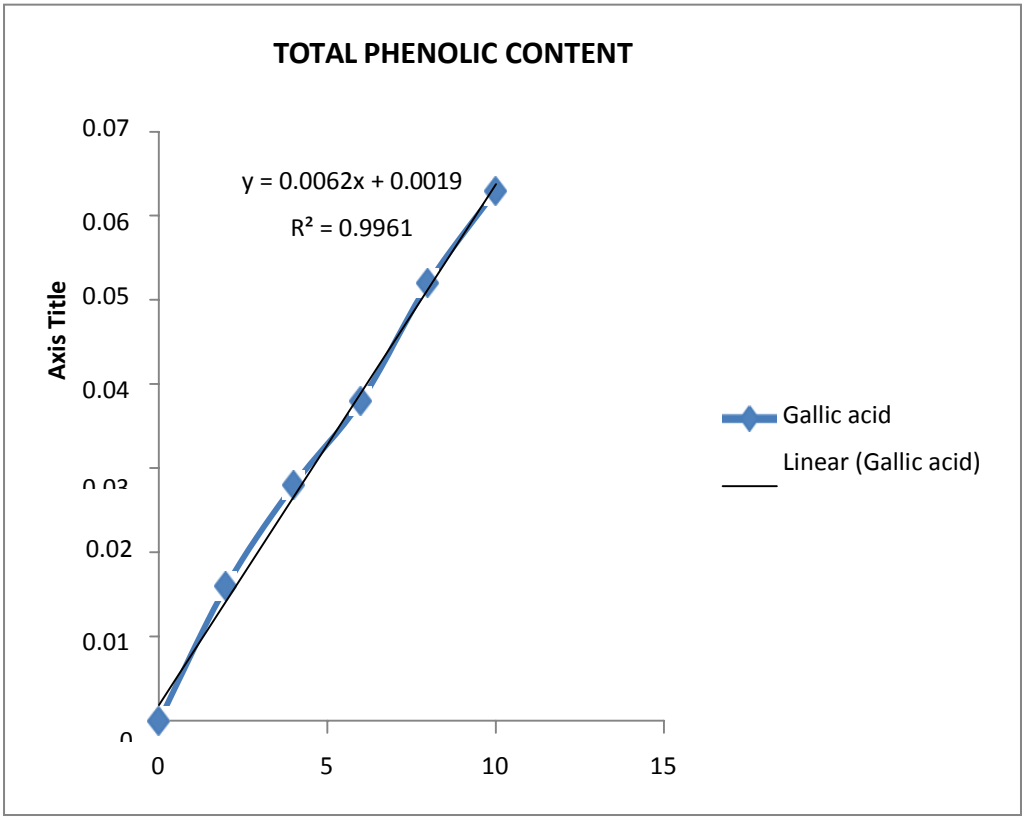


Fig 14: Calibration curve of Phenoilc conent

Comparison of standard (Gallic acid) absorbance at various concentration (2 µg to10 µg) and polyherbal formulation. Sample absorbance corresponds at standard absorbance at a concentration of 9µg. Hence the amount of flavanoid s present in formulation found to be 9µg.

TOTAL TANNIN CONTENT

Table:17 **Total tannin content**

S.NO	Concentration of solution(µg)	ABSORBANCE (510nm)
1	0	0
2	5	0.08
3	10	0.16
4	15	0.25
5	20	0.32
6	25	0.37
6	PHF	0.33

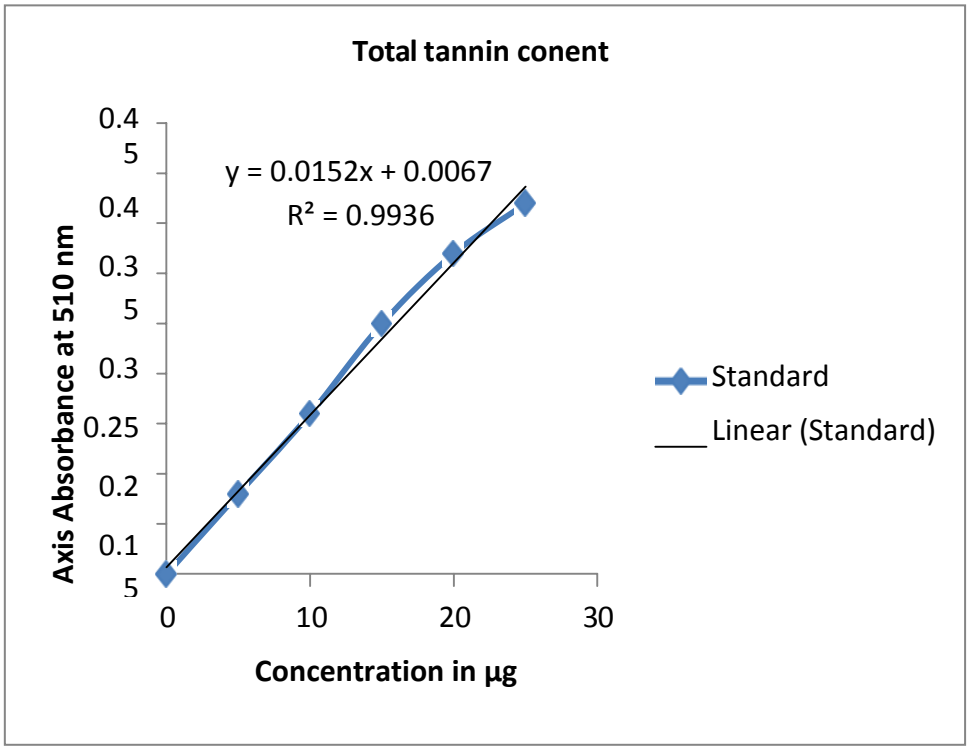


Fig 15: Calibration curve of total tannin conent

Comparison of standard absorbance at various concentration (5 µg to25 µg) and polyherbal formulation. Sample absorbance corresponds at standard absorbance at a concentration of 22µg. Hence the amount of tannin s present in formulation found to be 22µg.

TOTAL FLAVONOID CONTENT

Table 18: **Total flavonoid conent**

S.NO	Concentration of standard solution(µg)	ABSORBANCE (765nm)
1	20	0.11
2	40	0.14
3	60	0.17
4	80	0.22
5	100	0.26
6	PHF	0.17

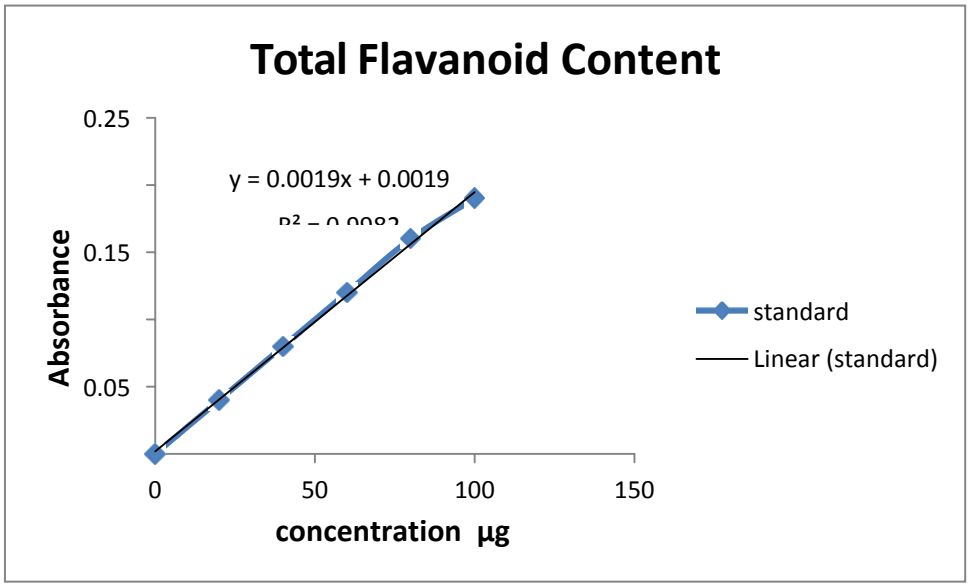


Fig 16: Calibration curve of total flavanoid conent

Comparison of standard (Quercertin) absorbance at various concentration (20 µg to100 µg) and polyherbal formulation. Sample absorbance corresponds at standard absorbance at a concentration of 63µg. Hence the amount of flavanoid s present in formulation found to be 63 µg

The estimated amounts of phenolics, Flavanoids, and Tannins were enumerated in the Table 34.

Table 19: Quantitative estimation of phytoconstituents

S.NO	PARAMETER	OBSERVATION (%w/w)
1	Total tannin content	0.54±0.15
2	Total flavonoid content	3.25±0.37
3	Total phenolic content	1.75±0.21

Result (n=3) are reported as Mean ± Standard deviation

From the results obtained it is determined that the average content of phenolics, Flavonoids and Tannins were present in the Polyherbal formulation.

HEAVY METALS ANALYSIS

Tablets were analyzed for the heavy metals which include Arsenic, Cadmium, Lead and Mercury. The results are as follows,

Table 20: Quantitative Heavy metals analysis

S.NO.	HEAVY METALS	OBSERVATION (in ppm)	LIMITS (in ppm)
1.	Arsenic	0.02	5
2.	Cadmium	0.02	0.3
3.	Lead	0.06	10
5.	Mercury	0.01	0.5

From the results it is shown that the Polyherbal formulation complies with the heavy metals limits of the WHO guidelines and hence it is safe to be taken internally.

PRE-COMPRESSION STUDIES [47]

The drug and the powder blends are evaluated for Pre-compression parameters. The results are given in the tables

Totally nine trials of formulation were carried out using different choices of excipients considering different facts of manufacturing problems as well as quality defects in mind. All the resultant formulations were evaluated for their flow property, uniformity of filling, uniformity of weight, moisture content and disintegration time.

Table 21: Evaluation of trial batches

Parameters	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7	Trial 8	Trial 9
Bulk density ($g/cm^{2)}$	0.36	0.39	0.42	0.43	0.45	0.47	0.47	0.50	0.51
Tapped density($g/cm^{2)}$	0.50	0.53	0.55	0.55	0.56	0.56	0.55	0.57	0.58
Compressibility index (%w/w)	26.75	26.40	23.63	21.81	19.64	16.07	14.54	12.28	12.06
Hausner's Ratio	1.35	1.36	1.32	1.29	1.26	1.20	1.19	1.15	1.13
Angle of repose (degrees)	46.05	45.66	43.03	42.03	41	38	37	34	32

STANDARDISATION OF THE FINISHED PRODUCT

The final formulation was analyzed for its quality control parameters in three trials. The mean value was obtained and Standard deviation was calculated. Wherever there were no official standard, limits for each parameter was established based on trial and error analysis of Trial 9 batch tablets.

EVALUATION OF TABLETS

1. Description

"Light brown" coloured tablets. The polyherbal tablets were evaluated for organoleptic characters which include colour, odour, taste and nature.

Table 22. Organoleptic Characters

S.NO	PARAMETER	OBSERVATION
1.	Description	Light brown colour tablet, round shape
2.	Colour	Light brown
3.	Odour	Characteristic odour
4.	Taste	Bitter taste

Table 23. Physical Parameters

S.NO	PARAMETER	OBSERVATION
1.	pH (1% aqueous solution)	7.33 ± 0.21
2.	Moisture content	3.98 ± 0.5%w/w
3.	Uniformity of weight	625.3 ±3.4mg

Results are reported as Mean ± Standard deviation.

- 1% aqueous solution of polyherbal formulation showed neutral pH.
- The average weight of the tablet was calculated as per I.P and the obtained value was within the limit (±7.5%).

Table 24. Formulation

Parameters	F1	F2	F3	F4	F5	F6	F7	F8	F9
Average weight	625.1	623.3	626.4	619.3	622.2	623.2	621.4	624.2	621.5
Hardness	4.5	4.51	4.53	4.37	4.31	4.54	4.57	4.70	4.83
Thickness	4.08	4.10	4.09	4.08	4.08	4.09	4.10	4.08	4.10
Friability	0.61	0.64	0.63	0.59	0.62	0.87	0.88	0.87	0.88
Disintegration time	18.55	17.35	17.10	16.25	16.20	15.20	14.35	14.20	14.00

POLY HERBAL TABLETS

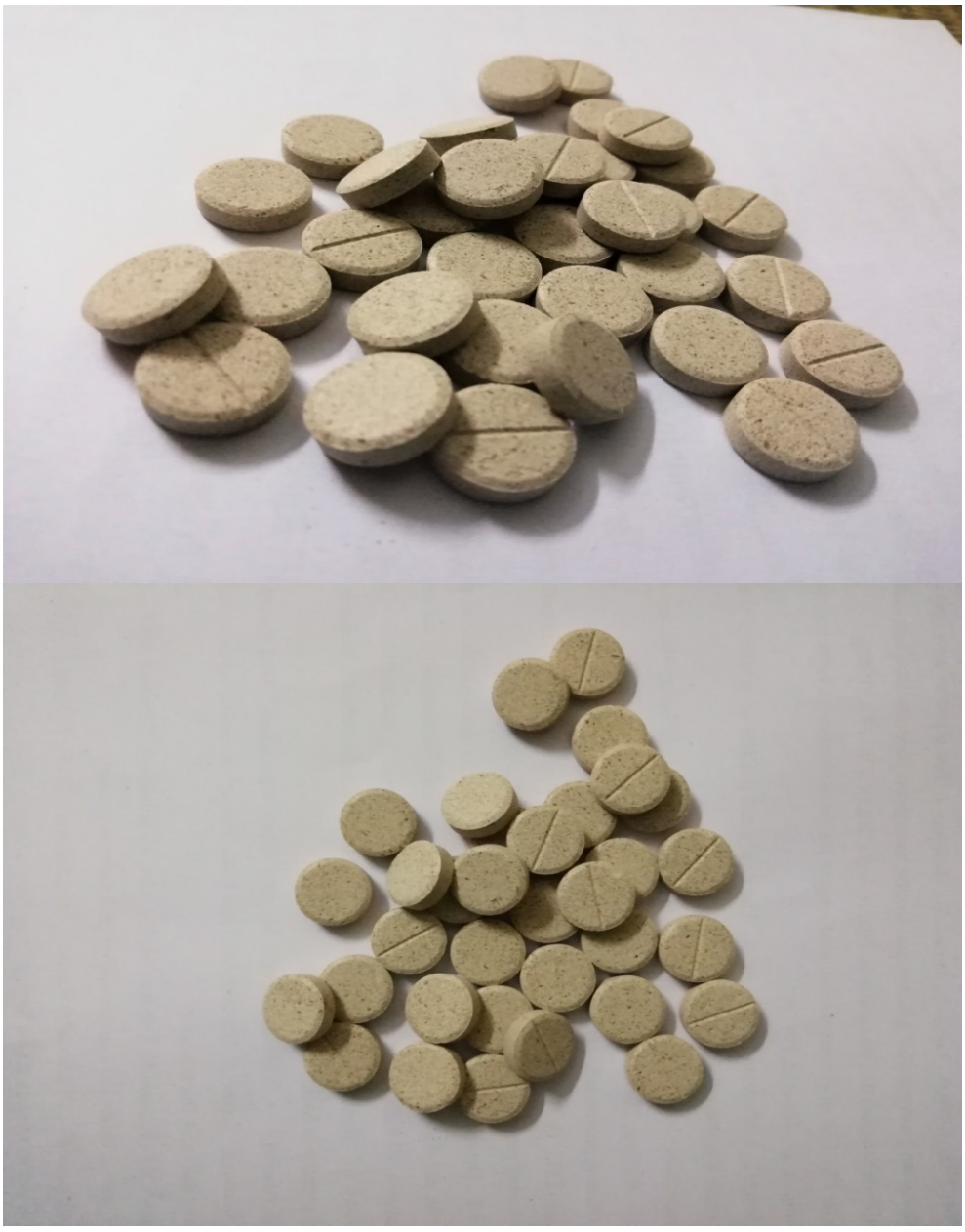


Figure: 17 Poly herbal tablet

OVERLAY OF HPTLC GRAPH

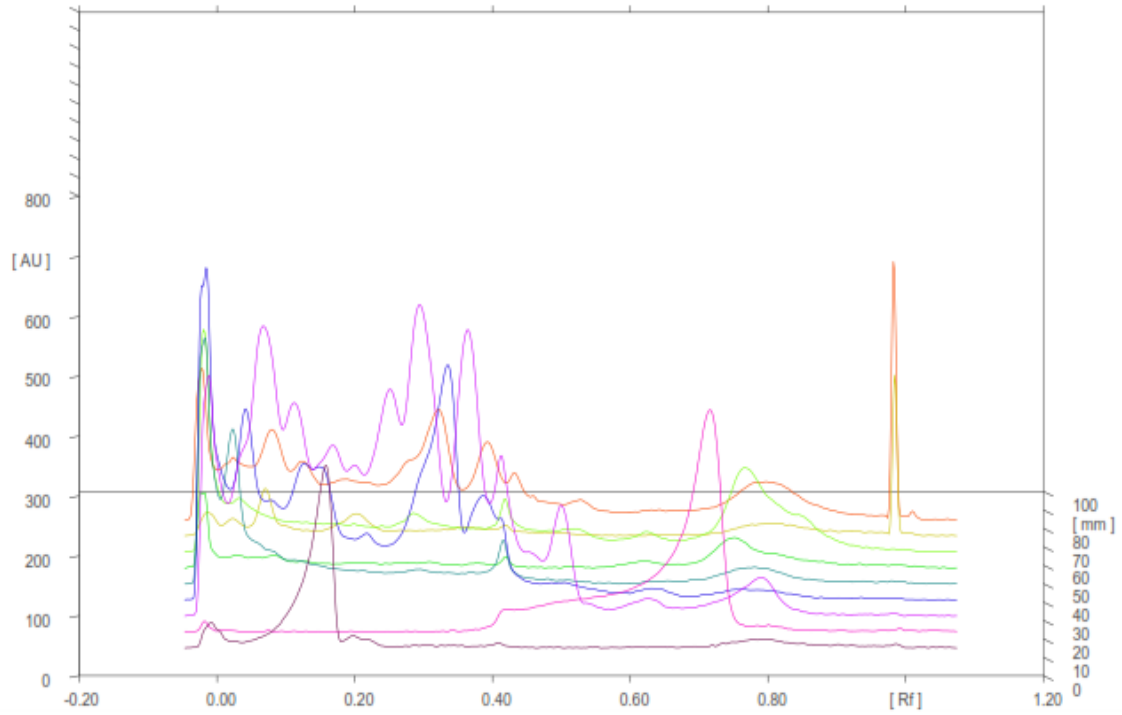


Figure 18 : Overlay graph of HPTLC

_____ Rutin
_____ Qucertin
_____ Fennugreek
_____ Moringa oleifera
_____ Nigella sativa
_____ Linum usitatissimum
_____ Cinnamum zeylanicum
_____ Macrotyloma uniflorum
_____ Formulation

Table 25. HPTLC Parameters

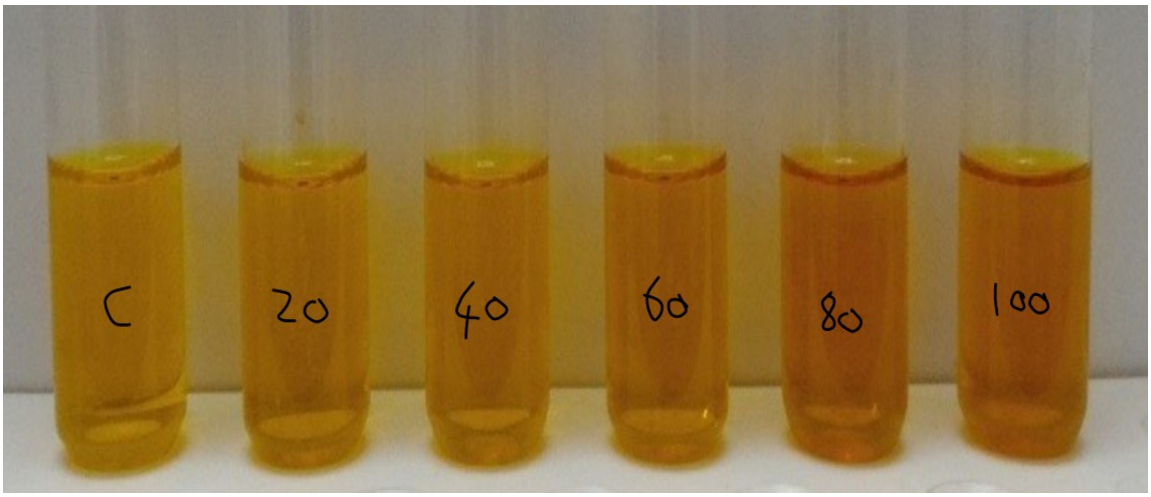
S.No.	Name of the Material	Rf value	Area	Rf value reference for formulation
1.	Rutin standard	0.04	8435.0	
2.	Qucertin standard	0.56	17587.5	
3.	Fennugreek	0.21	10761.3	
4.	Moringa oleifera	0.10	5366.7	
5.	Nigella sativa	0.38	1393.8	
6.	Linum usitatissimum	0.40	222.7	
7.	Cinnamum zeylanicum	0.50	1484.8	
8.	Macrotyloma uniflorum	0.97	1368.4	
9.	Formulation	0.05	4048.5	Rutin standard
		0.11	2299.8	Moringa oleifera
		0.23	7988.3	Fennugreek
		0.36	3482.1	Nigella sativa
		0.42	1635.4	Linum usitatissimum
		0.50	1082.9	Cinnamum zeylanicum
		0.70	5102.8	Qucertin standard
		0.97	2134.6	Macrotyloma uniflorum

PHARMACOLOGICAL STUDIES

INVITRO ANTI-DIABETIC ACTIVITY

α -Amylase Inhibition Assay

STANDARD ACARBOSE



POLYHERBAL FORMULATION (TRIAL 9)

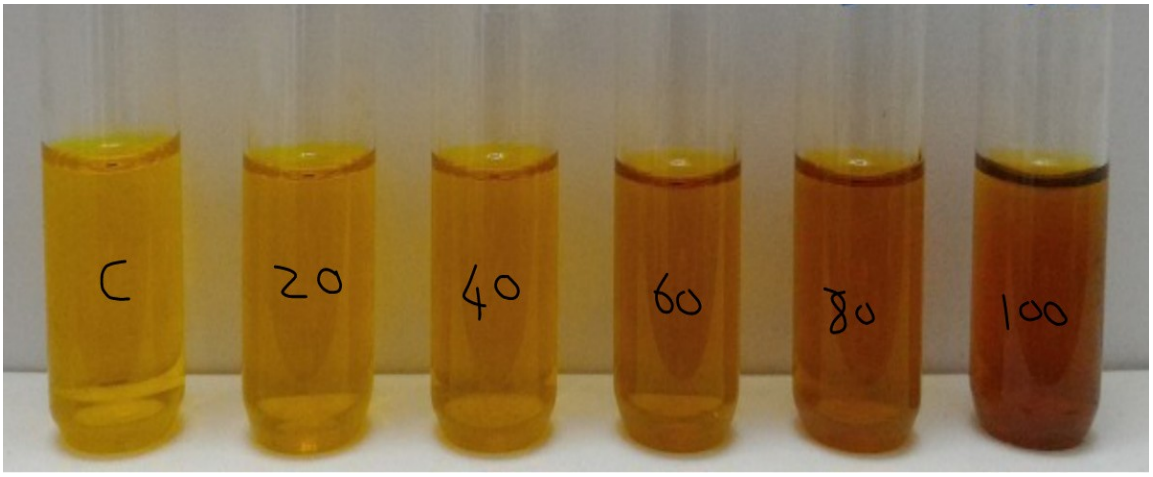


Figure : 19 α Glucosidase assay

Table : 26 α Glucosidase assay

CONCENTRATION OF SAMPLE (µg/ml)	% INHIBITION SAMPLE	% INHIBITION OF ACARBOSE
20	39	45
40	50	54
60	65	68
80	76	78
100	92	89

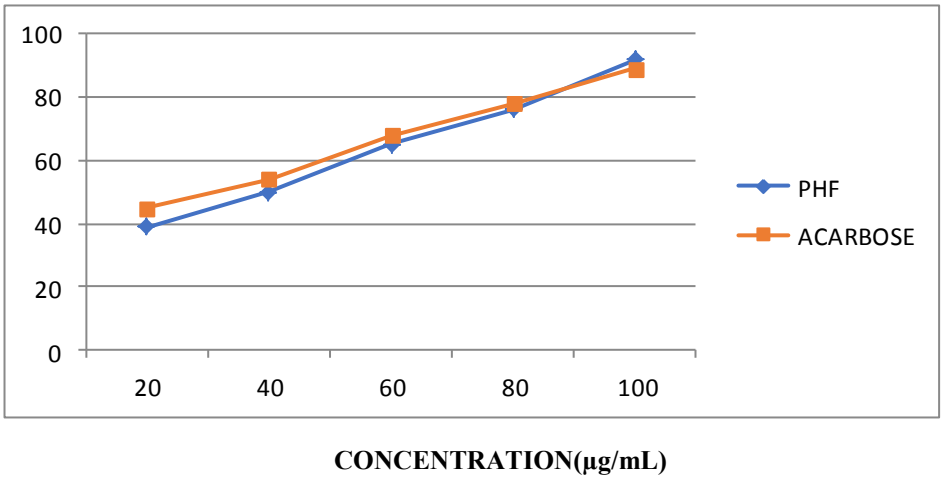


Figure 20 : Graphical representation of the α-glucosidase inhibition assay

DNS ASSAY GRAPH

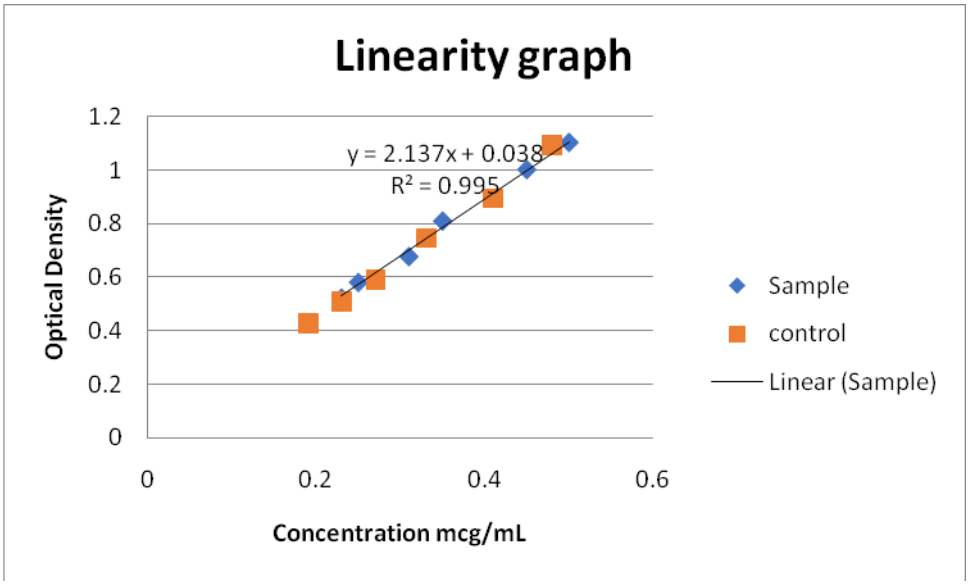


Figure 21 : Graphical representation for cell line DNS assay

Table 27: DNS ASSAY

S. No.	Samples	0th hr		5th hr		10th hr		15th hr		20th hr		24th hr	
		OD	Conc (µg)	OD	Conc (µg)	OD	Conc (µg)	OD	Conc (µg)	OD	Conc (µg)	OD	Conc (µg)
1	Control	1.102	0.500	1.001	0.450	0.810	0.350	0.621	0.310	0.582	0.250	0.524	0.230
2	Sample	1.094	0.480	0.895	0.410	0.746	0.330	0.592	0.270	0.511	0.230	0.428	0.190

8. SUMMARY AND CONCLUSION

Herbal medicines are the oldest form of health care known to mankind. A number of traditional herbal medicinal practices have been adopted for the diagnostic prevention and treatment of various diseases.

Based on the extensive review of literature, Six raw materials were selected for the formulated as polyherbal tablet and the antidiabetic potency was evaluated in cell line.

The herbal raw materials were analyzed for identity, quality and purity as per the standards prescribed by WHO and Ayurvedic Pharmacopeia of India.

The Physiochemical parameters like Loss on drying, ash values and extractive values were determined, which will help in preventing variation in quality of the drugs. Preliminary phytochemical investigation revealed the presence of various phytoconstituents such as alkaloid, steroids, glycosides, Flavonoids, Phenols, Tannins, and terpenoid in the raw materials.

The safety of the raw materials was analysed by heavy metals the results found within the standard limits given by WHO.

The extracts were dried by tray drying and used for the formulation.

HPTLC fingerprinting of the polyherbal formulation was performed and the resultant chromatogram showed the presence of peaks as same in HPTLC fingerprint of extracts. The chromatogram can be used as an index for the qualitative analysis of the formulation.

The dried polyherbal extract was optimized for its quality measures and its batch consistency by making nine different trial batches (Trial 1,2,3,4,5,6,7,8,9). The trials were subjected to preformulation parameters to confirm the uniformity and quality.

The result concludes that the trial 9 was excellent in all parameters and the values were found within the standard limits and it was used for formulate Polyherbal Tablet.

The developed polyherbal Tablets were standardized for its Description, uniformity of weight, disintegration time, moisture content, pH, Physiochemical parameters, and phytochemical studies.

Quantitative estimation of phytoconstituents was done for flavonoid, phenols, and tannins.

The heavy metal analysis was carried out in polyherbal formulation as per the WHO Guidelines and found within the limits.

In vitro anti-diabetic activity was done by using α-glucosidase inhibition assay method. It possesses significant antidiabetic activity as compared to standard Acarbose.

3T3-L1 cell line was performed. The formulation showed significant effect compared to the standard.

The phytochemical study showed the presence of flavonoids. This may be responsible for the potent anti diabetic activity.

Further studies are recommended for stability studies in the formulated polyherbal tablet and also clinical trials have to perform in future in Human Volunteers.

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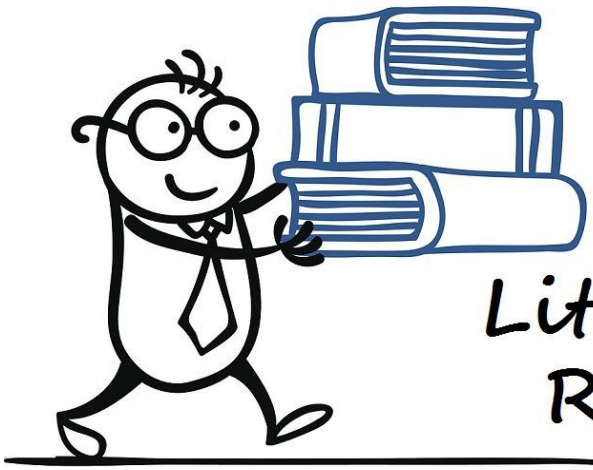
INTRODUCTIONS



Literature Review

Plant Profile

Aim & Objectives of the study

Plan of Work

Materials & Methods

Results & Discussion

Summary & Conclusion

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HISTORY
CONSTRUCTION
Corporate Finances
STATISTICS
DICTIONARY
ARCHITECTURE
DESIGN
Laptop

Annexures