1.1 ORAL DOSAGE FORM

For many years, oral drug delivery has been considered the most preferred method for systemic administration of medications across various pharmaceutical products with different dosage forms. The oral route is widely regarded as the most traditional and user-friendly method for delivering medications, primarily due to its affordability and simplicity, which in turn enhances patient adherence. Around half of all marketed drug products are taken orally, with tablets being the predominant dosage form [1]. The key objective in creating any dosage form is to guarantee that the drug is released correctly at the intended site to achieve the desired therapeutic effect. Factors such as the design of the dosage form, the tablet production process, and the overall chemical composition of the solid unit dosage play a crucial role in determining the drug's effectiveness.A total dosage form includes various excipients, and they most vital among all excipients is the active pharmaceutical ingredient. Their main additives are important to form a suitable dosage form, as single API cannot produce good formulation. During the process of mixing the excipients with the drug, the main active ingredient may mix with other ingredients in the blending process, so according to that, it is important to choose a specific procedure for the designing of the unit dosage form.[2]

1.2 HERBAL MEDICINE: A GROWING FIELD WITH A LONG TRADITION:

The World Health Organization defines traditional medicine as the collective knowledge, abilities, and practices rooted in the cultural theories, beliefs, and experiences of various societies. These approaches are employed to uphold health and to prevent, diagnose, alleviate, or manage physical and mental health conditions.The approaches in traditional medicine differ significantly, influenced by the local culture, environment, and historical background of each system (WHO, 2005).Despite this, these systems share a common philosophy that emphasizes health over disease and takes a holistic approach to life.Usually, the focus is on the patient's overall health rather than the specific illness or condition they are suffering from, and all conventional medical practices include the use of herbs.[3]

The primary justifications for the use of traditional medicine include its cost-effectiveness, better alignment with patients' cultural perspectives, reduced concerns about the side effects associated with synthetic medicines, the fulfillment of a desire for

personalized health care, and the dissemination of health information to the general public. Herbal medications are predominantly utilized for chronic diseases and health promotion rather than immediately life-threatening conditions. However, traditional medicines are more frequently resorted to when conventional treatments are ineffective, such as in cases of advanced diabetes and emerging infectious diseases. These remedies are frequently seen as safe and natural, but this belief can be deceptive. Negative interactions can occur when herbs are used alongside prescription drugs, over-the-counter treatments, or other herbs. To ensure safety and effectiveness, it is crucial for individuals to seek advice from healthcare professionals before adding herbal medications to their treatment plans.Mixing different types of treatments without proper guidance can lead to unforeseen complications and adverse reactions [4].

Herbs are commonly used to treat a variety of conditions, including inflammation, cardiovascular disease, prostate issues, depression, and to support the immune system. They are effective for both acute and chronic illnesses.For instance, traditional herbal remedies played a significant role in China's response to severe acute respiratory syndrome (SARS) in 2003, and the Africa flower has been traditionally used in Africa to alleviate symptoms associated with HIV-related wasting. Moreover, in many developed countries, pharmacies commonly stock herbal teas, essential oils, and extracts along with conventional medications. Herbal medicines are particularly popular in Europe, with Germany and France leading in over-the-counter sales among European nations [5].

Diabetes mellitus symptoms include hyperglycemia, hypertriglyceridemia, and hypercholesterolemia. Synthetic hypoglycemic medicines can cause significant adverse effects, such as hematological issues, liver and renal problems, and coma. Plant-based medications are often regarded as having lower toxicity and fewer adverse effects compared to synthetic drugs. This belief has spurred efforts to discover more potent and safer herbal treatments for diabetes. The World Health Organization states that herbal medicines are essential for primary healthcare for 80% of people worldwide.Throughout history, ethnobotanical remedies have been employed extensively to manage blood sugar disorders. In type 2 diabetes, elevated blood glucose levels stem from factors like poor dietary habits, lack of physical activity, inadequate insulin production following meals, and decreased insulin sensitivity in target tissues.By 2025, the global prevalence of chronic metabolic disorders, currently affecting approximately 150 million individuals, is

projected to rise to 300 million. While insulin and synthetic oral anti-diabetic drugs effectively reduce blood glucose levels, they do not address diabetic complications and have numerous side effects. Traditional medicinal plants are used worldwide to treat various diabetic complications. Historical literature documents numerous herbal medications and minerals used for treating diabetes mellitus, which are generally considered safer and have fewer adverse effects than synthetic drugs. The potential of medicinal plants has become increasingly important in providing humanity with viable herbal medicine alternatives [6].

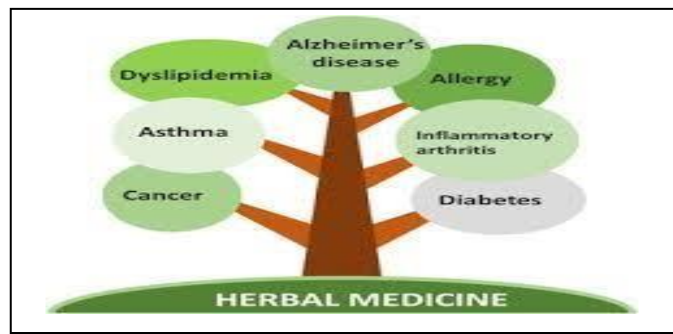


Figure No.1.1: Herbal Medicine used in diseases

1.3 IMPORTANCE

Ayurveda is a traditional medical system that utilizes various techniques to enhance health and well-being. The primary objective of Ayurvedic healthcare is to rebuild physical, mental, and emotional equilibrium in individuals. This approach aims to foster overall health, prevent diseases, and treat existing conditions. There is a growing trend among patients to seek herbal and alternative treatments, leading to a high demand for herbal medications in primary healthcare, particularly in developing countries. Herbal treatments are widely favored due to their affordability, cultural acceptance, compatibility with the human body, and typically minimal side effects [5].

Around 75 to 80 percent of people worldwide, especially in developing nations, rely mainly on herbal medicine for their primary healthcare. Despite the vast variety of plant species, estimated to be between 250,000 to 400,000, research has only been conducted on about 6% of them for their medicinal properties, with approximately 15% studied for their phytochemical characteristics. Therefore, it is crucial to conduct proper evaluations of herbs to ensure their safety and efficacy in primary healthcare. Expanding research to a

wider range of plant species can help unlock the full potential of herbal medicine for global health [6].

Several factors contribute to the use of herbal remedies. First, herbal medicine is an integral part of many cultures and belief systems for maintaining health and treating specific illnesses. Second, the lower cost of herbal products makes them more accessible to individuals with lower incomes. Thirdly, many people believe that herbal remedies are safe because they are natural, unlike modern pharmaceuticals, which are frequently linked to toxicity and adverse effects. Finally, the growing desire for more holistic and natural approaches to healthcare has also contributed to the increasing popularity of herbal remedies[7].

1.4 HERBAL FORMULATIONS

An herbal formulation is a specific type of dosage form containing one or more herbs or processed herbs in precise quantities to deliver particular nutritional or cosmetic benefits. These formulations are intended for various applications in humans or animals, including diagnosis, treatment, or mitigation of illnesses, as well as modifying their physiological functions. Typically, herbal formulations consist of an active ingredient combined with herbal preparations and other components derived from herbs. Transforming homegrown compounds into usable products involves several methods, including grinding, distillation, expression, fractionation, purification, concentration, and fermentation. The creation of these products utilizes various plant materials, which may be whole, divided, or cut, and includes parts of plants, algae, fungi, and lichen, typically in dried form, though sometimes fresh. Each herbal product is identified by its precise botanical name using the binomial nomenclature (genus, species, variety, and author) and the specific part of the plant used.These herbal preparations can take many forms, including tinctures, extracts, and essential oils, expressed juices, and processed exudates.

Unique chemical constituents or groups of constituents in herbal drugs, preparations, or medicinal products are referred to as markers. These markers play a critical role in quality control, regardless of their therapeutic value. By measuring these markers quantitatively in herbal substances or preparations, one can determine the amount of herbal substance or preparation within the herbal formulations.

The characterization of herbal formulations involves several elements such as formulation design and development, adherence to pharmacopoeial standards and criteria for acceptance, regular testing during production, criteria for product release and shelf-life, ongoing in-process testing, exploration of alternative manufacturing methods, adoption of emerging technologies, establishment of reference standards, and application of statistical principles [8].

1.5 TYPES OF HERBAL FORMULATIONS [8-10]:

Decoctions:

Decoctions are traditional herbal medicines made by boiling plant materials to extract water-soluble active ingredients. Typically, they are prepared using two to twelve different plant parts and should be consumed within 24 hours. However, they can be stored for up to 72 hours in a very cold environment. In Chinese herbal medicine, formulations such as the Sijunzi decoction comprise ingredients like Panax ginseng, Poria cocos, Atractylodes macrocephala, and Glycyrrhiza uralensis. Modern analytical methods such as chromatography-tandem mass spectrometry (LC/MS) have been used to identify active compounds such as ginsenosides, flavonoids, and triterpenoids in these mixtures, revealing differences in concentration compared to extracts from individual herbs.Studies have demonstrated that decoctions, such as Cassia fistula pod extract, can maintain chemical stability for extended periods when stored properly. The combination of herbs in decoctions can enhance the bioavailability and synergistic effects of active components, emphasizing the therapeutic benefits of traditional herbal formulations.

Tinctures:

Tinctures are extracts of plant materials made using alcohol or hydro-alcoholic solutions to dissolve various plant constituents. This process involves macerating plant materials in a water-ethanol solution to extract a variety of chemical compounds with different polarities. Ensuring the quality and effectiveness of pharmaceutical products is crucial. To achieve this, advanced analytical techniques like nuclear magnetic resonance (NMR) and mass spectrometry (MS) are utilized. These techniques help in identifying individual chemical components, ensuring batch consistency, and assessing product stability over time.These methods provide precise and reliable data crucial for maintaining consistent product quality and safety. Typically containing at least 20% v/v alcohol, tinctures are

preserved for several years. The alcohol content not only enhances the extraction of non-water-soluble compounds but also informs consumers of potential effects and storage requirements.

Herbal Glycerites:

Herbal glycerites use glycerine instead of alcohol for extraction, requiring a final product concentration of at least 50% to 60% glycerine for stability. They have a shelf life ranging from two to six years. Glycerine is effective in preserving fresh plant juices, maintaining their colour and suspension longer than alcohol. Glycerites are particularly suitable for pediatric formulations and remedies for soothing the throat, digestive system, and coughs. They are a preferable option for individuals sensitive to alcohol or with dietary restrictions against its consumption, despite having a shorter shelf life and lower potency compared to alcoholic extracts.

Herbal Alcoholic Beverages (Bitters and Wines):

Herbal alcoholic beverages are made using ethanolic or hydro-ethanolic extracts of plant materials and are traditional in various cultures, notably in African, Southeast European, and Mediterranean regions. The medicinal action of these beverages depends on the herbs used. Alcohol extends shelf life and provides preservative effects. Storage conditions significantly impact their antioxidant, antibacterial, and antifungal activities. For example, the stability of an alcoholic orange juice beverage was maintained in terms of acidity, pH, and alcohol content during 14-hour storage at different temperatures, although degradation markers and colour changes occurred over time.

Oxymels:

Oxymels are sweet and sour mixtures combining honey and vinegar, often used as carriers for herbal infusions, decoctions, and tinctures. Oxymels are versatile in their uses, functioning both as gargles and as vehicles for potent herbs like garlic, cayenne, and lobelia. Their durability depends on the exact proportions of honey, vinegar, and herbal extracts used, with storage methods and the quality of ingredients playing crucial roles in determining their durability.To preserve their effectiveness and prevent decay, it's recommended to store oxymels in a cool, dark environment.

Herbal Capsules:

Herbal capsules are solid dosage forms that contain powdered or granular herbal ingredients encased in either a hard or soft gelatine shell. They disintegrate easily after oral administration, masking the taste of their contents. Capsules provide consistent dosing and superior stability compared to liquid preparations, remaining stable at room temperature for up to two years. The gelatine shell protects active ingredients from degradation due to light, air, and moisture, making capsules a convenient and effective form for herbal supplements.

Herbal Tablets:

Herbal tablets are solid, compressed forms containing herbs and excipients like binders, lubricants, and disintegrants to ensure proper formulation and dissolution in the gastrointestinal tract. Tablets may be coated to mask taste, facilitate swallowing, and protect contents from stomach acid or environmental conditions. Proper coatings enhance stability and shelf life, preventing interactions between active components and moisture or oxygen, thus maintaining efficacy and quality over time.

Herbal Ointments:

Herbal ointments are semi-solid topical preparations designed for application to the skin, nasal mucosa, or rectal area.These hydrophobic preparations do not mix with skin secretions and incorporate extracted or finely sieved plant materials for emollient or medicinal purposes. Stability is a significant concern as natural ingredients may degrade quickly; for instance, ointments containing calendula and arnica tinctures for haemorrhoid treatment have a shelf life of one to two months under appropriate storage conditions and protection from light. These formulations harness the therapeutic properties of various herbs to treat localized conditions, providing a targeted approach to alleviate symptoms and promote healing.

Herbal Balms:

Similar to ointments, herbal balms are designed to relieve body aches and pains, containing herbal ingredients with rubefacient properties that increase blood flow and provide pain relief. Their stability is comparable to ointments, though the variety of herbs used can affect specific stability and effectiveness. Proper storage is crucial to maintain

efficacy and shelf life. Consulting a healthcare professional before use is recommended, particularly for individuals with sensitive skin or allergies.

Herbal Creams:

Herbal creams are typically semi-solid mixtures containing both oil and water, often enhanced with extracts from medicinal plants. These formulations typically include antimicrobial preservatives to enhance stability. However, owing to their water content, herbal creams generally have a shorter shelf life compared to ointments.Creams are hydrophilic and used for various skin conditions, requiring proper classification and labelling to distinguish them from hydrophobic ointments. Following storage instructions is essential to prevent spoilage, making them a versatile option for skincare routines due to their soothing and moisturizing properties.

Herbal Oils:

Herbal oils involve suspending or dissolving plant materials in an oil-based medium. Infused oils, distinct from essential oils, are typically used topically or externally and occasionally for oral consumption. Stability and shelf life depend significantly on the type of oil and extraction method. Store herbal oils in a cool, dark location to prevent oxidation and preserve their potency.Common herbal oils include lavender, tea tree, and chamomile, each offering unique benefits for skin and health.

Herbal Soaps:

Herbal soaps are created by saponifying fatty acids with a base like caustic soda and incorporating herbal components into the soap base. These soaps are used for their antibacterial and antifungal properties to treat skin conditions like eczema, ringworm, and dandruff. Antioxidants or preservatives can extend shelf life. Herbal soaps are popular for their natural fragrances and ability to nourish and moisturize the skin, preferred for their potential health benefits and environmentally friendly ingredients.

Herbal Pastes:

Herbal pastes are topical formulations containing up to 50% powder in a fatty base, used to focus the action of irritating or discolouring agents on the skin. They may also be formulated for oral use, such as herbal toothpaste, provided the ingredients are safe for consumption. The stability of herbal pastes is influenced by the type of base and herbal

ingredients, requiring storage in a cool, dry place to maintain efficacy. Consulting a healthcare professional before use is recommended for specific skin or oral conditions.

Herbal Teas:

Herbal teas are prepared for infusion or tea-making, with infusions needing immediate consumption due to poor storage stability. Herbal teas are offered in tea bags or as powdered herbs, and their longevity hinges on moisture levels and how they are stored. When kept in airtight containers, herbal teas can remain fresh for up to a year; however, tea bags have a shorter shelf life. To maintain their flavour and potency, herbal teas should be stored in a cool, dry place, away from direct sunlight. Proper storage can ensure that suppositories remain effective for several months to a year.

Herbal Suppositories:

Herbal suppositories are solid forms designed for insertion into the rectum, where they melt or dissolve upon reaching body temperature, resulting in either local or systemic effects. Made using a base like cocoa butter combined with powdered herbs or extracts, they treat conditions like constipation, swollen membranes, and inflamed nasal mucosa. Stability is affected by storage temperature and packaging, requiring cool, dry storage to prevent melting or degradation.

Herbal Pessaries:

Similar to suppositories, herbal pessaries are designed for vaginal insertion using bases like glycerated gelatin, which dissolve at body temperature to release herbal components. Stability is comparable to suppositories, providing targeted relief for specific conditions. Consulting with a healthcare provider or herbalist before use ensures safety and efficacy for individual needs.

Herbal Plasters and Poultices:

Poultices are therapeutic preparations crafted by crushing fresh herbs, encasing them in gauze, and applying them externally to alleviate inflammation and manage minor skin conditions such as insect bites and wounds. They leverage the natural properties of herbs to provide localized relief and promote healing. As they are made from fresh herbs, they should be used immediately. Herbal plasters are created by mixing powdered herbs with a substance such as clay or beeswax to form a paste. This paste is then spread onto a cloth

and placed on the skin. This method of preparation helps to extend the shelf life of the herbs by preserving them in this form.

Herbal Fomentations and Compresses:

Compresses involve soaking a cloth in an herbal infusion or tincture and applying it to the skin, used warm or cold, to alleviate pain and inflammation or improve circulation and muscle relaxation. Fomentations are similar but use hot herbal infusions, which are particularly effective for treating muscle aches, menstrual cramps, and chest congestion. Proper temperature testing before application is crucial to avoid burns.

Herbal Liniments:

Liniments are external applications used to relieve pain, often made by combining heat-producing herbs with alcohol or oil. They are applied as warm massage oils for aching muscles and ligaments but should not be used on broken skin. Liniments provide quick relief and are popular for athletes and those with chronic pain. Consulting with a healthcare professional before use is recommended, particularly for individuals with sensitive skin or allergies.

Herbal Baths:

Herbal baths involve adding fresh or dried herbs, or herbal tinctures and infusions, to bathwater. Fragrant herbs containing essential oils promote relaxation and reduce tension, improving circulation and detoxifying the body with regular use. Common herbs include lavender, chamomile, and rosemary.

Herbal Lozenges:

Lozenges are formulated to release therapeutic properties slowly in the mouth and are made by combining pulverized herbs with excipients like sugar, honey, and gums. They treat throat infections and soothe throat pain, with stability comparable to tablets. Proper storage in a cool, dry place is essential to maintaining effectiveness. Lozenges are preferred for their ease of consumption and pleasant taste.

Figure No.1.2: Standardization of Herbal Drug

1.6 WHO DEFINITIONS RELATED TO HERBAL MEDICINE:

The redefinition of certain terms by the World Health Organization (WHO) aims to establish consistency in defining key concepts related to herbal medicine. These definitions serve the purpose of establishing uniform terminology for use in assessing and researching herbal medicines. It is important to note that these definitions may differ from those found in the legislation of countries practicing traditional medicine. Hence, these definitions serve to enhance communication and understanding within the herbal medicine community, encompassing researchers, practitioners, and policymakers. They may evolve with ongoing advancements in herbal medicine research and practices. [10-11].

1.7 ADVANTAGES AND DISADVANTAGES OF HERBAL MEDICINES

Advantages

Minor ailments like scrapes, rashes, and burns can be treated with herbal medications. At a very cheap cost, they can also be used to treat depression, arthritis, and migraines, according to the University of New Hampshire. Herbal medicines can be purchased at local supermarkets or cultivated at home, which makes them far less expensive than pharmaceutical drugs. Christopher Golden, affiliated with the Harvard University Center for the Environment, suggests that transitioning from pharmaceuticals to herbal remedies could potentially reduce annual healthcare expenditures by 22–63%. Foods like rhubarb, ginger, and garlic are common examples of common place foods that contain herbal medicine. [10,11]

Disadvantages

There may be numerous benefits to using herbal medicines. It does, however, have certain drawbacks as well. For starters, herbal remedies take longer than prescription medications to start working. A person must be very patient if they choose to use herbs as a substitute for prescription medication.

Herbal medicine is frequently taken on its own. That's why there are no warnings or dosage listed. When taken simultaneously, herbal and prescription drugs may have detrimental interactions that are harmful to the user's health.

It is essential to recognize that herbal medicines derived from plants can sometimes cause poisoning rather than healing. Different parts of the same plant may have varying effects, with some being edible and others toxic. For example, the rhubarb plant has an edible stem and roots that are used as laxatives, but its leaves are poisonous. Individuals may fail to identify toxic plants accurately, thereby posing a risk of poisoning themselves or others. It is crucial to seek guidance from a knowledgeable herbalist or botanist before consuming any plant-based medicines. Proper identification and understanding of the potential risks associated with herbal remedies are necessary for safe usage. [10,11]

Herbs:

Herbs encompass a wide variety of botanical substances sourced from different parts of plants, such as leaves, flowers, fruits, seeds, stems, bark, roots, and rhizomes. These plant components may be utilized in their entirety, fragmented, or ground into powders for various medicinal and therapeutic applications. Herbal materials are often used in different forms like teas, tinctures, capsules, or extracts due to their medicinal benefits. Prior consultation with a healthcare professional is crucial before using herbal remedies to ensure safety and efficacy. [12]

Herbal Materials:

Herbal materials encompass a variety of substances derived from herbs, including fresh juices, gums, fixed oils, essential oils, and dry powders. These materials undergo preparation using techniques such as steaming, roasting, or stir-baking with honey, alcohol, or other agents, varying by region. Familiarity with these traditional methods is crucial for practitioners, as they can significantly impact the potency and efficacy of herbal materials. Furthermore, herbal materials are utilized in diverse forms such as teas,

tinctures, capsules, and topical applications. [10-12]

Herbal Preparations:

The foundation for finished herbal products lies in herbal preparations, which may consist of tinctures, extracts, commented or powdered herbal components, as well as fatty oils and extracts. These preparations are crafted using a range of physical or biological methods, including extraction, fractionation, purification, and concentration. Additionally, treatments involving scalding or soaking herbal components in alcohol, honey, or other substances are incorporated. Herbal formulations are carefully developed to maximize the extraction of beneficial compounds from plants, thus amplifying their medicinal properties. The versatility of herbal preparations allows for a broad spectrum of applications, rendering herbal medicine a versatile and efficacious healing modality. [10-12]

1.8 WHO GUIDELINES FOR STANDARDIZATION OF HERBAL FORMULATIONS:

Ensuring the safety and quality of polyherbal formulations is crucial. These mixtures, made from various herbs, aim to deliver targeted health benefits.

1. Standardization of polyherbal formulations plays a crucial role in ensuring their safety, efficacy, quality, and consistent performance across different batches.
2. First, standardization involves implementing quality control measures for raw herbal materials utilized in formulations. This process ensures that these materials conform to established criteria for identity, purity, and potency.
3. Secondly, plant preparations and the finished polyherbal products undergo stringent quality assessments.
4. Another crucial element of standardization is stability evaluation, which includes conducting studies to ascertain the shelf life of polyherbal formulations under different storage conditions.
5. Furthermore, safety assessments are conducted through a combination of historical usage data, traditional knowledge, and modern toxicological studies.
6. Lastly, the efficacy of polyherbal formulations is evaluated through ethno medical information and scientific studies assessing biological activities.[12-15]

1.9 HERBAL DOSAGE FORMS-POWDER

Herbal supplies can be prepared as powders, lehiyas, tailas, capsules, tablets, syrups, and more. The powder form, which offers dose accuracy, is the most adaptable. It is simpler to take a prepared powder with active components than it is to ingest the individual herbs.

These treatments consist of powdered herbal ingredients that can be used topically or added to food, drinks, insufflations, and wounds. They could contain carefully sifted herbal ingredients from different plant parts intended to have a specific therapeutic effect. The stability of the powder depends on the characteristics of the herbal ingredients and the moisture content in the packaging, similar to herbal teas.[15-17]

Advantages of Powders

1. There is a large selection of components, and determining the dosage for patient administration is simple.
2. Powders offer higher physicochemical stability and a longer shelf life as compared to liquid dose forms. For instance, after reconstituted with water, the shelf life of powdered antibiotic syrups is reduced to 1 to 2 weeks from the original 2 to 3 years.
3. Powders are found more acceptable as children and adults who have trouble swallowing tablets or capsules.
4. Powder can be used to administer a big dose that cannot be given in another form. For instance, it is occasionally impractical to create tablets to deliver a medicine to the patient at a dose of 1 to 5 g.
5. Medications administered in powder form are dispersed rapidly in the stomach compared to those in compressed form.
6. Oral powders containing water-soluble medications generally dissolve more quickly than tablets or capsules, as tablets and capsules require shell breakdown before drug absorption can occur.
7. A powder can be more easily swallowed by dispersing in water or another liquid.
8. Oral powders can be combined with a beverage or applesauce, just before using.
9. Powder dosage forms are easier to manufacture than other dosage forms, which results in lower product costs.
10. Powders provide solids compounders a lot of versatility.[17-21]

Disadvantages of Powders

1. Powders are not the preferred dose form for medications with bad tastes. This is due to the potential issue with this method of preparation in hiding disagreeable flavors.
2. Drugs that quickly lose their potency when exposed to air or an acidic pH shouldn't be given out as powders. For instance, since ferrous iron salts are readily oxidized, powder form should not be used for administration.
3. Powders are heavy and not ease to transport.
4. The administration of medications that are inactivated in the stomach or that can harm the stomach should not be done using powders as a dose form.
5. It might not be appropriate to dispense strong medications that require modest doses as powders (such as bulk powders). This is so that individual doses can be withdrawn from the bulk using a 5 ml spoon, which can be filled differently depending on whether it is level or heaping.
6. Dry or liquescent medications should not be dispensed using powders. [17-21]

1.10 ENDOCRINE SYSTEM

The endocrine system comprises glands that produce and release hormones directed towards various parts of the body. These glands are distributed throughout the body, including the neck, brain, and reproductive system, with some being as small as a rice or pea, while others, like the pancreas, can reach lengths of around 6 inches.[22-24]

- **Hypothalamus:** The hypothalamus, situated in the brain, plays a crucial role in regulating the endocrine system by directing other glands like the pituitary gland to synthesize hormones in response to neural signals. It oversees various physiological processes such as emotions, hunger, thirst, sleep-wake cycles, and reproductive functions.
- **Pituitary**: The pituitary gland, often referred to as the "master gland," is located at the base of the brain and is about the size of a pea. It releases hormones that regulate the functions of other glands, including the thyroid, adrenal glands, ovaries, and testes. This gland is crucial for physical growth and works in close cooperation with the hypothalamus to maintain hormonal balance.

- **Thyroid**: Situated in the front of the neck, this butterfly-shaped gland controls metabolism, body temperature, heart rate, and blood pressure. Dysfunction of the thyroid gland can lead to symptoms such as weight changes, fatigue, and mood swings.
- **Parathyroid**: These four tiny glands, barely larger than a grain of rice, regulate calcium levels in the body. They release parathyroid hormone to maintain calcium balance in the blood, which is crucial for the proper function of bones, the brain, the heart, and the kidneys.
- **Adrenal**: Two adrenal glands located above each kidney are responsible for metabolism, blood pressure regulation, sexual development, and stress response. They produce hormones like adrenaline and cortical, vital for the body's fight or flight response.
- **Pineal**: Mainly responsible for releasing melatonin, a hormone that regulates the sleep-wake cycle, this gland also influences circadian rhythms and mood. Dysfunction of the pineal gland can result in sleep disorders and mood disturbances.
- **Pancreas**: Acting as an endocrine and digestive gland, the pancreas produces insulin to regulate blood sugar levels and digestive enzymes for food breakdown. Dysfunction can lead to conditions like diabetes and pancreatic insufficiency.
- **Ovaries**: Found in females, these glands secrete sex hormones like estrogen, progesterone, and testosterone, which are crucial for regulating the menstrual cycle, fertility, and secondary sexual characteristics. They also release eggs for fertilization during ovulation.
- **Testes**: The male testes are responsible for producing testosterone and sperm, which influence sperm production, physical characteristics, and libido. Positioned in the scrotum outside the body, they are situated to maintain an ideal temperature conducive to sperm production.[22-24]

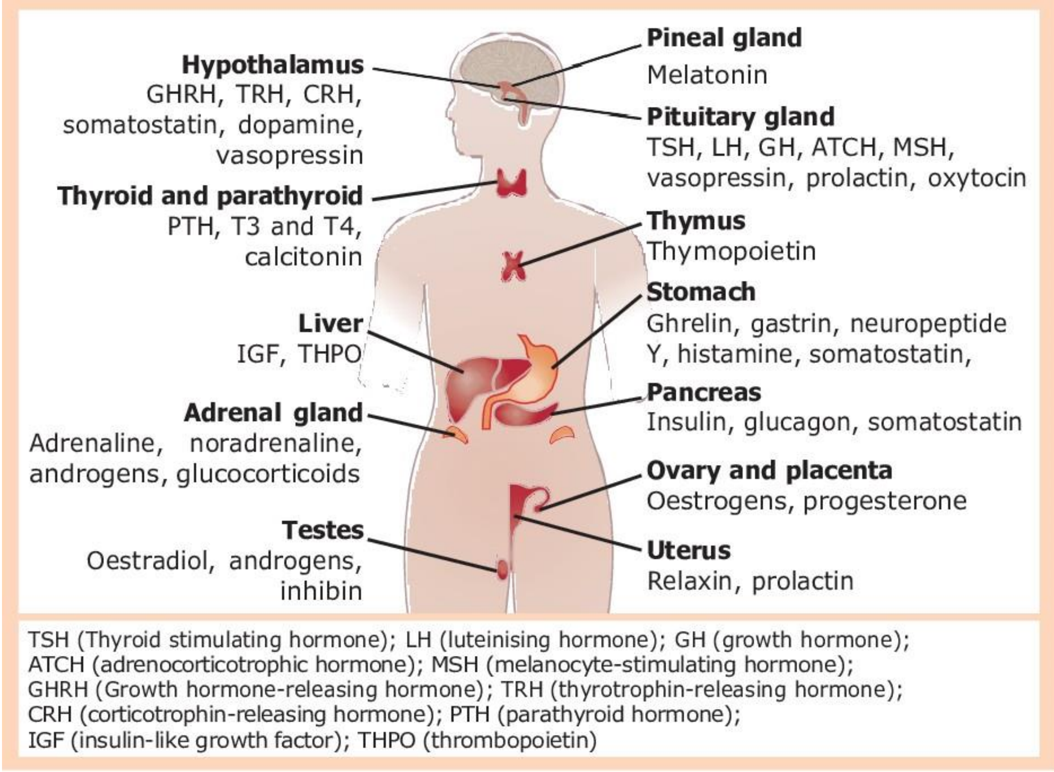


Figure No. 1.3: Major Endocrine glands, Tissues and their hormones [22]

1.11 DIABETES MELLITUS:

Diabetes mellitus remains a significant global health concern, characterized by disrupted insulin secretion and increased blood glucose levels due to liver glucose production and insulin resistance. Traditional medicines derived from medicinal plants are extensively utilized worldwide, with a notable reliance of approximately 60% of the global population on these therapies. In countries like India, diabetes mellitus poses a particularly acute health challenge, especially in urban settings.

Several approaches exist for handling diabetes and its complications. Herbal formulations are favored due to their reduced toxicity, minimal adverse effects, cost-efficiency, and perceived higher efficacy.Many medicinal plants have shown potential for lowering blood sugar levels, leading to continued research efforts. However, additional pharmacological and chemical studies are necessary to fully comprehend how these plants exert their hypoglycemic effects. [25-28]

1.12 TYPES OF DIABETES MELLITUS

1. **Type 1 diabetes** Type 1 diabetes results from the dysfunction or deterioration of pancreatic beta cells, responsible for producing insulin. When these cells are damaged or fail to function properly, insulin production becomes insufficient or ceases altogether. In most instances of type 1 diabetes, the immune system mistakenly attacks and destroys beta cells. To sustain life, individuals require insulin replacement therapy, which can be administered through subcutaneous injections using a syringe or insulin pump.[25-28]

Subcategories of Type 1 Diabetes Mellitus:

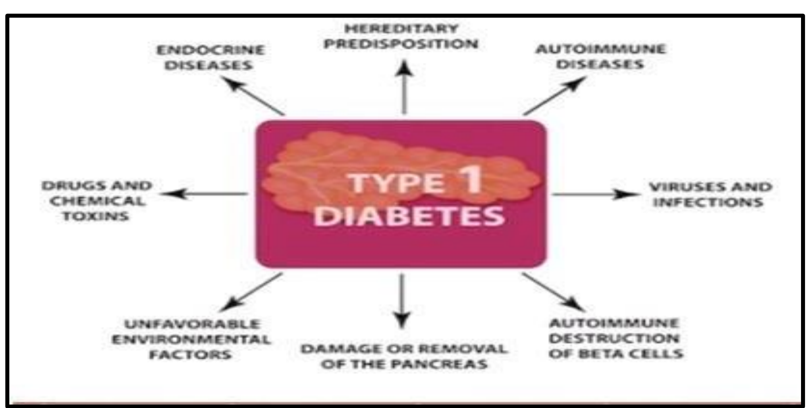


Figure No. 1.4: Type 1 Diabetes

A) Type 1A Diabetes (Including Latent Autoimmune Diabetes of Adults - LADA):

B) Type 1B (Idiopathic or non-immune-mediated diabetes)

2. **Type 2 diabetes:** Type 1B diabetes, alternatively referred to as idiopathic or non-immune-mediated diabetes, is a distinct subtype characterized by insulin deficiency that does not involve autoimmune destruction of beta cells. Unlike Type 1A diabetes, which is largely autoimmune, Type 1B diabetes is observed predominantly in individuals of African or Asian descent and is not linked to autoimmune mechanisms. The exact cause of Type 1B diabetes is not fully understood; however, it is believed to result from a combination of genetic predisposition and environmental factors.

   Patients diagnosed with Type 1B diabetes necessitate insulin therapy for effective blood glucose management and to mitigate associated complications like cardiovascular disease, neuropathy, and nephropathy. Treatment strategies for Type

1B diabetes emphasize personalized insulin regimens customized to meet each patient's unique requirements. The goal is to attain optimal glycemic control and enhance long-term health results.

Research into Type 1B diabetes aims to further elucidate its underlying mechanisms and identify potential genetic and environmental triggers that contribute to its development. This knowledge is crucial for developing targeted therapies and personalized treatment strategies to better manage and ultimately prevent Type 1B diabetes in susceptible populations. [25-28].

Causes of Type 2 Diabetes:

Herbal materials: The range of substances extends beyond just herbs. This includes fresh juices, gums, fixed and essential oils, as well as dry powders made from herbs. Regional preparation methods differ and may involve techniques such as steaming, roasting, or stir-baking, often incorporating honey, alcohol, or other ingredients.Understanding traditional preparation methods is essential for practitioners, as these techniques can influence the potency and effectiveness of herbal materials. Moreover, herbal materials are employed in diverse forms including teas, tinctures, capsules, or topical applications. [25-26]

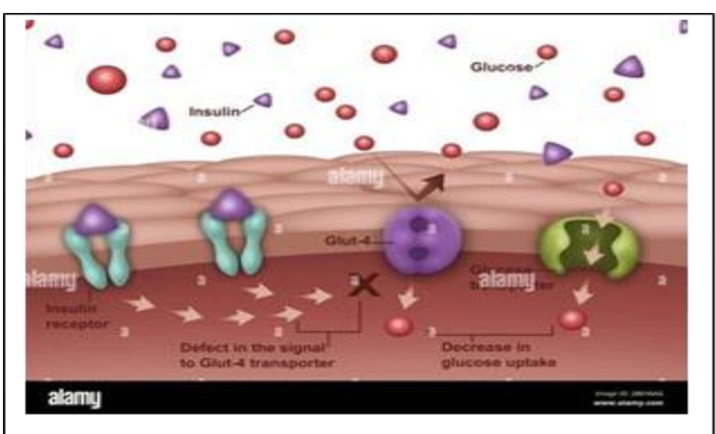


Figure No.1.5: Type 2 Diabetes

3. Gestational diabetes

Gestational diabetes is a form of diabetes that may occur during pregnancy in women who did not previously have diabetes. It affects approximately 2% to 10% of pregnancies annually in the US. Managing gestational diabetes is crucial to promoting a healthy pregnancy for both the mother and the unborn child.[25-26]

Causes of Gestational Diabetes:

Gestational diabetes occurs when the body does not produce enough insulin during pregnancy. The pancreas, which secretes insulin, is crucial for regulating blood sugar levels by helping glucose enter cells for energy production.Throughout pregnancy, hormonal fluctuations and physiological changes, including weight gain, contribute to insulin resistance, wherein cells become less responsive to insulin's actions. Consequently, insulin resistance heightens the body's demand for insulin.

While insulin resistance is a natural occurrence in late pregnancy for all expectant mothers, some women exhibit pre-existing insulin resistance before conception. These individuals necessitate increased insulin production at the onset of pregnancy, rendering them more susceptible to developing gestational diabetes. [25-26]

Epidemiology of Diabetes:

The global occurrence of diabetes is rising swiftly, presenting a substantial health challenge globally. In 2010, an estimated 285 million individuals were affected by diabetes, predominantly type II diabetes, which accounted for nearly 90% of cases. However, by 2013, the International Diabetes Federation reported a surge in diabetes prevalence, with approximately 381 million people afflicted worldwide. Projections indicate a continuous rise in diabetes cases, with an estimated 250 million individuals currently living with the condition globally. By 2030, this figure is anticipated to exceed 350 million. [25-26]

Pancreatic Pathophysiology:

The pancreas is essential for regulating metabolic processes, especially maintaining blood glucose levels. It functions both as an exocrine gland, secreting digestive enzymes, and as an endocrine gland, producing critical peptide hormones. These hormones—insulin, glucagon, and somatostatin—are crucial for controlling glucose metabolism. They are secreted by specialized cells within the pancreatic islets of Langerhans: β cells produce insulin, alpha cells produce glucagon, and δ cells produce somatostatin, each playing a vital role in metabolic regulation.[22-25]

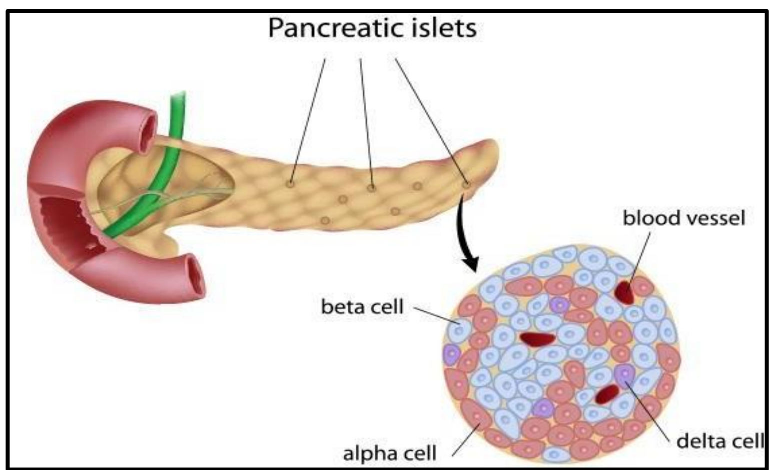


Figure No. 1.6: Islets of Langerhans

1.13 GENES OF DIABETES MELLITUS:

Diabetes mellitus is influenced by genetic predispositions, with two notable genes, MODY1 and MODYII, contributing to approximately 2% to 5% of cases, demonstrating clear heritability. [22-26]

MODY I Gene:

The MODY I gene is located on chromosome 20 and is responsible for encoding hepatocyte nuclear factor-4α (HNF-4α). This particular transcription factor plays a crucial role in regulating genes related to glucose metabolism and insulin secretion. Its functions are vital for preserving the functionality of pancreatic beta cells and ensuring proper hepatic glucose balance.Mutations in this gene can decrease HNF-4α production, resulting in pancreatic beta cell dysfunction and irregular insulin secretion. These mutations are associated with maturity-onset diabetes of the young (MODY), a form of diabetes marked by early-onset hyperglycemia, typically manifesting before the age of 25. [22-26]

MODY II Gene:

The gene associated with MODY II, situated on chromosome 12, encodes the hepatocyte nuclear factor-1α (HNF-1α), which acts as a transcription factor present in both liver cells and pancreatic beta cells.Its primary function involves the regulation of genes crucial for glucose metabolism, insulin secretion, and cellular differentiation. Mutations in the MODY II gene can interfere with HNF-1α function,

resulting in reduced insulin production and impaired glucose regulation.Individuals with MODY II typically present with mild hyperglycemia that is often asymptomatic or manifests later in life compared to other forms of diabetes. Understanding the genetic basis and function of HNF-1α is essential for diagnosing and managing MODY II and developing targeted therapies that restore normal beta cell function and glucose homeostasis.

Research into the MODY I and MODY II genes continues to uncover insights into the molecular mechanisms underlying monogenic diabetes.[25-26]

1.14 INSULIN

In 1921, Frederick Banting made a ground breaking discovery by isolating insulin and showing its ability to lower blood sugar levels through an extract obtained from the pancreas. The following year, in 1922, a significant milestone was achieved when a 14-year-old boy with severe diabetes mellitus was treated with this insulin extract, leading to remarkable improvements in his condition.This breakthrough led to the purification of insulin within a few years.

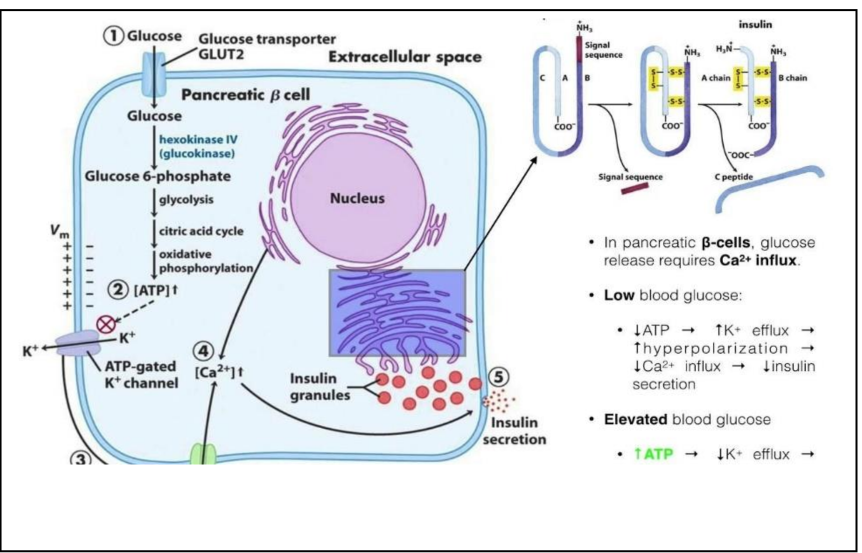


Figure No. 1.7: Secretion of Insulin in βcells

Beta cells, located within upon stimulation by rising glucose levels, insulin is released from these cells in two distinct phases. Initially, there is an immediate release of preformed insulin stored within secretory granules. This is followed by a subsequent release of newly synthesized insulin after a brief pause, which persists for

a longer duration.

The pancreas produces insulin, and these cells form clusters called "Islets of Langerhans," named after their discoverer, a German medical student. Insulin becomes active after release into the bloodstream but has a short half-life of about 6 minutes due to insulinase, an enzyme in the liver and kidneys that breaks it down. This enzymatic degradation leads to rapid fluctuations in blood insulin levels. [23-24]

Action of Insulin

Insulin exerts a wide range of effects across different time spans. When insulin binds to its receptors, it triggers processes in several tissues, particularly skeletal muscle and adipose tissue. This activation leads to the immediate expression of GLUT-4 glucose transporters, which facilitate glucose uptake in response to insulin. Moreover, insulin facilitates the movement of these transporters from internal storage sites to the cell membrane, thereby enhancing their functional capacity and numbers. However, certain tissues, including liver cells, red blood cells, gastrointestinal mucosa, renal cells, and neural cells, utilize alternative glucose transporters that operate independently of insulin regulation.Through the phosphorylation-mediated modulation of specific enzymes, insulin coordinate temporal changes in enzymatic activity spanning minutes to hours.

Moreover, insulin exerts long-term effects by upregulating the expression of numerous metabolic enzymes over several days. These alterations reflect enhanced gene transcription, mRNA synthesis, and enzyme production, thereby contributing to sustained metabolic regulation mediated by insulin. [23-24]

Insulin role on the body

Understanding the significance of insulin therapy becomes more accessible when comprehending the physiological role of this endogenous hormone and the implications of its deficiency in diabetes.

In a non-diabetic state, insulin facilitates two primary functions:

1. **Regulation of blood sugar levels:**

   After carbohydrates are digested into glucose, the pancreas regulates blood glucose levels by secreting insulin. Insulin is a hormone produced by beta cells in the

pancreatic Islets of Langerhans, which responds to increased blood glucose levels following carbohydrate digestion. [23-24]

2. Storage of excess glucose:

When insulin levels rise, such as post-meal, surplus glucose is stored in the liver as glycogen. In periods between meals, when insulin levels drop, the liver releases stored glucose into the bloodstream to uphold blood sugar levels. In cases of diabetes, inadequate insulin production or insulin resistance disrupts this process, resulting in elevated blood glucose levels. Those with type 1 diabetes cannot produce insulin and thus require insulin therapy to substitute the deficient hormone. In type 2 diabetes or gestational diabetes, where insulin production may be inadequate or ineffective, insulin therapy might be recommended if other medications fail to sufficiently manage blood glucose levels. The principal aim of insulin therapy is to sustain blood sugar levels within the recommended range, thereby averting complications associated with diabetes like kidney impairment, vision loss, and neuropathy. [23-24]

1.15 TYPES OF INSULIN

Various types of insulin are available, each with distinct durations and effects on blood sugar levels. Physicians often recommend combining different insulin types based on individual factors such as lifestyle, glucose levels, daily blood sugar fluctuations, and diabetes type.

The primary categories of insulin therapy comprise:

1. Long-Acting, Ultra Long-Acting, or Intermediate-Acting Insulin:

These forms of insulin are designed with extended half-lives to provide sustained glucose-lowering effects over prolonged periods, particularly during fasting or between meals. Examples include:

a. Glulisine (sold under brand names Lantus and Toujeo)

b. Detemir (marketed as Levemir)

c. Degludec (commercially available as Tresiba)

d. NPH (Neutral Protamine Hagedorn sold under various brands such as Humulin N, Novolin N, and Novolin ReliOn Insulin N)

Their duration of action ranges from eight to forty hours, making them suitable for basal insulin replacement therapy.

2. Rapid-Acting or Short-Acting Insulin:

These insulin types are characterized by their rapid onset of action, making them effective in managing post-meal glucose spikes. They typically begin to work within minutes after administration, ensuring timely control of blood sugar levels. Examples include:

a. Aspart (marketed as NovoLog and Fiasp)

b. Glulisine (sold under the brand name Apidra)

c. Lispro (available as Humalog and Admelog)

d. Regular (products such as Humulin R, Novolin R, Myxredlin, and ReliOn R)

Rapid-acting insulins have a shorter duration of action, typically lasting between two and four hours, thereby requiring frequent dosing to maintain optimal glycemic control throughout the day.Insulin is not available in tablet form due to its susceptibility to digestion in the gastrointestinal tract. However, several administration methods exist, and the most suitable option depends on individual preferences and lifestyle considerations. These methods include:

- **Injections (shots or pens):** Insulin administration involves injecting it into the layer of fat just beneath the skin using either a syringe with a needle or a pen-shaped device. The frequency of injections depends on factors such as the type of diabetes, blood glucose levels, and how often meals are consumed, varying from once daily to multiple times per day.
- **Insulin pump:** This apparatus delivers small, regular amounts of fast-acting insulin through a slender tube inserted under the skin. These amounts are distributed over the course of the day, and various types of insulin pumps are available for acquisition.Despite the difficulties linked to insulin treatment, like handling blood sugar swings, choosing a personalized insulin plan that suits one's lifestyle can aid in averting diabetes-related problems and enhancing overall health and well-being.[27-28]

1.16 DIABETES SYMPTOMS

Following are symptoms of Diabetes mellitus

- Frequent urination, particularly during the nighttime
- Excessive thirst
- Unintended weight loss
- Increased appetite
- Blurred vision
- Persistent fatigue
- Dry skin
- Slow-healing sores
- Increased susceptibility to infection.[27-28]

Symptoms of Type 1 Diabetes:

Individuals diagnosed with type 1diabetes often experience symptoms such as nausea, vomiting, or abdominal discomfort. These symptoms typically arise rapidly over a period of weeks to months and can be quite severe. Although type 1diabetes is frequently diagnosed in childhood, adolescence, or early adulthood, it can also manifest at any stage of life.[25-28]

Symptoms of Type 2 Diabetes:

Type 2 diabetes symptoms usually develop slowly over a period of years, with some people experiencing no symptoms initially. Although it traditionally affects adults, there are a growing number of cases being diagnosed in children and teenagers. Given its subtle symptomatology, understanding the risk factors for type 2 diabetes is crucial. If any symptoms are noticed, it is advisable to consult a healthcare professional promptly.[25-28]

Symptoms of Gestational Diabetes:

Gestational diabetes, which occurs during pregnancy, typically does not present obvious symptoms. It is recommended to screen for gestational diabetes between the 24th and

28th weeks of pregnancy.If detected, necessary measures can be implemented to safeguard the well-being of both the mother and the baby. [25-28]

Symptoms in Male:

Men suffering from diabetes may experience particular symptoms beyond those commonly observed. These can encompass reduced libido, erectile dysfunction (ED), and diminished muscular endurance. [25-28]

Symptoms in Female:

In contrast, females diagnosed with diabetes may encounter particular symptoms like lack of moisture in the vaginal area, urinary tract infections, yeast infections, and dry, irritated skin. [25-28]

1.17 DIABETES PREVENTION

Since an immune system problem is the root cause of type 1 diabetes, it cannot be prevented. You also have no control over some of the factors that contribute to type 2 diabetes, such as your age or DNA. However, many additional risk factors for diabetes are controllable. The majorities of diabetes prevention techniques call for minor dietary and exercise modifications. Here are some strategies to postpone or avoid type 2 diabetes if you have been diagnosed with prediabetes:

To reduce the chances of developing diabetes, people should engage in a minimum of 150 minutes of physical activity each week, such as walking or cycling. It is recommended to reduce intake of saturated fats, trans fats, and refined carbohydrates. Consuming more fruits, vegetables, and whole grains is advantageous, along with managing portion sizes by opting for smaller servings.Additionally, overweight or obese individuals should aim to reduce their body weight by 5% to 7%.

While these strategies are beneficial, exploring additional approaches is recommended to further prevent this chronic health condition. [25-28]

Treatment of Diabetes

Type 1 Diabetes

Type of Insulin	Onset Time	Duration of Action
Rapid-acting insulin	Within 15 minutes	2 to 4 hours
Short-acting insulin	Within 30 minutes	3 to 6 hours
Intermediate-acting insulin	Within 2 to 4 hours	12 to 18 hours
Long-acting insulin	2 hours after injection	Up to 24 hours
Ultra-long acting insulin	6 hours after injection	36 hours or more
Premixed insulin	Within 5 to 60 minutes	10 to 16 hours

Type 2 diabetes

Individuals with type 2 diabetes may get relief from Diabetes Mellitus by benefit from diet and exercise. You may need to take medicine to decrease your blood sugar if making lifestyle changes isn't enough. These medications reduce blood sugar in several ways, including:

Table 1.1 List of Chemical drugs used as Antidiabetic

Drug	How it works	Examples
Alpha-glucosidase inhibitors	slow your body's breakdown of sugars and starchy foods	Acarbose(Precose) andMiglitol
Biguanides	Reduce the amount of glucose your liver makes	Metformin (Glucophage,Riomet)
DPP-4 inhibitors	Improve your blood sugar without making it drop too low	Alogliptin(Nesina),Linagliptin (Tradjenta),Saxagliptin (Onglyza),and Sitagliptin(Januvia)
Glucagon-like	stimulate your pancreas	Semaglutide (Ozempic),

peptides	to produce more insulin; slow stomach emptying	Dulaglutide (Trulicity), Exenatide (Byetta),and Liraglutide(Victoza)
Meglitinides	Stimulate your pancreas to release more insulin	Nateglinide and Repaglinide
SGLT 2 inhibitors	Release more glucose in to the urine	Canagliflozin (Invokana), Dapagliflozin (Farxiga), Andempagliflozin (Jardiance)
Sulfonyl ureas	Stimulate your pancreas to release more insulin	Glyburide (Glynase), Glipizide (Glucotrol), and Glimepiride (Amaryl)
Thiazolidinediones	Help insulin work better	Pioglitazone (Actos) and Rosiglitazone

To effectively manage type 2 diabetes, it may be necessary to prescribe several medications concurrently. Additionally, some individuals with type 2 diabetes may require insulin therapy. [29-30]

Gestational diabetes

During pregnancy, it is crucial to regularly monitor blood sugar levels throughout the day. Adjustments in physical activity and diet are often sufficient to control high blood sugar levels. Nevertheless, research suggests that around 15% to 30% of expectant mothers diagnosed with gestational diabetes may require insulin therapy to effectively manage their blood sugar levels. Importantly, it should be noted that insulin treatment does not present risks to the developing foetus. [27-29]

Diabetes diagnosis

Diagnosing diabetes often includes screening for gestational diabetes during the second or third trimester of pregnancy. Healthcare providers use several blood tests to detect prediabetes and diabetes, including:

- **Fasting Plasma Glucose (FPG) Test**: Measures blood sugar levels after an overnight fast of at least 8 hours.
- **HbA1C Test:** Provides information about average blood sugar levels over the past three months.
- **Gestational diabetes:** Diagnosis typically involves healthcare professionals conducting blood sugar level assessments during weeks 24 to 28 of pregnancy. Two different types of tests are commonly employed for this evaluation.
- **Glucose challenge test:** An hour after consuming a sugary beverage, blood sugar is measured for a glucose challenge test. No further testing is necessary for typical findings. However, if your blood sugar levels are elevated, a glucose tolerance test is required.
- **Glucose tolerance test:** Your blood sugar is measured during a glucose tolerance test following an overnight fast. After receiving a sugar-filled beverage, blood sugar is checked again an hour later and again two hours later. In the event that any of these three blood sugar values are not high, gestational diabetes is diagnosed. [28-30]

1.18 DIABETES PREVENTION:

Type 1 diabetes develops due to an autoimmune disorder and cannot be prevented. On the other hand, factors like genetic predisposition, age, and others contribute to type 2 diabetes, which are not controllable. However, there are several modifiable risk factors for diabetes. Strategies for prevention focus on adjustments in diet and exercise.

For individuals with prediabetes, steps can be taken to delay or prevent progression to type 2 diabetes:

- Engage in at least 150 minutes of aerobic exercise weekly.
- Reduce intake of saturated fats, Tran's fats, and refined carbohydrates.

- Increase consumption of fruits, vegetables, and whole grains.
- Control portions during meals.
- Aim to lose 5% to 7% of body weight if overweight or obese. [25-29]

1.19 ROLE OF HERBAL MEDICINE IN DIABETES MANAGEMENT:

In the management of diabetes, traditional herbal medicines are commonly administered in extract form due to their observed efficacy. Numerous clinical studies have validated the anti-diabetic properties of medicinal plant extracts, which have been shown to aid in restoring the functionality of pancreatic β-cells. [31-32]

Allium sativum:

Garlic, scientifically identified as *Allium sativum* and part of the Liliaceae family, is recognized for its ability to lower blood sugar levels. Research indicates that garlic extract, when given at a dosage of 10 ml/kg/day, demonstrates significant hypoglycemic effects. It has been shown to be more efficacious in reducing blood glucose levels than Glibenclamide; a frequently prescribed medication for diabetes.Research conducted on rats treated with STZ has shown that different extracts of garlic (ethanol, ethyl acetate, and petroleum ether) possess anti-diabetic properties. Apart from its anti-diabetic effects, garlic is also recognized for its medicinal advantages, including antibacterial, anti-platelet, and properties that contribute to lowering blood pressure and cholesterol.

Aloe borbadensis:

Aloe vera, also known as Ghikanvar and belonging to the Liliaceae family, features cactus-like characteristics with thick, tapered, hairy, green leaves filled with a clear gel. Research indicates that administering 150 mg/kg of aloe vera aqueous extract orally significantly lowers blood glucose levels. Moreover, aloe vera gel provides various therapeutic benefits including anti-diabetic effects and antioxidant properties, while also increasing glutathione levels by up to fourfold in diabetic rats.

Azadirachta indica:

Azadirachta indica, commonly known as neem and belonging to the Meliaceae family, is native to India and Burma. Studies have indicated that both ethanolic and aqueous extracts of neem can effectively lower blood glucose levels, particularly in high dosages. This makes it a potential adjunct to allopathic drugs for managing type 2 diabetes when

conventional treatments alone are insufficient. Neem tablets derived from natural extracts are widely used in the treatment of diabetes globally, known for their ability to improve blood circulation by dilating blood vessels while reducing blood glucose levels in the body.

Brassica juncea:

It is a member of the Cruciferae family and is referred to as Rai.It's commonly used as a spice in a variety of dishes. In diabetic rats given alloxan, aqueous seed extract was shown to have blood sugar-lowering properties.The extract exhibits hypoglycemic action at dosages of 250, 350, and 450 mg/kg.

***Carica papaya*:**

Carica papaya, belonging to the Caricaceae family and commonly referred to as papaya, has shown positive impacts like lowering blood sugar levels, reducing lipids, and improving wound healing in diabetic rats induced with alloxan.Extracts derived from its seeds and leaves were utilized to illustrate these advantageous properties.

***Catharanthus roseus*:**

Catharanthus roseus, also known as Vinca roseus and belonging to the Apocynaceae family, has been found to exhibit hypoglycemic effects in alloxan-induced diabetic rats when treated with methanolic extracts from its leaves and twigs. Oral administration of a 500 mg/kg dose of these extracts effectively reduces blood glucose levels in animals. This effect is believed to occur through the stimulation of insulin synthesis from the β cells of Langerhans.

***Coriandrum sativum*:**

Coriandrum sativum, also known as coriander and belonging to the Umbelliferae family, is widely utilized as a spice in different culinary traditions. According to research results, the seed extract shows promising insulinotropic effects by enhancing the β cells of Langerhans and reducing serum glucose levels in alloxan-induced diabetic rats when administered at a dose of 200 mg/kg.

***Eugenia jambolana*:**

Eugenia jambolana, known as jamun and belonging to the Myrtaceae family, contains bioactive compounds such as malvidin 3-laminaribioside and ferulic acid. The dried seed

extract of *Eugenia jambolana* is used at a dose of 200 mg/kg for treating individuals with diabetes.

Gymnema sylvestre:

Gymnema sylvestre, also known as Gudmar and part of the Asclepidaceae family, has demonstrated potential in reducing blood sugar levels in streptozotocin-induced rats when treated with its leaf extract. This herb is widely utilized in traditional Indian ayurvedic medicine for diabetes management. Its constituents include alkaloids, flavonoids, saponins, and carbohydrates, which are also employed in treating conditions like cancer, inflammation, and microbial infections.

***Mangifera indica*:**

Mangifera indica, also known as mango and classified in the Anacardiaceae family, has been studied for its potential anti-diabetic properties. Studies have shown that administering a leaf extract at a dose of 250 mg/kg effectively lowers blood glucose levels. However, orally administering the aqueous extract did not significantly impact blood glucose levels in diabetic rats induced with alloxan.

Momordica charantia:

Momordica charantia, also known as bitter melon or karela, belongs to the Cucurbitaceae family. It contains bioactive compounds such as Momordic I, Momordic II, and Cucurbitacin B, which contribute to its hypoglycemic properties. These effects are believed to be mediated by a lectin that mimics insulin and interacts with body tissues. Research has shown significant hypoglycemic effects from an extract of M. charantia fruit administered at a dose of 200 mg/kg in studies.

Armillaria mellea:

Armillaria mellea, a frequently consumed edible mushroom, is recognized for its polysaccharides that exhibit anti-inflammatory, antioxidant, and immunomodulatory effects. Studies suggest that these polysaccharides may enhance insulin secretion in pancreatic cells exposed to alloxan by neutralizing free radicals.

Zingiber officinale:

Zingiber officinale, commonly known as ginger, exhibits glycemic control in diabetes mellitus by inhibiting critical enzymes involved in carbohydrate metabolism and enhancing insulin release and sensitivity. Its lipid-lowering effects improve insulin resistance, and it also protects against diabetic-related complications.

Sesbania grandiflora:

Sesbania grandiflora is employed for multiple purposes such as anticancer, antioxidant, cardioprotective, antiulcer, and hepatoprotective effects. It is rich in campesterol and beta-sitosterol found in both its pods and leaves. Research indicates that the aqueous leaf extract helps lower elevated blood glucose levels and lipid profiles in diabetic rats induced by STZ.

Beta vulgaris:

Beta vulgaris widely recognized as beetroot and beet leaves, has a history of medicinal use. It has been noted for its hypoglycemic, insulin-sensitizing, and antioxidant effects. Studies have demonstrated potential in decreasing hepatic steatosis and liver damage in rats with type 2 diabetes mellitus through augmentation of PPARα activity. [31-35]

1.20 FUTURE PERSPECTIVES OF HERBAL REMEDIES FOR DIABETES MELLITUS:

The utilization of herbal medicines continues to expand, with numerous traditional remedies incorporated into modern therapeutic practices. In many developing regions, especially rural areas, approximately 80% of the population relies on traditional medicine to fulfil their healthcare needs.Furthermore, developed countries are experiencing renewed interest in herbal medicines, driven by an increasing preference for natural products.It is crucial to differentiate between herbal remedies prescribed by healthcare professionals and those widely available for self-medication.

The increasing global prevalence of diabetes mellitus highlights the urgency of investigating alternative therapies. Recent breakthroughs in medical research have unveiled new bioactive compounds sourced from plants, demonstrating enhanced anti-diabetic efficacy compared to traditional oral hypoglycemic medications. There is a rising enthusiasm for identifying plants harboring potential anti-diabetic properties, which may

facilitate the future development of novel oral therapies for diabetes mellitus management.

Ongoing research in this area could lead to new methods for managing diabetes, providing additional natural remedy options to support current treatments. Utilizing the healing properties of herbal remedies may improve the overall effectiveness and outcomes of diabetes care. [35]

Kumar et al. (2015) Researchers carried out an investigation to examine the potential antidiabetic effects of a 70% alcoholic extract from *S. grandiflora* flowers in rats with alloxan-induced diabetes. The rats received oral doses of the extract, at 250 mg/kg and 500 mg/kg, daily for 28 days. The findings showed significant decreases in serum total cholesterol, triglycerides, SGOT, SGPT, and BUN levels when compared to the diabetic control group.Serum total cholesterol, triglycerides, SGOT, SGPT, and BUN was analyzed using semiautoanalyzers and biochemical kits.When compared to diabetic control, the alcoholic extract's anti-diabetic action was significantly increased ($p > 0.01$) at doses of 250 and 500 mg/kg of flower. Additionally, when compared to diabetic control rats, the extract of both dosages significantly ($p < 0.01$) decreased serum total cholesterol, triglycerides, SGOT, SGPT, and BUN levels. [36]

Gyawali et al. (2015) the potential antidiabetic effects of a methanolic extract derived from *Urtica dioica* were evaluated using streptozotocin-induced diabetic mice and compared with a commercial polyherbal compound called "Jamedachurna." This blend of multiple herbs, well-known for its significant presence of alkaloids, tannins, and terpenoids, demonstrated excellent effectiveness in both controlled laboratory experiments (in vitro) and studies involving live animals (in vivo). [37]

Mawlieh et al. (2020) assessed the anti-diabetic efficacy of two herbal products, ADD1 (Dia Areca) and ADD2 (Asanadi Kahsaya Choorna), using rat models of streptozotocin-induced diabetes. Both formulations effectively reduced elevated blood sugar levels.The current investigation is done to validate the blood sugar-lowering effectiveness of these formulations using rat models of diabetes caused by streptozotocin. Groups four and five received test drug ADD1 in two different doses, whereas groups six and seven received test drug ADD2 in two different doses. Streptozotocin was administered at a medium dose (40 mg/kg body weight) to cause diabetes. The results show both the test compounds has decreased the elevated blood sugar level. [38]

Sharma et al. (2011) conducted a study on the ethanolic extract derived from the seeds of *Alangium salvifolium* Linn to evaluate its potential in treating diabetes. Tablet formulations containing the extract successfully cleared multiple quality assessments, affirming its therapeutic efficacy against diabetes. [39]

Suruse et al. (2012) formulated and evaluated a herbal anti-diabetic capsule containing dried extracts of *Gymnema sylvestre, Mucuna pruriens, and Ginkgo biloba*. The refined formulation resulted in a notable reduction in serum glucose levels when compared with the conventional anti-diabetic drug. [40]

Sahu et al. (2018) the physicochemical properties and antidiabetic effects of a polyherbal formulation were investigated. This formulation consisted of hydroalcoholic extracts from *Andrographis paniculata, Azadirachta indica, and Moringa oleifera*. Results indicated a notable reduction in total cholesterol levels and an elevation in HDL, implying promising therapeutic efficacy. [41]

Nidhi et al. (2021) formulated a polyherbal anti-diabetic tablet and evaluated its physicochemical properties. The tablet exhibited good flow properties, moisture content, and disintegration time, indicating its suitability as a pharmaceutical formulation. [42]

Arora et al. (2013) the study explored the impact of a blend of natural ingredients including *Eugenia jambolana, Gymnema sylvestre, Tinospora cordifolia, Pterocarpus marsupium, and Terminalia bellerica* on diabetes. Results indicated promising effects in lowering blood sugar levels and exhibiting potential as an antidiabetic agent. [43]

Aziz et al. (2019) evaluated the antidiabetic activity of a polyherbal powder containing various medicinal plants. The powder exhibited good physicochemical properties and stability, suggesting its potential as a treatment for diabetes mellitus. Phytochemical qualitative analysis resulted identification of the presence of flavonoids, alkaloids, terpenoids, tannins, steroids, carbohydrates, and glycosides. According to physicochemical analysis, the polyherbal powder showed good flow properties and maintained stability throughout time. The multi-herbal powder was evaluated, which has a potential to treat diabetes mellitus. [44]

Uddandrao et al. (2020) the study explored the antioxidant and glucose-lowering capabilities of a polyherbal formulation (PHF) made from the fruits of *Piper nigrum*, bark of *Terminalia paniculata*, and bark of *Bauhinia purpurea*. Results showed that the PHF demonstrated notably superior antioxidant and glucose-lowering effects compared to the extracts from the individual plants. [45]

Shinde et al. (2021) formulated polyherbal pills using plant extracts and evaluated their antidiabetic potential. The tablets passed various quality tests, indicating their suitability

for further study.The mixes took longer than a minute to finally dissolve, leading one to the conclusion that further study is necessary to fully comprehend the underlying mechanism of action and long-term toxicity of the produced tablet. [46]

Alam et al. (2013) investigated the antilipidemic and antidiabetic effects of a polyherbal formulation known as Ziabeetein powder in diabetic rats induced by streptozotocin. The study found that the formulation successfully decreased blood glucose levels and enhanced lipid profiles. [47]

Suman et al. (2016) conducted a study to assess the effects of a polyherbal aqueous decoction tablet (PHADT) on antihyperglycemic and antihyperlipidemic properties in diabetic rats. The research findings indicated notable enhancements in multiple parameters related to diabetes management. [48]

Patel et al. (2017) aimed to create a polyherbal anti-diabetic pill with a faster disintegration rate. The developed polyherbal compound exhibited satisfactory post-compression parameters, indicating its potential as a solid dosage form. [49]

Kwakye et al. (2017) emphasized the importance of stability studies on herbal products to ensure product quality and patient safety. They discussed stability issues associated with various herbal dose forms, highlighting the need for standardized stability testing protocols. [50]

Parasuraman et al. (2014) reviewed the importance of polyherbalism in traditional medicine systems like Ayurveda. They emphasized the synergistic effects of combining multiple herbs to enhance therapeutic efficacy. [51]

Gupta et al. (2013) developed herbal effervescent granules using *Calliandra haematocephala* leaf extract and evaluated their physicochemical properties. The granules exhibited good flow characteristics and dissolution properties. [52]

Kothari et al. (2017) investigated the antidiabetic properties of *Sesbania grandiflora* extract and compared its effects to Acarbose. The findings revealed significant inhibition of amylase activity, highlighting its potential as a natural treatment for diabetes. [53]

Panigrahi et al. (2016) Studied was the potential anti-diabetic effects of *Sesbania grandiflora* extract in a rat model of type 2 diabetes induced by a high-fat diet and low-

dose streptozotocin. The findings showed significant reductions in blood glucose levels and improvements in insulin sensitivity following the extract's administration. [54]

Singh et al. (2014) conducted a study to investigate the antidiabetic effects of a hydro-alcoholic formulation containing extracts from *Luffa acutangula* and *Madhuca longifolia*. Their findings showed a significant decrease in fasting blood glucose levels in diabetic rats after administering the formulation. [55]

Sabale et al. (2020) presented an investigation into herbal medications for managing diabetic complications. Their study focused on the role of garlic, known for its antidiabetic properties due to the presence of allicin, in reducing blood glucose levels. Additionally, they explored the wound-healing and anti-hyperglycemic activities of neem. The researchers developed a natural diabetes medication combining neem and garlic extracts and evaluated its efficacy using various tests such as disintegration, dissolution, hardness, angle of repose, and friability. [56]

Shrivastava et al. (2017) examined the medicinal properties of Agaricus bisporus, an edible mushroom, through phytochemical screening. Their study aimed to detect various bioactive compounds, including alkaloids, carbohydrates, glycosides, proteins, flavonoids, saponins, phenolics, and steroids, in the methanolic extract of Agaricus bisporus. The researchers assessed the oral dosage form of a herbal tablet derived from Agaricus bisporus and reported satisfactory results in terms of pharmaceutical quality. [57]

Saifi et al. (2017) conducted a study to determine the dose-response relationship of individual and combined herbal extracts in the context of hypoglycemia. They identified optimal doses for extracts from the stem barks of *Ficus bengalensis*, the fruits of *Momordica charantia*, the seeds of *Trigonella foenum graecum*, and *Syzygium cumini*. The anti-diabetic effects of modified doses were investigated in rats with alloxan-induced diabetes. Based on the identified optimum doses, the researchers formulated a polyherbal mixture with significant antidiabetic properties, as evidenced by biochemical results on the 21st day of withdrawal of blood samples. [58]

Chauhan L et al (2018) the current study's objectives were to develop and assess **a** transdermal medication delivery system for a herbal antidiabetic medicine. Herbal extract transdermal patches were created using the solvent casting technique.Further assessments of the adjusted formulations included FESEM investigations, in vitro drug release, and

drug content.The study's findings demonstrated that the HPMC polymer-based herbal transdermal patch performed better in terms of physiochemical criteria like thickness, folding endurance, physical appearance, uniformity of weight, and moisture uptake investigations. The produced formulations had the lowest moisture content and moisture uptake and were determined to be uniform in thickness and folding durability.During the in vitro trials, the patches made with 30% w/v of plasticizer released more medication from the herbal transdermal patch in 24 hours than those made with 20 and 25% w/v. [59]

Gauttam et al. (2013) developed hydro-alcoholic extracts of *Momordica charantia, Trigonella foenumgraecum,* and *Withania somnifera* in a specific ratio and evaluated their efficacy in managing diabetes. They formulated lipids containing the optimal extract combination within phosphatidylcholine and cholesterol vesicles. The vesicles underwent various evaluations for morphology, entrapment efficiency, and release profile. In a 21-day trial on diabetic rats, the encapsulated formulation showed superior anti-diabetic potential compared to the unencapsulated extract and was comparable to metformin. [60]

Jyothi D. et al. (2017) aimed to develop antidiabetic formulations with enhanced oral hypoglycemic activity, reduced side effects, and improved patient compliance by incorporating crude extracts of fenugreek seeds into capsule formulations. The capsule formulations were prepared by encapsulating fenugreek seed extract granules with varying concentrations of sodium starch glycolate as a superdisintegrant (ranging from 0% to 5%). The finished capsule formulations were subjected to evaluations such as in vitro drug release, weight variation, disintegration time, drug content (trigonelline), and in vivo antidiabetic activity. Their findings suggest that incorporating fenugreek seed extracts into herbal dosage forms could offer advantages over using raw plant ingredients for diabetes treatment. [61]

Telapolu S. et al. (2018) conducted a study on MD-1, a polyherbal preparation used for treating diabetes mellitus (DM), to assess its physical and chemical characteristics for routine quality control. The study proposed that MD-1 might enhance glucose uptake and modulate insulin sensitivity through mild PPAR agonism, similar to natural chemical agonists. It was suggested that MD-1's ability to mitigate diabetes-related issues could be attributed to its unique binding modalities within the formulation compared to synthetic ligands like thiazolidinediones (TZD). [62]

Petchi R. R. et al. (2014) conducted a study aimed at formulating a polyherbal mixture using ethanol extracts from *Moringa indica* leaves, *Tribulus procumbens* whole plant, and *Gymnema pentaphylla* stem bark. The research focused on evaluating the formulation's efficacy in preventing diabetes in animal models. Their findings demonstrated significant antidiabetic effects comparable to glibenclamide, as evidenced by biochemical and histopathological analyses. [63]

Panda A et al. (2013) focused on polyherbal antidiabetic formulations, investigating their effectiveness in diabetic rats induced with Streptozotocin (STZ). The study synthesized five different combinations of eleven medicinal plants and compared their efficacy with glibenclamide. The results showed that these combinations effectively reduced elevated lipid levels and blood glucose levels, bringing them close to normal. Additionally, liver function parameters were restored to normal levels. [64]

Farghaly U et al. (2014) a study examined the antidiabetic properties of fenugreek, onion, and garlic in rats with elevated blood sugar levels. The results demonstrated significant decreases in blood glucose levels in the groups given onion and fenugreek, while garlic led to the most rapid reduction. Despite the herbal combination significantly lowering blood glucose levels compared to the control group, its effectiveness was not as substantial as the standard drug, glimepiride. [65]

Mandlik R. V. et al. (2008) examined the hypoglycemic and antidiabetic properties of a herbal preparation, DRF/AY/5001, in normal and hyperglycemic rats induced with epinephrine and alloxan. Their findings suggested that the herbal formulation exerted its effects through both pancreatic and extra-pancreatic mechanisms. Specifically, it increased enzymatic antioxidants in pancreatic tissue and inhibited lipid peroxidation. Furthermore, the study indicated that the herbal preparation demonstrated significant antidiabetic activity comparable to that of glibenclamide. [66]

Majumdar P. et al. (2016) focused on developing and evaluating a polyherbal tablet for diabetes. Their formulations met acceptable standards, suggesting the potential for developing potent and stable oral dosage forms for diabetes treatment and providing insights into the synergistic effects of herbal combinations. [67]

Mandal et al. (2016) Researchers investigated the potential effects of methanol extract derived from Beetroots. Their study focused on examining its influence on blood glucose

levels through an oral glucose tolerance test (OGTT). The results indicated a substantial decrease in glucose levels that correlated with the dosage of the extract administered. Additionally, the experiments revealed significant antinociceptive properties associated with the extract, implying potential therapeutic benefits. [68]

Al-Harbi LN et al. (2021) Conducted a study on the effects of methanolic Beetroot extract (BE) on dyslipidemia, liver fat accumulation, and liver damage in a rat model of type-2 diabetes mellitus (T2DM).Their research indicated that BE administration increased antioxidant levels and PPAR expression in normal and T2DM rats, leading to reduced hepatic steatosis and liver damage. The findings suggest that higher doses of BE may offer greater benefits, underscoring its potential in diabetes management. [69]

Kumar S. et al. (2020) investigated the antidiabetic and haematinic efficacy of *Beta vulgaris* juice using an alloxan-induced experimental animal model. The study found that Beta vulgaris juice demonstrated anti-diabetic efficacy over the course of treatment, albeit slightly less potent than insulin. The results indicate the potential of *Beta vulgaris* as a haemolytic and anti-diabetic agent. [70]

Chauhan N. N. et al. (2021) aimed to develop a multi-herbal anti-diabetic pill and evaluate its physicochemical characteristics compared to commercially available herbal tablets. The study combined powdered extracts of *Enicostemma littorale*, *Aconitum heterophyllum* roots, *Picrorhiza kurroa* rhizomes, and *Piper longum* fruits to create the polyherbal anti-diabetic tablet. Various tests were conducted to assess the quality parameters. [71]

Harshmali K et al. (2019) the study assessed the efficacy of herbal formulations described in "Thalpathe Piliyam" for managing type II diabetes. It conducted a comparison between patients treated with a powdered herbal combination and those receiving conventional allopathic treatment. The results indicated notable reductions in fasting blood sugar, blood pressure, and heart rate in both treatment groups. These findings suggest that the herbal formulation could be beneficial for managing type II diabetes. [72]

Manekar S S et al (2014) the study used control and test groups of healthy rats that had been randomly given the diabetes-inducing drug streptozotocin (STZ). 500 mg/kg and 1000 mg/kg of a herbomineral formulation were given to diabetic rats. The goal of the

current study was to determine whether a herbomineral formulation containing five different herbs and two minerals had any anti-diabetic effects on streptozotocin (STZ 50 mg/kg ip single dose)-induced diabetic rats. The effectiveness of the plant extract was comparable to that of the well-known hypoglycemic medication Glibenclamide and Insulin. [73]

Jain S et al. (2006) examined the hypoglycemic effects of herbal extracts in a type 1 diabetes animal model. Their study revealed varying degrees of hypoglycemic effects with different herbal extracts, suggesting their potential as alternative treatments for diabetes. [74]

Kumar et al. conducted a study to assess the effectiveness of a polyherbal vati formulation for managing diabetes. The vati formulation consisted of several herbal ingredients, such as Giloy, Neem, Chirayata, Gurmar, Ashwagandha, Gokshura, Haritaki Chhoti, Bahera, Amla, Bilva, Kachur, Vasaka, Haldi, Kutki, Jamun, Shuddha Shilajit, Karela, Methi, and Malabar tree. The formulation was standardized by evaluating various physicochemical parameters, such as water-soluble and alcohol-soluble extractive values, moisture content, bulk density, pH, water-soluble ash, acid-insoluble ash, loss on drying, and organoleptic characteristics. The results showed that these parameters were within acceptable limits, indicating the quality and potential efficacy of the formulation for its intended use. [75]

Mahajan et al (2018) and colleagues conducted a study aimed at developing polyherbal eyedrops containing antioxidant-rich herbal extracts. They assessed the efficacy of these eyedrops in preventing diabetic cataract induced by galactose. The study utilized formulations that contained extracts from *Ginkgo biloba* leaves, Beetroot *(Beta vulgaris),* and Amla (*Emblica officinalis*) fruits. Diabetic cataracts were induced in Wistar rats by giving them a daily dose of a 10% galactose solution for duration of 30 days.Prophylactic treatment with formulated eyedrops began concurrently with galactose administration and continued throughout the 30-day period. Examination using a slit lamp revealed clear lenses without signs of opacity in the group receiving prophylactic therapy. In contrast, rats treated solely with galactose exhibited dense nuclear opacity typical of diabetic cataract. The findings suggest that a polyherbal mixture with extracts from *Ginkgo biloba*, Beetroot, and Amla may be effective in preventing cataract development in individuals

with diabetes. [76]

Gilchrist et al. (2014) A new nitrate-depleted Beetroot juice was developed for potential clinical trials to explore its impact on cognitive function in patients with type 2 diabetes mellitus, as part of a broader investigation into dietary nitrate effects in this population. This newly formulated juice was found to have similar sweetness, proton NMR characteristics, and taste as regular beetroot juice. After a two-week trial period, participants who consumed the active juice showed significantly faster simple reaction times compared to those who received the placebo (mean difference 13.9 ± 25.6 ms; 95% CI 3.8–24.0 ms; p = 0.009). However, no significant differences were observed in other cognitive function tests between the two groups. The study concluded that the nitrate-depleted Beetroot juice placebo was suitable for use in dietary nitrate supplementation trials and highlighted that supplementing the diet with 7.5 mmol of nitrate significantly improved simple reaction times in patients with type 2 diabetes mellitus over the two-week period.[77]

Thissera et al. (2020) Researchers conducted a study to explore the potential anti-diabetic properties of *S. grandiflora*. They investigated its chemical composition and evaluated its impact on key enzymes involved in carbohydrate metabolism, specifically amylase and glucosidase. Through LC-HRMS dereplication analysis, they identified 32 metabolites in the leaves and twigs. Bio-guided fractionation and HPLC purification isolated 14 major metabolites, including two terpenoids, vomifoliol and loliolide, which exhibited glucosidase inhibitory activity (IC50 values of 64.5 µM and 388.48 µM, respectively). Additionally, the flavonoid quercetin showed the most potent glucosidase inhibition with an IC50 value of 17.45 µM. Molecular modeling studies supported significant binding interactions between these active compounds and the enzyme-binding pockets, affirming their inhibitory potential. Quantitative analysis indicated substantial concentrations of these inhibitors in the S. grandiflora extract, suggesting its potential as a dietary supplement for managing postprandial blood sugar levels. [78]

Reddy et al. (2019) investigated the therapeutic potential of a polyherbal formulation (PHF) in experimental animals with diabetic-induced nephropathy (DN). Diabetic rats were treated orally with PHF at doses of 250 and 500 mg/kg for approximately 16 weeks, while control groups received no treatment or a vehicle only. Following treatment,

various markers of inflammation, renal function, and lipid metabolism were assessed, along with histopathological examination of kidney tissues. Animals treated with PHF exhibited significant improvements in multiple parameters associated with DN, including lipid profiles,renal function markers, inflammatory cytokines, and histological signs of kidney injury. These findings suggest that PHF treatment may offer renoprotective effects in DN, possibly through its effects on inflammation, lipid metabolism, and renal function. [79]

Pari L et al. (2001), the study investigated the potential antihyperglycemic effects of Diamed in rats with alloxan-induced experimental diabetes. Diamed was administered orally for 30 days at doses of 1.39, 1.67, or 1.94 mL/kg. This treatment resulted in notable reductions in both blood glucose levels and glycosylated hemoglobin. Furthermore, it led to increases in plasma insulin and total hemoglobin levels. The highest dose, 1.94 mL/kg, exhibited the most significant effects and prevented weight loss in the rats. Compared to rats treated with glibenclamide at 600 mg/kg, Diamed-treated rats demonstrated significantly improved glucose tolerance in oral glucose tolerance tests. These findings underscore the potential of Diamed as an effective antihyperglycemic agent in experimental diabetes in rats. [80]

Mamatha M K et al (2020) Due to their lower side effect and affordable pricing, seasoner formulations have gained popularity recently, particularly in the treatment of type II DM. This review compares a suitable alternative polyherbal formulation with a monoherbal formulation to show the impact of polyherbal formulations on anti-diabetic activity. The group of illnesses known as diabetes mellitus may include hyperglycemia, which over time can seriously harm the intestines, blood vessels, eyes, kidneys, and nerves. [81]

Debnath B et al (2022) they wanted to create herbal neutraceutical tablets and test their effectiveness against diabetes in ob/ob mice. Using field survey techniques, five plant species were gathered based on oral interviews with Tripura's traditional healers. The findings imply that the newly created herbal pill might be suggested as a diabetic neutraceutical medication.[82]

Ghanshyam et al. (2016) a study investigated the effects of the methanolic extract of *Sesbania grandiflora* (MESG) on type 2 diabetic rats, which were induced using a

combination of low-dose streptozotocin and a high-fat diet. For 28 days, the diabetic rats were treated orally with either metformin (10 mg/kg) or MESG at doses of 200 and 400 mg/kg.The study concluded with assessments conducted on the pancreatic-to-body weight ratio and hepatic glycogen levels.Administration of MESG led to a significant decrease in elevated blood glucose levels in the diabetic rats ($P < 0.05$), as well as normalization of other measured parameters. These findings suggest that MESG may possess antihyperglycemic and antihyperlipidemic properties, potentially improving conditions associated with insulin resistance. [54]

Rajit Kumar et al. (2015) the antidiabetic properties of *Sesbania grandiflora* flower were examined using a 70% alcoholic extract in rats with alloxan-induced diabetes. Treatment with *S. grandiflora* extract at dosages of 250 and 500 mg/kg significantly reduced diabetic symptoms compared to untreated diabetic rats, with statistical significance ($P < 0.01$). Additionally, both dosages of the extract significantly decreased serum levels of total cholesterol, triglycerides, SGOT, SGPT, and BUN compared to diabetic control rats ($P < 0.01$). These findings suggest that the 70% alcoholic extract of *S. grandiflora* flowers exhibits a dose-dependent antidiabetic effect. [83]

Kanagavalli et al. (2023) assessed the efficacy of copper oxide nanoparticles (CuO NPs) synthesized using *S. grandiflora* leaves in inhibiting amylase and glucosidase enzymes and their potential antibacterial activity. The CuO NPs exhibited strong inhibitory effects against both enzymes, indicating potential antihyperglycemic activity. Moreover, the NPs demonstrated significant antibacterial activity against various pathogens, including Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa. The study highlighted the promising biological activities of Sg-CuO NPs, suggesting their potential for treating bacterial infections associated with hyperglycemia. [84]

Abdullah et al. (2023) developed and evaluated fortified yogurts containing extracts from *Cinnamomum verum, Elettaria cardamom, Beta vulgaris*, and *Brassica oleracea*. The sensory evaluation revealed favourable acceptability of the fortified yogurts, which also exhibited enhanced shelf life compared to plain yogurt. Phytochemical analysis showed the presence of bioactive compounds with angiotensin-converting enzyme (ACE) inhibitory activity in the flavoured yogurts. These findings suggested that the bioactive

substances in the fortified yogurts may have potential health benefits, including ACE inhibition. [85]

Al-Harbi et al. (2021) the research investigated the mechanisms by which methanolic Beetroot extract (BE) exhibits anti-dyslipidemic and hepato-protective effects in a rat model of type-2 diabetes mellitus (T2DM). The results demonstrated that treatment with BE led to significant reductions in serum levels of aspartate and alanine aminotransferases, hepatic malondialdehyde, tumor necrosis factor, interleukin-6, and mRNA expression of Bax, cleaved caspase-3, and SREBP 1/2. Additionally, BE markedly increased hepatic levels of total glutathione, superoxide dismutase, and mRNA expression of Bcl2 and PPAR in both normal and T2DM rats. These findings suggest that BE protects against hepatic steatosis and liver damage through its hypoglycemic, insulin-sensitizing, and antioxidant properties, and by upregulating PPARα. [86]

Kumar et al. (2020) the investigation focused on assessing the anti-diabetic and hematinic properties of *Beta vulgaris* juice in an experimental animal model induced with alloxan. Diabetes, which involves disturbances in glucose regulation, was the main focus of the study. The diabetic control rats showed notably higher fasting blood glucose levels in comparison to the normal control rats.However, rats administered Beta vulgaris juice demonstrated hematinic and anti-diabetic efficacy on days 7, 14, 21, and 28 post-treatment. The study indicated that the anti-diabetic effect of *Beta vulgaris* juice, while slightly less potent than insulin treatment, yielded statistically significant results ($p < 0.001$). [87]

Bolkent et al. (2020), the researchers investigated the impact of a traditional Chinese medicine herb, known for its hypoglycemic properties, on pancreatic β cells and blood glucose levels. According to the study, diabetic subjects exhibited a reduction in β cell count within the Islets of Langerhans, decreased secretion, wider intercellular spaces, and some β cells showed swelling of the granular endoplasmic reticulum cisternae. In streptozotocin-induced hyperglycemic rats, the herbal extract effectively decreased blood glucose levels without affecting body weight or blood glucose levels in non-diabetic subjects. The most significant reduction in blood glucose levels occurred after 42 days of treatment. Additionally, diabetic rats treated with the herb showed a notable increase in body weight compared to untreated diabetic rats. [88]

Isabela Micheletti Lorizola et al. (2021) in study explored the potential benefits of supplementing mice on a high-fat diet with Beetroot stalks and leaves, which are often discarded despite containing bioactive flavonoids known for their anti-inflammatory and antioxidant properties. The researchers used male Swiss mice aged six weeks, dividing them randomly into five groups: a standard diet group (CT) and a high-fat diet group (HF). The HF group was further subdivided into three treatment groups, each receiving beet stalks and leaves prepared using different methods (oven-dehydrated, lyophilized, or extracted). While hepatic triglyceride levels showed no significant changes, supplementation with beet stalks and leaves did result in a modest improvement in glucose homeostasis and a reduction in TNF protein levels. However, the precise mechanism underlying the observed glucose homeostasis enhancement with HFSL treatment remains uncertain, prompting further investigation into whether it involves increased pancreatic insulin production or enhanced glucose absorption by skeletal muscle and white adipose tissues. [89]

Helmy, S.et al.(2021) A study was conducted to investigate the potential anti-diabetic properties of leaf extracts from *Purslane (P), Chard (CHA), and Chicory (CHI)* in streptozotocin-induced diabetic rats. The study involved oral administration of various combinations of these extracts over a 40-day period, at a dose of 250 mg/kg body weight. A control group received Metformin at 100 mg/kg body weight for comparison. The study measured insulin levels, Fructosamine levels, fasting blood glucose, and oral glucose tolerance. Significant findings revealed that extracts rich in Purslane or Chicory demonstrated pronounced hypoglycemic effects compared to Metformin. Additionally, these extracts led to improvements in liver and pancreatic histology in diabetic rats. These findings suggest that the combination of leaf extracts may hold promise as natural treatments for managing diabetes. [90]

Haewook Han et al. (2015). In their review, Haewook Han et al. covered various aspects related to kidney stone formation, including epidemiology, mechanisms, diagnosis, pathophysiology, and techniques for assessing stone risks in both new and follow-up patients. The review concluded that preventing kidney stones involves individualized management of medical and dietary care, considering the unique risks associated with each type of stone. Identifying and addressing risk factors play crucial roles in preventing the recurrence of kidney stones. Dietary oxalate, found in foods such as Spinach, Beets,

and Rhubarb, may contribute to stone formation by increasing oxalate excretion in urine, thereby raising the risk of calcium oxalate stones. [91]

3.1 RESEARCH GAP:

The World Health Organization (WHO) emphasizes the substantial public health issue posed by the global diabetes epidemic, particularly in Southeast Asia and the Western Pacific regions. In India, approximately 77 million adults aged 18 and older are affected by type 2 diabetes, with an additional 25 million individuals at risk of developing the condition due to prediabetes. Frighteningly, over half of diabetes cases remain undiagnosed, which can result in serious health complications if left untreated.Projections indicate that the current 171 million global cases of diabetes could escalate to 366 million by 2030. Thus, urgent exploration of novel medications, preventive as well as therapeutic approaches is imperative to effectively manage this pervasive metabolic disorder.

Traditional synthetic treatments for diabetes, including Biguanide and Sulphonylurea, aim to enhance glucose absorption by peripheral cells or act as Insulinotropic Secretagogues for pancreatic cells. However, these treatments are often associated with significant limitations, such as high costs and adverse effects like Hypoglycemia, weight gain, gastrointestinal issues, and liver toxicity. Despite the variety of available therapies, achieving complete recovery from diabetes remains elusive.

The WHO has identified approximately 21,000 medicinal plants globally, many of which have demonstrated Antidiabetic properties and other related health benefits. Given the multifactorial nature of diabetes and its association with various disorders, a comprehensive therapeutic approach utilizing medicinal plants may hold promise for managing this complex condition.

Natural products are still considered a valuable source of pharmaceutical goods and one of the best depositories of novel structurally bioactive chemicals. Over 25 % of current medicine is comprised of natural ingredients.Merely 15% of higher plants have been studied for potential biological activity.Herbal treatments are an effective way to cure diabetes, even in the absence of modern antidiabetic drugs.Throughout the world,numerous herbal treatments are used to cure diabetes.The substances present in herbal remedies and plant based medications are typically thought to be less hazardous and safer than those found in synthetic challenger.Numerous medications are marketed today as a result of studies done on medicinal plants. The combination of medicinal plants has emerged as a significant approach to harnessing their medicinal potential. By

combining two or more medicinal plants, synergistic effects can be unlocked, leading to enhanced therapeutic outcomes. Many traditional systems of medicine, such as Ayurveda, have long utilized formulations combining various therapeutic plants. It is believed that these combinations work by exerting diverse mechanisms of action and potentially augmenting each other's effects.

In developing countries, there is a growing demand for herbal medicines for primary healthcare. This demand arises not just due to their affordability but also because of their societal and cultural approval, suitability for the human body, and reduced side effects in contrast to synthetic drugs.Consequently, traditional plants that are readily available, particularly those with synergistic effects when combined, are chosen for research and development.

When medicinal plant extracts are combined, the beneficial effects of the formulation are often amplified, while potential side effects, if any, are mitigated. This combination approach is deemed superior to using isolated ingredients alone. Hence, this study aimed to create and evaluate the effectiveness of a polyherbal blend containing phytochemicals sourced from *Sesbania grandiflora* leaf powder (Fabaceae) and *Beta vulgaris* root powder (Chenopodiaceae) in a predetermined proportion.

The objective was to enhance the formulation's antidiabetic properties through this novel combination. To date, there has been no formulation available containing these herbal drugs together for their antidiabetic activity. Thus, this study represents an attempt to develop an herbal formulation and investigate its antidiabetic properties, filling an important gap in existing research.

4.1 AIM:

The aim of the research work is to develop an Antidiabetic polyherbal formulation and to evaluate the pharmacognostic, phytochemical, HPTLC fingerprinting, and pharmacological aspects of antidiabetic activity.

4.2 OBJECTIVES:

The present research plan covers the following objectives:

- Selection of the appropriate plants that are useful in the treatment of diabetes mellitus, can assure easy availability of raw materials, and are cheap, through a literature survey of these plants, viz., *Beta Vulgaris* and *Sesbania Grandiflora*
- To obtain authentic standard raw material for selected drugs. Proper collection, identification of plants, and authentication.
- Evaluation of preliminary standardization parameters of crude drugs.
- Compatibility study and qualitative estimation of herbal extracts w.r.t. HPTLC
- Optimization of extract combination by performing OGTT test.
- *In* vivo antidiabetic activity of optimized combination.
- To design, develop, and formulate safe, efficient polyherbal formulations.
- Evaluation of Various Batches of Polyherbal Formulation
- Stability Study of Polyherbal Formulation

4.3 PLAN OF WORK:

1. Literature Survey
2. Procurement and authentication of the crude drugs
3. Standardization and Evaluation of crude drugs
    - Macroscopic examination
    - Microscopic examination

- Micromeretic parameters

4. Extraction of Phytoconstituents

- Physicochemical evaluation
- HPTLC Fingerprinting

5. Compatibility study

6. Optimization of combination of extract by OGTT

7. Acute toxicity study.

8. *Invivo* study of Polyherbal formulation

9. Development and Evaluation of suitable Polyherbal formulation

10. Stability studies.

PLANT PROFILE

Figure 5.1: ***Sesbania Grandiflora*** plants

5.1 MATERIAL USED

Sesbania grandiflora leaves powder, *Beta Vulgaris* root powder

Chemicals**:** Alcohol, Phloroglucinol and conc. HCL, Sudan red, Iodine solution, Picric acid etc.

5.2 EQUIPMENT AND INSTRUMENT USED:

Microscope with Camera Megvision, Bulk And Tapped Density Apparatus, Furnace, Hot Air Oven, Soxhlet Apparatus, Rotary evaporator, UV spectrophotometer, IR-spectrophotometer.

5.3 PLANT PROFILE

5.3.1 *Sesbania Grandiflora* [91-95]

Synonyms: - Vegetable Hummingbird, Katurai, Agati, or West Indian pea.

Family: Fabaceae

Common name: Hadga, Scarlet Wistaria Tree, Petai Belalang, Red Wisteria, Agathi, Sesban, and Vegetable Hummingbird.

Vernacular Names: Sesban, Vegetable Hummingbird, Bakphul, Corkwood Tree, Scarlet Wisteria

Plant taxanomy

Subfamily: Faboideae

Genus: Sesbania

Kingdom: Plantae

Order: Fabales

Tribe: Sesbanieae

Description:

Sesbania grandiflora is a swiftly growing, perennial leguminous tree that typically grows to a height of 10–15 meters. It can be either deciduous or evergreen and generally lives for about 20 years. The roots are heavily nodulated and can develop floating roots under waterlogged conditions. The trunk is straight with minimal branching. The leaves are pinnately compound, up to 30 cm long, consisting of 20–50 oblong leaflets measuring 1-4 cm in length and 0.5–1.5 cm in width. The flowers are found in axillary racemes and appear in various colors including white, yellowish, pink, or red.The pods are glabrous, hanging vertically, measuring 50–60 cm long, and contain 15–50 dark brown seeds, each approximately 5 mm long and 2.5–3 mm wide.

Parts Used: Bark, Leaves, Flowers, Tender fruits, whole plant.

Propagation and Cultivation:

Sesbania grandiflora flourishes in warm and humid conditions typical of lowland tropical regions. The ideal habitat for this species is typically found in regions below 1,000 meters above sea level. It thrives in areas where temperatures range from 22 to 30 °C annually, receiving between 2,000 to 4,000 mm of rainfall. However, it has been observed to survive in environments with as little as 800 mm of annual precipitation.This tree grows optimally in sunny spots with fertile, moist, and well-drained soil, but it is also adaptable to light sandy, medium, heavy clayey and low-fertility soils, thriving best in soil with a pH of around 5.5.

Agricultural Benefits:

Sesbania grandiflora stands out for its capacity to generate high-quality cellulose raw material efficiently, making it an ideal candidate for pulpwood production with a short

rotation period of 3–4 years. The tree is capable of blooming year-round, and there are several recognized varieties. It forms a symbiotic relationship with specific soil bacteria, which create nodules on its roots to fix atmospheric nitrogen. This nitrogen is utilized partly by the *Sesbania grandiflora* itself and also aids surrounding plants.

Flowering and Propagation:

Sesbania grandiflora, known for its year-round blooming with red, white, or near-white flowers, is typically spaced 10–12 feet (3–3.6 meters) apart during planting. Propagation primarily occurs through seeds, which generally exhibit quick germination without specific treatments, although scarification can enhance this process. Seedlings are occasionally nurtured in polythene bags or containers to facilitate better establishment. These trees are typically planted singly or in rows with a spacing of 1-2 meters, often along fence lines, field edges, and the edges of rice paddies to enhance agricultural productivity.

Properties and Uses:

Sesbania grandiflora is highly regarded for its value as a palatable fodder, particularly beneficial for ruminants due to its rich nitrogen content. The seeds contain up to 6.5% nitrogen, while the foliage ranges from 3.0% to 5.5%, making it an excellent supplement for low-quality roughages. The dry matter digestibility of the foliage falls between 65% and 73%, with relatively low crude fiber content (5–18%) and notable concentrations (0.30% to 0.45%) of saponins and tannins. While there is limited information on anti-nutritional factors, *Sesbania grandiflora* foliage is generally considered non-toxic, although caution is advised when feeding it to monogastric animals, as it has been linked to mortality in chickens. Each gram of *Sesbania grandiflora* typically contains 14–20 seeds.

The wood of *Sesbania grandiflora* is characterized as white, soft, and lacking durability, with a low specific gravity of 0.42 kg/dm2, making it unsuitable for use as fuel wood.

Ayurvedic description [95]

अगस्त्यम्

सिध्दोऽगस्तिः पंक्तिपत्रो मृदुशिंबी महारुहः ।

अगस्त्यस्योद्‌य यावत् सपुष्प इव दृश्यते ।। शि.

अगस्त्यः पित्तकफजिच्चातुर्थिकहरो हिमः ।

रुक्षो वातकरस्तिक्तः प्रतिश्यायनिवारणः ।।

तत्पुष्पं पीनसश्लेष्मपित्तनक्तान्ध्यनाशनम् । भा. प्र.

अगस्त्यपत्रं कटुकं सतिक्तं गुरूकृमिघ्नं विशदं कफघ्नम् ।

कण्डूहरं शोणितपित्तहारि स्यात् सूक्ष्ममुष्णं मधुरं विषघ्नम् ।। (कै. नि.)

मुनिशिंबी सरा प्रोक्ता बुध्दिदा सचिदा लघुः ।

पाककाले तु मधुरा तिक्ता चैव स्मृतिप्रदा ।।

त्रिदोषशूलकफहृत् पांडुरोगविषापनुत् ।

शोषगुल्महरा प्रोक्ता सा पक्वा रूक्षपित्तला ।। नि. र.

वृषागस्त्ययोः पुष्पाणि तिक्तानि कटुविपाकानि क्षयकासहराणि च ।

अगस्त्यं नातिशीतोष्णं नक्तान्धानां प्रशस्यते । सु. सू. ४६.

Chemical constituent:

The plant is rich in various chemical constituents such as tannins, coumarone, steroids, triterpenes, isoflavonoids (including isovestitol and sativan), betulinic acid, flavonoids, and medicarpin. Cyanidin and delphinidin glucosides are found specifically in the flowers, while alpha-ketoglutaric, oxaloacetic, and pyruvic acids are present in pollen and pollen tubes. The primary chemical constituents found in the plant include alkaloids, flavonoids, glycosides, tannins, anthraquinones, steroids, pholoba tannins, and terpenoids. Key compounds found in the plant contribute to its diverse medicinal properties. These compounds include isovestitol, medicarpin, and sativan, which are isoflavonoids, along with betulinic acid, classified as tannin. These components collectively provide antibacterial, antifungal, antioxidant, anti-urolithiatic, anticonvulsant, anxiolytic, and hepatoprotective properties. Moreover, the plant extract contains alkaloids, phenolics, tannins, triterpenoids, and sterols, highlighting its broad pharmacological potential.

Roots- Isovestitol, Medicarpin, Sativan, Betulinic acid.

Bark- Compounds such as β-amyrin, lupeol, stigmasta-4, 2, 2-dien-3-one, stigmast-4-en-3-one, kaurenoic acid, and stigmasterol have been identified. [96-97]

Table 5.1: Chemical constituents present in *Sesbania Grandiflora* leaves

Sr. No.	Chemical Constituent	Structure	Activity
1	Kaempferol	HO, O, OH, OH, OH, O	Significant inhibitory effects on α-amylase, alongside antibacterial and anxiolytic properties.
2	Astragalin	HO, O, OH, O, OH, O, O, OH, HO, OH, OH	Effective remedy for diabetic testicular function impairment, with antibacterial and anti-inflammatory actions
3	Quercetin	HO, O, OH, OH, OH, OH, O	Antidiabetic and antioxidant agent
4	Callitrin	O, O	Antidiabetic and antioxidant agent
5	Rhamnocitrin	O, O, OH, OH, OH, O	Potent anti-tumor activity
6	Chrysin-dimethylether	O, O, O, O	Potent efficacy against tumors.

7	Sativan		Antituberculotic action
8	Loliolide		Exhibits antidiarrheal, antibacterial, anti-inflammatory, and anthelmintic properties.
9	Isovestitol		Antibacterial, antifungal, anti-urolithic, anticonvulsant, anxiolytic, hepatoprotective
10	Medicarpin		Antibacterial, antifungal, anti-urolithic, anticonvulsant, anxiolytic, hepatoprotective
11	Betulinic acid		Antibacterial, antifungal, anti-urolithic, anticonvulsant, anxiolytic, hepatoprotective
12	Scarbroside		Strong anti-inflammatory activity
13	Carisone		Bioactivity not reported
14	Kolavonic acid		Bioactivity not reported

Ethnomedical uses of *Sesbania grandiflora* L. [98-102]

In the Siddha system of Indian traditional medicine, *Sesbania grandiflora* is used for treating various health conditions such as anaemia, bronchitis, fever, headache, ophthalmia, nasal catarrh, inflammation, leprosy, gout, and rheumatism.

- **Pods and Leaves**: These elements demonstrate properties such as fighting cancer, acting as antioxidants, protecting the heart, preventing ulcers, safeguarding the liver, reducing inflammation, combating worms, and managing diabetes.Furthermore, they find application in treating colic and various skin conditions.
- **Seeds:** The seeds possess stimulant, emmenagogue, and astringent properties. They are also employed to treat diarrhoea, act as a diuretic, induce vomiting, reduce fever, relieve constipation, and as a tonic.
- **Roots:** used as a poultice to reduce inflammation and fever. The root juice, along with honey, is used as an expectorant for catarrh.
- **Barks:** It is used to treat smallpox and other eruptive fevers. It is regarded for its tonic and febrifuge characteristics and is used to treat ulcers in the oral cavity and gastrointestinal system, as well as thrush and infantile gastric diseases.
- **Leaves**: In Ayurvedic medicine, leaves are utilized to address a wide range of conditions. They are employed to manage epileptic fits, act as a tonic and anthelmintic, and possess properties such as antigout, antileprosy, diuretic, laxative, antioxidant, antiurolithiatic, anticonvulsive, antiarthritic, anti-inflammatory, antibacterial, and anxiolytic effects. The leaf juice finds application in treating bronchitis, cough, vomiting, wounds, ulcers, diarrhoea, and dysentery. Additionally, leaves are traditionally used for nasal catarrh, nyctalopia, and cephalalgia.
- **Flowers**: Flowers contain beneficial nutrients like calcium, iron, and Vitamin B, which contribute to their use in treating various conditions such as nasal catarrh, headaches, and stuffy noses. They have properties that make them effective emollients and laxatives. The juice extracted from flowers is known to enhance vision. Furthermore, flowers show potential in the development of anti-plaque dental products like toothpaste and mouthwash. Research has also found that a specific

compound from the flowers can selectively induce programmed cell death in leukemic cells.

- **Fruits**: In Ayurveda, fruits are used for their therapeutic benefits in treating conditions like anemia, bronchitis, fever, and tumors. They are recognized for their laxative properties, cognitive enhancement abilities, and are frequently suggested for pain relief and thirst quenching.

  Dosage: Use 10-20 ml of leaf juice, 50-100 ml of decoction, and 5-10 grams of flowers.

Anti-Diabetic Activity: The aqueous extract derived from *Sesbania grandiflora* leaves has been demonstrated to lower elevated blood glucose levels and enhance lipid profiles in diabetic rats induced by streptozotocin (STZ). Notably, this effect is exclusive to diabetic rats and does not notably affect normal rats. Furthermore, the substance has been noted to lower blood sugar and levels of glycosylated haemoglobin, alongside boosting insulin and haemoglobin levels.

Anti-Ulcer Activity: The ethanolic extract of the bark of *Sesbania grandiflora* has been shown to prevent acute gastric injury in rats. The extract significantly prevents lesions induced by stress and nonsteroidal anti-inflammatory drugs.

Antioxidant and Anti-Urolithiatic Activity: The study investigated the potential of *Sesbania grandiflora* in preventing kidney stone formation by assessing calcium and oxalate deposition in the kidneys, kidney weights, and urinary excretion of calcium and oxalate. It also evaluated in vivo antioxidant parameters including lipid peroxidation, glutathione reductase, and catalase activity. Results indicated that *Sesbania grandiflora* juice exhibited significant scavenging activity against nitric oxide and 2-diphenyl-2-picrylhydrazyl free radicals. Additionally, the leaf juice demonstrated effective anti-urolithiatic effects against calcium oxalate stones and exhibited antioxidant properties.

Anticancer and Chemopreventive Activity

The study examined the anticancer effects of SF2 (Sesbania Fraction 2), a protein fraction extracted from *Sesbania grandiflora* flowers. SF2's impact was assessed on murine ascites tumor cell lines as well as different human cancer cell lines. Results indicated that SF2 significantly suppressed cell proliferation and triggered apoptosis in both Dalton's

lymphoma ascites (DLA) cells and SW-480 colon cancer cells. This apoptotic process was characterized by DNA fragmentation and the externalization of phosphatidylserine.In animal trials using ascites and solid tumor models, SF2 administration extended the lifespan of tumor-bearing mice and decreased tumor volume, underscoring its potential as an anticancer treatment. These results suggest that SF2 shows promise as a candidate for further development in anticancer therapies.

Anxiolytic and anticonvulsive activity

Researchers investigated the anticonvulsant properties of *Sesbania grandiflora* leaves using various animal seizure models. They employed bioassay-guided separation to identify the active fraction responsible for these effects. The study results showed that the benzene acetate (BE) fraction, obtained from the acetone-soluble part of a petroleum ether extract, significantly extended the seizure latency period in mice triggered by pentylenetetrazole (PTZ) and strychnine (STR).Additionally, it decreased the duration of tonic hind leg extension observed during seizures induced by maximum electroconvulsive shock (MES).Additionally, a triterpene-containing fraction from *S. grandiflora* exhibited broad-spectrum anticonvulsant activities along with anxiolytic properties.

Hepatoprotective Activity

The study aimed to investigate the hepatoprotective effects of an ethanolic extract derived from *Sesbania grandiflora* leaves, administered orally at a dose of 200 mg/kg/day over a period of 15 days. The research focused on mitigating hepatotoxicity induced by erythromycin estolate (800 mg/kg/day) in rodent models. Results indicated that treatment with the *sesbania* extract significantly reduced elevated levels of serum enzymes (aspartate transaminase, alanine transaminase, and alkaline phosphatase), bilirubin, cholesterol, triglycerides, phospholipids, free fatty acids, plasma thiobarbituric acid reactive substances, and hydroperoxides observed in rats treated solely with erythromycin estolate.These findings suggest that sesbania extract provides significant protection against erythromycin estolate-induced hepatotoxicity, comparable to the hepatoprotective effects of silymarin, a well-known liver protection agent.

Antimicrobial Activity

This study explored the antimicrobial properties of three traditional Thai flower vegetables—*Sesbania grandiflora, Senna siamea,* and *Telosma minor*—for their potential

use in treating gastrointestinal issues. The flowers were subjected to a water extraction procedure at a ratio of 1:2 (flower to water), with continuous shaking over a week to obtain crude extracts. These extracts underwent assessment for antimicrobial properties against Bacillus cereus, Escherichia coli, and Staphylococcus aureus through the disc diffusion technique. It was observed that the antimicrobial efficacy of the extracts reached its maximum after seven days across all three types of flowers.Notably, the extract demonstrated a particularly strong inhibitory effect on Staphylococcus aureus, showing the largest inhibition zone in the tests.

Analgesic and antipyretic activity

The study aimed to explore the analgesic and antipyretic properties of *Sesbania grandiflora* flowers. Various extracts (petroleum ether, ethyl acetate, and ethanol) were prepared and assessed for their analgesic effects in albino rats using the Hot Plate and Tail Flick methods. According to the results, the ethyl acetate extract demonstrated significant analgesic and antipyretic effects compared to the petroleum ether and ethanol extracts.

Antibacterial activity

The antibacterial effectiveness of *Sesbania grandiflora* leaf extracts was assessed to determine their inhibitory activity against various bacterial strains and their minimum inhibitory concentrations (MICs). Significant inhibitory activity was observed across all extracts against the tested bacterial strains. The ethanol extract displayed notable effectiveness particularly against methicillin-resistant strains and dermatophytes. Additionally, the antimicrobial effectiveness increased proportionally to the concentration of the extracts, with the ethanol extract displaying the most potent inhibitory properties. This heightened efficacy can be attributed to the presence of alkaloids, tannins, saponins, phenols, and steroids in the plant extracts, which are widely recognized for their antibacterial properties.

5.3.2 *Beta Vulgaris* [92,93, 94,103-104]

Synonyms: *Beta vulgaris* L. (red beet), *Beta cicla* (chard), *Beta maritime*.

Family: Chenopodiaceae/Amaranthaceae.

Common name: Beet, Beetroot, Chard, Sugar beet, Spinach, Swiss chard.

Vernacular Names: Beet (Punjabi), Bit (Malayalam), Bita (Marathi), Bitagacha

(Bengali), Carkkarai valikilan kuceti (Tamil), Cuqandar (Hindi), Dumpamokka (Telugu), Gajarugadde (Kannada), Salada (Gujarati)

Plant taxanomy

Subfamily: Betoideae

Genus: Beta

Kingdom: Plantae

Order: Caryophyllalles

Tribe: Beteae

Figure 5.2: *Beta Vulgaris* L.Root

Parts Used: Root, Leaves

Types of beetroot: Each of the four distinct types of *Betavulgaris* isused differently:

Table 5.2: Different types of *Beta Vulgaris L*

Sr. No	Type of Beet Root	Description	Photo	Important source
1	Garden beet	Thick fleshy globular to long and tampered taproot, dark purpulish Red skin colour		Riboflavin ,manganese, antioxide
2	Swiss beet, leaf beet group	Leaves are green, white to yellow to red stalks		Riboflavin, iron, vitaminA,C
3	Sugar beet	A taproot characterized by its conical shape, white color, and fleshy texture, featuring a flat crown.		Rich source of sucrose
4	Mangel wurzel	These roots, typically large and spherical in shape, come in white, yellow, or orange hues, and primarily cultivated as animal feed.		Rich source of sucrose

Description

Beta vulgaris is an herbaceous biennial plant, although it may sometimes exhibit perennial characteristics. It typically has leafy stems that reach heights of 1-2 meters. The leaves are generally heart-shaped, with wild varieties measuring 5–20 cm in length, while cultivated varieties can have considerably larger leaves. The plant produces flowers in dense spikes; each individual flower is small, with a diameter of 3–5 mm, green or occasionally tinged reddish, and consists of five petals. These flowers are wind-pollinated. The fruit manifests as a cluster of hard nutlets. The roots of *Beta vulgaris* are typically deep red-purple, although less common varieties can have golden yellow or red-and-white striped roots.

Propagation and Cultivation

Climate

Beta vulgaris grows best in cool climates, yielding high-quality roots known for their high sugar content and deep internal color. Nonetheless, extended periods of cold weather can impede the plant's development.The plant exhibits moderate frost tolerance. Roots developed at relatively high excellent colour and quality.

Soil Requirements

Beta vulgaris prefers well-drained loams and sandy loams, with an optimal pH range of 6.3 to 7.5. The plant flourishes in neutral, moist, and fertile soil, provided it is not excessively limey or acidic.

Propagation

Beta vulgaris is typically propagated by seeds. Plant the seeds at a depth of around 1.5 cm and space them approximately 7 cm apart.The rows should be spaced 30 to 40 cm apart. Once the seedlings reach a height of 3 to 5 cm, they should be thinned to a spacing of 7 to 10 cm, ensuring only one seedling remains in each spot by removing the weaker ones. *Beta vulgaris* typically takes about two months to mature, from sowing to harvest.

Table 5.3: Description of *Beta vulgaris*

Plant	Sprawling perennial plant upto 60cm (2 ft) high
Leaves	Darkgreen,leathery,shiny rosette leaves with wavy & rough Triangular lower leaves and narrow and oval upper leaves. Grow 20–40cm(7.9–15.7in) in length
Fruit	Enclosed by the leathery and in curved perianth, and is immersed in the swollen, hardened perianth base
Root	Swollen and fleshy long main red root
Flowers	Green and tiny with the sepals thickening and hardening
Seed	The horizontal seed is lenticular,2–3 mm, with a red-brown, Shiny seed coat. The seed contains annular embryo and copious perisperm

Chemical Constituent:

Beetroot contains a variety of naturally dynamic compounds, such as Betalains (counting betacyanins and betaxanthins), Flavonoids, Polyphenols, Saponins, and Inorganic nitrate (NO3). Moreover, it may be a great source of fundamental minerals counting Potassium, Sodium, Phosphorus, Calcium, Magnesium, Copper, Zinc, and Manganese.

Table 5.4: Chemical constituent present in *Beta Vulgaris* root

Sr. No	Chemical constituent	Structure	Activity
1	Kaempferol	HO, O, OH, OH, OH, O	Antibacterial properties and has been found to possess anxiolytic effects. Additionally, it acts as a notable inhibitor of a-amylase.

2	Catechol		Antioxidant
3	Gallicacid		Antioxidant ,anti-inflammatory,antineoplastic
4	Ferullicacid		antioxidant
5	Myrecitin		Stronganti-oxidant, anticancer,antidiabetic and anti-inflammatory activities
6	Neringenin		Anti-dyslipidemic, anti-obesity anti-diabetic, antifibrotic.
7	Apigenin		Muscle relaxation, sedation, antioxidant,antiinflammatory, antiamyloidogenic, neuroprotective
8	Cinnamic acid		Antioxidant,antimicrobial , anticancer, neuroprotective, anti-inflammatory, antidiabetic.
9	Vulgaxanthin-I		Antioxidant pigments

10	Vulgaxanthin -II		Antioxidant pigments
11	Indicaxanthin		Antioxidants
12	Isobetanin		Antioxidants
13	Betaxanthin		Antioxidant ,anti-inflammatory, detoxification support

Ethno medicinal uses of *Beta Vulgaris* L:

These substances demonstrate promising therapeutic advantages for various metabolic conditions such as high blood pressure, diabetes, insulin resistance, and kidney impairment. They have the capability to lower both systolic and diastolic blood pressure, inhibit platelet aggregation, boost vascular and endothelial performance, enhance insulin sensitivity, and provide protective benefits for kidney function.

Dose:

There are no official dosage recommendations, but as per research, dose upto 500 mg Juice.

5.4 EXCIPIENT PROFILE [105]

5.4.1 Magnesium stereate

Table 5.5: Description of Magnesium stereate

Synonyms	Dibasic magnesium stearate, magnesium distearate; magnesi stearas magnesium octadecanoate, octadecanoic acid, magnesium salt, Stearic acid, magnesium salt, Synpro 90.
Chemical Name and CAS Registry Number	Octadecanoic acid magnesium salt [557-04-0]
Description	Fine, light white, precipitated or milled, having a faint odor of stearic acid and a characteristic taste, greasy to the touch and readily adheres to the skin.
Functional Category	Tablet and capsule lubricant.(0.25%and5.0%w/w)
Density (bulk)	0.159 g/cm^3
Density (tapped)	0.286 g/cm^3
Flowability	Poorly flowing, cohesive powder.
Specific surface area	1.6-14.8m^2/g
Solubility	Practically insoluble in ethanol, ethanol (95%), ethe and water; slightly soluble in warm benzene and warm ethanol (95%).
Melting range	117-150^{0}C
Stability and Storage Conditions	Magnesium stearate is stable and should be stored ina well-closed container in a cool, dry place.
Incompatibility	Incompatible with strong acids, alkalis, and iron salts

5.4.2 Talc

Table 5.6: Description of Talc

Synonyms	Altale, ES53b,hydrous magnesium calcium silicate; hydrous magnesium silicate magnesium hydrogen metasilicate
Chemical Name and CAS Registry Number	Talc [14807-96-6)
Description	very fine, white to grayish-white, odorless, impalpable, unctuous, crystalline powder
Functional Category	Anticaking agent, glidant, tablet and capsule diluent, tablet and capsule lubricant. Tablet and capsule diluents (5.0-30.0)
Specific surface area	2.41-.42 m^2 / g1
Solubility	Practically insoluble in dilute acids and alkalis, organic solvents and water.
Specific gravity	2.7-2.8
Stability and Storage Conditions	Talc should be stored in a well-closed container in acool dry place.
Incompatibility	Incompatible with quaternary ammonium compounds

5.4.3 Lactose, Anhydrous

Table 5.7: Description of Lactose, Anhydrous

Synonyms	Anhydrous 60M; Anhydrous Direct Tableting, Lactopress Anhydrous, SuperTab 21 AN,.Super Tab 22 AN
Chemical Name and CAS RegistryNumber	3O-f-o-Galactopyranosyl-(1-4)-8-D-glucopyranose [63-42-3]
Description	White to off-white crystalline particles or powder.
Functional Category	Directly compressible tablet excipient; dry powder inhaler carrier; lyophilization aid
Solubility	Soluble in water; sparingly soluble in ethanol (95%) and ether
Melting point	232.0°C
Stability and Storage Condition	It should be stored in a well-closed container in a cool, dry place.
Incompatibility	Lactose anhydrous is incompatible with strong oxidizers

5.4.4 Microcrystalline cellulose

Table 5.8: Description of Microcrystalline cellulose

Synonyms	Avicel PH; Cellets,Celex; cellulose gel, Celphere ,Ceolus KG ,crystalline cellulose E460
Chemical Name and CAS Registry Number	Cellulose [9004-34-6]
Description	a white, odorless, tasteless, crystalline powder composed of porous particles.
Functional Category	Adsorbent, suspending agent, tablet and capsule, diluents, tablet, disintegrant.
Solubility	Slightly soluble in 5% w/v sodium hydroxide solution; practically insoluble in water, dilute acids,and most organic
Uses	Tablet disintegrant-5-15
Stability and Storage Condition	Material should be stored in a well-closed container ina cool, dry place.
Incompatibility	Microcrystalline cellulose is incompatible with strong oxidizing agents.

5.5 EXPERIMENTAL DETAILS

Throughout the various experiments, the following drugs, excipients, chemicals, and reagents were used:

5.5.1 Materials

Table 5.9: List of Materials used

Name of materials	Supplied / Gifted by
Beta Vulgaris Root	Local market, Ale Junnar.
Sesbania Grandiflora Leaves	Rural area, Ale, Junnar.
Methanol	Research-Lab FineChem, Mumbai
Phloroglucinol	Research-Lab FineChem, Mumbai
Hydrochloric acid	Research-Lab FineChem, Mumbai
Potassium Mercuric Iodide	Research-Lab FineChem, Mumbai
Iodine	Hilab Chemicals, Shrirampur
Potassium Iodide	Hilab Chemicals, Shrirampur
Picricacid	Research-LabFine Chem, Mumbai
Potassium bismuth iodide	Hilab Chemicals, Shrirampur
α-napthol	Reliance Scientific, Pune
Sulphuric acid	Research-Lab Fine Chem, Mumbai
Fehling solution A	Reliance Scientific, Pune
Fehling solution B	Reliance Scientific, Pune
Copper acetate	Ecolab, Pune
Glacial acetic acid	Research-Lab Fine Chem, Mumbai
Benedict’s Solution	Research-Lab Fine Chem, Mumbai
Chloroform	Molychem, Mumbai
Ammonia	Research-Lab Fine Chem, Mumbai
Pyridine	Research-Lab Fine Chem, Mumbai
Sodium nitroprusside	Sahydri Scientific, Islampur
Sodium hydroxide	Research-Lab Fine Chem, Mumbai
Millon’sreagent	Research-Lab Fine Chem, Mumbai
Copper sulphate	Sayadri Scientific Islampur

Potassium hydroxide	Research-Lab Fine Chem, Mumbai
Ninhydrin reagent	Research-Lab Fine Chem, Mumbai
Acetone	Research-Lab Fine Chem, Mumbai
Potassium hydroxide	Research-Lab Fine Chem, Mumbai
Phenolphthalein	Research-Lab Fine Chem, Mumbai
Ferric chloride	Pure chem., Pune
Gelatin	Research-Lab Fine Chem, Mumbai
Lead acetate	Research-Lab Fine Chem, Mumbai
Bromine	Research-Lab Fine Chem, Mumbai
Potassium bromide	Research-Lab Fine Chem, Mumbai
Silicagel	Molychem, Mumbai
Ethanol	Vighnahar Karkhana,Shiroli
Ether	Molychem, Mumbai
Quercetin	Ajinkya enterprices, Pune
GallicAcid	Ajinkya enterprices, Pune
Starch	Sayadri Scientific Islampur
Phosphate Buffer	Research-Lab Fine Chem, Mumbai
Toluene	Research-Lab Fine Chem,
Formicacid	Research-Lab Fine Chem,
Ethyl acetate	Research-Lab Fine Chem, Mumbai
Anisaldehyde	Sayadri Scientific Islampur
Potassium Iodide	Hilab Chemicals, Shrirampur
Streptozotocin	Shree chemicals, Pune
Lactose	Sayadri Scientific, Islampur
Microcrystalline cellulose	Sayadri Scientific, Islampur
Talc	Sayadri Scientific, Islampur
Magnesium stereate	Sayadri Scientific, Islampur
Glibenclamide tablet	Emcure Pharmaceuticals Ltd.

5.5.2 Instrument/Equipment

Table 5.10: List of Instruments / Equipment's used

Name of the Equipment / Instrument	Make	Model
Electronic Balance	Shimadzu	AY220
Soxhlet assembly	Sahydri Scientific	1000
Rotary evaporator	Medica instruments	Evator
Chromatography visualization (UV Cabinet)	Camag-winCATS	-
Digital melting point apparatus	Veego	Vpmds,Nashik
Sonicator	Citizen	CD-4820
pH meter	Elico,India	LI613
UV-visible double beam spectrophotometer	Shimadzu	UV-1800
FTIR spectrophotometer	PerkinElmer	Spectrum65
Chromatogram development chambers	Camag	
Microscope	Imicron,India	RadicalRM-3
Centrifuge	Remi	R8C
High speed centrifuge	Beckman Coulter	Allegra 64RC centrifuge USA
Precoated silicagel 60F254 TLC plates(10×10cm, layerthickness 0.2mm)	E. Merk KGaA, Darmstadt,Germany	-
The HPTLC system consisted of Linamat V Autosprayer connected to a nitrogen cylinder,a twint rough chamber(10×10cm),aDerivation chamber and a plate heater	Camag,Muttenz,Switzerland	-
Refractometer	Abbe	-
Binocular dissecting microscope	Labomed	Vision2000
Magnetic Stirrer	RemiMotar	1MLH

Rheometer	Stress-Tech	Reologica,Sweden
Viscometer	Brookfield Engineering Lab	MLVT115
Stability chamber	Biotechno lab	BTL
Immersion device for derivatisation	Camag	-
TLC / HPTLC plate heater	Camag	-
Digital Homogeniser	Remi	RQT-127A/D

5.5.3 Software

1. Graphpad Instatv3.1.
2. winCATS Windows Xp version1.4.6

5.6. COLLECTION AND AUTHENTICATION OF PLANTS

The plant materials utilized in this study included leaves of *Sesbania Grandiflora* sourced from the local area of Ale, and roots of *Beta Vulgaris* Linn purchased from the local market in Alephata, Pune.

Authentication of Plant:

The plant materials were identified and authenticated by Dr. Ranangdale Savita Sanjay Kumar, who holds a M.Sc. and Ph.D. and is a Fellow of the Indian Association of Angiosperm Taxonomy (FIAAT) and the Academy of Agri-Artists (FAA). Dr. Kumar is a botanist at the Department of Botany, Balasaheb Jadhav College of Arts, and Commerce & Science, affiliated with Pune University in Maharashtra. Additionally, the authentication was corroborated by Dr. R. K. Chaudhary, a Senior Scientist at the Agharkar Institute.Herbarium collection numbers 619 (*Beta vulgaris*) and 622 (*Sesbania grandiflora* Linn.) were assigned to these specimens. Voucher specimens with numbers 23-93 (*Sesbania grandiflora*) and 23-94 (*Beta vulgaris*) have been deposited in the laboratory.

5.7 PHARMACOGNOSTIC EVALUATION [106-107]

5.7.1 Morphological and Microscopical Evaluation

The morphological characteristics of specific plant parts including color, scent, size, shape, and taste were examined. Microscopic sections of *Sesbania Grandiflora* leaves and *Beta Vulgaris* roots were prepared and stained with various reagents to confirm their identification. These sections were then observed using a compound microscope.

5.7.2 Powder Microscopy and Fluorescence Analysis

Microscopy

Insert needle tip deeply into coarse powder after wetting with water. Gently press the needle tip into the water drop on the glass slide, mix it well, and then cover it with the cover slip. Using filter paper, remove any extra water from the cover slip's edge. This was seen under magnification.

Fluorescence Analysis

The fluorescence properties of leaf and root powder were investigated using visible and ultraviolet light. This process included soaking the powder in different reagent solutions and examining its reaction under specific wavelengths with a UV chamber.

5.8 MICROMETRIC EVALUATION [108-109]

5.8.1 Angle of repose:

Angle of repose:

The flow characteristics of the physical mixtures in all formulations were evaluated using the angle of repose technique at a consistent height. A funnel with an inner diameter of 10 mm was positioned 2 cm above a flat surface. Approximately 10 grams of each sample was slowly poured through the funnel until it formed a cone-shaped heap at the funnel's outlet. The base of the heap was marked, and the average radius of the powder cone was measured. Subsequently, the angle of repose (θ) was determined using the formula:

$$\tan\theta = \frac{h}{r}$$

Where: θ is the angle of repose,
h is the height of the powder cone,
r is the average radius of the base of the powder cone.

Density apparatus:

Density measurements were performed using a custom density apparatus comprising a graduated cylinder attached to a mechanical tapping device driven by a rotating cam mechanism. A carefully measured quantity of powder sample was introduced into the cylinder under controlled circumstances.

Bulk density:

Bulk density measurements were performed as follows: 25 g of the specimen was carefully transferred through a glass funnel into a 100 ml graduated cylinder. The volume displaced by the specimen was then measured, and the bulk density was calculated using the formula:

$$\text{Bulk density} \left(\frac{\text{g}}{\text{ml}}\right) = \frac{\text{Weight of sample in grams}}{\text{Volume occupied by the sample}}$$

Tapped density:

The tapped density of each specimen was assessed by transferring 25 grams of the substance into a 100 ml graduated cylinder via a glass funnel. Similarly, the barrel was gently tapped from a height of 2 inches until the volume stabilized. The volume of the sample was then recorded, and the tapped density was calculated using the following formula:

$$\text{Tapped density} \left(\frac{\text{g}}{\text{ml}}\right) = \frac{\text{Weight of sample in grams}}{\text{Volume occupied by the Sample}}$$

5.9. PHYSICOCHEMICAL EVALUATION [106-109]

5.9.1 Determination of Foreign Particles

Procedure:Dispense 100 grams of the designated drug sample as per the monograph and spread it evenly across a thin surface.Examine the sample visually or with a 6X magnifying lens to detect any extraneous material. Remove the foreign matter, weigh it separately, and determine its percentage in relation to the total sample weight.

5.9.2 Determination of Moisture Content

Procedure: First, measure around 10 grams of the substance and transfer it into an evaporating dish whose weight has been measured beforehand. Heat the sample at 105 °C for 5 hours, and then measure its weight. Continue this process, drying the sample and measuring its weight hourly, until consecutive measurements show no more than a 0.25% difference, indicating a stable weight.To confirm the constant weight, dry the sample for an additional 30 minutes, cool it in a desiccator for 30 minutes, and ensure that successive weights differ by no more than 0.01 grams.

Figure 5.3: Determination of loss of Drying (Moisture Content)

5.9.3 Determination of extractive values [106]

Figure 5.4: Alcohol soluble extractive value

A) Alcohol soluble extractive value:

Measure out 4 grams of the dried plant material, usually in powdered form, and place it in a glass-stoppered flask. Add 100 ml of 90% ethanol to the flask. Allow the mixture to steep for 24 hours, unless otherwise specified. Shake the flask intermittently during the first 6 hours and then let it stand without disturbance for the remaining 18 hours. After the steeping period, evaporate 25 ml of the filtrate in a tarred flat-bottomed Petri dish using a water bath until dry. Dry the residue at 105°C for one hour in a hot air oven and then cool it in a desiccator before weighing. Repeat this drying and weighing process until a constant weight is achieved. Finally, calculate the percentage of ethanol-soluble extractives based on the initial weight of the dried plant material using the appropriate formula.

$$\text{\% of Alcohol soluble extractive value} = \frac{B - A \times 4}{W} \times 100$$

Where,

W= wt. of plant material taken (g)

B= wt. of dish + residue (g)

A= empty wt. of the dish (g)

B) Water Soluble Extractive Value:

Figure 5.5: Water Soluble Extractive Value

Procedure:

Measure out 4 grams of the dried plant material, usually in powdered form, and place it in a glass-stoppered flask. Add 100 ml of 90% ethanol to the flask. Allow the mixture to macerate for 24 hours, unless otherwise specified. Shake the flask occasionally during the first 6 hours, and then let it stand undisturbed for the remaining 18 hours. After maceration, evaporate 25 ml of the solution to dryness in a tarred flat-bottomed petri dish using a water bath. Dry the resulting residue at 105°C for one hour in a hot air oven, and then cool it in a desiccator before weighing. Repeat the drying and weighing until a constant weight is achieved. Calculate the percentage of ethanol-soluble extractives relative to the initial weight of the dried plant material using the appropriate formula.

$$\text{\% of Water Soluble Extractive Value } = \frac{B - A \times 4}{W} \times 100$$

Where,

A = empty wt. of the dish (g).

B = wt. of dish + residue (g)

5.9.4: Determination of Ash Values [107]

Ash consists of inorganic compounds including phosphates, carbonates, and silicates of sodium, potassium, magnesium, and calcium. Sometimes, the crude drug contains additional inorganic constituents like calcium oxalate, silica, and carbonates, which can impact the "total ash value." To address this, these substances are eliminated through acid treatment, as they are soluble in hydrochloric acid. The resulting "acid-insoluble ash value" is subsequently calculated.

Figure 5.6: Furnace for Ash value

a. **Determination of Total Ash Values**

Two grams of powdered materials, namely *Sesbania grandiflora* leaves and *Beta vulgaris* Linn roots, were separately placed in a silica crucible that had been preheated and weighed. The powdered substances were distributed evenly and accurately weighed. The samples were incrementally combusted, with temperature not exceeding 550°C, until complete carbon liberation. Following cooling in a desiccator, the crucible underwent reweighing, and the ash content was determined by subtracting the crucible's empty weight from the total ash-laden weight.

b. **Determination of Acid Insoluble Ash**

To determine the acid-insoluble ash content, 1 gram of the sample underwent ashing, followed by blending with 25 ml of diluted hydrochloric acid.This mixture was gently heated to a temperature range of 70–80°C for duration of 5 minutes. After heating, the solution was filtered through ashless filter paper, rinsed with hot water, and heated until a consistent weight was reached. The acid-insoluble ash percentage was subsequently calculated based on the weight of the air-dried sample.

c. **Determination of water-soluble Soluble Ash**

Moreover, 1 gram of the ash sample was subjected to bubbling with 25 ml of water for 5 minutes. The resulting insoluble residue was filtered using ash-free filter paper, washed with hot water, and subsequently heated at a temperature below 550°C for 15 minutes. The weight of this residue was subtracted from the initial ash weight to determine the water-soluble ash content. Based on these measurements, the percentage of water-soluble ash relative to the air-dried material was calculated.

5.9.5 Extraction of Phytochemicals [106-108]

Sesbania Grandiflora

The leaves of *Sesbania Grandiflora* were harvested and dried in the shade. Once dried, they were coarsely powdered using a blender and then sieved through a 100-mesh sieve. The resulting powder was stored in an airtight container. One hundred grams of the powder were subjected to extraction using various solvents including water, acetone, ethanol, and methanol, using the Soxhlet extraction method until the powder loss its color

completely. The extracts were concentrated under reduced pressure using a rotary evaporator set at 40°C. The concentrated material was then freeze-dried at -20°C for 12 hours using a lyophilizer. The resulting lyophilized extracts were stored in sealed containers within a desiccator for further analysis.

Beta vulgaris

The root material of *Beta Vulgaris* was acquired from a local market and prepared by thorough cleaning and peeling. After drying, the root pieces were coarsely powdered using a mixer grinder and sifted through a 100-mesh sieve. The resulting powder was stored in an airtight container. Subsequently, 100 grams of this powder underwent extraction with solvents such as water, acetone, ethanol, and methanol using the Soxhlet extraction method until the powder was fully decolorized. The extracts were concentrated under vacuum using a rotary evaporator at 40°C. Subsequently, the concentrated extract underwent freeze-drying at -20°C for 12 hours followed by further lyophilization using a lyophilizer. The resulting lyophilized extracts were stored in sealed containers within a desiccator for future analysis.

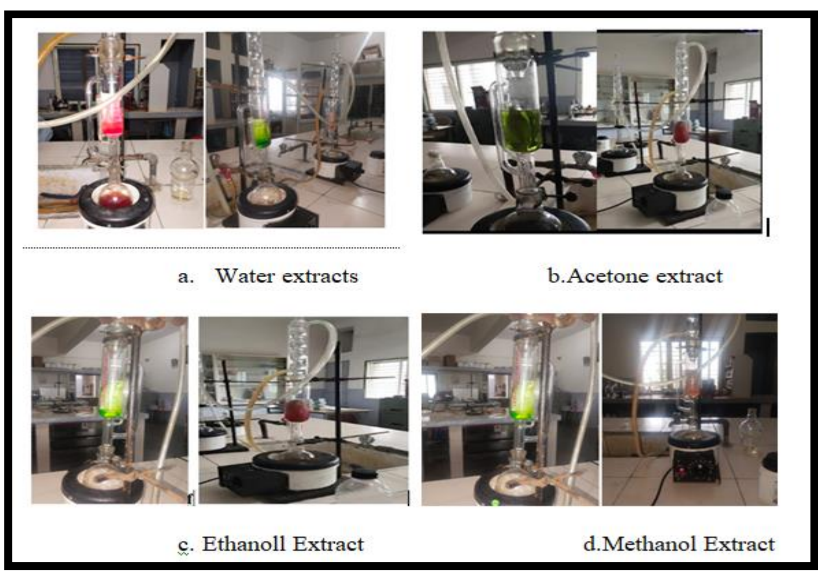


Figure 5.7: Extraction of *Sesbania grandiflora* and *Beta vulgaris* powder.

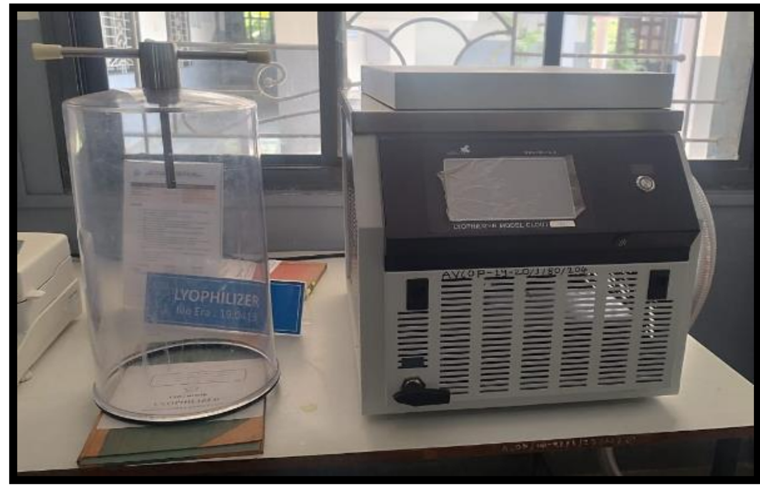


Figure 5.8: Lyophillizer

5.9.6 Phytochemical Evaluation [106-107]

Chemical analyses of the drug extracts were conducted to detect the presence of various compounds such as alkaloids, carbohydrates, glycosides, saponins, proteins, amino acids, phytosterols, fixed oils, fats, phenolic compounds, tannins, gums, mucilages, and flavonoids. These analyses yielded comprehensive data on the phytochemicals contained within the crude drug samples.

Procedure:

5.9.6.1 Detection of Alkaloids

Table 5.11: Chemical tests for Detection of Alkaloids

Test	Principle	Observation
Dragendorff's reagent	Extract reacts with Dragendorff's reagent (Potassium-bismuth-iodide solution)	Reddish-brown precipitate.
Mayer reagent	Extract reacts with Mayer reagent (Potassium-mercuric-iodide solution)	Cream colour precipitate.
Wagner reagent	Extract reacts with Wagner reagent (Iodine-potassium-iodide solution)	Brown colour precipitate.
Hager reagent	Extract reacts with Hager reagent (Saturated solution of picric acid)	Yellow colour precipitate.

5.9.6.2 Detection of Carbohydrates

Table 5.12: Chemical tests for Detection of Carbohydrates

Test	Principle	Observation
Molish test	Treatment with alcoholic α napthol and concerntrated sulphuric acid	Violet ring formed at junction of two liquids
Fehling's Test	Boil extract with Fehlings Solution A and Fehlings solution B	Red precipitate formed
Benedict's Test	Few ml of a sample solution is placed in a test tube. Two ml of Benedict's reagent (a solution of sodium citrate and sodium carbonate mixed with a solution of copper sulfate) is added. The solution is then heated in a boiling water bath for three minutes	A reddish precipitate will form within three minutes.
Barfoed's Test	Heat extract with Barfoed's reagent (copper acetate in acetic acid)	Monosaccharides produce the red precipitate in 2 to 3 minutes; disaccharides produce the precipitate in 10 minutes.

5.9.6.3 Detection of Glycosides

Table 5.13: Chemical tests for Detection of Glycosides

Test	Principle	Observation
Brontrager's Test	Powdered drug is dissolved in few ml dilute sulphuric acid and mixture is boiled. Filtered the solution, filtrate is then extracted with organic solvent like chloroform. Chloroform layer is separated and to that ammonia is added.	The ammonia layer gives rose pink colour
Legal's Test	This test is performed by using pyridine and alkaline sodium nitroprusside	Red colour solution

5.9.6.4 Detection of Saponins

- The extracts were agitated with distilled water. The presence of foam suggests saponins are present.

5.9.6.5 Detection of Proteins and Amino Acids

Table 5.14: Chemical tests for Detection of Proteins

Test	Principle	Observation
Biuret Reaction	Sample solution is mixed with 10% sodium hydroxide and 0.1% copper sulphate solution.	The solution becomes violet or pink colour.
Ninhydrin Tes	Sample solution is mixed with 0.1% freshly prepared Ninhydrin solution and then boil	Violet or purple colour.
Millons test	Treat extract with Millons reagent	Formation of white precepitate

5.9.6.6 Detection of Phytosterols

Table 5.15: Chemical tests for Detection of Phytosterols

Test	Principle	Observation
Salkowski's Test	On adding a few drops of conc. Sulphuric acid and allowing the solution to stand	Formation of brown ring
Liebermann Burchard's test	The extract was treated with few drops of acetic anhydride, boiled and cooled and add conc. sulphuric acid	Formation of a bluish green colour solution

5.9.6.7 Detection of Fixed Oils and Fats

Table 5.16: Chemical tests for Detection of Fixed Oils and Fats

Test	Principle	Observations	Indication
Saponification Test	Treatment with alcoholic potassium hydroxide and phenolphthalein	Formation of soap	Presence of fixed oils and fats
Spot Test	Pressing extracts between filter papers	Oil stains observed	Presence of fixed oils

5.9.6.8 Detection of Tannins

- **Ferric Chloride Test:** To identify phenolic compounds and tannins, the extracts were combined with a 5% ferric chloride solution. The appearance of a blue-black or green-black color indicated the presence of these compounds.
- **Gelatin Test:** The extracts were combined with a 1% gelatin solution in a 10% sodium hydroxide mixture. The presence of tannins was confirmed by the formation of a white precipitate.

5.9.6.9 Detection of Phenolic Compounds

- **Lead Acetate Test:** To detect phenolic compounds, the extracts were treated with a 10% lead acetate solution. A significant white precipitate indicates their presence.
- **Alkaline Reagent Test:** The presence of flavonoids is confirmed by a yellow fluorescence when the extracts are treated with a 10% ammonium hydroxide solution.
- **Aqueous Bromine Test:** Tannins are identified by adding an aqueous bromine solution to the extracts, resulting in the formation of a yellow precipitate.

5.9.6.10 Detection of Gums and Mucilage

- The extracts were dissolved in a mixture of distilled water and alcohol. The appearance of a white precipitate suggested the presence of gums and mucilage.

5.9.6.11 Detection of Flavonoids

- **Alkaline Reagent Test:** Treatment with magnesium hydroxide solution followed by dilute acid shows a colour change from intense yellow to colourless, indicating the presence of flavonoids.
- **Shinoda's Test:** The observed color changes (from pink to crimson red, then green to blue) during treatment with magnesium and concentrated hydrochloric acid suggest the presence of flavonoids.

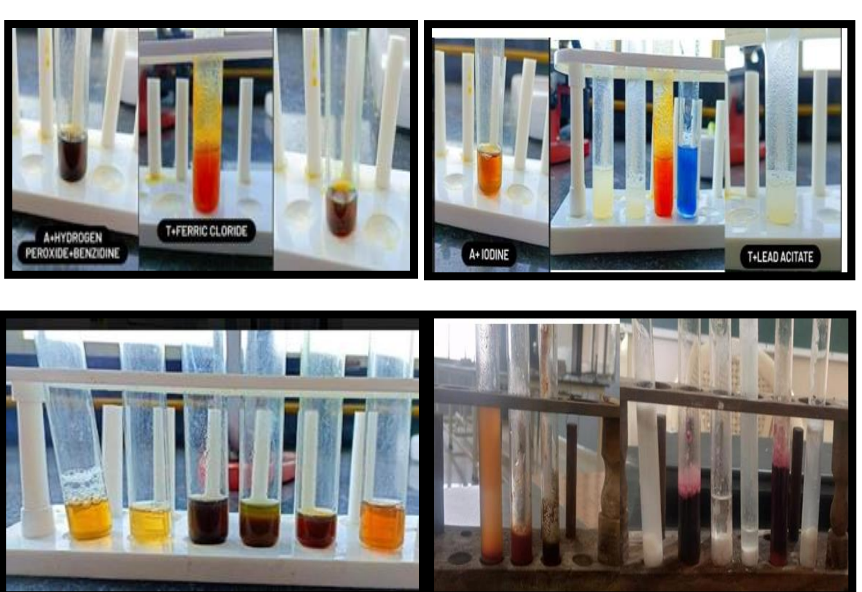


Figure 5.9: Preliminary Phytochemical Screening of Plant Extracts

5.9.7 pH of Extracts[108]

To ascertain the pH of the powdered extract, 1 gram of the powder was mixed with 10 milliliters of freshly prepared and cooled distilled water in a volumetric flask. The suspension was vigorously shaken for 5 minutes, followed by measurement of the pH of the resulting solution using a digital pH meter.

Figure 5.10: pH meter

5.10 DETERMINATION OF TOTAL PHENOLIC CONTENT [111]

The total phenolic content was assessed utilizing the Folin-Ciocalteu technique. To begin, each extract was dissolved in methanol, and the presence of phenolic compounds was signaled by a blue color change, with the absorbance being recorded at 760 nm via a spectrophotometer. The experiment was performed in triplicate for each extract. Gallic acid solutions with concentrations spanning 10 to 100 µg/ml were used to create standard curves. The total phenolic content was subsequently calculated using the linear regression equation derived from the calibration curve and reported as grams of Gallic acid equivalents per gram of extract (g GAE/g).

5.11 DETERMINATION OF TOTAL FLAVONOID CONTENT[111-112]

Total flavonoid content was determined spectrophotometrically at 510 nm. Each extract was dissolved in methanol, and the color change of the solution was monitored. Quercetin solutions ranging from 10 to 100 µg/ml were used to establish a standard curve. Analysis was conducted in triplicate for each extract. The total flavonoid content was quantified using the linear regression equation obtained from the calibration curve and presented as Quercetin equivalents per gram of extract (g QE/g).

Method:-

Fourier Transform Infrared (FTIR) spectroscopy is a widely used analytical technique that was employed using the KBr disc method for interpreting IR spectra. Approximately 1 mg of the compound under study was meticulously weighed and then ground with 70 mg of potassium bromide in a pristine agate mortar until achieving a fine powder consistency. Subsequently, the mixture was pressed into a pellet utilizing a potassium bromide holder. The IR spectra of the compounds were recorded over the spectral range of 400 to 4000 cm. FTIR spectroscopy facilitated the identification of specific absorption peaks in terms of their corresponding wave numbers, providing valuable insights into the molecular structure and functional groups present in the compounds analyzed.

Figure 5.11: FTIR spectroscopy

5.13 HPTLC FINGERPRINTING [110,113-117]

Purpose

The prime objectives of carrying out the study work were as follows:

- Beneficial for locating bioactive substances and indicators.
- Chromatographic fingerprints to identify the key components that make up a plant's active ingredients individually as well as in polyherbal formulation.

5.13.1 Introduction:

HPTLC is a useful technique for expanding chromatographic fingerprints to identify the key active components in medicinal plants. It offers better separation and resolution, yielding more reliable and reproducible results compared to TLC. A crucial aspect of accurate identification is analyzing the crude extract. HPTLC is particularly valuable in plant taxonomy for identifying species based on their secondary metabolites and serves as a phytochemical marker. Herbal identification through HPTLC fingerprinting is known to be linear, precise, and accurate. These fingerprints are essential for ensuring the purity of herbal products and identifying adulterants, thereby aiding in the assessment of plant constituents across the plant kingdom. Gallic acid (PubChem CID: 370) and Quercetin (PubChem CID: 5280343) are significant phenolic compounds commonly present in various mangrove plants, known for their potential health-promoting properties.This compounds have reported several pharmacological benefits like antioxidant, anti-inflammatory, antineoplastic gastrointestinal, neuropsychological, metabolic, and cardiovascular disorders including antidiabetic activity. Consuming foods high in polyphenols has been linked to a number of multitarget antioxidative activities thus far. In particular, GA, the chemical compound that all polyphenols share, has demonstrated encouraging outcomes in the treatment of DM and its comorbid

problems.

It has been proven that Quercetin lowers serum cholesterol levels as well as blood glucose, liver enzyme levels, and glucose content.Additionally, it has been reported to reduce oxidative harm, improving pancreatic-cell regeneration and result in generation of insulin.It is Quercetin is a promising template for the development of new antidiabetic drugs.

5.13.2 HPTLC chromatographic conditions

The drugs were applied onto precoated silica gel 60F254 plates (E. Merck, Darmstadt, Germany) using a LinomatV (CAMAG, Muttenz, Switzerland), with plates having a thickness of 0.2 mm and backed with aluminum. The bands were applied with a thickness of 6 mm using a solvent mixture composed of acid (5:4:1, v/v/v). The development was conducted using a CAMAG µL syringe (20 cm × 10 cm) in a twin trough glass chamber (CAMAG, Muttenz, Switzerland) containing a solvent mixture of toluene, ethyl acetate, and formic acid. Prior to development, the chamber was equilibrated with the solvent mixture for 15 minutes at room temperature (25 ± 2 °C) and a relative humidity of 60% ± 5%.

Sample preparation: The drugs were dissolved in methanol and incubated overnight for 24 to 48 hours. The sample was then concentrated using a rotary evaporator method to obtain the crude extract for analysis.

Chromatographic conditions for all samples

The following samples were taken for HPTLC fingerprintring

- *Sesbania grandiflora* leaf methanolic extract.
- *Beta vulgaris* L. root methanolic extract.
- Combination of *Beta vulgaris L. root and Sesbania grandiflora* leaf methanolic extract.

The conditions were chosen to enable simultaneous detection and confirmation of bioactive compounds, such as Quercetin and Gallic acid, which are commonly found in the environment and have significant pharmacological benefits in the medicinal field.

Table 5.17: Chromatographic condition

Solvent system	Toluene: ethyl acetate: formic acid (5:4:1)
Layer	Precoated silica gel 60 F 254 with a thickness of 0.2 mm aluminium backed plate.
Syringe	CAMAG μL syringe
Application volume	5
B and thickness	6mm
Development mode	Linear ascending
Chamber	20cm×10cm twin trough glass chamber
Standards	Gallic acid, Quercetin, Kameferol, Betalain
Nanometer	254nm and 366nm
Analysis	Lane analysis
Sample syringe	Linomat V
Running time	20 min
Software	Camag Linomate

5.14 *INVIVO* STUDIES

To study was conducted to evaluate efficiency of optimized polyherbal combination for Antidiabetic activity in Streptozotocin induced Diabetic Wistar Albino Rats

Objectives

The primary objectives of present investigation include:

- To prepare extracts of leaves *Sesbania Grandiflora* and root of *Beta Vulgaris* Linn.
- To perform OGTT for optimizing the combination of extracts leaves *Sesbania Grandiflora* and root of *Beta Vulgaris* Linn. for different ratio.
- To perform acute toxicity study.
- To study efficiency of polyherbal combination for antidiabetic activity along histopathology in Streptozotocin induced Diabetic Wistar Albino Rats.

5.14.1 Procurement of Animals

The study was conducted following approval from the Institutional Animal Ethics Committee (IAEC) of Vishal Institute of Pharmaceutical Education and Research, Brew. The study was registered under protocol number 1409/PO/RE/S/11/IAEC/2020-2021/07/01 and adhered to guidelines established by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), under the Ministry of Environment and Forests, Government of India. All procedures involving animals were conducted in accordance with ethical standards and followed the approved protocol number PIPH07/01 at Vishal Institute of Pharmaceutical Education and Research, Ale.

5.14.2 Standardisation and Optimization of Best Combination of Drug Ratio for Designing Formulation

For formulation designing various ratio of extract combination is considered and optimized combination will be considered for designing formulation containing the combination. The antihyperglycemic potential of different combinations of methanolic extracts from *Sesbania Grandiflora* and *Beta Vulgaris* (labeled as PHF1, PHF2, and PHF3) was assessed in the OGTT model using normal Albino Wistar rats at a dosage of 1000mg/kg. Among these extracts, PHF2 demonstrated the highest antihyperglycemic activity.Therefore, PHF2 drug combination with maximum antihyperglycemic was considered as optimized batch for designing of formulation further studies used different extract combinations were prepared for the formulation development and necessary excipients were added to design the formulation.

Table 5.18: Drug combination ratio

Formulation code	Combination of drug	Ratio
PHF1	MESG + MEBV	2:1
PHF2		1:1
PHF3		1:2

This study defines MESG as the methanolic extract obtained from *Sesbania grandiflora*, while MEBV represents the methanolic extract sourced from *Beta vulgaris*. An oral glucose tolerance test (OGTT) was conducted on rats that had fasted overnight. After the fasting period, glucose was administered at a dose of 2 g/kg to induce hyperglycemia. The rats were divided into five groups, each consisting of six animals (n=6):

- Group I, the normal control, received a pretreatment of 0.5% w/v carboxymethyl cellulose (CMC) solution.
- Group II was administered a glucose load and Glibenclamide at a dose of 5 mg/kg.
- Groups III, IV, and V were given single oral doses of 1000 mg/kg of PHF1, PHF2, and PHF3, respectively.

  - PHF 1 consisted of MESG and MEBV in a ratio of 2:1.

  - PHF 2 contained equal amounts of MESG and MEBV (1:1 ratio).

  - PHF 3 comprised MESG and MEBV in a ratio of 1:2.

Thirty minutes after administration, all animals were given an oral glucose dose of 2 g/kg. Blood samples were collected from the tail vein before the initial dosing and subsequently at 30, 60, 90, and 120 minutes after administering the glucose.The combination demonstrating the most significant antihyperglycemic effect, according to the OGTT results, was selected for further formulation. *Beta vulgaris* contains oxalic acid, which can combine with other compounds to potentially form kidney stones. To address this, urine samples were examined microscopically for the presence of uric acid and calcium oxalate crystals.[118-119]

5.14.3 Acute toxicity studies.

Acute toxicity studies were conducted on a polyherbal formulation in accordance with the revised draft guidelines 423 of the Organization for Economic Co-operation and Development (OECD). The study employed male Wistar rats, with three animals assigned to each dose group.

The procedure involved oral administration of escalating doses of plant extracts and the polyherbal formulation: 5, 50, 300, and 2000 mg/kg body weight. Rats were fasted overnight prior to dosing and monitored continuously for 24 hours. Behavioral observations included alertness, restlessness, irritability, fearfulness, as well as neurological assessments such as spontaneous activity, reactivity, response to touch and pain, and gait. Autonomic functions like defecation and urination were also monitored throughout the study period.

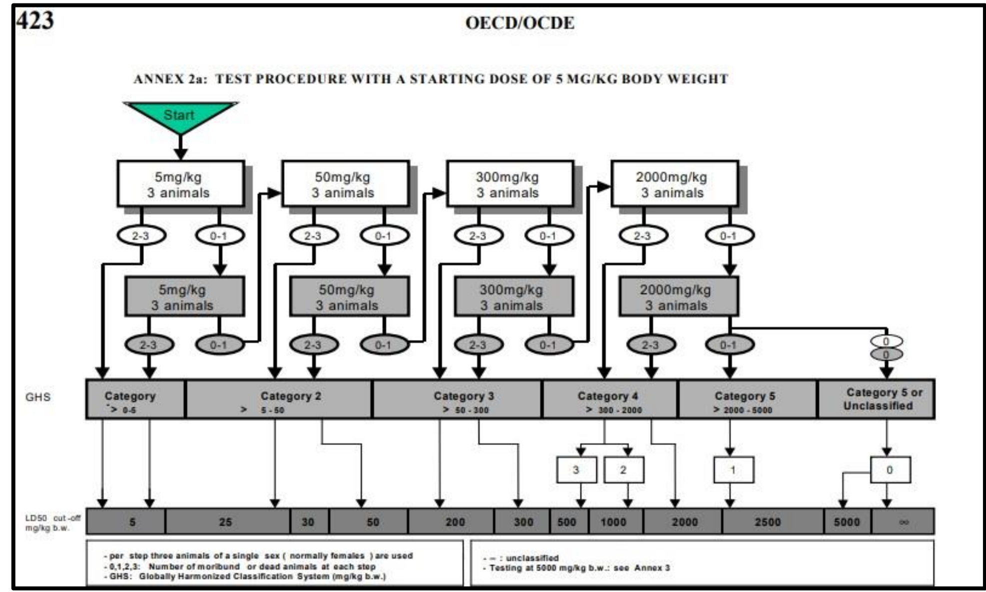


Figure 5.12: OECD guidelines for acute toxicity studies

After the initial 24-hour observation period, the animals were further monitored for mortality and general health for an additional 14 days. This study design allows for the assessment of acute toxicity effects, ensuring sufficient data collection to classify the test substance based on its acute toxicity profile. [120-121]

5.14.4 Experimental Design for Acute Toxicity Study

The acute toxicity investigation in Wistar albino rats adhered to the guidelines outlined by the Organization for Economic Co-Operation and Development 423 (OECD-423). Three male Wistar rats, each weighing between 210-250 g, were used in the study. These rats were housed in well-ventilated polypropylene cages under controlled environmental conditions (temperature: 21 ± 3 °C, humidity: 55 ± 10%, with a 12-hour light-dark cycle). The rats underwent a 7-day adaptation period with access to standard rodent pellets and water ad libitum before the experimentation phase. Prior to administration of the polyherbal formulation, the rats were fasted overnight. The formulation, suspended in 2 ml of sterile water, was orally administered at doses of 300 and 2000 mg/kg to the experimental groups, while the control group received water alone. Observations involved monitoring the rats individually for 30 minutes and periodically over the initial 24 hours to assess their behavior (alertness, irritability, anxiety), neurological responses (spontaneous movement, responsiveness, reaction to touch and pain), and autonomic changes (defecation and urination) throughout the 14-day experimental period.Parameters such as body weight on the 7th and 14th days, mortality, and other signs of toxicity were also recorded. [120-121]

5.14.5 *Invivo* Study for Antidiabetic Activity of Optimized Combination Experimental Design

A study conducted with a refined polyherbal blend in Wistar rats followed the guidelines outlined by the Organization for Economic Co-Operation and Development 423 (OECD-423). Male Wistar rats weighing between 210-250 g were selected and divided into five groups, each consisting of 6 rats, totaling 30 animals. The rats were housed in well-ventilated polypropylene cages under controlled environmental conditions: temperature maintained at 22±3°C, humidity at 55±10%, and a 12-hour light-dark cycle. Before the experiment began, the rats underwent a 7-day acclimatization period during which they were fed standard rodent pellets (Lab diet) and provided with unrestricted access to water. Throughout the 30-day experimental period (excluding acclimatization and euthanasia), the rats were fasted overnight prior to administration of the polyherbal formulation.

Diabetes was induced in overnight-fasted Wistar albino rats for the experiment. This was

achieved by administering a single intraperitoneal (i.p.) injection of Streptozotocin (STZ) at a dose of 45 mg/kg, dissolved in 0.1 M citrate buffer (pH 4.5). To prevent hypoglycemic mortality, a 5% w/v glucose solution was administered to the rats 12 hours after the STZ injection, followed by a return to a normal diet. Diabetes was confirmed by measuring fasting blood glucose levels 48 hours after the STZ injection. Glibenclamide was administered orally once daily for 30 consecutive days to assess its effects.Haemoglobin A1C (HbA1c) levels were measured 90 days after treatment to evaluate long-term glucose management.[123-125]

Administration of Optimized Combination:

According to OECD 423 guidelines and the outcomes of acute toxicity studies, the PHF 2 extract was prepared by suspending it in 1 mL of sterile water. This preparation was then orally administered to rats at reduced doses of 200 mg/kg (1/10th of the acute toxicity study dose) and 400 mg/kg (1/20th of the acute toxicity study dose) daily for duration of 30 days. A control group received sterile water as a vehicle.

Throughout the study duration, rats were provided unrestricted access to food commencing 4 hours subsequent to each administration of PHF 2. This experimental arrangement was designed to assess the subchronic toxicity characteristics of PHF 2 at reduced dosages over an extended timeframe, thereby ensuring the safety and examining the potential outcomes of prolonged exposure to this polyherbal formulation.

Grouping of Animals: To confirm diabetes in STZ-treated rats, fasting blood glucose levels were measured and rats with levels over 250 mg/dL were considered diabetic. These rats were then randomly assigned to Groups 2 through 5 for further experiments.

Group 1: Normal group: Water (Vehicle)

Group 2: Diseased Control: 45 mg/kg Streptozotocin (STZ).

Group 3: F200:45mg/kg Streptozotocin (STZ) & 200mg/kg of PHF 2 (F200) extract (1/10th of the dosage of acute toxicity studies.)

Group 4:F400: 45mg/kg Streptozotocin (STZ) & 400mg/kg of PHF 2 (F400) extract (1/20th of the dosage of acute toxicity studies.)

Group 5: Standard: 45 mg /kg Streptozotocin (STZ) & 5mg/kg Glibenclamide.

During the experimental phase, the weights of all animals were consistently documented. The observations encompassed monitoring for any significant alterations in skin condition, fur appearance, eye health, and mucous membrane integrity, as well as the presence of secretions or excretions and autonomic activity. Researchers also noted any changes in posture or unusual behaviors exhibited by the animals. The investigation aimed to evaluate signs of toxicity, if present, and track mortality rates and changes in body weight as measures of overall health and potential adverse effects associated with the test substance administration. [120,121,126-128]

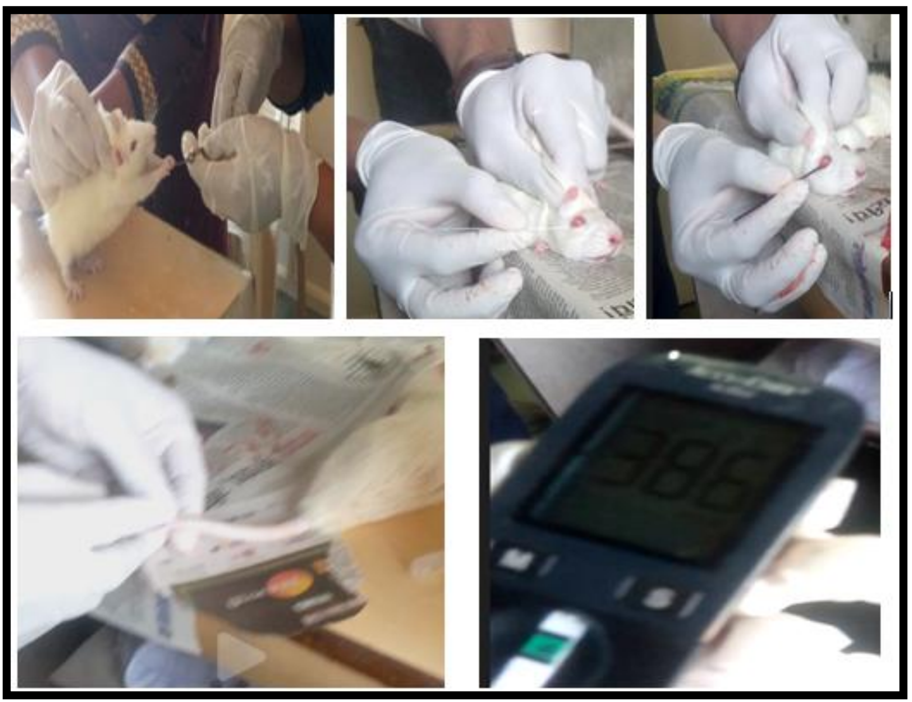


Figure 5.13 *Invivo* Studies

5.14.5.3 Haematological and Biochemical analysis

At the conclusion of the sub-acute toxicity study, all animals underwent an overnight fasting period and were euthanized in accordance with CPCSEA guidelines. Blood samples were collected using heparinized tubes from retro-orbital sinuses to evaluate a range of hematological parameters. These parameters included Total RBC Count, Hemoglobin levels, Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Total WBC Count, as well as differential counts for Polymorphs, Lymphocytes, Eosinophils, Monocytes, Basophils, and Platelet count.Blood samples were collected from groups treated with Control, PHF 2 (F200 and F400) at both low and high doses.

The samples were placed in sterile vials and analyzed for various biochemical parameters including Blood Glucose levels, Serum Creatinine, Serum Protein, Serum Albumin, Alanine transaminase (ALT), Aspartate amino transferase (AST), Blood Urea Nitrogen (BUN), Total Cholesterol, Triglycerides, HDL, LDL, VLDL Cholesterol, and Cholesterol/HDL Ratio.[128-139]

5.14.5.4 Histopathology Analysis

After completing the acute toxicity study, all animals were fasted overnight and euthanized in accordance with CPCSEA guidelines. The absolute weights of organs including the pancreas, liver, kidney, and spleen were measured for the Control group, as well as for the Low and High doses of PHF 2 treated groups. Samples of each organ were then fixed in 10% Formalin for further analysis. [128-139]

5.15 Development and evaluation of Polyherbal Tablet

5.15.1 Preparation of tablet

Figure 5.14: Tablet compression machine

The formulation development process included optimizing the combination of extracts to achieve effective ratios. Trial batches were prepared using excipients such as lactose, microcrystalline cellulose, talc, and magnesium stearate. Specifically, variations in the concentration of microcrystalline cellulose were investigated to evaluate their impact on

disintegration time. The formulation process began with trials to adjust binder ratios and determine appropriate excipient quantities, ultimately leading to procedure optimization. Methanolic extracts from *Sesbania Grandiflora* leaves and *Beta Vulgaris* roots were combined with a lactose binder. The mixture obtained was sieved through a mesh size 22 to achieve granules, which were dried using a tray dryer at 45 °C. After drying, magnesium stearate was added to lubricate the granules, forming a powder. Finally, tablets were prepared using the direct compression technique. [140-146]

Table: 5.19 Composition of Polyherbal formulation

Sr. No.	Ingredient	Quantity (in mg)				Uses
		F1	F2	F3	F4	
1.	***Sesbania Grandiflora* Extract**	100	100	100	100	Antidiabetic
2.	***Beta Vulgaris* Extract**	100	100	100	100	Antidiabetic
3.	Lactose	q.s	q.s	q.s	q.s	Diluent
4.	Microcrystalline Cellulose	40	50	60	70	Disintegrating agent
5.	Magnesium stearate	10	10	10	10	Lubricant
6.	Talc	10	10	10	10	Lubricant
7.	Total weight	500	500	500	500	

5.15.2 Preformulation studies [140-149] (Lachman-Liberman1999, USP2007)

The granules' preformulation parameters were ascertained, including bulk density, tap density, Hausner's ratio, Carr's index, and angle of repose.

5.15.2.1 Angle of repose

The flow characteristics of the physical blends were analyzed by determining their angle of repose using a fixed height method. In this approach, a tube with an inner diameter of 10 mm was positioned 2 cm above a surface. Approximately 10 grams of the sample were gently poured down the inner wall of the tube, forming a conical pile that extended to the tube's edge. A circle was marked around the base of the resulting powder cone, and its diameter was measured. The angle of repose was then calculated using the average diameter and the following formula:

$$\tan(\theta) = r\,h$$

Where: θ = Angle of repose.

r= Average radius of the powder cone.

h = Height of the pile.

Table 5.20: Flow properties and corresponding Angle of Repose

Angle of repose(θ)	Type of flow
25-30	Excellent
31-35	Good
36-40	Fair-aid not needed
41-45	Passable but may hang up
46-55	Poor
56-65	Very poor

Bulk Density:-

The bulk density was determined by placing 25 grams of the sample into a 100 ml graduated cylinder. The volume displaced by the sample was recorded, and the bulk density was then calculated using the appropriate formula.

$$\text{Bulk density (g/ml)} = \frac{\text{Weight of sample in grams}}{\text{Volume occupied by the sample}}$$

Tapped density

The tapped density of the substance was determined by transferring 25 grams through a glass funnel into a 100 ml graduated cylinder. The cylinder was gently tapped from a height of 2 inches until the volume stabilized. The final tapped volume of the sample was recorded, and the tapped density was then calculated using the formula:

$$\text{Tapped density (g/ml)} = \frac{\text{Weight of sample in grams}}{\text{Volume occupied by the sample after tapping}}$$

Compressibility index

The compressibility index assesses the flow properties of a powder by correlating its bulk density to its tapped density. Carr's compressibility index provides a useful standard for performing this assessment.

$$\text{Carr's index} = \frac{\text{TD} - \text{BD}}{\text{TD}} \times 100$$

Table 5.21: Grading of powders for their flow properties

(Carr's index)	Flow
5-15	Excellent
15-16	Good
*18-21	Fair to Passable
*23-35	Poor

Table 5.22: Compressibility Index and Hausner's Ratio for powder flow

Compresibility Index	Flow	Hausner's Ratio
<10	Excellent	1.00-1.11
11-15	Good	1.12-1.18
16-20	Fair	1.19-1.25
21-25	Passable	1.26-1.34
26-30	Poor	1.35-1.45
31-35	Very poor	1.46-1.59
>35	Very Very poor	>1.60

Hausner ratio

The Hausner ratio is a parameter utilized to evaluate the compaction tendency caused by vibration in the feed hopper. It is calculated by dividing the tapped density of a material by its bulk density. A lower Hausner ratio indicates better flowability, while a higher ratio indicates poorer flow properties.

$$\text{Hausner ratio} = \frac{\text{Tapped density}}{\text{Bulk Density}}$$

5.15.3 Post compression Evaluation of Polyherbal tablet

Post-compression evaluation of polyherbal tablets involved the examination of their physical appearance, consistency in dosage, variability in weight, disintegration time, and conformity with Indian pharmacopoeial criteria.

5.15.3.1 Organoleptic Characters

Organoleptic characteristics of PHF2 were examined for attributes such as color, appearance, odor, and taste, and recorded accordingly.

5.15.3.2 pH

pH levels were measured using a pH meter to determine the acidity or alkalinity of the tablets.

5.15.3.3 Friability Test

Friability testing was performed using a Roche friabilator. Twenty tablets were gathered for each test, collectively weighed, and subjected to rotational forces at 25 rpm for 4 minutes. Following the rotation, the tablets underwent thorough cleaning to remove any dust particles before being re-weighed. The percentage weight loss (% friability) was then calculated by comparing the initial weight with the final weight after testing.

$$\% \text{ friability} = \frac{a - b}{a} \times 100$$

Where,

a= collection weight before friability and

b=collective weight after friability**.**

5.15.3.4 Weight variation

Weight variation assessment involved individually weighing twenty tablets and calculating their average weight. Each tablet's weight was expected to fall between 90% and 110% of this average weight to meet standard requirements.

Table 5.23: The limits of the weight variation

Average weight of tablet	% deviation
80 mg or less	10
More than 80 mg but less than 250 mg	**7.5**
250 mg or more	**5**

5.15.3.5 Thickness and diameter

The thickness and diameter of each tablet were measured individually with a Digital Vernier Caliper (model Absolute Digimatic CD-6 11 CSX). These measurements ensure that the tablets maintain uniformity in size and shape.

5.15.3.6 Hardness

Tablets need to exhibit sufficient strength and resistance to breakage during manufacturing, packaging, and transportation processes. Hardness typically measures the tablet's ability to withstand crushing forces. The hardness of 10 tablets was evaluated using a Monsanto hardness tester, and the average hardness was calculated.

5.15.3.7 Disintegration time

The disintegration test utilized a digital microprocessor-based apparatus manufactured by Electrolab in Mumbai, India. For each tablet, it was positioned within a tube alongside a disk, and this assembly was immersed in a 1000 ml beaker filled with water. The water level was maintained at a consistent height, approximately 25 mm above the bottom of the beaker and 25 mm below the water surface. Throughout the test, the apparatus maintained a stable temperature of 37±2°C to replicate physiological conditions.

5.15.3.8 Stability Studies

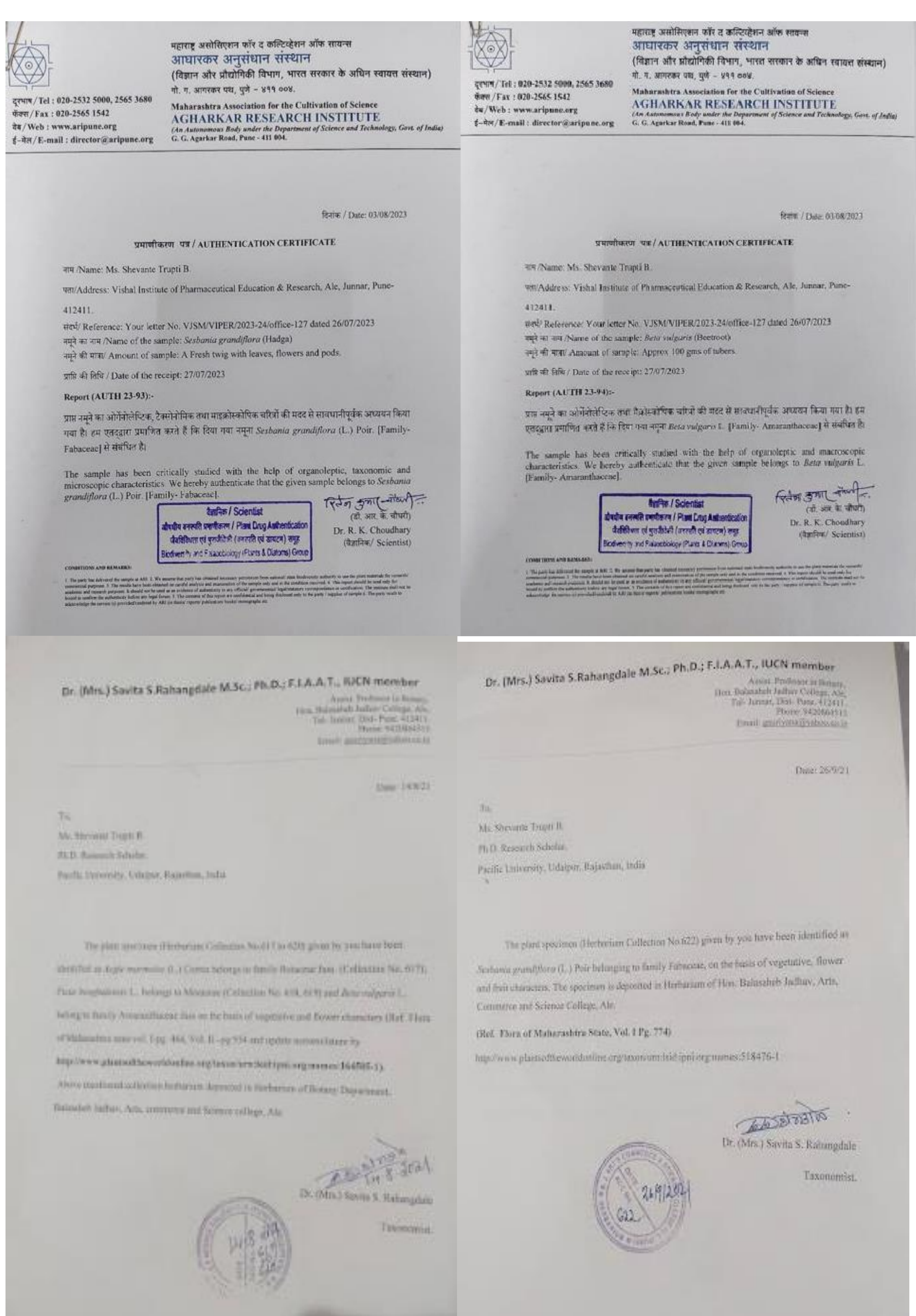
The stability investigations assess how well a dosage form maintains its characteristics over time. For the polyherbal tablets in this study, stability tests were conducted over a 30-day period under two distinct conditions: ambient temperature (25°C ± 2°C, relative humidity 60 ± 5%) and accelerated temperature (40°C ± 2°C, relative humidity 75 ± 5%). Evaluations were conducted on days 7, 15, and 30 during the study.

5.15.4 FTIR compatibility study

FTIR analysis was performed to assess the compatibility between the active ingredients and excipients. The procedure confirmed their compatibility, revealing functional groups such as phenolic, alcohol, alkenes, and nitride.

6.1 COLLECTION AND AUTHENTICATION OF PLANTS:

The leaves of *Sesbania Grandiflora* Linn were harvested locally in Ale, while the roots of *Beta Vulgaris* Linn were obtained from the Ale market in Pune. The authenticity of these plant materials was confirmed through identification by Dr. Ranangdale Savita Sanjaykumar, M.Sc., Ph.D., FIAAT, FAAB Sc, from the Department of Botany at Balasaheb Jadhav College of Art, Commerce & Science, affiliated with Pune University, Maharashtra. Further authentication was performed by Dr. R.K. Chaudhary, Senior Scientist at Agharkar Research Institute, a DST autonomous body in Pune under the Government of India. The herbarium collection assigned numbers 619 (*Beta Vulgaris*) and 622 (*Sesbania Grandiflora*) were used for the authenticated specimens, which are stored as laboratory voucher specimens numbered 23-93 for *Sesbania Grandiflora* and 23-94 for *Beta Vulgaris*.



महाराष्ट्र असोसिएशन फॉर द कल्टिव्हेशन ऑफ सायन्स
आघारकर अनुसंधान संस्थान
(विज्ञान और प्रौद्योगिकी विभाग, भारत सरकार के अधिन स्वायत्त संस्थान)
गो. ग. आगरकर पथ, पुणे - ४११ ००४.
Maharashtra Association for the Cultivation of Science
AGHARKAR RESEARCH INSTITUTE
(An Autonomous Body under the Department of Science and Technology, Govt. of India)
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दिनांक / Date: 03/08/2023

प्रमाणीकरण पत्र / AUTHENTICATION CERTIFICATE

नाम /Name: Ms. Shevante Trupti B.

पता/Address: Vishal Institute of Pharmaceutical Education & Research, Ale, Junnar, Pune-412411.

संदर्भ/ Reference: Your letter No. VJSM/VIPER/2023-24/office-127 dated 26/07/2023

नमूने का नाम /Name of the sample: *Sesbania grandiflora* (Hadga)

नमूने की मात्रा/ Amount of sample: A Fresh twig with leaves, flowers and pods.

प्राप्ति की तिथि / Date of the receipt: 27/07/2023

Report (AUTH 23-93):-

प्राप्त नमूने का ओर्गेनोलेप्टिक, टैक्सोनोमिक तथा माइक्रोस्कोपिक चरित्रों की मदद से सावधानीपूर्वक अध्ययन किया गया है। हम एतद्द्वारा प्रमाणित करते हैं कि दिया गया नमूना *Sesbania grandiflora* (L.) Poir. [Family-Fabaceae] से संबंधित है।

The sample has been critically studied with the help of organoleptic, taxonomic and microscopic characteristics. We hereby authenticate that the given sample belongs to *Sesbania grandiflora* (L.) Poir. [Family- Fabaceae].

वैज्ञानिक / Scientist
औषधीय वनस्पति प्रमाणीकरण / Plant Drug Authentication
जैवविविधता एवं पुराजैविकी (वनस्पति एवं डायटम) समूह
Biodiversity and Palaeobiology (Plants & Diatoms) Group

(डॉ. आर. के. चौधरी)
Dr. R. K. Choudhary
(वैज्ञानिक/ Scientist)

CONDITIONS AND REMARKS:

महाराष्ट्र असोसिएशन फॉर द कल्टिव्हेशन ऑफ सायन्स
आघारकर अनुसंधान संस्थान
(विज्ञान और प्रौद्योगिकी विभाग, भारत सरकार के अधिन स्वायत्त संस्थान)
गो. ग. आगरकर पथ, पुणे - ४११ ००४.
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दिनांक / Date: 03/08/2023

प्रमाणीकरण पत्र / AUTHENTICATION CERTIFICATE

नाम /Name: Ms. Shevante Trupti B.

पता/Address: Vishal Institute of Pharmaceutical Education & Research, Ale, Junnar, Pune-412411.

संदर्भ/ Reference: Your letter No. VJSM/VIPER/2023-24/office-127 dated 26/07/2023

नमूने का नाम /Name of the sample: *Beta vulgaris* (Beetroot)

नमूने की मात्रा/ Amount of sample: Approx 100 gms of tubers.

प्राप्ति की तिथि / Date of the receipt: 27/07/2023

Report (AUTH 23-94):-

प्राप्त नमूने का ओर्गेनोलेप्टिक तथा मैक्रोस्कोपिक चरित्रों की मदद से सावधानीपूर्वक अध्ययन किया गया है। हम एतद्द्वारा प्रमाणित करते हैं कि दिया गया नमूना *Beta vulgaris* L. [Family- Amaranthaceae] से संबंधित है।

The sample has been critically studied with the help of organoleptic and macroscopic characteristics. We hereby authenticate that the given sample belongs to *Beta vulgaris* L. [Family- Amaranthaceae].

वैज्ञानिक / Scientist
औषधीय वनस्पति प्रमाणीकरण / Plant Drug Authentication
जैवविविधता एवं पुराजैविकी (वनस्पति एवं डायटम) समूह
Biodiversity and Palaeobiology (Plants & Diatoms) Group

(डॉ. आर. के. चौधरी)
Dr. R. K. Choudhary
(वैज्ञानिक/ Scientist)

CONDITIONS AND REMARKS:

Dr. (Mrs.) Savita S.Rahangdale M.Sc.; Ph.D.; F.I.A.A.T., IUCN member

To,
Ms. Shevante Trupti B.
Ph.D. Research Scholar
Pacific University, Udaipur, Rajasthan, India

Dr. (Mrs.) Savita S. Rahangdale
Taxonomist.

Dr. (Mrs.) Savita S.Rahangdale M.Sc.; Ph.D.; F.I.A.A.T., IUCN member

Date: 26/9/21

To,
Ms. Shevante Trupti B.
Ph.D. Research Scholar
Pacific University, Udaipur, Rajasthan, India

The plant specimen (Herbarium Collection No.622) given by you have been identified as *Sesbania grandiflora* (L.) Poir belonging to family Fabaceae, on the basis of vegetative, flower and fruit characters. The specimen is deposited in Herbarium of Hon. Balasaheb Jadhav, Arts, Commerce and Science College, Ale.

(Ref. Flora of Maharashtra State, Vol. I Pg. 774)

http://www.plantsoftheworldonline.org/taxon/urn:lsid:ipni.org:names:518476-1

Dr. (Mrs.) Savita S. Rahangdale
Taxonomist.

Figure 6.1: Authentication Letter of Drugs

Preparation of Powders

Sesbania Grandiflora linn leaves powder

The plant leaves were dried naturally in the shade, ground into coarse powder, and kept in airtight containers. This coarse powder (60#) of the dried plants was stored in airtight containers for future pharmacognostic, physicochemical, and phytochemical analyses.

Beta Vulgaris linn root powder

The purchased Beet roots were washed to eliminate any adhering dirt, and the outer layer was peeled off before chopping the roots into small pieces. These pieces were then dried, ground into a coarse powder, sieved, and stored in airtight containers for subsequent pharmacognostic, physicochemical, and phytochemical evaluations.

Figure 6.2: *Sesbania Grandiflora* leaves powder and *Beta Vulgaris* root powder

6.2 PHARMACOGNOSTICAL EVALUATION

6.2.1 Morphological Evaluation:

Sesbania Grandiflora Linn

Morphological studies of *Sesbania grandiflora* Linn leaves indicate that they are regular and rounded, measuring 15-20 cm in length, with 10-20 or more pairs of opposite leaflets. The organoleptic evaluation of the powdered *Sesbania grandiflora* leaves revealed that the powder is dark green in color, has a slight odor, and possesses a characteristic taste.

Table 6.1: Description of *S.Grandiflora L.*

Tree	Small soft wooded upto 3–8m (10–26 ft) tall.
Fruit	Inversely arranged, measuring 2–4 cm in length and 10–15 mm in breadth. They are linear, oblong, mucronate, and annual, with lance-shaped or setaceous deciduous stipules. A mature compound leaf typically contains 10–20 pairs of leaflets.
Flowers	Oblong, ranging from 1.5 to 10 cm (1–4 inches) long, with two to four flowers arranged in a lax manner.
Pods	Slender, either falcate or straight, measuring 30–45 cm (12–18 inches) in length. The pods have a thick suture and contain approximately 30 seeds, each about 8 mm (0.3 inches) in size.

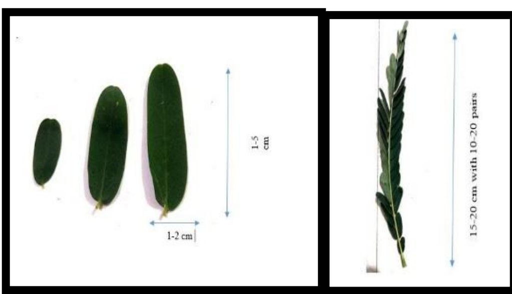


Figure 6.3: ***Sesbania Grandiflora*** linn leaves

Beta Vulgaris

Figure 6.4: ***Beta Vulgaris*** linn Root**.**

The morphological studies give the information about *Beta Vulgaris root* is red in colour, binneal or perinnieal upto 120-150 cm plant swollen and fleshy long main red root.

Table 6.2: Description of *Beta vulgaris* Root.

Plant	Sprawling perennial plant upto 60 cm (2 ft) high
Leaves	Dark green,leathery,shiny rosette leaves with wavy & rough Triangular lower leaves and narrow and oval upper leaves. Grow 20–40cm (7.9–15.7inch) in length.
Fruit	Enclosed by the leathery and in curved perianth, and is immersed in the swollen, hardened perianth base.
Root	Swollen and fleshy long main red root.
Flowers	Green and tiny with the sepals thickening and hardening reach1–2 m (3.3–6.6ft) in height.
Seed	The horizontal seed is lenticular 2–3 mm; with a red-brown, Shiny seed coat. The seed contains an annular embryo and copious perisperm.

6.2.2 Microscopical evaluation

A medicine can be examined in greater detail under a microscope, and its histological characteristics can be used to identify it.

Sesbania Grandiflora Linn

The cross-section of the leaf displays a dorsiventral structure, characterized by single-layered upper and lower epidermal cells covered by a thin cuticle. Adjacent to the upper epidermis are 2-3 layers of closely packed angular collenchyma cells and 1-4 layers of round-bottomed parenchyma cells. The midrib contains a collateral vascular bundle where metaxylem faces downwards and protoxylem upwards. Towards the lower leaf region, there are 2-3 layers of angular collenchyma followed by 1-3 layers of parenchyma cells.

Surrounding the vascular bundles, 1–4 layers of collenchymatous cells are present. The leaf blade consists of single-layered upper and lower epidermises, a palisade parenchyma layer of 2 cells, and loosely arranged spongy parenchyma cells. Anisocytic stomata are distributed on both leaf surfaces, with a higher concentration on the lower surface.

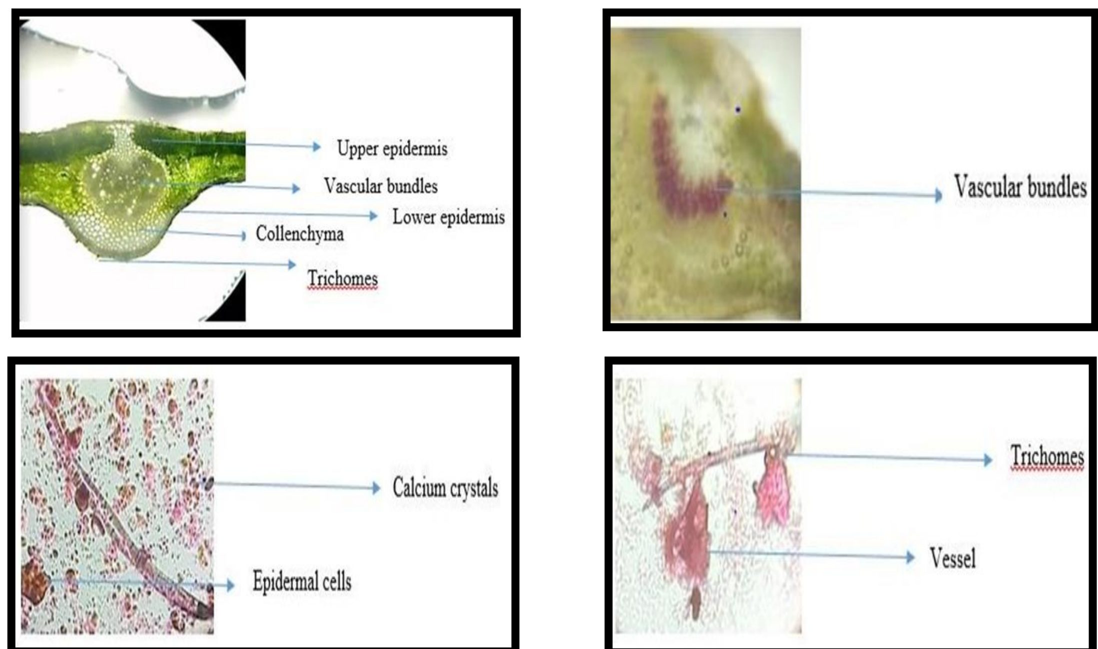


Figure 6.5: Microscopic Evaluation of *Sesbania Grandiflora Linn*

Beta Vulagaris Root

The transverse section of *Beta vulgaris* reveals distinctive features, including deeply red vacuoles within the cortical parenchyma and the pith, while the rhizodermis and conductive vessels remain uncolored. Both sugar beet and red beet roots lack starch and exhibit a secondary root structure characterized by concentric circles of conductive tissues interspersed with parenchyma, forming wide rays. These parenchyma cells possess cellulose walls. In the beet root cross-section, well-defined xylem vessels are observed, with the protoxylem located towards the pith and the metaxylem positioned outward. Beneath the phloem lies the cambium, which is connected to the pith rays and the pericycle. The majority of the root's thickness is attributed to this cambium, which produces a greater amount of secondary xylem compared to secondary phloem.

The vascular bundles in *Beta vulgaris* are of the collateral type, with the phloem positioned at the back. Meristematic tissues, known as the cambium, persist between the primary tissues and are responsible for the formation of secondary xylem and phloem.

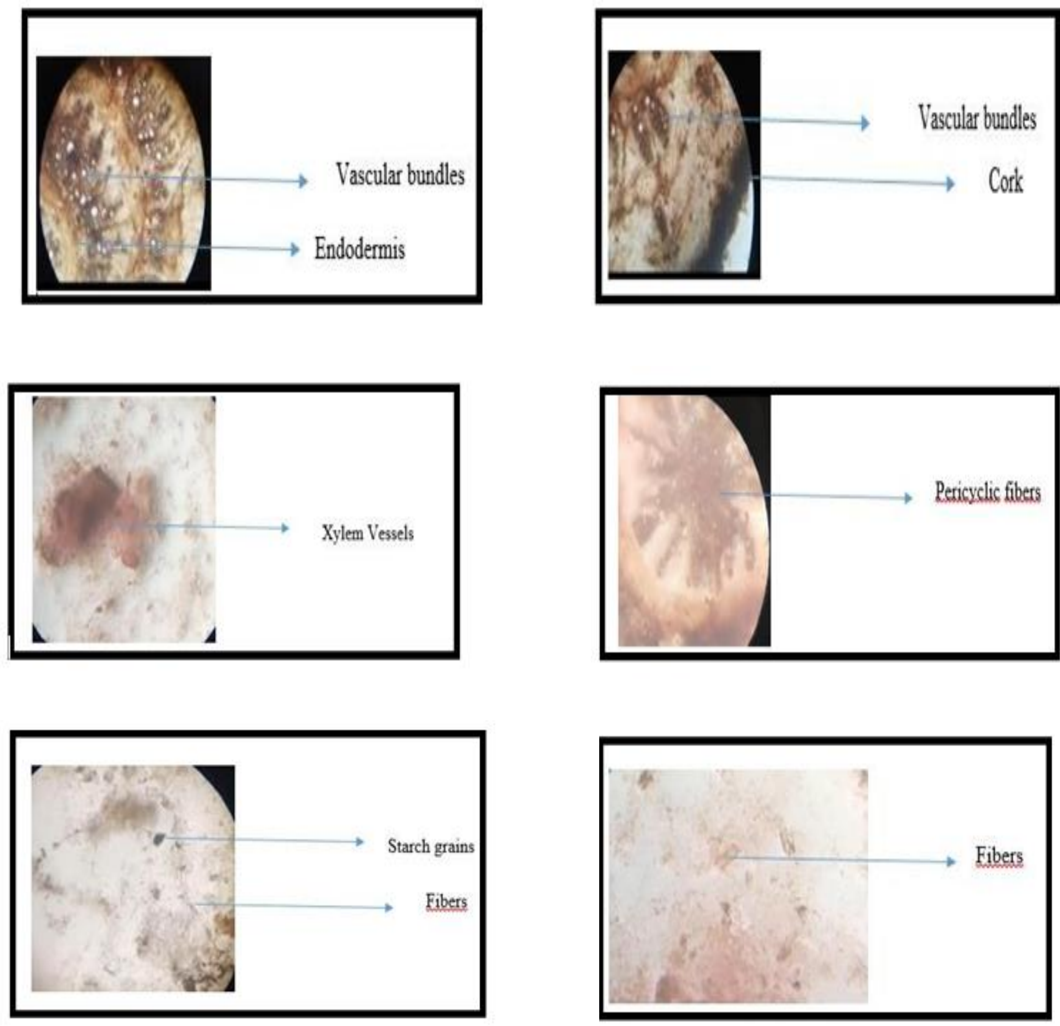


Figure 6.6: Microscopic Evaluation of *Beta Vulgaris* Root

6.2.3 Fluorescence Analysis

When exposed to different reagents, powders emitted varying colors of light under UV and visible light, facilitating the identification of the drug in its powdered state.

Table 6.3: Ultra–Violet analysis of leaves *Sesbania Grandiflora*

Powder +reagent	Orinary light	254 nm short UV wave	365 nm short UV wave
Only powder	Green	Green	Fluroscent green
Powder+ 1N NaOH	Green	Dark green	Yellowish brown
Powder + CH_3COOH	Dark brown	Dark green	Orange
Powder + 50% KOH	Green	Dark green	Orange
Powder + 50% HNO_3	Brown	Green	Brown
Powder + 50% H_2SO_4	Blue	Greenish blue	Light green
Powder + Water	Green	Green	Yellow green

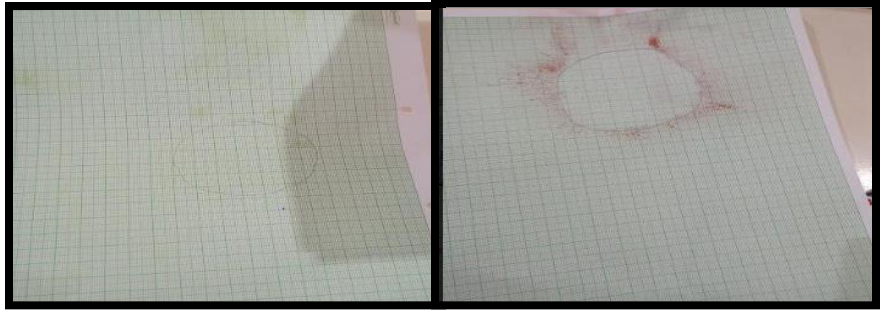
Table 6.4: Ultra–Violet analysis of root of *BetaVulgaris*

Powder +reagent	Ordinary light	254 nm short UV wave	365 nm short UV wave
Only powder	Dim red	Green	Dim green
5 % NaOH	Green	Dim green	Yellowish brown
Chloroform	Yellow	Red	Green
1 % KOH	Reddish yellow	Blue	Dim blue
Conc. HNO_3	Pale yellow	Green	Black
H_2SO_4	Brown	Deep brown	Brown
Acetone	Light green	Red	Light green
$FeCl_3$	Black	Blue	Blue

6.3 MICROMETRIC PARAMETERS:

Micrometric parameters like Angle of repose, bulk density, and tapped density for *Sesbania Grandiflora leaves and Beta Vulgaris Root* was determined. The angle of repose of *Sesbania Grandiflora leaves* is 32.5**7** it is good**,** *Beta Vulgaris* root powder is 28.37 gives excellent results.The bulk density and tapped density of *Sesbania Grandiflora is 0.532 and 0.727 and Beta Vulgaris* ***r***oot powder 0.553 and 0.702**.**

6.3.1 Angle of Repose:



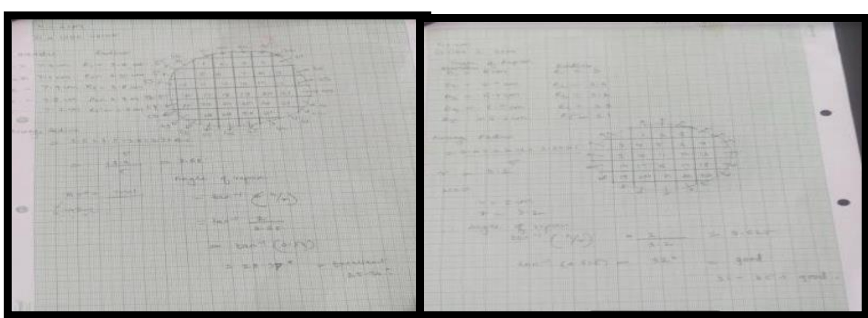


Figure 6.7: Angle of Repose of *Sesbania Grandiflora* leaves powder and *Beta Vulgaris* Root powder

Table 6.5: Angle of Repose of *Beta Vulgaris* Root powder and *Sesbania Grandiflora* leaves powder

Sr. No.	Name of plant	Angle of repose (degree)			Bulk Density	Tapped Density
		Obtain	Std.	Flow property		
1	***Beta Vulgaris***	28.37	25-30	Excellent	0.532	0.727
2	***Sesbania Grandiflora***	32	31-35	Good	0.553	0.702

6.4 PHYSICOCHEMICAL EVALUATION:

6.4.1 Determination of Ash Value

Ash values play a critical role in assessing the quality and purity of crude drugs by identifying impurities such as carbonates, oxalates, and silicates. The water-soluble ash measurement indicates the quantity of inorganic compounds present, while acid-insoluble ash, primarily silica, signifies contamination by gritty materials. Acid-insoluble ash specifically quantifies silica content, often originating from sand, whereas water-soluble ash denotes the fraction that dissolves in water. Reduced ash values indicate lower levels of inorganic substances and silica in crude drugs, reflecting higher purity.

6.4.2 Determination of Solvent Extractive Value:

The determination of extractive values was conducted for *Sesbania Grandiflora and Beta Vulgaris L*. The alcohol-soluble extractive values obtained were 10.20% w/w and 14.59% w/w for *Sesbania Grandiflora and Beta Vulgaris L*, respectively. Similarly, the water-soluble extractive values were found to be 4.07% w/w and 19.11% w/w for *Sesbania Grandiflora and Beta Vulgaris L,* respectively.

Figure 6.8: Alcohol soluble Extractive

Figure 6.9: Water Soluble Extractive.

The assessment of raw botanicals is crucial for their identification and for setting benchmarks concerning their excellence and cleanliness. To uphold this, authoritative guidelines for botanical substances must be formulated. These guidelines allow quality assurance experts to meticulously scrutinize and authorize the substances. Evaluating physical properties is essential for identifying any contamination or mishandling of the substances, guaranteeing the credibility and safety of the ultimate products.

6.4.3 Determination of Foreign Organic Matter: The percentage of foreign organic matter in *Sesbania Grandiflora* was found to be 1.58%, and in *Beta vulgaris*, it was 0.07%.

6.4.4 Determination of Loss on Drying (Moisture Content):

The moisture content was determined to be 2.98% for *Sesbania Grandiflora* and 8.74% for *Beta vulgaris*. This measurement is crucial for evaluating the chemical composition of the raw material and estimating the quantities of specific components that can dissolve in certain solvents. Additionally, it was noted that the highest extraction yields from *Sesbania Grandiflora* leaves and *Beta vulgaris* roots were achieved using methanol as the extracting solvent.

The percentage of bioactive compounds in the raw material is calculated on a basis of air-dried weight. It is essential to minimize moisture content to prevent degradation from chemical reactions or microbial contamination, and to inhibit the proliferation of bacteria, yeast, or fungi during storage.Additionally, foreign organic matter, which includes any plant parts not specified in the drug's definition and description, must be kept within the

maximum allowable limit outlined in the monograph of crude drugs. Exceeding this limit can compromise the quality of the drug.

Physicochemical characterizations of the powders of *Sesbania Grandiflora leaves and Beta vulgaris roots* are presented in Table 6.6.

Table 6.6: Physicochemical Parameters of leaves *Sesbania Grandiflora* and root of *Beta Vulgaris*

Sr.No.	Physicochemical Parameters	*SG*	*BV*
1.	**AshValues**		
	Total ash	8.13%(w/w)	13.51%(w/w)
	Acid insoluble ash	1.67%(w/w)	1.21%(w/w)
	Water soluble ash	1.83%(w/w)	6.90%(w/w)
2.	**Extractive Values**		
	Alcohol soluble extractive	10.23%(w/w)	14.59%(w/w)
	Water soluble extractive	4.07%(w/w)	19.11%(w/w)
3.	**Moisture Content**	2.98%(w/w)	8.74%(w/w)
4.	**Foreign organic matter**	0.93%(w/w)	0.07%(w/w)

1.4.5 Extraction

The extracts underwent a physical examination to assess their color and consistency. Following this, they were concentrated using a rotary evaporator at a controlled temperature of 40°C under vacuum conditions to remove the alcohol solvent effectively. The resulting concentrated extracts were further processed by freeze-drying at -20°C for a period of 12 hours. Subsequently, the freeze-dried extracts were lyophilized using a lyophilizer to obtain powdered form. These lyophilized extract powders were then stored in an airtight container within a desiccator until required for further analysis or experimentation.

Figure 6.10: Rotatory Evaporator

Table 6.7: Extraction of leaves of *Sesbania Grandiflora and root of Beta vulgaris L*

Sr. No.	Extracts	Parts	Solvent	Colour	Sense Touch of extract	%Yield
1	***Sesbania Grandiflora***	**Leaves**	Aqueous	Green	Sticky	12.6%
			Ethanol	Green	Sticky	11.5%
			Acetone	Light green	Sticky	03.5%
			Methanol	Green	Sticky	17.8%
2	***Beta Vulgaris***	**Root**	Aqueous	Dark red	Sticky	23.61%
			Ethanol	Dark red	Sticky	21.49%
			Acetone	Dark red	Sticky	09.7%
			Methanol	Dark red	Sticky	25.37%

Figure 6.11: Aqueous Extract leaves of Sesbania Grandiflora and root of Beta vulgaris L

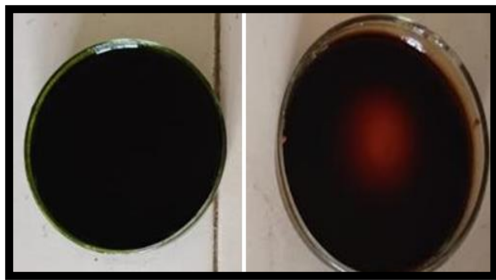


Figure 6.12: Methanolic Extract of leaves of Sesbania Grandiflora and root of Beta vulgaris L

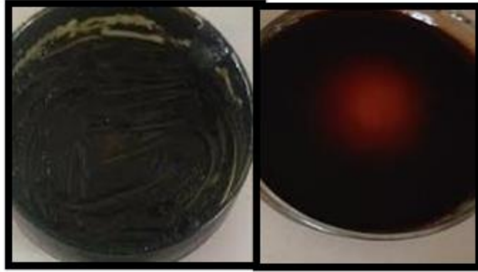


Figure 6.13: Acetone Extract of leaves of Sesbania Grandiflora and root of Beta vulgaris L

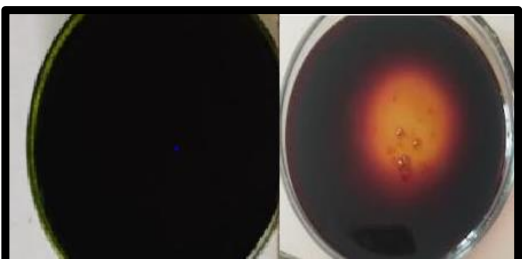


Figure 6.14: Ethanolic extract of leaves of Sesbania Grandiflora and root of Beta vulgaris L

6.4.6 Phytochemical Evaluation

The purpose of phytochemical evaluation is to identify the different phytoconstituents that are present in crude medicinal extracts as well as to create a profile of a specific extract's chemical constituents. Several tests have been carried out to identify the phytochemical components.The findings demonstrated that every phytochemical has the capacity to be extracted using various solvents.Depending on how polar the solvent is, this could vary.

The fluid extraction analysis confirmed the presence of proteins, tannins, alkaloids, and carbohydrates. Methanolis widely employed as a solvent in Ayurvedic practices for extracting bioactive compounds because of its effectiveness in isolating a broad range of chemicals from plant materials.Its rapid evaporation rate makes methanol an ideal solvent for active extraction purposes. Methanolic extracts are particularly chosen for subsequent investigations.

Sesbania Grandiflora

Initial screening of various extracts from *Sesbania Grandiflora* revealed the presence of phytoconstituents such as alkaloids, carbohydrates, glycosides, phytosterols, flavonoids, proteins, saponins, amino acids, and tannins, as well as fixed oils and fats, gums, mucilage, and volatile oils. Methanolic extracts exhibited the highest concentration of these components, indicating effective extraction. Methanol was identified as an optimal solvent for extraction due to its ability to extract a wide range of compounds, coupled with moderate evaporation rates.

Table 6.8: Qualitative Phytochemical Tests of leaves *Sesbania Grandiflora*

Sr.No.	Tests	Aqueous	Ethanol	Methanol	Acetone
1.	**Tests for Alkaloids**				
	Mayer's test	+	-	+	+
	Wagner's test	+	+	+	+
	Hager's tests	+	-	+	+
	Dragendorff's test	+	-	+	+
2.	**Tests for Carbohydrates**				
	Molish test	_	+	+	+
	Fehling test	+	-	+	+
	Barfoed's test	+	-	+	-
	Benedict's test	+	+	+	+
3.	**Tests for Glycosides**				
	Borntrager'stest	-	-	+	+
	Legal'stest	-	-	+	+
4.	**Test for Saponins**				
	Test solution + 20 ml distilled H20	-	+	+	+
5.	**Tests for Proteins & amino acids**				
	Millon's test	-	-	-	+
	Biuret test	+	-	-	-
	Ninhydrin test	+	-	-	-

6.	**Test for Phytosterol**				
	Libermann-Burchard's test	-	-	+	-
7.	**Tests for Fixed oils & fats**				
	Spot test	-	-	-	-
	Saponification test	-	-	-	-
8.	**Tests for Tannins**				
	Ferric chloride test	+	+	+	+
	Gelatin test	+	+	+	+
	Aqueous bromine test	+	+	+	+
9.	**Tests for Flavonoids**				
	Lead acetate	-	-	-	-
	Alkaline reagent test	-	-	-	-
10.	**Test for Gums & Mucilages**				
	Ext. + dis.H_20+abs.alc.+stirring	+	+	+	-
11.	**Test for Volatile oil**				
	50gm.of powder subjected to hydro-distillation	-	-	-	-

This table contains various tests conducted on aqueous, ethanol, methanol, and acetone extracts to detect phytochemical constituents in the plant material. Each test's reaction outcome (+ for positive, - for negative) is indicated for each solvent type.

Similarly, preliminary screening of various extracts from *Beta Vulgaris* roots identified alkaloids, carbohydrates, glycosides, Fixed oils, phytosterols, gums, Tannins, Flavonoids, Proteins and amino acids, saponins and fats, gums, Mucilage, and Volatile oil as phytochemical constituents. Methanolic extracts from the roots also showed a high presence of these constituents, suggesting efficient extraction. The table clearly demonstrates that methanol can serve as the primary solvent for extraction, with the added benefit of easy evaporation. Phytochemical constituents detected in the crude extracts of *Beta Vulgaris* roots are summarized in Table 6.9.

Table 6.9: Qualitative Phytochemical Tests of root of *Beta Vulgaris*

Sr.No.	Tests	Aqueous	Ethanol	Methnol	Acetone
1.	**Tests forAlkaloids**				
	Mayer's test	-	+	+	-
	Wagner's test	-	+	+	-
	Hager's tests	-	+	+	-
	Dragendorff's test	-	+	+	-
2.	**Tests for Carbohydrates**				
	Molish test	+	+	+	-
	Fehling test	+	+	+	-
	Barfoed's test	+	+	+	-
	Benedict's test	_	+	+	-
3.	**Tests for Glycosides**				
	Borntrager's test	+	+	+	+
	Legal's test	+	+	+	+

4.	**Test for Saponins**				
	Test solution+20ml distilled H20	+	+	-	+
5.	**Tests for Proteins & amino acids**				
	Millon's test	-	-	-	-
	Biuret test	-	-	-	-
	Ninhydrin test	-	-	-	-
6.	**Test for Phytosterol**				
	Libermann-Burchard's test	+	-	+	+
7.	**Tests for Fixed oils & fats**				
	Spot test	-	-	-	-
	Saponification test	-	-	-	-
8.	**Tests for Tannins**				
	Ferric chloride test	+	_	+	+
	Gelatin test	+	_	+	+
	Aqueous bromine test	+	_	+	+
9.	**Tests for Flavonoids**				
	Lead acetate	+	+	+	+
	Alkaline reagent test	+	+	+	+
10.	**Test for Gums & Mucilages**				
	Ext.+dis.H20+abs.alc.+stirring	-	-	-	-
11.	**Test for Volatile oil**				
	50gm.of powder subjected to hydro-distillation	-	-	-	-

This table contains various tests conducted on aqueous, ethanol, methanol, and acetone extracts to detect phytochemical constituents in the plant material. Each test's reaction outcome (+ for positive, - for negative) is indicated for each solvent type.

6.5.7 pH of Extracts methanolic extract of leaves of Sesbania Grandiflora and root of Beta vulgaris L

pH of *Sesbania Grandiflora* leaves extract and *Beta Vulgaris* Root extract, mentioned in the table:

Table 6.10: pH of Extracts

Sr.No.	Extract	pH
1.	***Sesbania Grandiflora* leaves**	6.7
2.	***Beta Vulgaris* Root**	7.1

6.6 Total Phenolic Content

Table 6.11: Absorbance of Gallic acid at 760nm

Concentrations (µg/ml)	Absorbance
10	0.0712
20	0.098
40	0.209
60	0.397
80	0.521
100	0.631

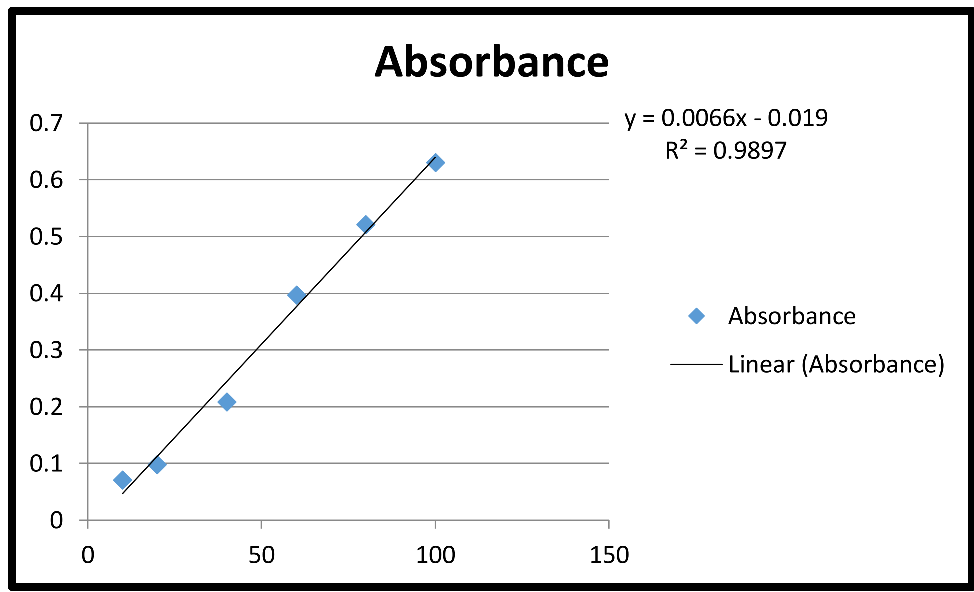


Figure 6.15: Standard curve of Gallic acid at 760 nm

The table summarizes the determination of total phenolic content in extracts from *Sesbania Grandiflora* leaves and *Beta Vulgaris* roots using different solvents. The total phenolic content varied from 3.0 to 11.23 mg of Gallic Acid Equivalent per gram (GAE/g) for *Sesbania Grandiflora* leaf extracts and from 17.35 to 187.5 mg GAE/g for *Beta Vulgaris* root extracts. The calculation was based on a standard curve equation derived from Gallic acid standards ($y = 0.0066x - 0.019$, $R^2 = 0.9897$), which was constructed using various concentrations of Gallic acid solutions.

In both plant extracts, the highest total phenolic content was observed in the Methanolic extracts, followed by Aqueous, Ethanol, and Acetone extracts in decreasing order of concentration. This indicates that Methanol was the most effective solvent for extracting phenolic compounds from both *Sesbania Grandiflora* leaves and *Beta Vulgaris* roots.

6.7 Total Flavonoid Content

Table 6.12: Absorbance of Quercetin at 510nm

Concentration (µg/ml)	Absorbance
10	0.023
20	0.043
40	0.096
60	0.167
80	0.243
100	0.302

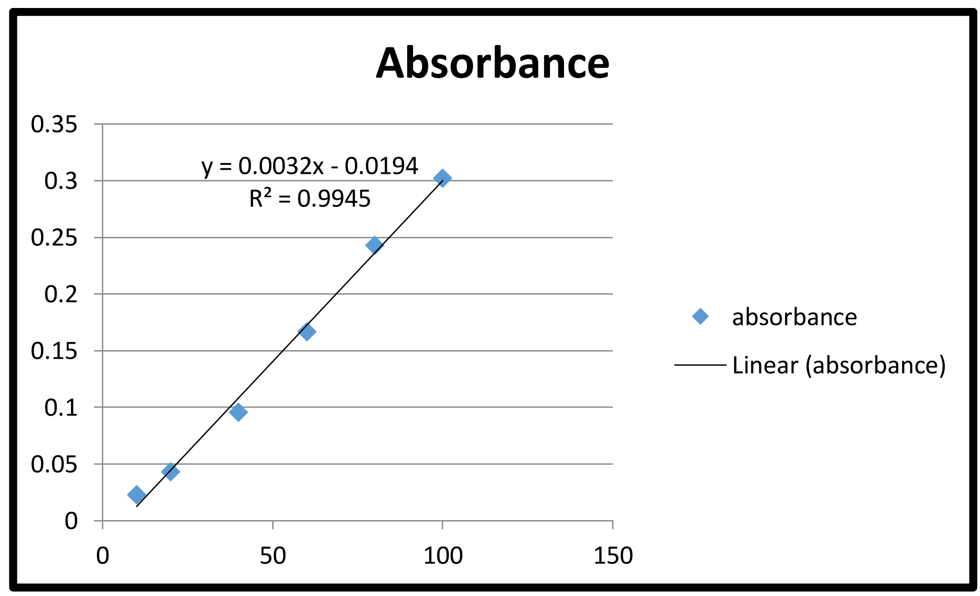


Figure 6.16: Standard curve of Quercetin at 510nm

Determination of total flavonoid content in various extract solvent varied from 5.13 to 19.24 mg/Qeg for leaves extracts of *Sesbania Grandiflora* and 8.37 to 15.45 mg/Qeg for root extracts of *Beta Vulgaris*. Total phenolic content was calculated using a standard curve equation of Quercetin y = 0.0032x - 0.0194, R2 = 0.9945. Quercetin standard curve was obtained from various concentrations of Quercetin solution.The highest total

flavonoid content in extracts of leaves of *Sesbania Grandiflora* was in increasing sequence Methanolic >Aqueous >Ethanol >Acetone. The same result was revealed for root extracts of *Beta Vulgaris*. Thus it indicates that total flavonoid content determined highest in Methanolic extracts of both plants.

Table 6.13: Total Phenolic and Flavonoid Content leaves extracts of *Sesbania Grandiflora*

Total Phenolic and Flavonoid content of leaves extracts of *Sesbania Grandiflora*				
Parameters	**Aqueous**	**Ethanol**	**Acetone**	**Methanol**
Total Phenolic content (mg/GAEg)	9.23±0.07	5.43±1.27	3±0.12	11.23±1.03
Total Flavonoid content (mg/Qeg)	13.07±0.12	10.23±1.19	5.13±0.03	19.24±0.82

Table 6.14: Total Phenolic and Flavonoid Content content of root extract of *Beta Vulgaris* L

Total Phenolic and Flavonoid content of root extract of *Beta Vulgaris* L				
Parameters	**Aqueous**	**Ethanol**	**Acetone**	**Methanol**
Total Phenolic Content (mg/GAEg)	165.3±1.47	175.23±1.75	17.35±0.19	187.5±1.91
Total Flavonoid Content (mg/Qeg)	13.49±0.83	14.07±0.14	8.37±0.92	15.45±1.36

6.8 IR-Interpretation of *Sesbania Grandiflora Leaves and Beta Vulgaris Root*

Fourier Transform Infrared (FTIR) spectroscopy was employed to analyze the samples and identify the presence of functional groups, thereby elucidating the significance of specific bioactive components present. The peak values and corresponding functional groups identified are illustrated in Figure 6.17.

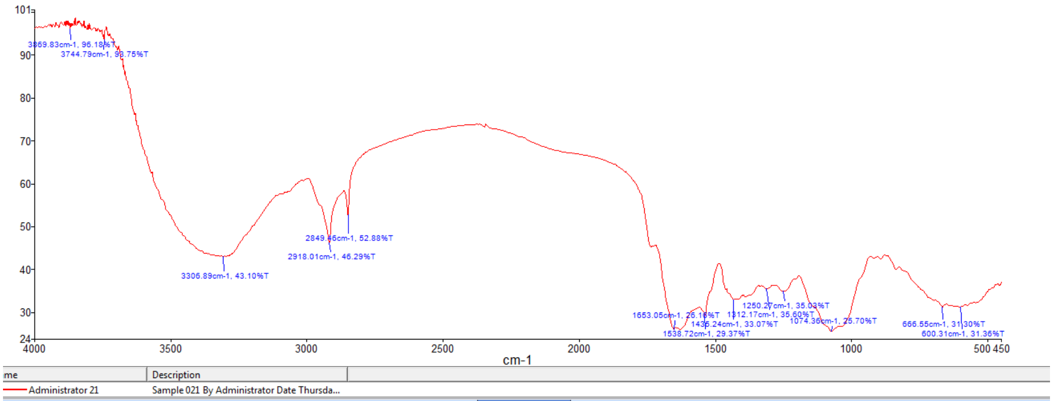


Figure 6.17: I.R Spectra of methanolic extract of *Sesbania Grandiflora* leaves

Table 6.15: Functional group in methanolic extract of *Sesbania Grandiflora* leaves

Functional group	Vibrations	Peak
Alkane	Streching	2918.01cm^{-1}, 2849.46cm^{-1}
Aldehyde C=O	Stretching	1722cm^{-1}
Amine N-H	Bending	1653.05cm^{-1}
Sulfoxide S=O	Stretching	1074.38cm^{-1}
Alkene C=C	Bending	812.21cm^{-1}
Halo C-Br	Stretching	600.31cm^{-1}

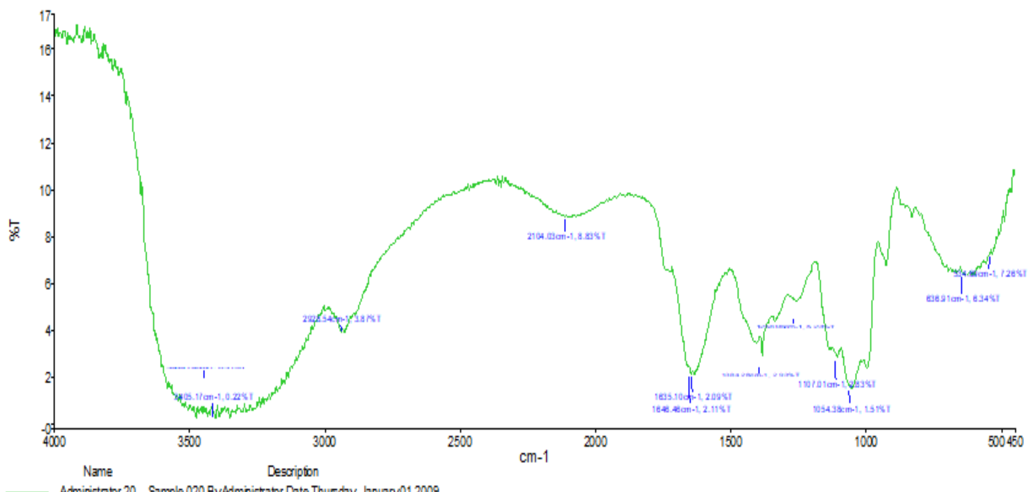


Figure 6.18:I.R Spectra of methanolic extract of *Beta Vulgaris* root.

Table 6.16: Functional group in methanolic extract of *Beta Vulgaris* Root

Functional group	Vibrations	Peak
(O-H) bond	Stretching	3405.17 cm^{-1}
C-H	Stretching	2928.54 cm^{-1}
Carbonyl group (C,O)	Stretching	1646.46 cm^{-1}
Aromatic (C,C) bond	Bending	1304.20 cm^{-1}
(C–O) bond	Stretching	1107.01 cm^{-1}
Amine	Stretching	636.91 cm^{-1}

6.9 HPTLC FINGERPRINTING

Sterols, tannins, flavonoids, amino acids, glycosides, phenolic compounds, carbohydrates, saponins, and alkaloids were found by primary phytochemical screening. Methanolic extracts have also been the subject of thin layer chromatography investigations. HPTLC fingerprinting is a useful technique for detection of the phytochemicals found in herbal medicine. Fingerprinting using HPTLC demonstrating that the methonolic extract contains Gallic acid, Quercetin, Betalin, Kaempefrol.Value of Rf is computed and reported.

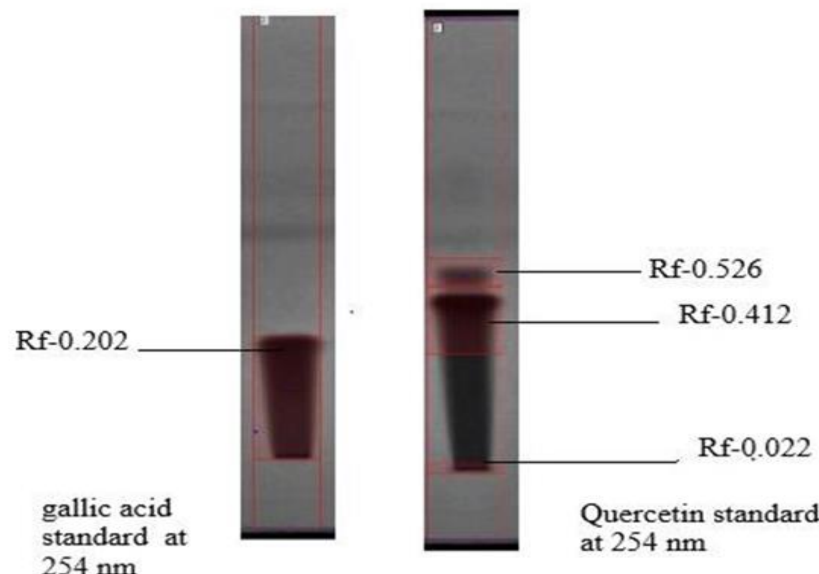


Figure 6.19: HPTLC finger printing of Gallic acid and Quercetine at 254 nm and 366 nm

6.9.1 HPTLC fingerprinting of Methanolic extract of leaves of *beta vulgaris* at 254 nm and 366nm

Table 6.17: HPTLC fingerprinting of Methanolic extract of leaves of *beta vulgaris* at 254nm and 366nm

Test extracted	Solvent system	Number of spots	Rf values
Methanolic extract of leaves of *Beta Vulgaris*	Toluene;Ethyl acetate; Formic acid Volume 5:4:1	15	0.01, 0.014 ,0.16, 0.18 , 0.24, 0.32, 0.43 0.44, 0.46, 0.47, 0.50, 0.511, 0.54, 0.74, 0.75

Table 6.18: R_fValue of Methanolic extract of root of *Beta Vulgaris*

	Rf Value		Assigned substances
	254 nm	366 nm	
1	0.526, 0.265, 0.126, 0.210	0.692, 0.542, 0.471, 0.238	Methanolic extract of *Beta Vulgaris L* root
2	0.202	0.238, 0.455, 0.452,	Gallic acid
3	0.526, 0.412,	0.551, 0.452	Quercetin
4	0.455	0.455	Flavanoids

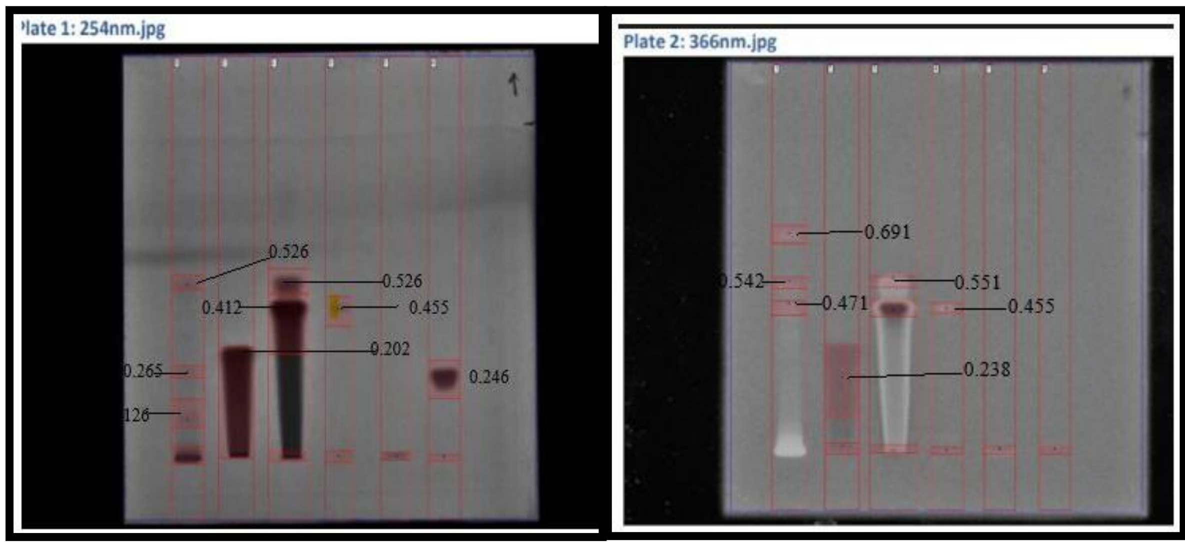


Figure 6.20: HPTLC finger printing of Methanolic extract of root of *Beta Vulgaris* at 254 nm and 366 nm

Table 6.19: Peak table of Methanolic extract of root of *Beta Vulgaris* with R_f Values at 254nm and 366nm

Bands

Lane ID	Band ID	Rf	Area	Volume	Displayed Volume	Notes
1	1	0.526	1794	2908350	29.08	DTPL-55 (A)
1	2	0.265	1656	1335702	13.36	DTPL-55 (A)
1	3	0.126	3726	4058304	40.58	DTPL-55 (A)
1	4	0.016	2277	23211393	232.11	DTPL-55 (A)
2	1	0.202	15808	252106136	2521.1	GALIACID
3	1	0.526	3910	17193715	171.94	QUERECETIN
3	2	0.412	9520	92020065	920.2	QUERECETIN
3	3	0.022	1700	7138555	71.39	QUERECETIN
4	1	0.455	3190	2960430	29.6	FLAVONOIDS
4	2	0.017	1155	759550	7.6	FLAVONOIDS
5	1	0.017	990	3127806	31.28	TANNINS
6	1	0.246	4968	37781709	377.82	TERPENOIDS
6	2	0.016	828	211140	2.11	TERPENOIDS

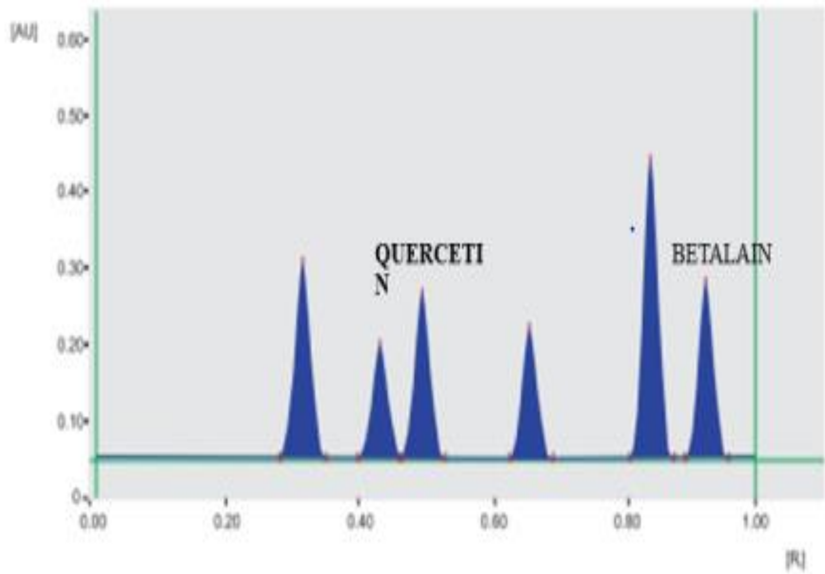


Figure 6.21: HPTLC Densitogram of Methanolic root Extract of *Beta Vulgaris*

Table 6.20: Peak table of Betalain in root of *Beta Vulgaris* with R_f Values at 254nm and 366nm

Track	Peak Start	Peak End	Area in %
1	0.348	0.351	100
2	0.436	0.439	100
3	0.512	0.514	100
5	0.649	0.657	100
6	0.833	0.837	100
7	0.941	0.946	100
7 (Betalain)	0.340	0.343	100

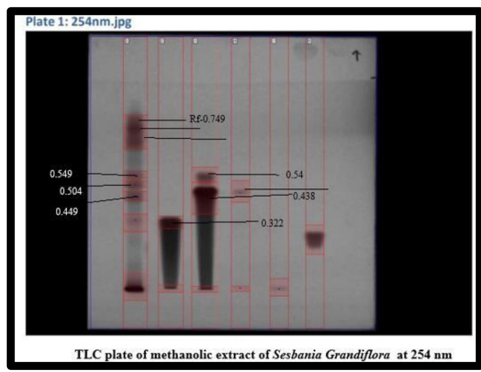
6.9.2 HPTLC fingerprinting of Methanolic extract of leaves of *Sesbania Grandiflora* at 254nm and 366nm

Table 6.21: HPTLC fingerprinting of Methanolic extract of leaves of *Sesbania Grandiflora* at 254nm and 366nm

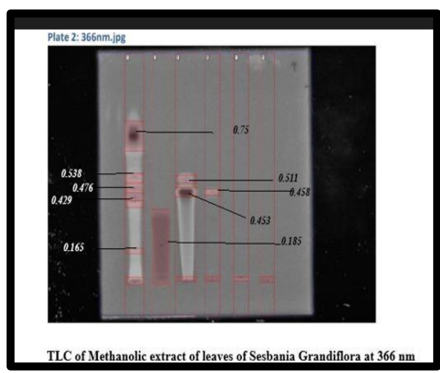
Test extracted	Solvent system	Number of spots	Rf values
Methanolic extract of leaves of *Sesbania Grandiflora*	Toluene; Ethyl acetate; Formic acid Volume 5:4:1	15	0.01, 0.014 ,0.16, 0.18 , 0.24, 0.32, 0.43 0.44, 0.46, 0.47, 0.50, 0.511, 0.54, 0.74, 0.75

Table 6.22: R_f Value of Methanolic extract of leaves of *Sesbania Grandiflora*

Band number	Rf Value		Assigned substances
	254 nm	366 nm	
1	0.504, 0.449, 0.336	0.538, 0.476, 0.429,0.165	Methanolic extract of *Sesbania Grandiflora* leaves
2	0.322	0.185	Gallic acid
3	0.438	0.511,0.453	Quercetin
4	0.468	0.458	Flavanoids



TLC plate of methanolic extract of *Sesbania Grandiflora* at 254 nm



TLC of Methanolic extract of leaves of Sesbania Grandiflora at 366 nm

Figure 6.22: HPTLC densitogram of Methanolic extract of leaves of *Sesbania Grandiflora* at 254nm and 366nm

Table 6.23: Peak table of Methanolic extract of leaves of *Sesbania Grandiflora* with R_f Values at 254nm and 366nm

Bands

Lane ID	Band ID	Rf	Area	Volume	Displayed Volume	Notes
1	1	0.749	7300	76505387	765.05	DTPL - 55 (B)
1	2	0.549	1606	5464415	54.64	DTPL - 55 (B)
1	3	0.504	1314	7098155	70.98	DTPL - 55 (B)
1	4	0.449	1971	9972384	99.72	DTPL - 55 (B)
1	5	0.336	3869	2617269	26.17	DTPL - 55 (B)
1	6	0.025	6132	55362105	553.62	DTPL - 55 (B)
2	1	0.322	2775	33678900	336.79	GALIACID
2	2	0.019	1650	0	0	GALIACID
3	1	0.54	3772	8076262	80.76	QUERECETIN
3	2	0.438	7708	82330132	823.3	QUERECETIN
3	3	0.014	1476	1206384	12.06	QUERECETIN
4	1	0.468	3575	7727555	77.28	FLAVONOIDS
4	2	0.014	825	831105	8.31	FLAVONOIDS
5	1	0.011	2964	3572247	35.72	TANNINS
6	1	0.245	4872	51044952	510.45	TERPENOIDS

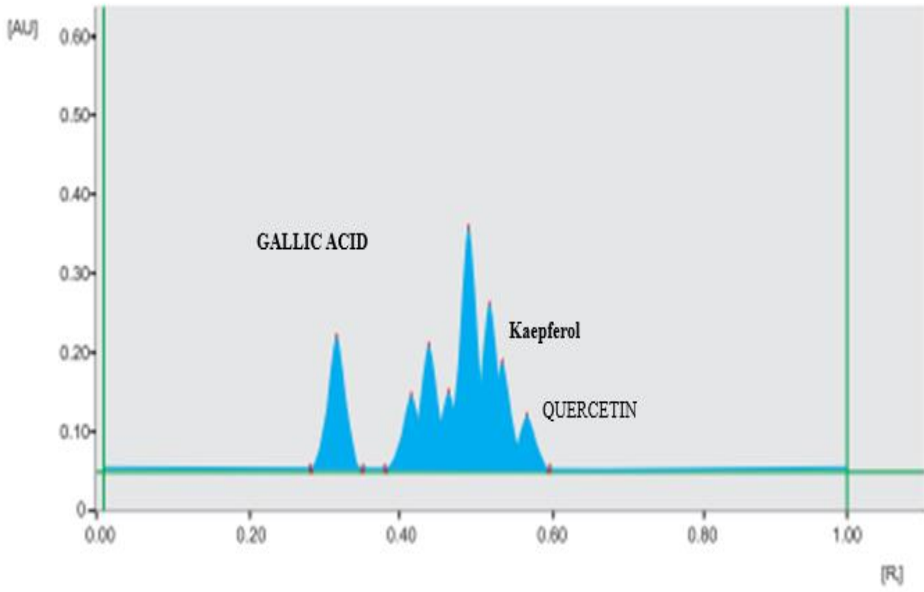


Figure 6.23: Densitogram of Methanolic Leaves Extract of *Sesbania Grandiflora*

Table 6.24: Peak table of Kaepferol and Quercetin in leaves of Sesbania Grandiflora with R_f Values at 254nm and 366nm

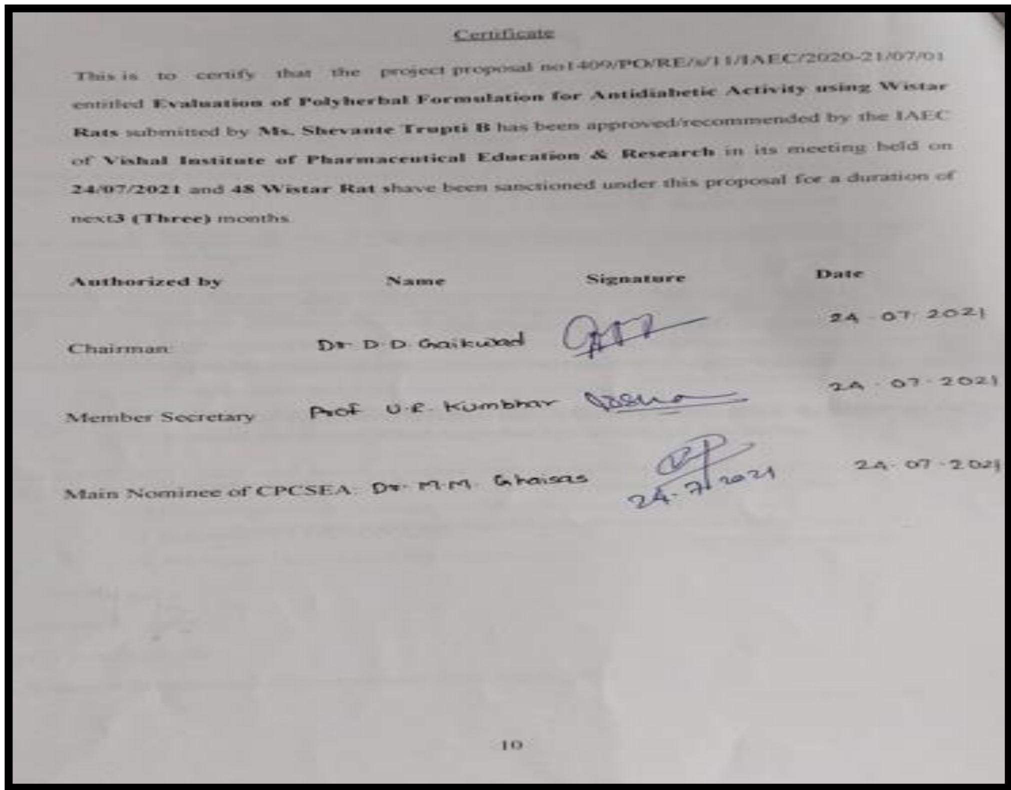
Track	Peak Start	Peak End	Area in %
1	0.336	0.342	100
2	0.429	0.434	100
3	0.449	0.454	100
4	0.476	0.481	100
5	0.504	0.513	100
6	0.538	0.545	100
7 (Kaepferol)	0.583	0.587	100
8 (Quercetin)	0.511	0.523	100

Fingerprinting techniques applied to the polyherbal formulation showed successful separation when using wavelengths of 254 nm and 366 nm. This analysis confirmed that the active compound found in the polyherbal formulation matched those identified in all three extracts.

6.10 *INVIVO* STUDIES

6.10.1 Procurement of Animals

Adult male Wistar rats weighing between 220-250 grams were sourced from LACHMI in Pune, Maharashtra, India. These rats were housed in large polyacrylic cages under controlled laboratory conditions with a 12-hour light/dark cycle. They had ad libitum access to water and were fed a standard rodent diet from Nutrivate Pvt. Ltd., Bangalore, India. The study received approval from the Institute Animal Ethics Committee of the Vishal Institute of Pharmaceutical Education and Research, Ale (registration number 1409/PO/RE/S/11/IAEC/2020-2021/07/01), and all procedures followed the guidelines established by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India.



Certificate

This is to certify that the project proposal no1409/PO/RE/s/11/IAEC/2020-21/07/01 entitled **Evaluation of Polyherbal Formulation for Antidiabetic Activity using Wistar Rats** submitted by **Ms. Shevante Trupti B** has been approved/recommended by the IAEC of **Vishal Institute of Pharmaceutical Education & Research** in its meeting held on **24/07/2021** and **48 Wistar Rat** shave been sanctioned under this proposal for a duration of next**3 (Three)** months.

Authorized by	Name	Signature	Date
Chairman	Dr D.D. Gaikwad		24.07.2021
Member Secretary	Prof U.E. Kumbhar		24.07.2021
Main Nominee of CPCSEA	Dr M.M. Ghaisas	24.7.2021	24.07.2021

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Certificate

This is to certify that the project proposal no1409/PO/RE/s/11/IAEC/2020-21/07/01 entitled **Evaluation of Polyherbal Formulation for Antidiabetic Activity using Wistar Rats** submitted by **Ms. Shevante Trupti B** has been approved/recommended by the IAEC of **Vishal Institute of Pharmaceutical Education & Research** in its meeting held on **24/07/2021** and **48 Wistar Rat** shave been sanctioned under this proposal for a duration of next**3 (Three)** months

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Chairman	Dr. D.D. Gaikwad		24.07.2021
Member Secretary	Prof U.E. Kumbhar		24.07.2021
Main Nominee of CPCSEA	Dr. M.M. Ghaisas	24.7.2021	24.07.2021

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Figure 6.24: Animal Ethical letter for *Invivo* Studies for Antidiabetic Activity

6.10.2 Standardization and Optimization of best combination of drug ratio for designing formulation.

6.10.2.1 Optimization of combination of drug ratio for designing formulation

Different combinations of methanolic extracts from Sesbania Grandiflora and Beta Vulgaris, designated as PHF1, PHF2, and PHF3, were evaluated for their potential to lower blood glucose levels in an oral glucose tolerance test (OGTT) using normal Albino Wistar rats. A single dose of 1000 mg/kg was administered, and among the tested formulations, the PHF2 extract demonstrated the most significant antihyperglycemic activity.

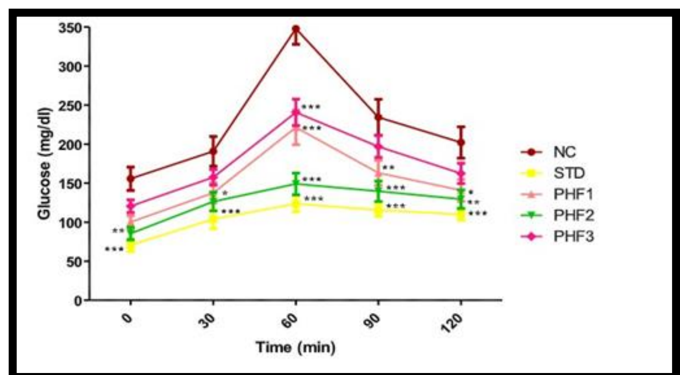


Figure 6.25: Effect of PHF 1, PHF 2, and PHF 3 extracts on blood glucose levels (mg/dl) in a group of experimental rats subjected to an oral glucose load.

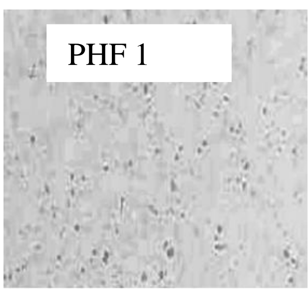
The data are presented as Mean±SEM (n = 6) and were analyzed using a two-way ANOVA, with *** indicating statistical significance at $p < 0.001$.

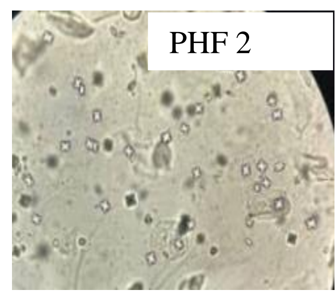
6.10.2.1 Microscopical Urine analysis

Diet is recognized as a significant environmental factor influencing the common medical condition nephrolithiasis. Dietary adjustments have become essential in the medical management of kidney stone disease due to their crucial role in its development and recurrence. The primary goal of dietary recommendations is to reduce urine super-saturation, thereby lowering the risk factors associated with kidney stones such as uric acid, calcium phosphate, and calcium oxalate. Current guidelines recommend reducing intake of animal proteins and sodium, increasing consumption of fruits and fibers, maintaining adequate fluid intake, and ensuring a balanced calcium intake.

Figure 6.26: Metabolic cages for Urine collection

Microscopic urine analysis was conducted on all three combinations, revealing that PHF1 exhibited minute crystals of calcium oxalate and uric acid compared to PHF2 and PHF3.





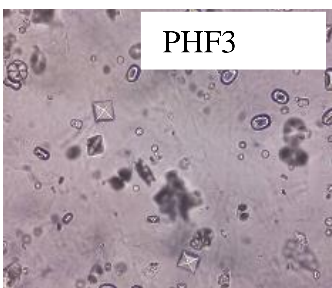


Figure 6.27: Microscopical examination of Urine collected after treatment of PHF1, PHF2, PHF3

Therefore, PHF2 drug combination with maximum antihyperglycemic activity and absence of uric acid crystals and calcium oxalate crystals was considered as optimized batch for designing of formulation further studies used different extract combinations were prepared for the formulation development and necessary excipients were added to design the formulation.[151-152]

6.10.3 Acute toxicity studies

PHF 2 was given to Wistar albino rats, and they were monitored for any clinical symptoms and changes in body weight after 14 days. Throughout the study, all rats remained healthy, displaying no notable clinical symptoms, unusual behavior, or mortality.

Table 6.25: Observation of changes in Clinical Signs in PHF (2000mg/kg) administered in Acute Toxicity Group

Observation	30 mins	4 hrs	14 hrs	24 hrs
Body weight	No change	No change	No change	No change
Preterminal deaths	No	No	No	No
Motor activity	No change	No change	No change	No change
Convulsions	No change	No change	No change	No change
Salivation	No change	No change	No change	No change
Skin colour	No change	No change	No change	No change
Diarrhoea	No change	No change	No change	No change
Aggression	No change	No change	No change	No change
Sedation	No change	No change	No change	No change
Excitation	No change	No change	No change	No change

The acute toxicity study results, detailed in the table, indicate that animals administered with polyherbal extract up to a dosage of 2000 mg/kg showed no signs of illness, death, or notable physiological alterations.

6.10.4 Evaluation of the in vivo antidiabetic effects of a polyherbal formulation in diabetic rats induced by streptozotocin.

6.10.4.1 Hematological Parameters

6.10.4.1.1 Impact of Polyherbal Formulation (PHF) doses of 200 and 400 on Total Red Blood Cell Count (millions/Cu mm) in Streptozotocin-Induced Diabetic Rats

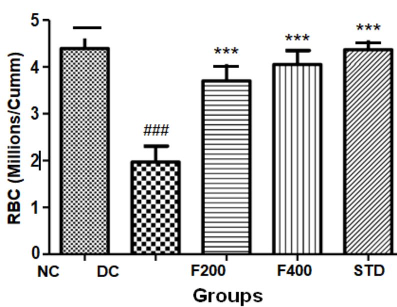


Figure 6.28 presents the Effect of PHF2 doses (F200 and F400) on the red blood cell (RBC) count (Million/Cu mm) in STZ-induced diabetes in rats.

The results are displayed as Mean ± SEM (n = 6) and were analyzed using One-way ANOVA followed by Tukey's Kramer test. Statistical significance is indicated by ###p < 0.001 whencompared to normal control (NC) rats and ***p < 0.001 when compared to diabeticcontrol (DC) rats.

Another study, shown in Figure 6.28 demonstrated that administration of STZ (45 mg/kg,i.p.) significantly increased the total RBC count in STZ-induced diabetic rats comparedto DC rats (p < 0.001). Furthermore, treatment with PHF2 (200 and 400 mg/kg, p.o.) and Glibenclamide (5 mg/kg, p.o.) significantly increased the total RBC count compared toDC rats (p < 0.001).

6.10.4.1.2 Effect of PHF2 F200and F400 on Hemoglobin (g/dl) in STZ induced Diabetes in Rats

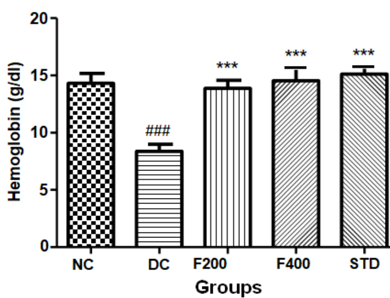


Figure 6.29 shows the effect of PHF2 at doses of F200 and F400 on haemoglobin levels (g/dl) in rats with streptozotocin-induced diabetes.

The results are presented as mean ± SEM (n = 6) and were statistically analyzed using one-way ANOVA followed by Tukey's post hoc test. Statistical significance is indicated as ###p < 0.001 compared to normal control (NC) rats and ***p < 0.001 compared to diabetic control (DC) rats.

In rats injected with streptozotocin (STZ, 45 mg/kg, i.p.), there was a significant reduction in hemoglobin levels compared to the control group (p < 0.001). Conversely, treatment with PHF2 (at doses of F200 and F400 mg/kg, administered orally) and Glibenclamide (5 mg/kg, administered orally) resulted in a significant (p < 0.001) increase in hemoglobin levels compared to the control rats.

6.10.4.1.3 Effect of PHF2 F200 and F400 on Packed Cell Volume (%) in STZ induced Diabetes in Rats

Administration of Streptozotocin (45 mg/kg, intraperitoneally) significantly reduced PCV levels compared to NC rats (p < 0.001). However, treatment with both doses of PHF2 (200 mg/kg and 400 mg/kg) and Glibenclamide (5 mg/kg, orally) significantly increased PCV levels compared to DC rats (p < 0.001).

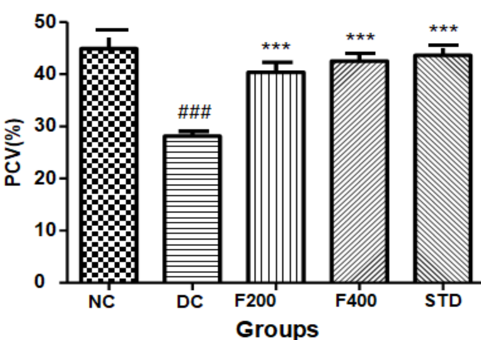


Figure 6.30 illustrates the effect of PHF2 at doses of 200 mg/kg (F200) and 400 mg/kg (F400) on packed cell volume (PCV) in rats with streptozotocin-induced diabetes.

The results are presented as mean ± SEM for six rats (n = 6) and were analyzed using one- way ANOVA followed by Tukey's Kramer post hoc test. Statistical significance is indicated by ### ($p < 0.001$) compared to normal control (NC) rats and *** ($p < 0.001$) compared to diabetic control (DC) rats.

6.10.4.1.4 Effect of PHF2 F200 and F400 on Mean Corpuscular Volume (fl) in STZ induced Diabetes in Rats

The effects of PHF2 F200 and F400 on MCH (%) in STZ induced diabetes in rats are shown inFigure 6.31. The treatment of rats with STZ (45 mg/kg, i.p.) induced significant ($p<0.001$) decrease in MCH count when compared with DC rats. However, the treatment of rats with PHF2 (F200 and F400 mg/kg, p.o.) and Glibenclamide (5 mg/kg, p.o.) exhibited significant ($p<0.01$; $p<0.001$) increase in MCH count when compared with DC rats.

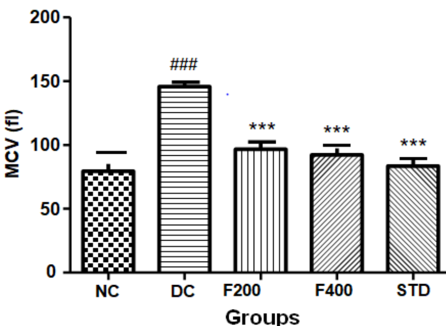


Figure 6.31 illustrates the effect of PHF2 at dosages of F200 and F400 on mean corpuscular volume (MCV, fl) in rats with streptozotocin-induced diabetes.

Administration of streptozotocin (45 mg/kg, i.p.) resulted in a significant increase in MCV levels compared to the diabetic control (DC) group ($p<0.001$).

6.10.4.1.5 Effect of PHF2 F200 and F400 on Mean Corpuscular Hemoglobin (%) in STZ induced Diabetes in Rats

Administration of streptozotocin (45 mg/kg, i.p.) significantly reduced MCH levels compared to diabetic control (DC) rats ($p < 0.001$). However, treatment with PHF2 at both 200 mg/kg and 400 mg/kg, along with orally administered Glibenclamide at 5 mg/kg, significantly increased MCH levels compared to DC rats ($p < 0.01$ and $p < 0.001$, respectively). These findings suggest that PHF2 has potential as a therapeutic agent for increasing MCH levels in diabetic conditions.

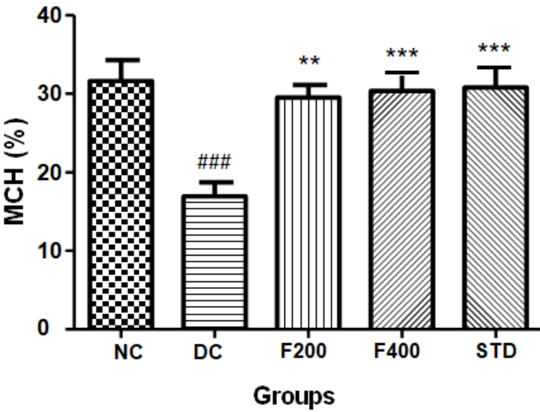


Figure 6.32 presents the effects of PHF2 at doses of 200 mg/kg (F200) and 400 mg/kg (F400) on mean corpuscular hemoglobin (MCH) in rats with streptozotocin-induced diabetes.

The data, shown as mean ± SEM for six rats per group, were statistically analyzed using one-way ANOVA followed by Tukey's post hoc test.

6.10.4.1.6 Effect of PHF2 F200 and F400 on Mean Corpuscular Hemoglobin Concentration (%) in STZ induced Diabetes in Rats.

Treatment with PHF2 at doses of 200 mg/kg and 400 mg/kg, along with Glibenclamide at 5 mg/kg orally, notably increased MCHC levels compared to DC rats ($p < 0.001$). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test.

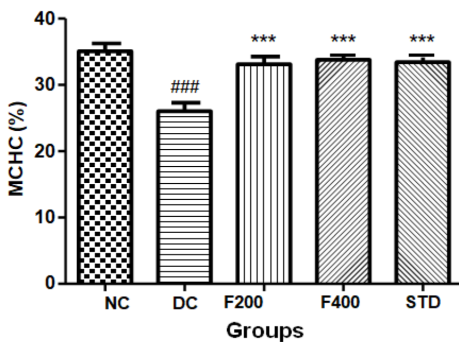


Figure 6.33 illustrates the effect of PHF2 on mean corpuscular hemoglobin concentration (MCHC) in rats with diabetes induced by streptozotocin.

The streptozotocin was administered at a dose of 45 mg/kg intraperitoneally, leading to a significant reduction in MCHC levels when compared to diabetic control (DC) rats ($p < 0.001$).

6.10.4.1.7 Effect of PHF2 F200 and F400 on Total WBC Count (millions /Cu mm) in STZ induced Diabetes in Rats

The data, presented as mean ± SEM (n = 6), underwent statistical analysis using one-way ANOVA followed by Tukey's post hoc test. Administration of streptozotocin (45 mg/kg, i.p.) significantly reduced the WBC count compared to the diabetic control (DC) group ($p < 0.001$).

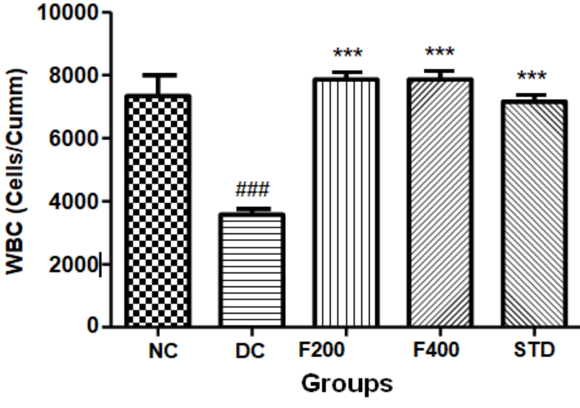


Figure 6.34 illustrates the effect of PHF2 at doses of 200 mg/kg (F200) and 400 mg/kg (F400) on white blood cell (WBC) count in rats with streptozotocin-induced diabetes.

In contrast, treatment with PHF2 (F200 and F400 mg/kg, orally) and Glibenclamide (5 mg/kg, orally) significantly increased the WBC count relative to DC rats ($p < 0.001$), indicating the immunomodulatory effects of PHF2 under diabetic conditions.

6.10.4.1.8 Effect of PHF2 F200 and F400 on Polymorphs (%) in STZ induced Diabetes in Rats

The results are presented as mean ± SEM (n = 6) and were analyzed using one-way ANOVA followed by Tukey's post hoc test. Administration of streptozotocin (45 mg/kg, i.p.) significantly decreased the polymorph count compared to diabetic control (DC) rats ($p < 0.001$).

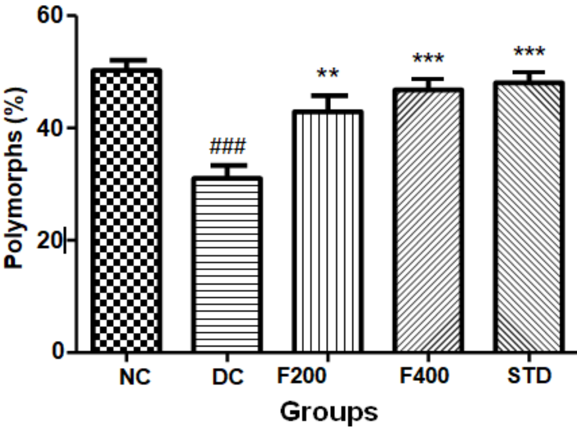


Figure 6.35 illustrates the effect of PHF2 at doses of 200 mg/kg (F200) and 400 mg/kg (F400) on polymorphonuclear leukocytes (polymorphs) in rats with streptozotocin- induced diabetes.

However, treatment with PHF2 (200 and 400 mg/kg, orally) and Glibenclamide (5 mg/kg, orally) significantly increased the polymorph number compared to DC rats ($p < 0.01$; $p < 0.001$), suggesting the immunostimulatory effects of PHF2 in diabetic conditions.

6.10.4.1.9 Effect of PHF2 F200 and F400 on Lymphocytes (%) in STZ induced Diabetes in Rats

The data are presented as Mean ± SEM with a sample size of six rats per group and were analyzed using one-way ANOVA followed by Tukey's post hoc test.

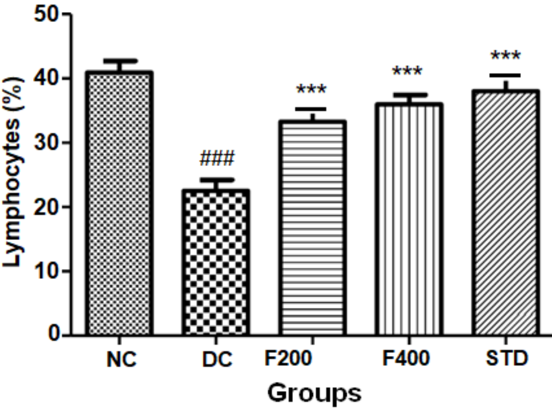


Figure 6.36 illustrates the effect of PHF2 at doses of 200 mg/kg (F200) and 400 mg/kg (F400) on lymphocyte levels in streptozotocin-induced diabetic rats.

Administration of streptozotocin at 45 mg/kg intraperitoneally resulted in a significant decrease in lymphocyte counts compared to the diabetic control (DC) group ($p<0.001$).

6.10.4.1.10 Effect of PHF2 F200 and F400 on Eosinophils (%) in STZ induced Diabetes in Rats

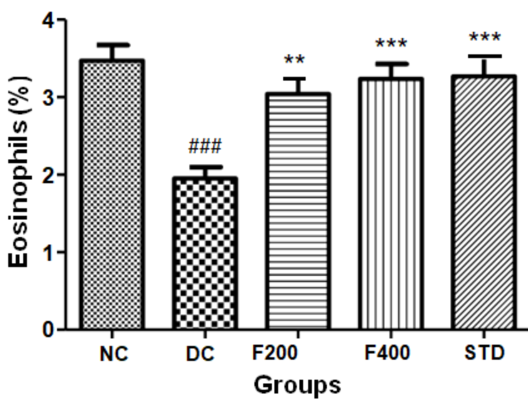


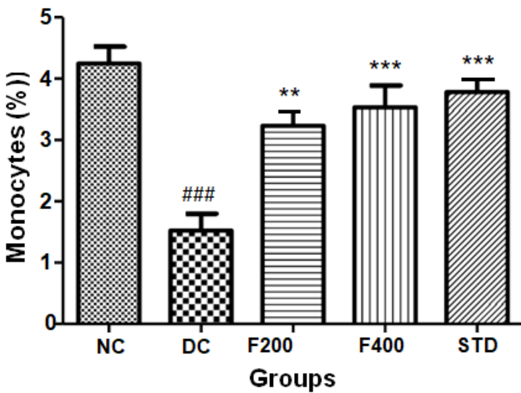
Figure 6.37 depicts the effect of PHF2 administered at doses of 200 mg/kg (F200) and 400 mg/kg (F400) on eosinophil levels in rats with streptozotocin-induced diabetes.

The results, shown as Mean ± SEM (n = 6), underwent statistical analysis using One-way

ANOVA followed by Tukey's post hoc test. Eosinophil counts exhibited a significant decrease in rats with streptozotocin-induced diabetes following injection (45 mg/kg, i.p.) compared to diabetic control (DC) rats ($p < 0.001$). In contrast, treatment with both doses of PHF2 (200 mg/kg and 400 mg/kg) and Glibenclamide (5 mg/kg, orally) significantly increased eosinophil counts compared to DC rats ($p < 0.01$; $p < 0.001$). These findings suggest that PHF2 may have an immunomodulatory effect in diabetic conditions.

6.10.4.1.11 Effect of PHF2 F200 and F400 on Monocytes (%) in STZ induced Diabetes in Rats

Following the administration of streptozotocin (45 mg/kg, i.p.), there was a significant decrease in monocyte count compared to diabetic control (DC) rats ($p < 0.001$).



In Figure 6.38, the effects of PHF2 administered at doses of F200 and F400 on monocyte levels in rats with streptozotocin-induced diabetes are illustrated.

In contrast, treatment with PHF2 (F200 and F400 mg/kg, orally) and Glibenclamide (5 mg/kg, orally) notably increased monocyte count compared to DC rats ($p < 0.01$; $p < 0.001$). These results are presented as Mean ± SEM ($n = 6$) and were analyzed using One-way ANOVA followed by Tukey's post hoc test.

6.10.4.1.12 Effect of PHF2 F200 and F400 on Basophils (%) in STZ induced Diabetes in Rats

The results are presented as mean ± SEM (n = 6) and were statistically analyzed using one-way ANOVA followed by Tukey's post hoc test.

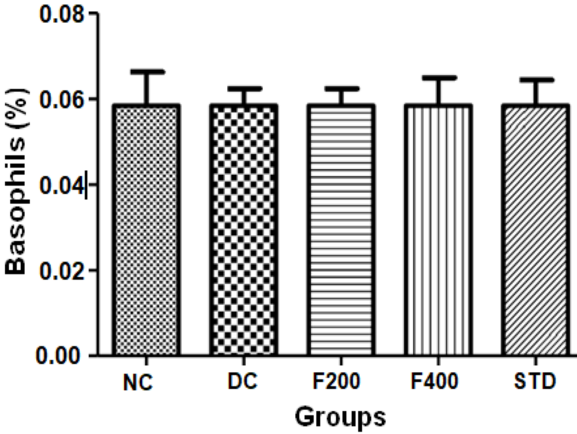


Figure 6.39 illustrates the effects of PHF2 administered at doses of 200 mg/kg (F200) and 400 mg/kg (F400) on basophil counts in streptozotocin-induced diabetic rats.

Following administration of streptozotocin (45 mg/kg, i.p.), PHF2 (F200 and F400 mg/kg, orally), and Glibenclamide (5 mg/kg, orally), no significant alteration in basophil counts was observed compared to normal control (NC) rats. These findings indicate that these treatments did not significantly impact basophil levels under the described test conditions.

6.10.4.1.13 Effect of PHF2 F200and F400 on Platelet Count (Lakhs/Cumm) in STZ induced Diabetes in Rats

The results are presented as mean ± SEM (n = 6), and statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test.

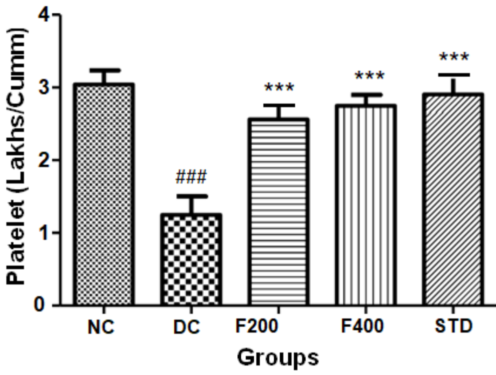


Figure 6.40 illustrates the effect of PHF2 at doses of F200 and F400 on platelet count (Lakhs/Cumm) in rats with streptozotocin-induced diabetes.

Diabetes was induced in the rats by administering streptozotocin (45 mg/kg, i.p.), resulting in a significant decrease in platelet count compared to diabetic control (DC) rats ($p < 0.001$). Treatment with PHF2 (F200 and F400 mg/kg, orally) and Glibenclamide (5 mg/kg, orally) markedly increased platelet count compared to DC rats ($p < 0.01$; $p < 0.001$).

6.10.4.2 Biochemical Parameters

6.10.4.2.1 Effect of PHF2 F200 and F400 on Blood Glucose (mg/dl) in STZ induced Diabetes in Rats

Two doses of PHF2 were investigated: 200 mg/kg (F200) and 400 mg/kg (F400). Blood glucose levels (mg/dl) are presented as mean ± SEM (n = 6). Statistical analysis involved one-way ANOVA followed by Tukey's post hoc test. Initially, streptozotocin administration (45 mg/kg, i.p.) significantly elevated blood glucose levels compared to diabetic control (DC) rats ($p < 0.001$).

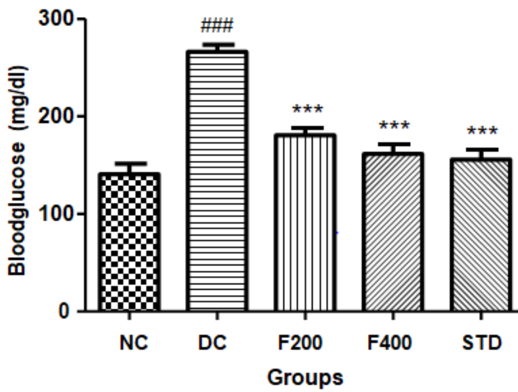


Figure 6.41.The effect of PHF2 administration on blood glucose levels in rats with streptozotocin-induced diabetes.

Treatment with PHF2 (F200 and F400 mg/kg, orally) and Glibenclamide (5 mg/kg, orally) markedly reduced blood glucose levels compared to DC rats ($p < 0.01$; $p < 0.001$). These findings suggest that PHF2 effectively lowers blood glucose levels in this experimental diabetic model, highlighting its potential as an antihyperglycemic agent.

6.10.4.2.2 Effect of PHF2 F200 and F400 on Serum Creatinine (mg/dl) in STZ induced Diabetes in Rats

The data are presented as mean ± SEM (n = 6) and were statistically analyzed using One-way ANOVA followed by Tukey's post hoc test. Streptozotocin injection (45 mg/kg, i.p.) significantly increased creatinine levels compared to diabetic control (DC) rats ($p < 0.001$).

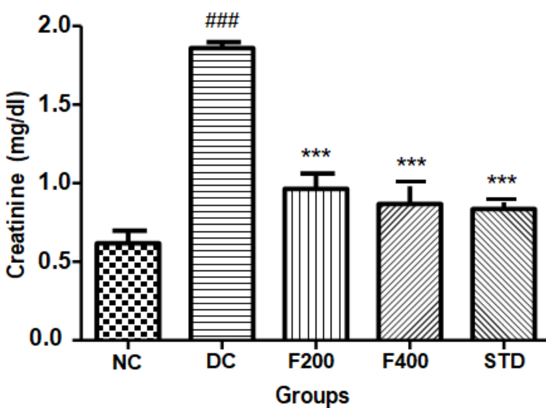


Figure 6.42 illustrates the effect of PHF2 administered at doses of 200 mg/kg (F200) and 400 mg/kg (F400) on creatinine levels (mg/dl) in rats with streptozotocin-induced diabetes.

The data are presented as mean ± SEM (n = 6) and were statistically analyzed using One-way ANOVA followed by Tukey's post hoc test. Streptozotocin injection (45 mg/kg, i.p.) significantly increased creatinine levels compared to diabetic control (DC) rats ($p < 0.001$).

However, treatment with PHF2 (F200 and F400 mg/kg, orally) and Glibenclamide (5 mg/kg, orally) significantly reduced creatinine levels compared to DC rats ($p < 0.001$). These findings indicate that PHF2 demonstrates protective effects against streptozotocin-induced creatinine elevation, suggesting its potential in improving diabetes-associated renal dysfunction.

6.10.4.2.3 Effect of PHF2 F200 and F400 on Serum Protein (g/dl) in STZ induced Diabetes in Rats

The data, presented as Mean ± SEM (n = 6), underwent statistical analysis using One-way ANOVA followed by Tukey's Kramer test. Injection of streptozotocin (45 mg/kg, i.p.) significantly reduced serum protein levels compared to diabetic control (DC) rats ($p < 0.001$).

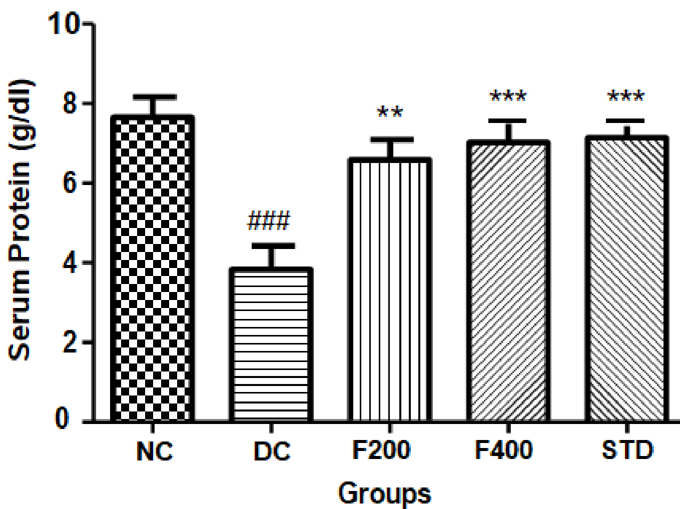


Figure 6.43: illustrates the effect of PHF2 at doses of 200 mg/kg (F200) and 400 mg/kg (F400) on serum protein levels (mg/dl) in rats with streptozotocin-induced diabetes.

Conversely, treatment with PHF2 at both 200 mg/kg and 400 mg/kg doses, as well as Glibenclamide at 5 mg/kg (administered orally), substantially elevated serum protein levels compared to DC rats ($p < 0.01$; $p < 0.001$). These results suggest that PHF2 effectively restored serum protein levels in this experimental diabetic model, indicating its potential to ameliorate diabetes-induced protein abnormalities.

6.10.4.2.4 Effect of PHF2 F200 and F400 on Alanine transaminase (IU/L) in STZ induced Diabetes in Rats

Administration of streptozotocin (45 mg/kg, i.p.) markedly elevated ALT levels compared to the diabetic control (DC) group ($p<0.001$).

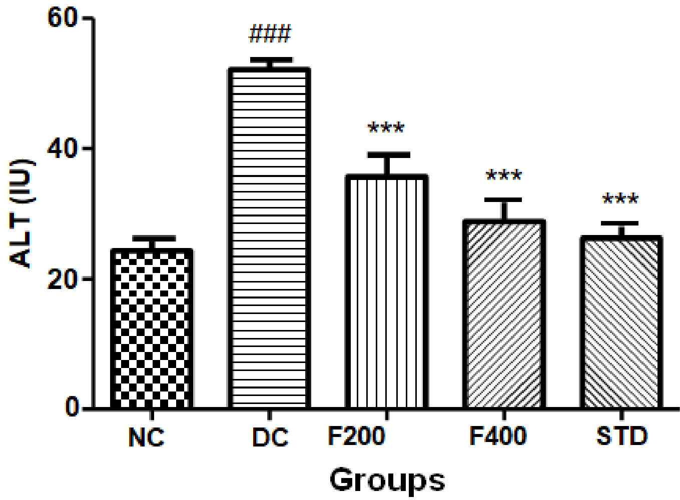
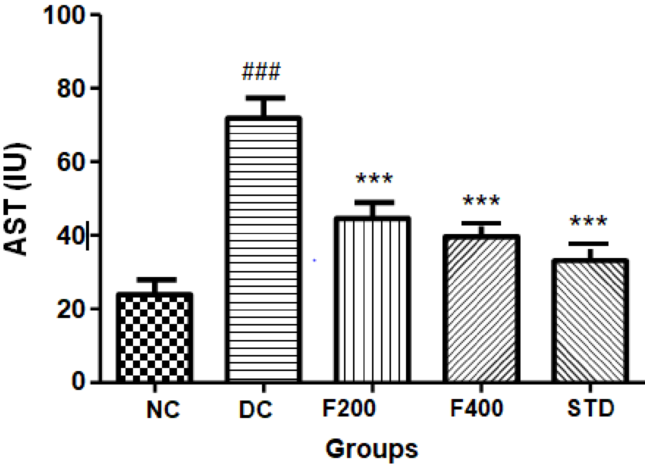


Figure 6.44: illustrates the effects of PHF2 at doses of 200 mg/kg (F200) and 400 mg/kg (F400) on alanine transaminase (ALT) levels (IU/L) in rats with streptozotocin-induced diabetes.

However, both doses of PHF2 (200 mg/kg and 400 mg/kg) as well as Glibenclamide at 5 mg/kg (all administered orally) significantly reduced ALT levels compared to the DC group ($p<0.001$). The results are presented as mean ± SEM (n = 6) and were statistically analyzed using one-way ANOVA followed by Tukey's post hoc test.

6.10.4.2.4 Effect of PHF2 F200 and F400 on Aspartate amino transferase (IU/L) in STZ induced Diabetes in Rats.

Diabetes was induced in rats using streptozotocin (45 mg/kg, i.p.), resulting in a significant increase in aspartate transaminase levels compared to the diabetic control (DC) group ($p < 0.001$).These results are presented as Mean ± SEM (n = 6) and were analyzed using One- way ANOVA followed by Tukey's post hoc test.



In Figure 6.45, the effects of PHF2 at doses of F200 and F400 on aspartate transaminase levels (IU/L) in rats with streptozotocin-induced diabetes are illustrated.

However, treatment with PHF2 (F200 and F400 mg/kg, orally) and Glibenclamide (5 mg/kg, orally) markedly reduced aspartate transaminase levels compared to DC rats ($p < 0.001$).

6.10.4.2.6 Effect of PHF2 F200 and F400 on Blood Urea Nitrogen (mg/dl) in STZ induced Diabetes in Rats

Streptozotocin induction (45 mg/kg, i.p.) significantly elevated BUN levels compared to diabetic control (DC) rats ($p<0.001$).The results are expressed as Mean ± SEM (n = 6) and were analyzed using One-way ANOVA followed by Tukey's post hoc test.

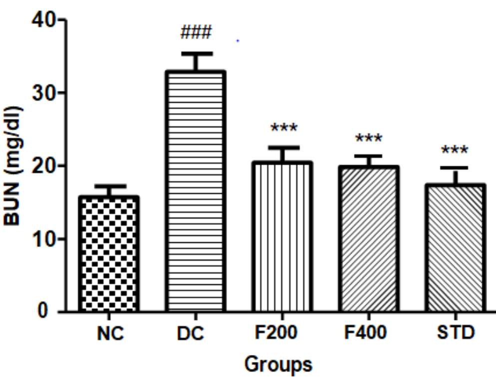


Figure 6.46 depicts the effect of PHF2 at doses F200 and F400 on blood urea nitrogen (BUN) levels (mg/dl) in rats with streptozotocin-induced diabetes.

Treatment with PHF2 (F200 and F400 mg/kg, orally) and Glibenclamide (5 mg/kg, orally) significantly reduced BUN levels compared to DC rats ($p<0.001$).

6.10.4.2.7 Effect of PHF2 F200 and F400 on Total Cholesterol (mg/dl) in STZ induced Diabetes in Rats

The data are presented as Mean ± SEM (n = 6) and were statistically analyzed using One-way ANOVA followed by Tukey's post hoc test. Administration of streptozotocin (45 mg/kg, i.p.) significantly increased total cholesterol levels compared to diabetic control (DC) rats ($p < 0.001$).

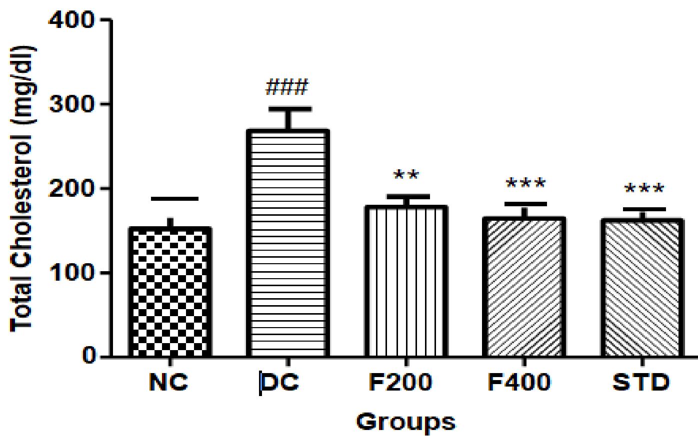
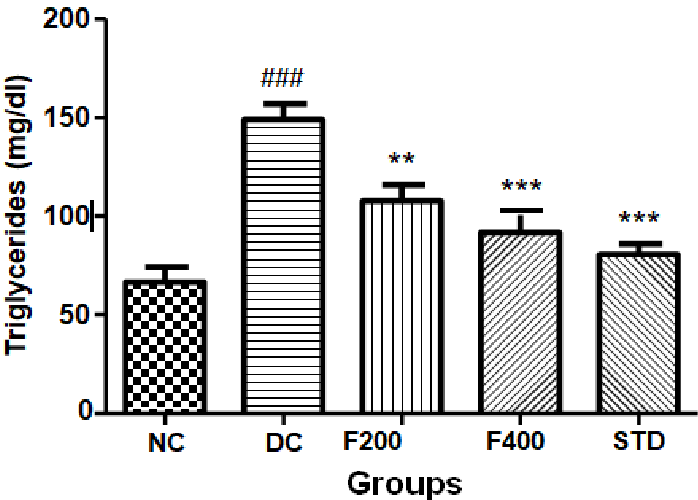


Figure 6.47 illustrates the effects of PHF2 administered at doses of 200 mg/kg (F200) and 400 mg/kg (F400) on total cholesterol levels (mg/dl) in rats with streptozotocin-induced diabetes.

Conversely, treatment with PHF2 (F200 and F400 mg/kg, orally) and Glibenclamide (5 mg/kg, orally) significantly decreased total cholesterol levels compared to DC rats ($p < 0.01$; $p < 0.001$). These results suggest that PHF2 may effectively lower elevated cholesterol levels associated with streptozotocin-induced diabetes, indicating its potential as a therapeutic agent for managing dyslipidemia in diabetic conditions.

6.10.4.2.8 Effect of PHF2 F200 and F400 on Triglycerides (mg/dl) in STZ induced Diabetes in Rats

Following the administration of streptozotocin (45 mg/kg, i.p.), there was a significant increase in triglyceride levels compared to diabetic control (DC) rats ($p < 0.001$). In contrast, treatment with PHF2 (F200 and F400 mg/kg, orally) and Glibenclamide (5 mg/kg, orally) markedly reduced triglyceride levels compared to DC rats ($p < 0.01$; $p < 0.001$).



In Figure 6.48, the effect of PHF2 administered at doses of F200 and F400 on triglyceride levels (mg/dl) in rats with streptozotocin-induced diabetes is depicted.

The results are presented as Mean ± SEM (n = 6) and were analyzed using One- way ANOVA followed by Tukey's post hoc test.

6.10.4.2.8 Effect of PHF2 F200 and F400 on HDL Cholesterol (mg/dl) in STZ induced Diabetes in Rats

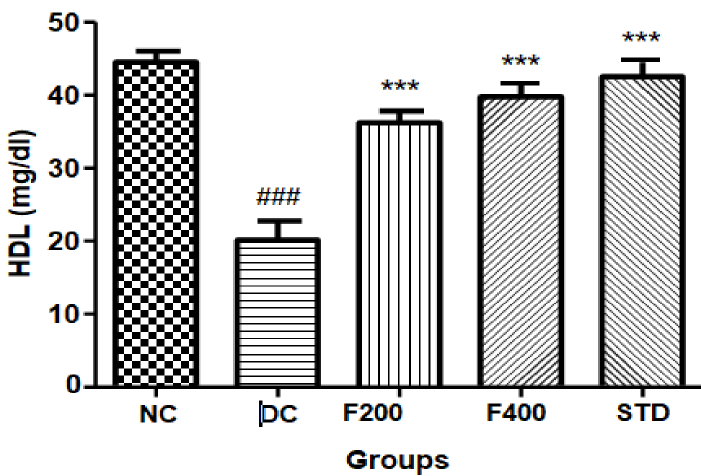


Figure 6.49 shows the effect of PHF2 at doses of F200 and F400 on high-density lipoprotein (HDL) levels (mg/dl) in rats with streptozotocin-induced diabetes.

Administration of streptozotocin (45 mg/kg, i.p.) markedly decreased HDL levels compared to diabetic control (DC) rats ($p<0.001$). In contrast, treatment with PHF2 (F200 and F400 mg/kg, orally) and Glibenclamide (5 mg/kg, orally) significantly increased HDL levels compared to DC rats ($p<0.001$). The data are presented as Mean ± SEM (n = 6) and were analyzed using One-way ANOVA followed by Tukey's post hoc test.

6.10.4.2.9 Effect of PHF2 F200 and F400 on LDL Cholesterol (mg/dl) in STZ induced Diabetes in Rats

The results are presented as Mean ± SEM (n = 6) and were assessed using One- way ANOVA followed by Tukey's Kramer test. Administration of streptozotocin (45 mg/kg, i.p.) markedly increased LDL levels compared to diabetic control (DC) rats ($p < 0.001$).

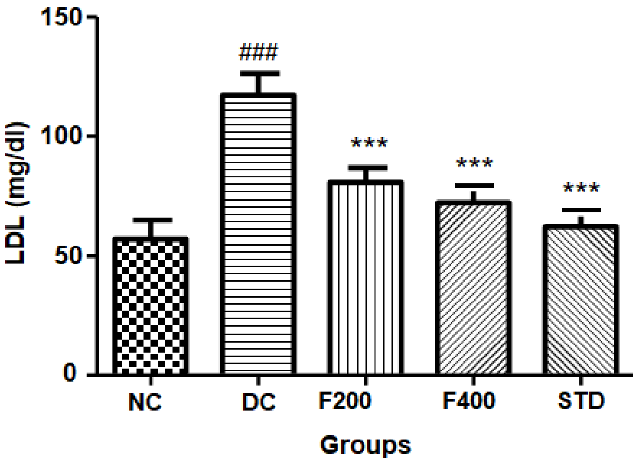


Figure 6.50 depicts the effect of PHF2 given at doses of 200 mg/kg (F200) and 400 mg/kg (F400) on low-density lipoprotein (LDL) levels (mg/dl) in rats with streptozotocin-induced diabetes.

Conversely, treatment with PHF2 (F200 and F400 mg/kg, orally) and Glibenclamide (5 mg/kg, orally) significantly reduced LDL levels compared to DC rats ($p < 0.001$). These observations indicate that PHF2 may effectively alleviate the rise in LDL cholesterol linked with streptozotocin-induced diabetes, suggesting its potential as a therapeutic agent for managing dyslipidemia in diabetic conditions.

6.10.4.2.10 Effect of PHF 200 and 400 on VLDL Cholesterol (mg/dl) in STZ induced Diabetes inRats

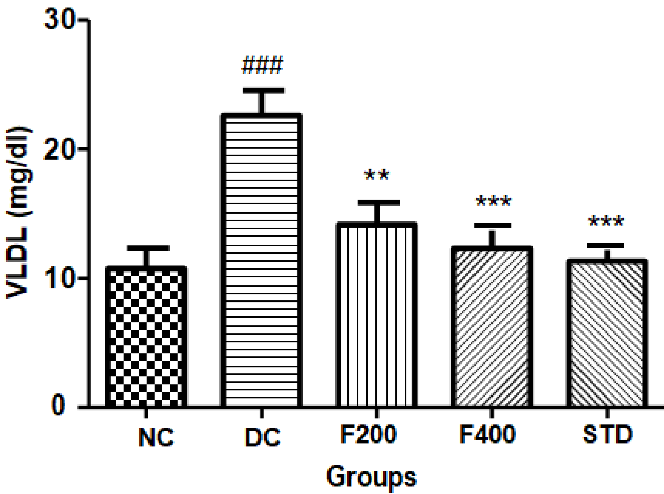


Figure 6.51 presents the effects of PHF2 administered at doses of 200 mg/kg (F200) and 400 mg/kg (F400) on very low-density lipoprotein (VLDL) levels (mg/dl) in rats with streptozotocin (STZ)-induced diabetes.

The data are expressed as Mean ± SEM (n = 6) and were analyzed using One-way ANOVA followed by Tukey's Kramer test.

Administration of STZ (45 mg/kg, i.p.) significantly increased VLDL levels compared to diabetic control (DC) rats ($p < 0.001$). In contrast, treatment with PHF2 (F200 and F400 mg/kg, orally) and Glibenclamide (5 mg/kg, orally) significantly decreased VLDL levels compared to DC rats ($p < 0.01$; $p < 0.001$). These results indicate that PHF2 may effectively mitigate the elevation of VLDL cholesterol associated with STZ-induced diabetes, suggesting its potential therapeutic utility in managing dyslipidemia in diabetic conditions.

6.10.4.2.11 Effect of PHF 200 and 400 on HbA1c levels in STZ induced Diabetes in Rat

The data are expressed as Mean ± SEM (n = 6) and were analyzed using One-way ANOVA followed by Tukey's Kramer test.

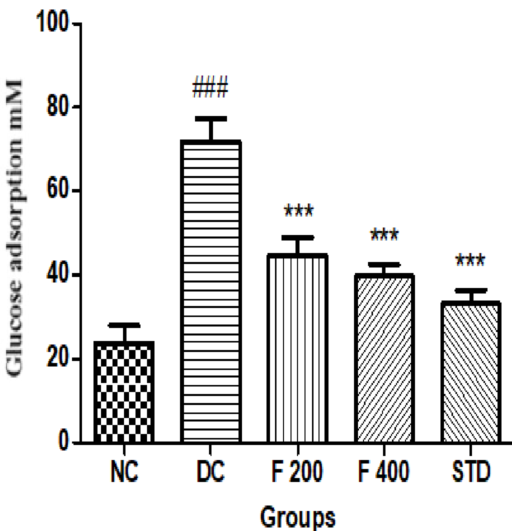


Figure 6.52 presents the effects of PHF2 administered at doses of 200 mg/kg (F200) and 400 mg/kg (F400) on Glucose absorption levels mM in rats with streptozotocin (STZ)-induced diabetes.

The treatment of rats with STZ (45 mg/kg, i.p.) induced significant ($p<0.001$) increment in HbA1c levels when compared with DC rats. However, the treatment of rats with PHF (200 and 400 mg/kg, p.o.) and Glibenclamide (5 mg/kg, p.o.) exhibited significant ($p<0.01$;$p<0.001$) decrement in HbA1c levels when compared with DC rats.

6.10.4.2.12 Effect of PHF 200 and 400 on HbA1c levels in STZ induced Diabetes in Rat

The data are expressed as Mean ± SEM (n = 6) and were analyzed using One-way ANOVA followed by Tukey's Kramer test.

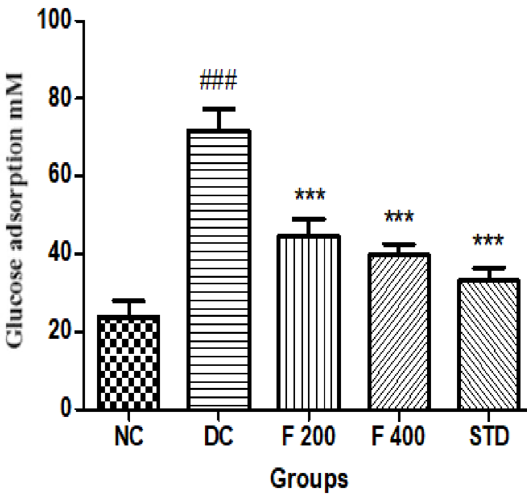


Figure 6.53 presents the effects of PHF2 administered at doses of 200 mg/kg (F200) and 400 mg/kg (F400) on HbA1c level in rats with streptozotocin (STZ)-induced diabetes.

The treatment of rats with STZ (45 mg/kg, i.p.) induced significant ($p<0.001$) increment in HbA1c levels when compared with DC rats. However, the treatment of rats with PHF (200 and 400 mg/kg, p.o.) and Glibenclamide (5 mg/kg, p.o.) exhibited significant ($p<0.01$; $p<0.001$) decrement in HbA1c levels when compared with DC rats.

6.10.4.2 Histopathological analysis

6.10.4.3.1 Histopathology of Pancreas: Pancreas histology revealed normal cells and acini in the islets of Langerhans in the normal control pancreas (A). In diabetic animals receiving prolonged in diabetic rats (B), damage to the islets of langerhans and reduced islet dimensions were noted. Treatment with PHF2 400 mg/kg (D) restored the islets' cellular population size to normal.

Table 6.26: Histopathology of Pancresae in groups

ORGAN	GROUP	HISTOLOGY	OBSERVATION
PANCREASE	NORMAL		No fibrosis or inflammation was present, and the pancreas displayed normal histoarchitecture with wells of acini containing healthy islet cells.
	DISESED		Islet cell shrinkage and atrophy associated with inflammatory alterations
	PHF2 F200MG		regenerated pancreatic cells with normal islet cells
	PHF2 F400MG		no atrophy or necrosis and fibrotic changes appear
	STANDARD		mild to severe atrophy, slight necrosis, and modest fibrotic alterations

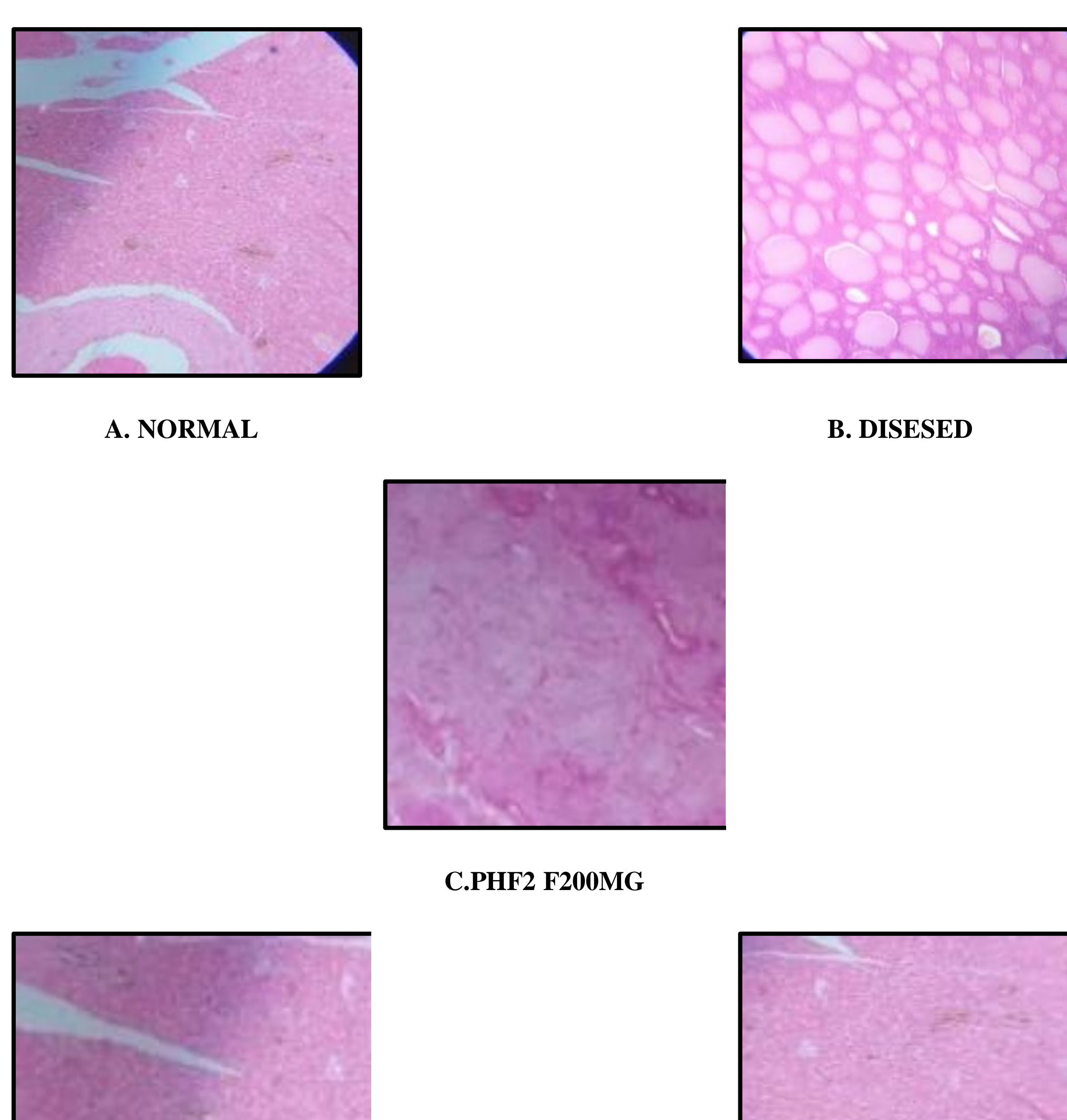


Figure 6.54: Histopathology of Pancresae in groups

Since streptozotocin is known to cytotoxically affect pancreatic islet B-cells, it has been widely used to produce type 1 diabetes in rats. It tampers with the oxidative and metabolic processes of the cell.A growing body of research, drawn from both experimental and clinical investigations, indicates the significance of oxidative stress in the onset and advancement of both forms of diabetes mellitus.

Because of the oxidation of glucose, non-enzymatic protein glycation, and the ensuing oxidative degradation of glycation proteins, diabetes causes an excess of free radical production. Diabetes often results in a decline in antioxidant defenses. In cases of streptozotocin-induced diabetes, glibenclamide is frequently employed as a standard anti-diabetic treatment to assess the efficacy of other hypoglycemic drugs. In this work, the hypoglycemic action of PHF in rats with diabetes was investigated. The number of beta-cells in the islets increased in diabetic rats (Group III and Group IV) treated with PHF and Glibenclamide, suggesting beta cell rejuvenation. The pancreatic slice of a rat with streptozotocin-induced diabetes showed a substantial reduction in the quantity and size of Langerhans islets. Along with mononuclear cell invasion, there was a significant decrease in the number of secretory cells per islet. The pancreatic slice of diabetic rats given PHF2 treatment (Group V) showed a large number and abundance of islets. There was infiltration of mononuclear cells and natural secretory cells with granular, pale cytoplasm in the isolated islets. The pancreas of a diabetic rat (Group V) receiving glibenclamide showed a significant increase in the size of the Langerhans islets and an increase in secretory cells. The pancreatic sections of Group I animals showed signs of normal histology. In group II rats, STZ-induced hyperglycemia resulted in pancreatitis, necrosis, islet hyperplasia, degeneration, and inflammatory cell filtration. In rats with interstitial pancreatitis, PHF therapy resulted in virtually normal pancreatic histology with less fibrosis, normal architecture, and less inflammatory cell filtration. The Glibenclamide-treated Group V rats had normal Langerhans islets, as well as normal architecture and histology.

Group I: Normal Control–arrow indicates the proper arrangement of the pancreatic cells.

Group II: Diabetic control induced with STZ (45mg/kg BW)–arrow indicates the pancreatic cells were swollen, with spotty necrosis with the accumulation of lipids.

Group III: STZ treated with PHF2 (200mg/kg)–arrow indicates partly recovered pancreatic cells.

Group IV: STZ treated with PHF2 (400mg/kg)–arrow indicate recovered normal arrangement pancreatic cells.

Group V: STZ treated with glibenclamide (45mg/kg)–arrow indicates recovered normal arrangement pancreatic cells

Pathohistological changes were employed in the pancreas of normal and diabetic rats to examine PHF2 and its role in diabetes. The pancreas of diabetic rats showed a decrease in islets cells and an increase in adipose tissue. Less fatty acid infiltration was seen in the pancreas of diabetic rats. PHF2 protects beta cells from free radical damage and restores the antioxidant status of the pancreas. The pancreatic of rats treated with PHF2 did not show any pathological alterations, indicating that the chemical does not normally have a negative effect on the pancreas. We observed vascular congestion, intertubular bleeding, and mononuclear cellular infiltration of hepatocytes in the liver and kidneys of experimental diabetic rats. When PHF2 was administered to diabetic rats, these alterations were less noticeable.

6.10.4.3.2 Histopathology of Liver

Result revealed typical hepatic cells with the central vein (A), nucleus, and cytoplasm well intact. The typical lobular organization of the group II diabetic rats was maintained. The major vein was clearly visible and notably clogged. There were also noticeable focal foci of bleeding. It was obvious that the fat had changed.
The portal tracts showed up as expected (B). The portal tracts and central veins of the hepatocytes in group IV (diabetes rats with PHF 400mg/kg) seem normal (D).

Table 6.27: Histopathology of Liver in groups

ORGAN	GROUP	HISTOLOGY	OBSERVATION
LIVER	NORMAL		Hepatic cells in normal condition, with the central vein, cytoplasm, and nucleus all intact.
	DISEASED		**Necrosis and Inflammation**
	PHF2 200MG		**Regeneration**
	PHF2 400MG		**No Necrosis and Inflammation**
	STANDARD		**Slight necrosis**

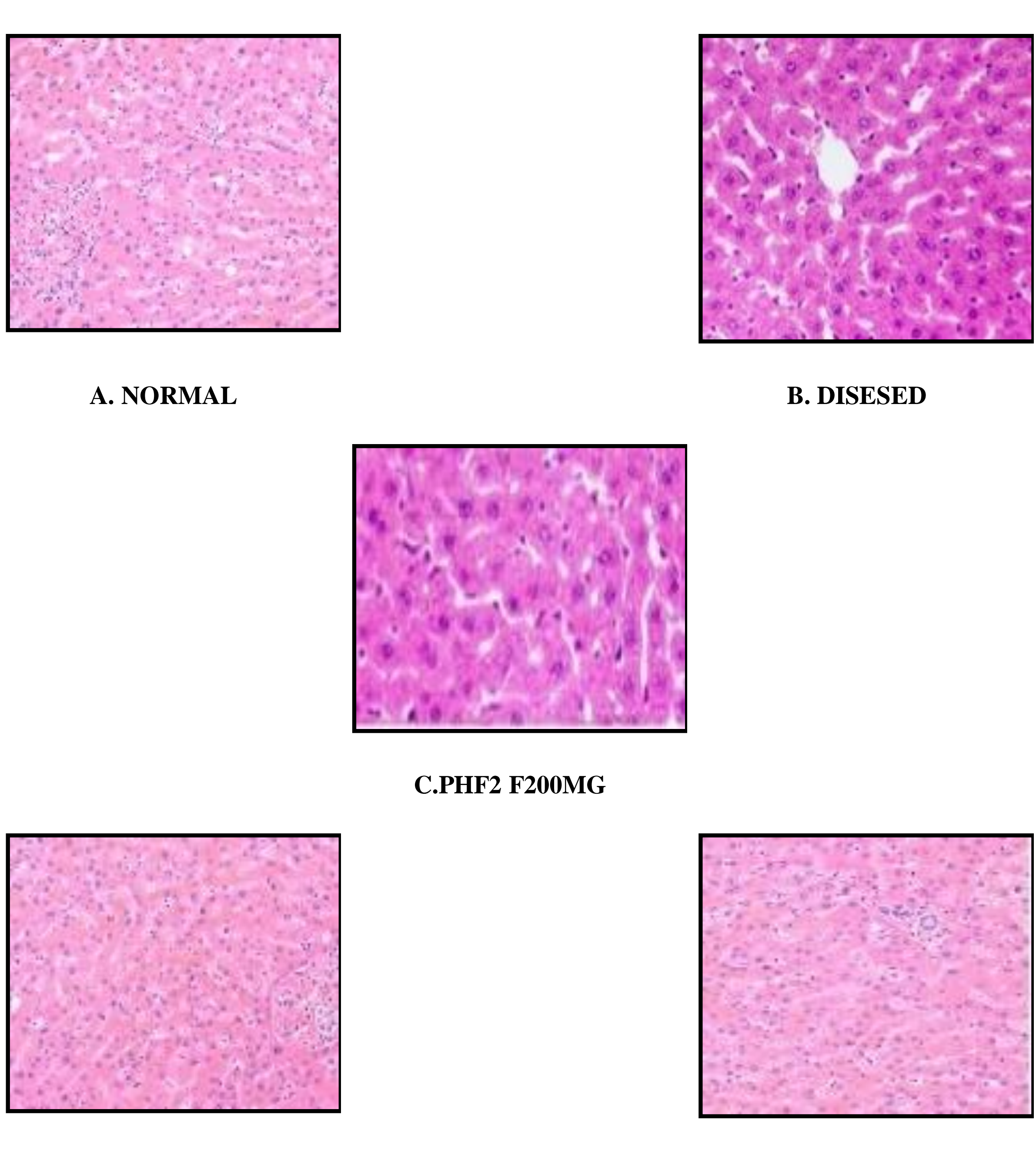


Figure 6.55: Histopathology of Liver in groups

The livers of the diabetic rats who received treatment showed very noteworthy outcomes; tissue samples from these experimental groups were used in histological investigations. Histopathology results showed that while control animals (Group-I) had perfect hepatocytes in their liver tissue, animals treated with streptozotocin had severely damaged liver hepatocytes, major blood sinusoids had undergone degeneration, and internal blood

cysts were seen in animals with streptozotocin-induced diabetes (Group II). Groups III through V, who were treated with glibenclamide and plant extract, demonstrated reversible tissue regeneration with a notable hepatocyte component during this interim period.

In order to determine if the polyherbal treatments had a dosage effect on the STZ-induced diabetic liver tissues, a light microscopy study was carried out. The histological composition of the liver in Group 1 (the control group) was normal. The modifications in Group II's liver's histological structure were greatly influenced by the STZ therapy. Complete (painful) hepatocyte death was seen in severe congestion with nuclear condensation, loss of hepatic lobules, and congested hepatic inflammation in histological sections of diabetic rats treated with STZ. The discovered anomalies in the liver histological sections of diabetic rats generated by streptozotocin may lead to hyperglycemia, which escalates the generation of free radicals and ultimately causes the degeneration of liver cells.

The effects of the diabetogenic drug STZ may first have an influence on the increase in oxygen free radicals in diabetes, and then blood glucose levels. After receiving 200 mg/kg BW PHF for 45 days, diabetic rats (Group V) exhibited a rather normal hepatocyte shape and no hepatic abnormalities. Treatment with PHF markedly enhanced liver healing in STZ diabetic rats. PHF-treated diabetic rat livers showed clean bile canaliculi, complete cell recovery, and a typical hepatic cord arrangement in the centrolobular area. PHF therapy for diabetic rats resulted in the correct distribution of kupffer and sinusoidal cells throughout the liver.

In diabetic rats, PHF exhibited an anti-diabetic effect. This effect could be attributed to insulin mimetic action or to alternative mechanisms, such as increased glucose uptake by peripheral tissue, decreased endogenous glucose production, or increased gluconeogenesis in the liver and muscle. Histological investigation revealed that the liver tissue of diabetic rats treated with 45 mg/kg BW of Glibenclamide in group VI did not show aberrant hepatocyte abnormalities reflecting the effects on diabetic rats. The medication glibenclamide's ability to secrete insulin may have played a major role in the management of diabetes.

Group I: Normal Control–arrow indicates the proper arrangement of the hepatic cells.

Group II: Diabetic control induced with STZ (45mg/kg BW)–arrow indicates the hepatic cells were swollen, with spotty necrosis with the accumulation of lipids.

Group III: STZ treated with PHF2 (200mg/kg)–arrow indicates partly recovered hepatic cells.

Group IV: STZ treated with PHF2 (400 mg/kg)–arrow indicate recovered normal arrangement hepatic cells.

Group VI: STZ treated with Glibenclamide (45 mg/kg)–arrow indicates recovered normal arrangement hepatic cells.

Histological examinations revealed that PHF produced the most effective liver and renal rehabilitation in STZ-induced diabetic rats, acting in a manner akin to that of animals treated with Glibenclamide Severe tubular epithelial atrophy, mild mesangial proliferation, restricted sclerotic changes in the glomerulus, and moderate capillary congestion were all seen in the kidneys of diabetic rats.

Due to increased catabolic processes such glycogenolysis, lipolysis, and proteolysis—which are brought on by insulin and/or cellular sugar shortages in the liver organ cells—a drop in body weight is seen in the livers of diabetic experimental animals. However, overindulging in sugar raises the stress on the kidneys and intensifies protein synthesis, lipogenesis, and glycogen production. These changes may lead to several metabolic changes and severe microvascular renal issues in the development of diabetic nephropathy. In our investigation, the appropriate dosage of extract prevented the liver state from getting back to normal. Giving alkaloids to diabetic rats was found to drastically reduce weight and liver and renal system disease while restoring normal texture.

6.11 DEVELOPMENT AND EVALUATION OF POLYHERBAL FORMULATION.

6.11.1 Preparation of tablet

Optimized ratio of extract combination was used to develop formulation. Trial batches were formulated by taking excipients like Lactose, Microcrystalline cellulose, Talc, Magnesium stearate. The concentration of microcrystalline was differed to study the effect of change concentration on disintegration time.

Table 6.28: Composition of Polyherbal formulation

Ingredient	Quantity(in mg)				Uses
	F1	F2	F3	F4	
Sesbania Grandiflora Extract	100	100	100	100	Antidiabetic
Beta Vulgaris Extract	100	100	100	100	Antidiabetic
Microcrystalline Cellulose	40	50	60	70	Disintegrating agent
Magnesium stearate	10	10	10	10	Lubricant
Talc	10	10	10	10	Lubricant
Lactose	Q.S.	Q.S.	Q.S.	Q.S.	Diluent
Total weight	**500**	**500**	**500**	**500**	

Figure 6.55: F1 powder **Figure 6.56: F2 powder**

Figure 6.57: F3 powder **Figure 6.58: F4 powder**

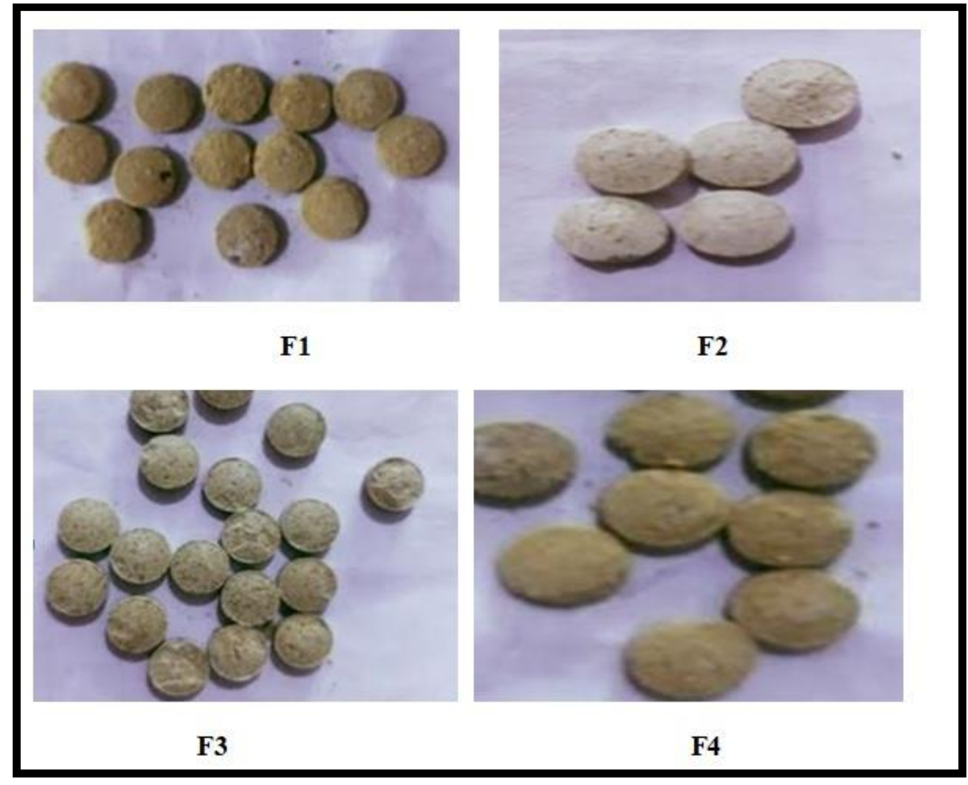


Figure 6.59: F1 TO F4 TABLETS

Table 6.29: Organoleptic characters of Polyherbal formulation

Parameters	F1	F2	F3	F4
Color	Darkbrown	Buff colored	Greyish brown	Greenish brown
Odour	Unique characteristic	Unique characteristic	Unique characteristic	Unique characteristic
Taste	Bitter	Bitter	Bitter	Bitter
Texture	Smooth	Smooth	Smooth	Smooth

6.11.2 FTIR compatibility study

FTIR study for compatibility of the active ingredient and excipients was done and it wasfound that they were compatible with each other.FTIR showed the occurrence of functional groups such as phenolic, alcohol, alkene, andnitride.

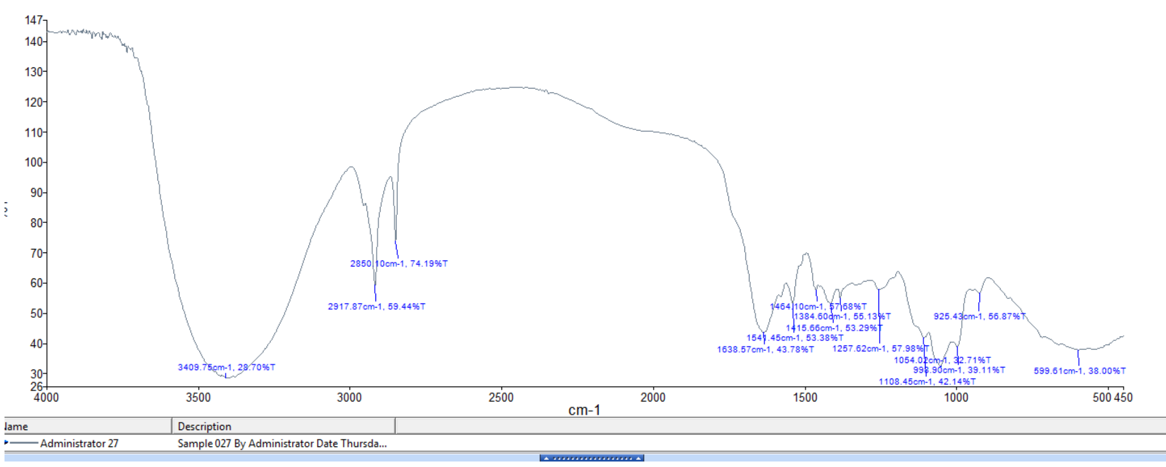
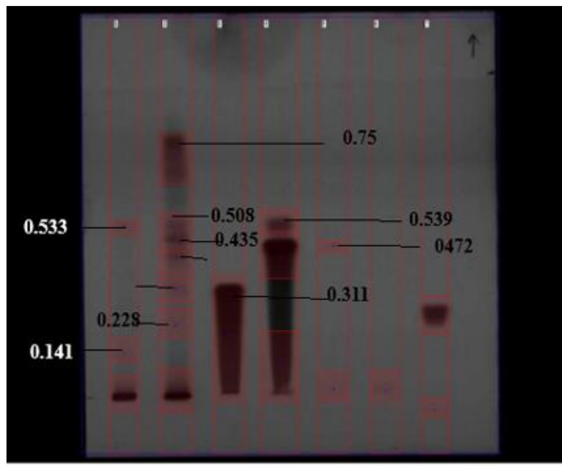


Figure 6.60: FTIR of Polyherbal formulation

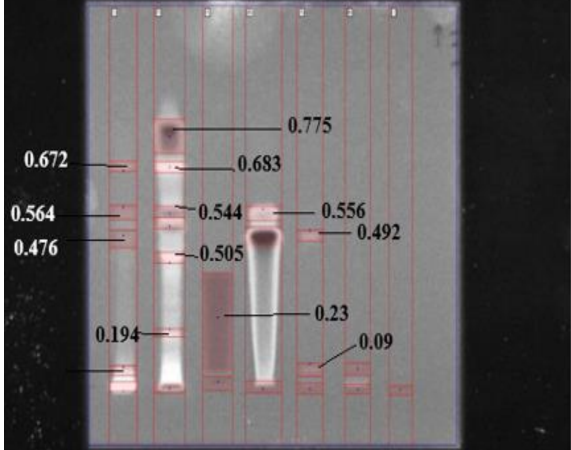
Table 6.30: Functional group in Polyherbal Formulation

Functional group	Vibrations	Peak
Alkane	Streching	2917.87cm^{-1}, 2850.10cm^{-1}
Amine N-H	Bending	1653.05cm^{-1}
Sulfoxide S=O	Stretching	1074.38cm^{-1}
Alkene C=C	Bending	925.43cm^{-1}
Carbonyl group (C,O)	Stretching	1638.57 cm^{-1}
Aromatic (C,C) bond	Bending	1384.20 cm^{-1}
(C–O) bond	Stretching	1108.45 cm^{-1}
Amine	Stretching	599.61 cm^{-1}

6.11.3 HPTLC fingerprinting of Polyherbal formulation



TLC of methanolic extract of PHF



TLC OF METHANOLIC EXTRACT OF PHF AT 366 NM

Figure 6.61: HPTLC finger printing of Methanolic extract of root of *PHF 2* at 254

nm and 366 nm

Table 6.31: HPTLC fingerprinting of Methanolic extract combination in equal ratio *at* 254nm and 366nm

Test extracted	Solvent system	Number of spots	Rf values
Methanolic extract of PHF at 254 nm	Toluene: Ethyl acetate; Formic acid Volume 5:4:1	06	0.533, 0.228,0.75,0.508,0.435,0.34
Methanolic extract of PHF at 254 nm		08	0.672,0.564,0.476,0.775,0.683,0.544,0.505,0.395

Table 6.32: R_fValue of Methanolic extract of root of *Beta Vulgaris*

	Rf Value		Assigned substances
	254 nm	366 nm	
1	0.533,0.228, 0.508,0.435, 0.34	0.672,0.564,0.476,0.775,0.683,0.544,0.505,0.395,0.194	Methanol extract of PHF
2	0.2	0.23	Gallic acid
3	0.539,0.439	0.556,0.469	Quercetin
4	0.472	0.492	Flavanoids

6.11.4 Pre-formulation studies

Table 6.33: Pre-formulation studies

Formulation	Angle of repose	Bulk density (gm/ml)	Tapped Density (gm/ml)	Carr's index (%)	Hausners ratio
F1	37±0.52	0.55±0.42	0.68±0.51	18.24±0.21	1.21±0.11
F2	39±0.51	0.52±0.61	0.64±0.35	18.44±0.38	1.22±0.08
F3	31±0.24	0.47±0.71	0.57±0.34	17.13±0.2	0.1±0.52
F4	30±0.45	0.45±0.31	0.55±0.73	14.65±0.25	0.08±0.37

Powders of Polyherbal formulations

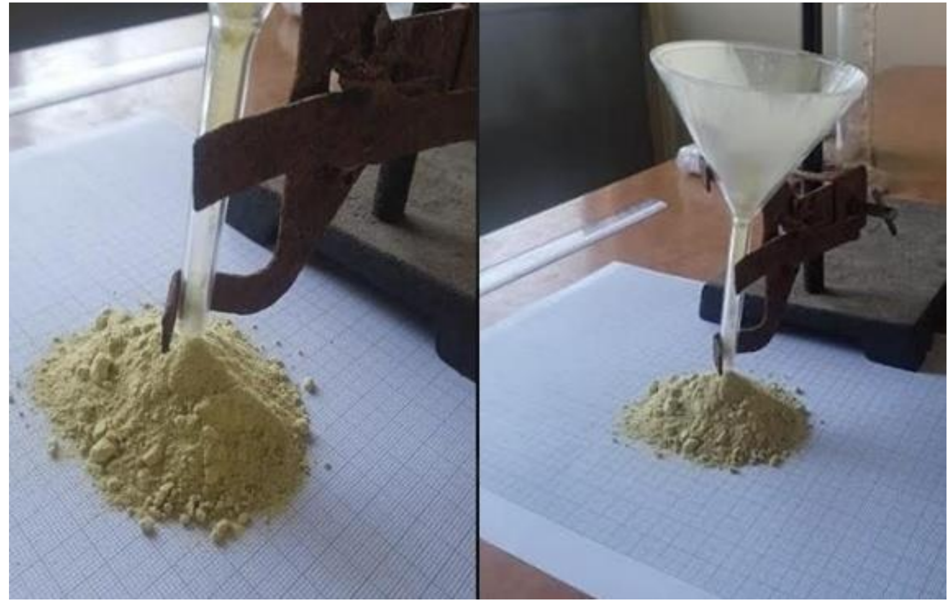


Figure 6.62: Angle of repose of PHF powder

Figure 6.63: Bulk and Tapped Density

6.11.5 Post compression studies of Polyherbal Formulation

Table 6.34: Results of Post compression studies of Polyherbal Formulation

Parameters	F1	F2	F3	F4
Average weight (mg)	500.11±0.91	498.5±0.87	501.11±0.51	498.32±0.32
Hardness (kg/cm^2)	4.93±1.21	4.84±1.19	5.14±1.26	5.06±0.95
Thickness (mm)	3.23±0.12	3.26±0.15	3.28±0.21	3.26±0.23
% Friability	0.92±0.41	0.84±0.52	0.81±0.54	0.86±0.56
Disintegration time(min.)	2.1±0.07	1.9±0.09	1.5±0.1	1.7±0.08

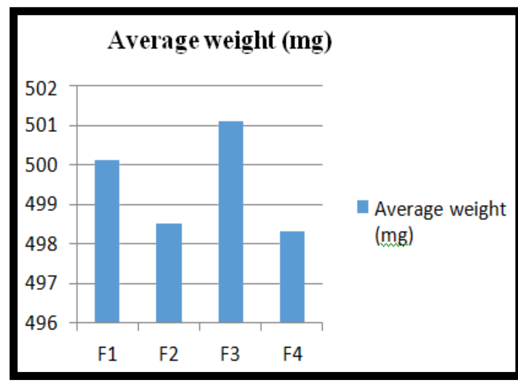


Figure 6.64: Average Weight

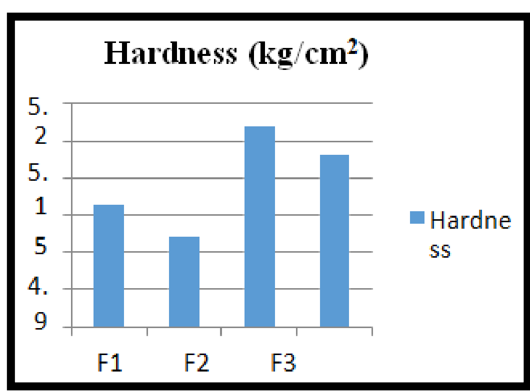


Figure 6.65: Hardness

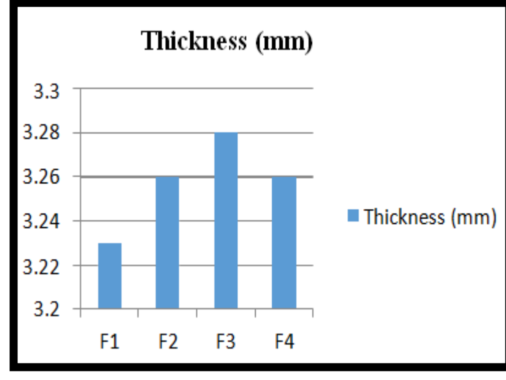


Figure 6.66: Thickness

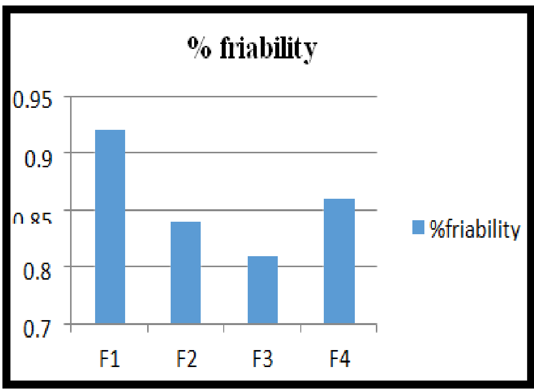


Figure 6.67: Friability

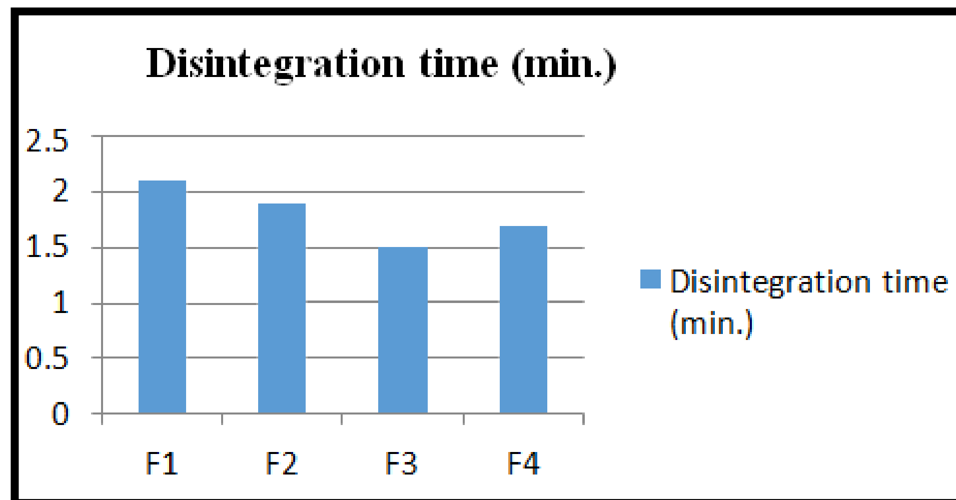


Figure 6.68Disintegration Time

An analysis of the tablet's formulation F3 revealed that its average weight, hardness, thickness, and % friability were all well optimized.

6.11.6 Stability study:

A 30-day stability study was conducted on formulation F3 under two distinct sets of conditions: ambient temperature (25 °C ± 2 °C, 60 ± 5% relative humidity) and accelerated conditions (40 °C ± 2 °C, 75 ± 5% relative humidity). The objective of this study was to assess the stability of the polyherbal tablets. Results indicated that the formulation maintained stability under both conditions for the entire duration of the study.

Table 6.35: Results of stability study of F3 Polyherbal tablet

Parameters	**F3**	**Room temperature 25 °C ± 2 °C/RH 60 ± 5%**		
	0th	**7th**	**15th**	**30th**
Hardness (kg/cm^2)	5.14±1.26	5.15±0.06	5.15±0.16	5.15±0.51
Friability (%)	0.81 ± 0.54	0.81±0.03	0.82±0.07	0.82±0.13
Disintegration time (min.)	1.5±0.1	1.6±0.23	1.6±0.18	1.6±0.11
Parameters	F3	Accelerated temperature 40 °C ± 2 °C/RH 75 ± 5%		
	0th	7th	15th	30th
Hardness (kg/cm^2)	5.14±1.26	5.15±0.17	5.15±0.21	5.15±0.20
Friability (%)	0.81±0.54	0.81±0.09	0.82±0.15	0.82±0.1
Disintegration time (min.)	1.5±0.1	1.6±0.14	1.6±0.25	1.6±0.21

7.1 SUMMARY AND CONCLUSION

A variety of metabolic disorders, including diabetes mellitus, are marked by hyperglycemia as well as impaired lipid, carbohydrate, and protein metabolism. The pandemic of diabetes is widespread and difficult to treat. The number of people with diabetes has more than doubled over the past 20 years. The disease's rapid expansion of type 2 diabetes in young adults, whose risk factor is enhanced by lifestyle, is its most alarming symptom. Epidemiological statistics show that disease is continuously spreading, hurting global health expenditures, and has been dubbed "the diabetes apocalypse".The prevention of disease should come before the treatmen to fit.

Since ancient times, humans have relied on plants and natural substances to support their health and well-being. References in Vedic literature and other ancient Indian texts highlight the use of specific plants for managing different ailments. The current study focuses on investigating a combination of two herbal plants: Sesbania Grandiflora leaves and Beta Vulgaris root, aiming to develop an antidiabetic formulation.

The usage of herbal medicines and associated items is widespread and has been growing exponentially over time. The study mentioned various utilities in the standardisation of crude drugs by pharmacognostic, physiochemical, phytochemical analysis and compatibility studies. High performance thin layer chromatography and phytochemical analysis are useful for confirm identity, quality and purity.

The results of the current *Invivo* investigation show that this polyherbal extract mixture has given significant ($p<0.05$) anti-diabetic properties and aids in maintaining stable glycemic and metabolic levels. In both healthy and experimentally induced hyperglycaemic (Streptozotocin caused) rats, the herbal preparation produces antidiabetic effects. It was found to be nontoxic up to 2000 mg/kg BW in acute toxicity testing conducted under OECD guidelines. The herbal formulation may exert its effects via extra-pancreatic as well as pancreatic mechanisms. In Streptozotocin-induced diabetic rats, the extract markedly lowered blood sugar levels by decreasing lipid peroxidation and enhancing enzymatic antioxidants in pancreatic tissue. Long-term therapy also demonstrated mitigation of Streptozotocin-induced histological damage to the islets of Langerhans, as confirmed by histopathological investigations.

The 400mg/kg dose of the herbal formulation nearly had the same inhibitory effects on biochemical and histological measures as the standard medication Glibenclamide (5mg/kg). This excerpt demonstrated enhancements in various metrics such as body weight, food intake, organ mass, and biochemical markers, suggesting its potential value in managing diabetes. The formulation of a polyherbal blend was optimized by determining the optimal ratio of methanolic extracts from Sesbania Grandiflora leaves and Beta Vulgaris roots. The synergistic effects of this combination offer superior therapeutic benefits compared to each plant extract used individually.

Optimized ratio of both extracts is obtained by OGTT on Wistar albino rat which are used further for developing formulation. The developed dispersible polyherbal formulation is directly compressed tablet and preformulation and tablet evaluation is performed for fourdesigned batched which differ from each other by different concentration of MCC.

Based on pre- and post-formulation testing findings and stability data of tablets from variousbatches, it is decided that batch no. **F3's** results are excellent compared to those of otherbatches.

7.2 FUTURE SCOPE

In both developing and developed nations of the world, the market for herbal medicines andother herbal healthcare goods is in a phase of rising demand. Due to their distinctive qualities, herbal materialshave thus opened up a brand-new, interesting subject for future research in all sciences, particularly in medicine.

The active components found in plants have quickly accelerated up the development of phytochemistry and pharmacognosy and are used against diabetes, cancer, and other diseases avoiding heart disease and reducing the ageing process.

1. Kour P, Bilandi A, and Kataria MK, Swami H. Sustained release matrix tablets of miglitol: techniques implemented and patents. International Journal of Institutional Pharmacy and LifeSciences.2015; 5(1):88-100.
2. Kara D. D., Krishna V. T., Pai G. K. A Review on Manufacturing of Tablets by Using Various Granulation Techniques // J. Global Pharma Technol. – 2017. – V. 10, N 9. – P. 05–10.
3. Wachtel GS, Benzie IFF. Herbal Medicine: An Introduction to its History, Usage, Regulation, Current Trends, and Research Needs. Herbal Medicine: BiomolecularandClinicalAspects 2011.2ndedition.
4. Canter P. H, Ernst E. Herbal supplement use by persons aged over 50 years inBritain: Frequently used herbs, concomitant use of herbs, nutritional supplementsandprescriptiondrugs,rateofinformingdoctorsandpotentialfornegativein teractions.Drugs Aging.2004;21:597–605.
5. Tilburt J. C, Kaptchuk T. J. J. Herbal medicine research and global health: An ethical analysis. Bull World Health Organ. 2008; (8):594–9.
6. Shinde, et al.: Formulation and evaluation of antidiabetic polyherbal tablets. AsianJournalofPharmaceutics 2021;15(2):250-53
7. Hasan S, Vedant Misra, Swati Singh, Garvita Arora, Sunita Sharma and Sarika Sharma,Current status of herbal drugs and their future perspectives, Biological Forum–An International Journal 2009;1(1),12-17
8. Cragg G M, Newman D J,Sander K M,Natural products in drug discovery and development, Journal of Natural Products 1997;60:52-60.
9. Prabhakar Reddy Veera Reddy.Herbal drugs and formulations. Biochem & Pharmacol 2013; 2(4):213.
10. Kwakye KO, Doriskumadoh. Dosage forms of herbal medicinal products and their stability considerations –an overview.J CritRev 2017;4(4):1-8
11. Siddiqui, Ameena & Shukla, Sudhir. (2015). Conservation of Plant Genetic Resources and Their Utilisation in Global Perspective. LS: International Journal of Life Sciences. 4. 10.5958/2319-1198.2015.00007

12. Sarangi MK, Padhi S. Novel herbal drug delivery system: An overview. Arch Med Health Sci 2018;6:171-9

13. https://blogs.baruch.cuny.edu/herbal and pharmaceutical medicines.

14. http://1epir.com/en/pages/mdex/id

15. Neeraj Choudhary, Bhupinder Singh Sekhon. An over view of advances in the standardization of herbal drugs.J PharmEducRes.2011;2(2):55-70.

16. Calixto J B. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (Phytotherapeutic agents) Brazilian Journal of Medical and Biological Research 2000; 33(2):179-189.

17. http://www.pharmainfo.net/Herbal medicines and its standardization.

18. Kumadoh, Doris, Ofori-Kwakye, Kwabena. Dosage forms of herbal medicinal products and their stability considerations-an overview. Journal of Critical Reviews 2007; 4:1-8.

19. https://www.researchgate.net/publication/319242213 Dosage forms of herbal medicinal products and their stability considerations an overview.

20. Balandrin MF, Klocke JA, Wrtele EA,Boilinger WH. Content and purity of extracts Olasodine in some available species of Solanum, Science and culture 1985; 56(5) ,214-216.

21. https://www.Pharmapproach.com/advantages-and-disadvantages-of powders.

22. Knight J. Endocrine System 1: Overview of the endocrine system and hormones. Nursing Times [online] 2021; 117(5):38-42

23. Michelle A, Clark, Richard Finkel, Jose A Rey, Karen Whalen. Lippincott's Illustrated reviews Pharmacology.5th edition.

24. Williams Textbook of Endocrinology. 12th edition. Elsevier Publishers. 22

25. https://www.mayoclinic.org/diseases-conditions/diabetes/in-depth/diabetes-treatment/art/20044084

26. https://www.cdc.gov/diabetes/basics/type2.html

27. Jain R, Jain P, Jain P.A Review on Treatment and Prevention of Diabetes Mellitus.

Int J Curr Pharm Res, Vol.8, Issue 3,16-18.

28. https://www.healthline.com/health/diabetes/Treatment,diagnosis,prevention.

29. https://www.niddk.nih.gov/health-information/diabetes/overview/insulin-medicines-treatments.

30. Marín-Peñalver, J. J., Martín-Timón, I., Sevillano-Collantes, C., & Del Cañizo-Gómez, F. J. (2016). Update on the treatment of type 2 diabetes mellitus. *World journal of diabetes*, *7*(17), 354–395. https://doi.org/10.4239/wjd.v7.i17.354

31. Hussain S A, Namilikonda M G, Chandra T K, Pasha M A. A Review on Medicinal Plants with Anti-Diabetic Activity.Int.J.Adv.Res.2020; 8(03):902-917.

32. Verma S, Gupta M, Popli H, Aggarwal G. Diabetes Mellitus Treatment Using Herbal Drugs: A review. International Journal of Phytomedicine 2018;10(1):1-10

33. Abdulmohsen N, Swedan B, Amer M G, Robert A A. The Role of Herbal Medicines in the Treatment of Diabetes: A Short Review. International Journal of Science and Research (IJSR).2021;10(11):1294-97

34. Modak, M., Dixit, P., Londhe, J., Ghaskadbi, S., & Devasagayam, T. P. (2007). Indian herbs and herbal drugs used for the treatment of diabetes. *Journal of clinical biochemistry and nutrition*, *40* (3), 163–173.

35. Kambale, E. K., Quetin-Leclercq, J., Memvanga, P. B., & Beloqui, A. (2022). An Overview of Herbal-Based Antidiabetic Drug Delivery Systems: Focus on Lipid- and Inorganic-Based Nanoformulations. *Pharmaceutics*, *14*(10), 2135.

36. Kumar R, Janadri S, Kumar S, Dhanajaya DR, Swamy S. Evaluation of antidiabetic activity of alcoholic extract of flower *Sesbania grandiflora* in alloxan induced diabetic rats. Asian Journal of Pharmacy and Pharmacology 2015; 1(1):21-26.

37. Gyawali R & Acharya G & Pokharel M & Shah A & Silwal A. Evaluation of Antidiabetic Polyherbal Formulations. Advance Research Journal of Medicinal and Clinical Sciences2015; 1(1):33-37.

38. Mawlieh B, Shastry S & Chand S. Evaluation of Anti-diabetic Activity of two marketed Herbal Formulations. Research Journal of Pharmacy and Technology

2020; 13(02):664-668

39. Sharma A, Kaushik K. Formulation and Evaluation of Herbal Antidiabetic Tablet 2011; Journal of Drug Delivery &Therapeutics; 2011; 1(1):65-67.

40. Suruse P B, Kale M K, Duragkar N J, Gundawar A. Formulation and Evaluation of Antidiabetic Herbal Capsules. Research J. Pharma. Dosage Forms and Tech. 2011; 4(2):113-118.

41. Sahu MK, Singh VK, S Rao PS. Development and Evaluation of Antidiabetic Potential of Polyherbal Formulation in Streptozotocin Induced Animal Model. Int J cell Sci & Molbiol.2018;5(2):0029-0037

42. Nidhi,N.C.,Rujuta,M.,Drasti,M.,Ismail,U.S.,Ezaj,D.andVaishnavi,C.P.Formulation , Evaluation and Comparison of the Poly Herbal Anti-Diabetic Tablet with the Commercial Tablets. Journal of Pharmaceutical Research International 2021; 33 (37A):252-263.

43. Arora R,Mittal A, Jha K K. Formulation and Evaluation of Herbal Anti-diabetic Formulation containing Eugenia jambolana, Gymnema sylvestre, Tinospora cordifolia,Pterocarpus marscipum, Terminalia bellerica & Emblica officinalis.ThePharma Innovation–Journal 2013;2(5):210-216.

44. Aziz Namra,Wal P,Wal Ankita, Saxena S. Evaluation of a Polyherbal Powder for Treatment of Alam S, Baig A, Reddy S K, Reddy M K, Mohiuddin M, Reddy M V, Gupta R K. Antidiabetic and Diabetes Mellitus .Indian J Pharma Sci 2019;81(6):1070-1077.

45. Uddandrao S V V, Bramhanaidu P, Ganapathy S. Evaluation of the Antioxidant and Antidiabetic Potential of the Polyherbal Formulation: Identification of Bioactive Factors. Cardiovascular & Hematological Agents in Medicinal Chemistry. 2020; 18(2):111-123.

46. Shinde S A, Jain S, Chavan S, Shukla K. Formulation and Evaluation of Antidiabetic Polyherbal Tablets.Asian Journal of Pharmaceutics.2021;15(2):250

47. Alam S et al. Antihyperlipidemic Effects of Aqueous Extract of Polyherbal Formulation (ZiabeeteinPowder) in Experimental Animals. Int. J. harm. Phytopharmacol. Res. 2013,2(4):263-267

48. Suman M, Shivalinge G K P, Paul U, Priyanka S.Evaluation of Antidiabetic and Antihyperlipidemic Activity Of Newly Formulated Polyherbal Antidiabetic Tablets In Streptozocin-Induced Diabetes Mellitus In Rats. Asian Journal of Pharmaceutical and Clinical Research2016; 9(1):202-207.

49. Patel K, Hingorani L, Jain V. Formulation Development and Evaluation of AntidiabeticPolyherbalTablet.Int.J.Pharm.Sci.Rev.Res.2017;42(2):146-151.

50. Kumodh D, Ofori-Kwakye K. Dosage Forms of Herbal Medicinal Products and Their Stability Considerations- An Overview. Journal of Critical Reviews 2017; 4(4):1-8.

51. Parasuraman S, Thing GS, Dhanaraj SA. Polyherbal formulation: Concept of Ayurveda.Pharmacognosy Revision.2014;8(16):73-80.

52. Gupta R et.al. Formulation and evaluation of herbal effervescent granules incorporated with Calliandra haematocephala leaves extract. IndoAmerican Journal of Pharm Research.2013; 3(6):4366-4371.

53. Kothari S, Thangavelu L, Roy A. Anti-diabetic activity of Sesbania grandiflora – alpha amylase inhibitory effect.J Adv Pharm Edu Res 2017;7(4):499-502

54. Panigrahi G, Panda C, Patra A. Extract of *Sesbania grandiflora* Ameliorates Hyperglycemia in High Fat Diet-Streptozotocin Induced Experimental Diabetes Mellitus.Scientifica 2016:1-10.

55. Singh H, Arora S, Mani M, Mahaur K, Phool C. Development of multicomponent formulation of herbal drugs for evaluation of Antidiabetic activity. Der Pharmacia Lettre, 2014, 6(1):219-223.

56. Sabale K D, Sabale K D, Kathawate G S, Mane S S. Formulation and Evaluation of Herbal Antidiabetic Tablet.AsianJ.Res.Pharm.Sci.2020; 10(3):145-148.

57. Shrivastava S. Panda P, Vishwakarma D K, Verma N K, Nayak J. Formulation and evaluation of herbal tablets containing Agaricus bisporus powder. International Journal of Advances in Pharmaceutics 2017; 06(02):63-69.

58. Saifi A, Chauhan R, Dwivedi J. Development of a polyherbal formulation FMST and evaluation for antidiabetic activity in alloxan induced diabetic rats. Asian J. Pharm. Res.2017; 7(1):1-7.

59. Chauhan L, Vashisht S. Formulation and evaluation of novel herbal antidiabetic transdermal patch.Innov Pharm Pharmacother 2018;6(4):61-64.

60. Gauttam V K, Kalia A N. Development of polyherbal antidiabetic formulation encapsulated in the phospholipids vesicle system. JAdvPharmTechnol Res. 2013; 4(2):108-17.

61. Jyothi D, Koland M, Sneh P, James J P.Formulation of Herbal Capsule Containing Trigonella Foenum-Graecum Seed Extract for the Treatment of Diabetes.Journal of Young Pharmacists, 2017; 9(3):352-356.

62. Telapolu S, Kalachavedu M, Punnoose AM, Bilikere D. MD-1, a poly herbal formulation indicated in diabetes mellitus ameliorates glucose uptake and inhibits adipogenesis - an invitro study. BMC Complement Altern Med. 2018 Apr2; 18(1):113.

63. Petchi R R, Chockalingam V, Parasuraman S. Antidiabetic Activity of Polyherbal Formulation in Streptozotocin – Nicotinamide Induced Diabetic Wistar Rats. Journal of Traditional and Complementary Medicine, 2014; 4(2):108-117.

64. Panda A, Jena S, Sahu PK, Nayak S, Padhi P. Effect of Polyherbal Mixtures on theTreatment of Diabetes.ISRN Endocrinology 2013:1-5.

65. Farghaly U, Kamel M S, Elzahwey A S, Mangoura S A. Design and Formulation of Novel Pharmaceutical Capsules of Herbal Origin for Diabetes Mellitus. International Journal of PharmaceuticalSciences and Research, 2014;5(4):1474-81.

66. Mandlik R V, Desai S K, Naik S R. Antidiabetic activity of a polyherbal formulation (DRF/AY/5001). Indian Journal of Experimental Biology, 2008; 46(8): 599-606.

67. Majumdar P, Paridhavi M. Physiocochemical Standardization and Formulation Development of Poly-herbal Tablet for Diabetes. British Journal of Pharmaceutical Research.2016; 12(3):1-17.

68. Mandal, I., Jannat, H., Rahman, S., Jahan, R., Khan, T., Mou, S. M., & Rahmatullah, M. (2014). Antihyperglycemic and Antinociceptive Activity Tests with Beta Vulgaris L. SSP. Vulgaris Roots: A Preliminary Report. *World Journal of Pharmaceutical Research*, *3*(9), 109-118.

69. Al-Harbi LN, Alshammari, Al-Dossari AM, Subash-babu P, Binobead MA, AlhussainMH, Alsedairy SA, Al-Nouri Doha, Shamlan G. *Beta vulgaris* L. (Beetroot) Methanolic Extract Prevents Hepatic Steatosis and Liver Damage in T2DM Rats by Hypoglycemic, Insulin-Sensitizing, Antioxidant Effects, and Upregulation of PPARα. MDPI biology.2021; 10(12):1306.

70. Kumar S, Shachi K, Dubey NK. Anti-Diabetic and Haematinic Effects of Beet Root Juice(Beta vulgaris L.) in Alloxan Induced Type-1 Diabetic Albino Rats. Journal of DiabetesResearchandTherapy.2020;6(1):1-3.

71. Chauhan N N, Mistry R, Mandale D, Ismail U S, Ezaj D, Patel V C. Formulation, Evaluation and Comparison of the Polyherbal Anti-Diabetic Tablet with the Commercial Tablets.Journal of Pharmaceutical Research International. 2021; 33(37A):252-263.

72. Harshmal Kadt, Sakunthala H S. Evaluation of the effect and efficacy of herbal powder preparation derived from 'Thalpathe Piliyam'in the management of type II Diabetes mellitus. Young Ayurveda Researchers' and Innovators' Symposium (YARIS – 2019), Institutional Research Committee, Gampaha Wickramarachchi Ayurveda Institute, University of Kelaniya, Yakkala, and SriLanka.2019:40.

73. Manekar S S, Rangari V D, Agrawal M N, Rathod S M. Formulation and Anti-Diabetic Activity Studies of Herbomineral Formulation for Treatment of Diabetes. International Journal of Pharmaceutical Sciences and Research.2014;5(9):3912-3917.

74. Jain S, Pandhi P,Singh A P,Malhotra S. Efficacy of Standardized Herbal Extracts in Type 1 Diabetes - An Experimental Study.Afr.J.Traditional,Complementary and Alternative Medicines.2006; 3(4):23-33.

75. Kumar, Ajeet & Kumar, Rajesh.Formulation and evaluation of ayurvedic anti-diabetic drug. International Journal of Pharmaceutical Sciences and Drug Analysis 2022; 2(1): 30-37

76. Mahajan N, Lokhande B, Thenge R, Gangane P, Dumore N.Polyherbal formulation containing antioxidants may serve as a prophylactic measure to diabetic cataract: Preclinical investigations in rat model. Pharmacognosy

Magazine. 2018; 14(58): 572-577.

77. Gilchrist M, Winyard P G, Fulford J, Angela C A. Shore, Benjamin N. Dietary nitrate supplementation improves reaction time in type 2 diabetes: Development and application of a novel nitrate-depleted beetroot juice placebo.Elsevier.2014;40:67-74.

78. Thissera B, Visvanathan R, Khanfar M A, Qader M M, Hassan M H A, Hassan H M, Bawazeer M, Behery F A, Yaseen M, Liyanage R, Abdelmohsen U R, Rateb M E. Sesbania grandiflora L. Poir leaves: A dietary supplement to alleviate type 2 diabetes through metabolic enzymes inhibition, South African Journal of Botany. Elsevier 2020; 130:282-299.

79. Reddy KS, Sudheer A, Pradeepkumar B, Reddy CS. Effect of a polyherbal formulation in streptozotocin-induced diabetic nephropathy in wistar rats.Indian J Pharmacol 2019;51:330-6

80. L Pari, R Ramakrishnan, S Venkateswaran,ntihyperglycaemic effect of Diamed, a herbal formulation, in experimental diabetes in rats, Journal of Pharmacy and Pharmacology, 200;53(8):1139–1143.

81. Mamatha M K, Suma US, Annegowda HV. The Ascent of Polyherbal Formulation in the Treatment of Diabetes Mellitus. Res.J.Pharmacognosy and Phytochem.2020; 12(4):256-260.

82. Debnath B, Manna K. Formulating anti-diabetic nutraceutical tablets based on edible plants from Tripura, India.Foods and Raw Materials.2022; 10(2):227–234.

83. Panigrahi G, Panda C, Patra A. Extract of Sesbania grandiflora Ameliorates Hyperglycemia in High Fat Diet-Streptozotocin Induced Experimental Diabetes Mellitus.Scientifica (Cairo).2016; 2016:4083568.

84. Kumar R, Janadri S, Kumar S, Swamy S. Evaluation of antidiabetic activity of alcoholic extract of flower Sesbania grandiflora in alloxan induced diabetic rats. Asian Journal of Pharmacy and Pharmacology.2015;1(1):21-6.

85. Ramasubbu K, Padmanabhan S, Al-Ghanim K A, Nicoletti M, Govindarajan M, Sachivkina N, Rajeswari V D. Green Synthesis of Copper Oxide Nanoparticles Using Sesbania grandiflora Leaf Extract and Their Evaluation of Anti-

Diabetic,Cytotoxic,Anti-Microbial,andAnti-Inflammatory Properties in an In-Vitro Approach. Fermentation.2023Mar27;9(4):332.

86. Abdullah R, Arshad H, Kaleem A, Iqtedar M, Aftab M, Saleem F. Assessment of angiotens in converting enzyme inhibitory activity and quality attributes of yoghurt enriched with Cinnamomum verum, Elettaria cardamomum, Beta vulgaris and Brassica oleracea.Saudi Journal of Biological Sciences. 2023 Feb1;30(2):103556.

87. Al-Harbi L N, Alshammari G M, Al-Dossari A M, Subash-Babu P, Binobead M A, Alhussain M H, AlSedairy S A, Al-Nouri D M, Shamlan G. *Beta vulgaris* L. (Beetroot) Methanolic Extract Prevents Hepatic Steatosis and Liver Damage in T2DM Rats by Hypoglycemic, Insulin-Sensitizing, Antioxidant Effects, and Upregulation of PPARα. *Biology*.2021; 10(12):1306.

88. Dubey, Nagina Kumar & Kumar, Sanjeev &Shachi, Kumari & Dubey, Usha. (2020).Anti-Diabetic and Haematinic Effects of Beet Root Juice (Beta vulgaris L.) in Alloxan Induced Type-1 Diabetic Albino Rats. Journal of Diabetes Research and Therapy. 6.10.16966/2380-5544.150.

89. Türkyilmaz I, Bayrak B, Saçan Ö, Kabasakal L, Sener G, Yanardağ R.Chard (beta vulgaris l. varcicla) extract inhibits polyopathway and hyperglycaemia-induced oxidative stress in ratlens.farmacia.2022;70(1).

90. Isabela Micheletti Lorizola, Josiane Érica Miyamoto, Ana Luiza FigueiredoVieira, Beatriz Rocchetti Sumere, Rosângela Maria Neves Bezerra, Marcio Alberto Torsoni, Adriana Souza Torsoni, Mauricio Ariel Rostagno, Marciane Milanski, Caroline Dário Capitani, Beet (Beta vulgaris L.) stalk and leaf supplementation changes the glucose homeostasis and inflammatory markers in the liver of mice exposed to a high-fat diet, Food Chemistry:Molecular Sciences,2021;2:2666-5662.

91. Helmy, S. A., Morsy, N. F. S., Elaby, S. M., & Ghaly, M. A. H. A. (2024). Antidiabetic Effect of Combined Leaf Extracts of Portulaca oleracea L., Beta vulgaris L., and Cichorium intybus L. in Streptozotocin-Induced Diabetic Rats. *Journal of Medicinal Food*, *27*(4), 339-347.

92. Han, Haewook & Segal, Adam & Seifter, Julian & Dwyer, Johanna. (2015). Nutritional Management of Kidney Stones (Nephrolithiasis). Clinical nutrition

research. 4. 137-52. 10.7762/cnr.2015.4.3.137.

93. https://ntbg.org/database/plants/detail

94. https://www.planetayurveda.com/library.

95. Sushrut Samhita S.S.Su.46/281,282

96. Hasan,N.,Osman,H.,Mohamad,S.,Chong,W.K.,Awang,K.,& Zahariluddin S.M. (2012). The Chemical Components of *Sesbania grandiflora* Root and their Antituberculosis Activity.Pharmaceuticals, 5(8), 882–889.

97. Aung, A.A. Chemical investigation and antimicrobial lactivities of Sesbania grandiflora L.UnivResJ, 2011;4(1),337-350.

98. Arfan, Nafisa & Julie, Azima & Mohiuddin, Abdul Kader & Khan, Shah. (2016). Medicinal Properties of the *Sesbania grandiflora* Leaves. Ibnosina J Med BS. Ibnosina J Med BS 2016. 271-277. 10.4103/1947-489X.210243.

99. Padmalochana, K., &Rajan, M. D. (2014). Antimicrobial activity of Aqueous, Ethanol and Acetone extracts of *Sesbania grandiflora* leaves and its phytochemical characterization. Int. J. of Pharma Sciences and Research, 2014; 5(12):957-962.

100. Mohiuddin, A. K. Medicinal and therapeutic values of *Sesbania grandiflora*. J Pharm Sci Exp Pharmacol, 2019, 81-86.

101. Ananta Worasakul, P., Hamamoto H., Sekimizu K., & Okonogi, S.(2017). Biological activities and antibacterial biomarker of Sesbania grandiflora bark extract.Drug Discoveries & Therapeutics,11(2),70–77.

102. Rateb, Mostafa E. *Sesbania grandiflora* L. Poir leaves: A dietary supplement to alleviate type 2 diabetes through metabolic enzymes inhibition. South African Journal of Botany,2020;130:282–299

103. Ninfali, P., & Angelino, D. (2013). Nutritional and functional potential of Beta vulgaris cicla and rubra.Fitoterapia,89,188–199

104. El-Sohaimy Sobhy, Eman Mohamed Abdo, Omayma El-Sayed Shaltout, Ahmed Elsaid Abdalla, & AhmedM. Zeitoun. (2020). Nutritional Evaluation of Beetroots (Beta vulgaris L.) and Its Potential Application in a Functional Beverage.Plants, 9(12), 1752–1752.https://doi.org/10.3390/plants 9121752

105. Rowe, R.C., Sheskey, P.J. and Quinn, M.E. (2009) Handbook of Pharmaceutical Excipients. 6th Edition, Pharmaceutical Press, 359,404,767.

106. Khadabadi SS, Deore SL. Experimental pharmacognosy, a comprehensive guide. Nirali Prakashan. 2013; 2: 1.3-1.4.

107. Khandelwal KR. Practical Pharmacognosy –Technique and Experiments. Nirali Prakashan. 2015; 25:1-25.

108. The Ayurvedic Pharmacopoeia of India. Part- I, 1st Edition, Vol-II, Government of India, Ministry of Health And Family Welfare Department Of Indian system of medicine and homoeopathy. 1989; 142-143.

109. Lohar DR. Protocol for Testing Ayurvedic, Siddha and Unani Medicine,Government of India, Department of AYUSH, Ministry Of Health And Family Welfare. Pharmacopoeia Laboratory for Indian Medicines Ghaziabad New Delhi.

110. Quality Control Methods for Medicinal Plant Materials. World Health Organization. Geneva WHO. 1998; 8-30.

111. Waterhouse, A. L. (2002). Determination of total phenolics. *Current protocols in food analytical chemistry*, *6*(1), I1-1.

112. Shirazi, O. U., Khattak, M. M. A. K., & Shukri, N. A. M. (2014). Determination of total phenolic, flavonoid content and free radical scavenging activities of common herbs and spices. *Journal of pharmacognosy and phytochemistry*, *3*(3), 104-108.

113. Harborne JB. Phytochemical Methods: A Guide to Modern Technique of Plant Analysis. Chapamn and Hall: London. 2007; 1(37)69:125-75

114. Thakker, V. Y., Shah, V. N., Shah, U. D., & Suthar, M. P. (2011). Simultaneous estimation of gallicacid, curcumin and quercetin by HPTLC method.*J Adv Pharm Educ Res*, *1*, 70-80.

115. Wagh, V.D., Wagh, K.V., Tandale, Y.N., & Salve,S.A.(2009). Phytochemical, pharmacological and phytopharmaceutics aspects of Sesbania grandiflora (Hadga): A review. Journal of Pharmacy Research, 2(5), 889-892.

116. Sethuraman, V., Janakiraman, K., Krishnaswami, V., Natesan, S., & Kandasamy,

R. (2021).Combinatorial analysis of quercetin and resveratrol by HPTLC in Sesbania grandiflora phyto-based nanoformulations. *Natural product research*, *35*(13), 2243-2248.

117. Jain, R., Hait, M., & Jain, S. K. (2022). Quantification of β-Sitosterol in Sesbania grandiflora Bark using High Performance Thin Layer Chromatography. *ES Food & Agroforestry*, *9*, 39-44.

118. Gauttam VK, Kalia AN. Development of polyherbal antidiabetic formulation encapsulated in the phospholipids vesicle system. J Adv Pharm Technol Res. 2013 Apr;4(2):108-17. doi: 10.4103/2231-4040.111527. PMID: 23833751; PMCID: PMC3696222.

119. Manik, S., Gauttam, V., & Kalia, A. N. (2013). Anti-diabetic and antihyperlipidemic effect of allopolyherbal formulation in OGTT and STZ-induced diabetic rat model.

120. S. Parasuraman,Toxicological screening,J Pharmacol Pharmacother, 2 (2011), pp. 74-79

121. ECD (2002), Test No. 423: Acute Oral toxicity - Acute Toxic Class Method, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264071001

122. Ghauri, A. O., Mohiuddin, E., Rehman, T., & Siddiqui, H. S. M. (2022). Acute and subacute toxicity studies of a poly herbal formulation used for diabetes. Pakistan Journal of medical sciences, 38(6), 1668–1673. https://doi.org/10.12669/pjms.38.6.5928

123. Majhi, S., Singh, L., Verma, M., Chauhan, I., & Sharma, M. (2022). In-vivo evaluation and formulation development of polyherbal extract in streptozotocin-induced diabetic rat. *Phytomedicine Plus*, *2*(4), 100337.

124. Chaudhuri, A., & Sharma, S. (2016). Evaluation of antidiabetic activity of polyherbal formulation in streptozotocin-induced diabetic rats. *Pharmaceutical and Biosciences Journal*, 01-06.

125. Kumar, C. H., Kumar, J. S., Ishaq, B. M., Rani, G. U., & Prkash, K. V. (2010). Antidiabetic activity of a polyherbal preparation. Pharmacologyonline, 2(1), 780-

87.

126. Petchi, R. R., Vijaya, C., & Parasuraman, S. (2014). Antidiabetic activity of polyherbal formulation in streptozotocin–nicotinamide induced diabetic Wistar rats. Journal of Traditional and Complementary medicine, 4(2), 108-117.

127. Arijit Chaudhuri & Shalini Sharma. (2016). Evaluation of Antidiabetic Activity of Polyherbal Formulation in Streptozotocin-Induced Diabetic Rats. Pharmaceutical and Biosciences Journal, 01-06. https://doi.org/10.20510/ukjpb/4/i5/113983

128. Ghanshyam Panigrahi, Chhayakanta Panda, Arjun Patra, & Extract of Sesbania grandiflora Ameliorates Hyperglycemia in High Fat Diet-Streptozotocin Induced Experimental Diabetes Mellitus & Scientifica, vol. 2016, Article ID 4083568, 10 pages, 2016. https://doi.org/10.1155/2016/4083568

129. Wroblewska,M.,Juskiewicz,J.,&Wiczkowski,W.(2011).Physiological properties of beetroot crisps applied in standard and dyslipidaemic diets of rats. Lipids in Health and Disease,10(1),178

130. Lorizola I, Furlan C, Portovedo M, Milanski M, Botelho P, Bezerra R, SumereB, Rostagno M, Capitani C. 2018. Beet stalks and leaves (Beta vulgaris L.) protect against high-fat diet-induced oxidative damage in the liver in mice. Nutrients 10 (7):872.

131. Rasica,L,Porcelli,S.,Marzorati,M.,Salvadego,D.,Vezzoli,A.,Agosti,F.,Grassi, B. (2018). Ergogenic effects of beetroot juice supplementation during severe-intensity exercise in obese adolescents. American Journal of Physiology Regulatory, Integrative and Comparative Physiology, 315(3), R453–R460.

132. El Gamal, A. A., AlSaid, M. S., Raish, M., Al-Sohaibani, M., Al-Massarani, S.M., Ahmad, A., Hefnawy, M., Al-Yahya, M., Basoudan, O. A., & Rafatullah, S. (2014). Beetroot (Beta vulgaris L.) extract ameliorates gentamicin-induced nephrotoxicity associated oxidative stress, inflammation, and apoptosis in rodent model.Mediators of inflammation, 2014, 983952.

133. Rateb, Mostafa E. *Sesbania grandiflora* L. Poir leaves: A dietary supplement to alleviate type 2 diabetes through metabolic enzymes inhibition. South African Journal of Botany,2020;130:282–299

134. Visvanathan, Rizliya; Khanfar, Mohammad A.; Qader, M. Mallique; Hassan,Marwa H.A.; Hassan, Hossam M.; Bawazeer, Majed; Behery, Fathy A.; Yaseen,Mohammed; Liyanage, Ruvini; Abdelmohsen, Usama R.; Rateb, Mostafa E. (2020). Sesbania grandiflora L. Poir leaves: A dietary supplement to alleviate type 2 diabetes through metabolic enzymes inhibition. South African Journal of Botany, 130, 282–299.

135. Thissera, B., Visvanathan, R., Khanfar, M. A., Qader, M. M., Hassan, M. H., Hassan, H. M., & Rateb, M. E. (2020). *Sesbania grandiflora* L. Poir leaves: A dietary supplement to alleviate type 2 diabetes through metabolic enzymes inhibition. *South African Journal of Botany*, *130*,282-299.

136. Beals, J.W., Binns, S.E., Davis, J.L., Giordano, G.R., Klochak, A.L., Paris,H. L., Bell, C. (2017). Concurrent beet juice and carbohydrate ingestion: Influence on glucose tolerance in obese and nonobese adults. Journal of Nutrition and Metabolism, 2017, 6436783.

137. Karole, S., Shrivastava, S., Thomas, S., Soni, B.,Khan, S., Dubey, J., Dubey, S.P., Khan, N., & Jain, D. K. (2019). Polyherbal Formulation Concept for Synergic Action: A Review. *Journal of Drug Delivery and Therapeutics*, *9*(1-s), 453-466.https://doi.org/10.22270/jddt.v9i1-s.2339

138. Anantaworasakul, P., Hamamoto, H., Sekimizu, K., & Okonogi, S. (2017). In vitro antibacterial activity and in vivo therapeutic effect of Sesbania grandiflora in bacterial infected silkworms. *Pharmaceutical biology*, *55*(1), 1256–1262. https://doi.org/10.1080/13880209.2017.1297467

139. Bolkent, Ş., Yanardağ, R., Tabakoğlu-Oğuz, A., &Özsoy-Saçan, Ö. (2000). Effects of chard (Beta vulgaris L. var. cicla) extract on pancreatic B cells in streptozotocin-diabetic rats: a morphological and biochemical study. Journal of ethnopharmacology, 73(1-2), 251-259.

140. Leon Lachman, Herbert A. Lieberman, Joseph L. Kanig: The hypothesis and Practice of Modern Drug store, Varghese distribution house, 3d release, 1990, 293-373

141. Mahapatra, S. K. &Verma, S. (2023).Formulation and Evaluation of Polyherbal

Tablet for Better Therapeutic Efficacy. *Research Journal of Pharmacy and Technology*, *16*(2), 835-838.

142. Gaikwad, B. B., Rane, B. R., & Jain, A. S. (2022). Formulation and Evaluation of Orodispersible Tablet of Sulindac. *European Journal of Pharmaceutical Research*, *2*(3), 11–19. https://doi.org/10.24018/ejpharma.2022.2.3.41

143. Sadaf Anwar, Mohd. Adnan Kausar, KehkashanParveen, Aqeela Zahra, Abrar Ali,Riadh Badraoui, Mejdi Snoussi, Waseem A. Siddiqui, Mohd Saeed, Polyherbal formulation: The studies towards identification of composition and their biological activities, Journal of King Saud University - Science, Volume 34, Issue 7,2022,102256,ISSN 1018-3647

144. Pradhan, A., Subba, M., Asif, M., & Sharma, C. (2022).Formulation and evaluation of Polyherbal tablet using Carica papaya, Emblica officinalis, Foeniculum vulgare. *Journal of Pharmacognosy and Phytochemistry*, *11*(5), 211-214.

145. Gunde, M., Sonule, R., & Suruse, P. (2022, November). Formulation and Evaluation of Polyherbal Antidiabetic Tablet. *2022 International Conference on Emerging Trends in Engineering and Medical Sciences (ICETEMS)* (pp.5-8). IEEE.

146. Paul,S.,Dey,T.,Koirala,P.,Tamang,S.,Bhattacharya,S.,&Das,R.(2023). Formulation and evaluation of Polyherbal tablet by using Neem, Tulsi, Turmeric and Ginger extract. *Journal of Drug Delivery and Therapeutics*, *13* (7), 46-51.

147. Umesh, A, Kumudhavalli, M.V., Kumar, M. & Venkateswarlu,B.S.Formulation And Evaluation Of Polyherbal Formulation Containing Indigenous Medicinal Plants.

148. Ray, A., Prasad, S., &Yadav, R.(2022).Formulation and Evaluation of Polyherbal Formulation of Aegle Marmelos & Pedalium Murex Extract for Anti-Diabetic Activity.*Journal of Pharmaceutical Negative Results*,7708-7720.

149. Mishra, R., Ray, A., Singh, A., Tripathy, S., Prasad, S., & Yadav, R. (2023). Development And Evaluation Of Anti Diabetic Activity In Polyherbal Tablets Of Local Herbs. *Journal of Pharmaceutical Negative Results*, 1418-1426.

150. ICH harmonized tripartite guideline. Stab Test New Drug Subst Prod. Q1A (R2). Geneva: International Conference of Harmonization; 2009.

151. Ferraro, P. M., Bargagli, M., Trinchieri, A., & Gambaro, G. (2020). Risk of Kidney Stones: Influence of Dietary Factors, Dietary Patterns, and Vegetarian-Vegan Diets. *Nutrients*, *12*(3), 779. https://doi.org/10.3390/nu12030779

152. https://www.mayoclinic.org/diseases-conditions/hyperoxaluria/symptoms-causes/syc-20352254.