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 ।

अगस्त्यस्योद्वय यावत् सपुष्प इव दृश्यते ।। शि.

अगस्त्यः पित्तकफजिच्चातुर्थिकहरो हिमः ।

स्क्षो 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 मथुरा तिक्ता चैव स्मृतिप्रदा ।।

त्रिदोषशूलकफहत् पांडुरोगविषापनुत् ।

शोषगुल्महरा प्रोक्ता सा पक्का रूक्षपित्तला ।। नि. र.

वृषागस्त्ययोः पुष्पाणि तिक्तानि कटुविपाकानि क्षयकासहराणि च ।

अगस्त्यं नातिशीतोष्णं नक्तान्धानां प्रशस्यते । सु. सू. ४६.

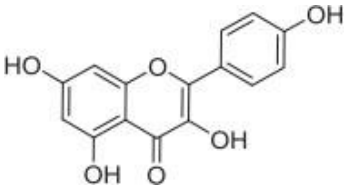
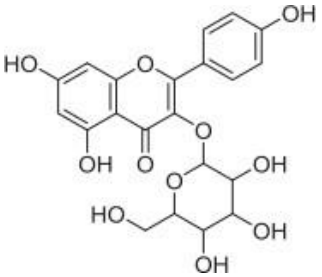
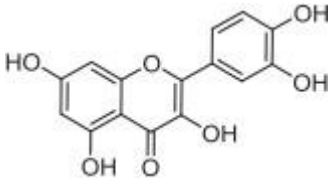
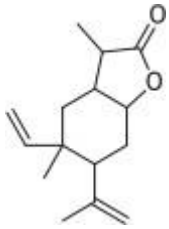
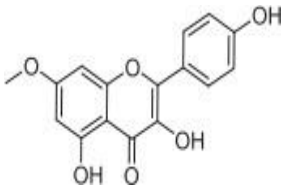
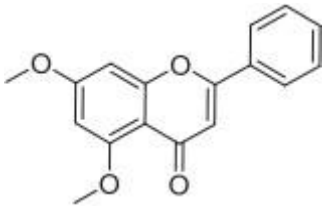
Chemical constituent:

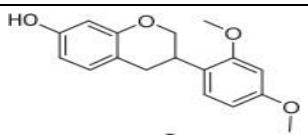
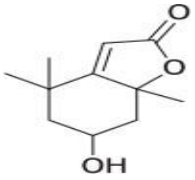
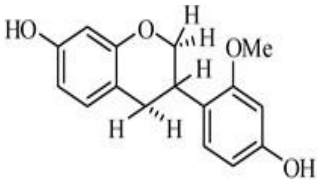
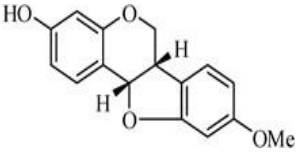
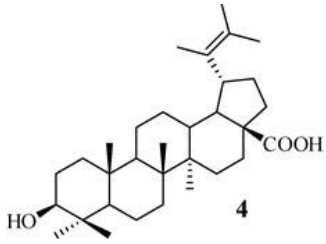
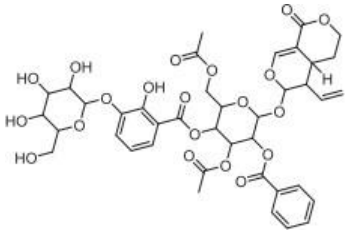
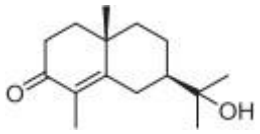
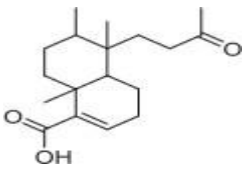
The plant is rich in various chemical constituents such as tannins, coumarone, steroids, triterpenes, isoflavonoids (including isovestitol and sativan), betulinic acid, flavonoids, and medicarpin. Cyanidin and delphinidin glucosides are found specifically in the flowers, while alpha-ketoglutaric, oxaloacetic, 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		Significant inhibitory effects on α-amylase, alongside antibacterial and anxiolytic properties.
2	Astragalin		Effective remedy for diabetic testicular function impairment, with antibacterial and anti-inflammatory actions
3	Quercetin		Antidiabetic and antioxidant agent
4	Callitrin		Antidiabetic and antioxidant agent
5	Rhamnocitrin		Potent anti-tumor activity
6	Chrysin-dimethylether		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.

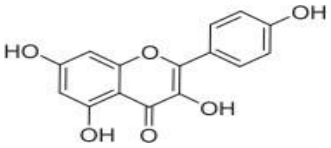
Table 5.3: Description of *Beta vulgaris*

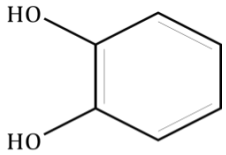
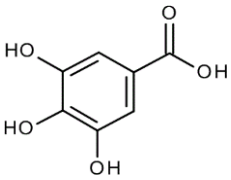
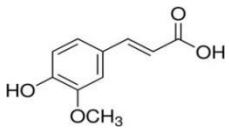
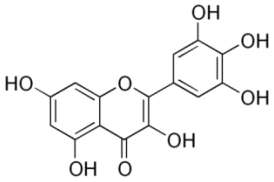
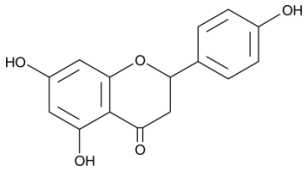
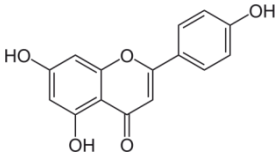
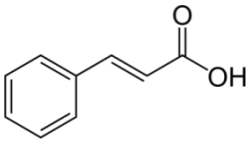
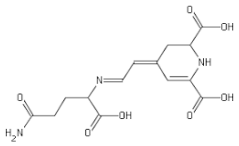
Plant	Sprawling perennial plant upto 60cm (2 ft) high
Leaves	Darkgreen,leathery,shiny rosette leaves with wavy & rough Triangular lower leaves and narrow and oval upper leaves. Grow 20–40cm(7.9–15.7in) in length
Fruit	Enclosed by the leathery and in curved perianth, and is immersed in the swollen, hardened 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

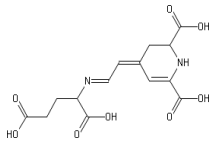
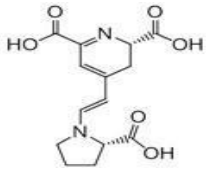
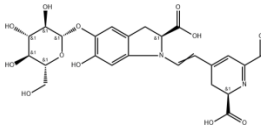
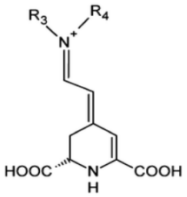
Chemical Constituent:

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		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.

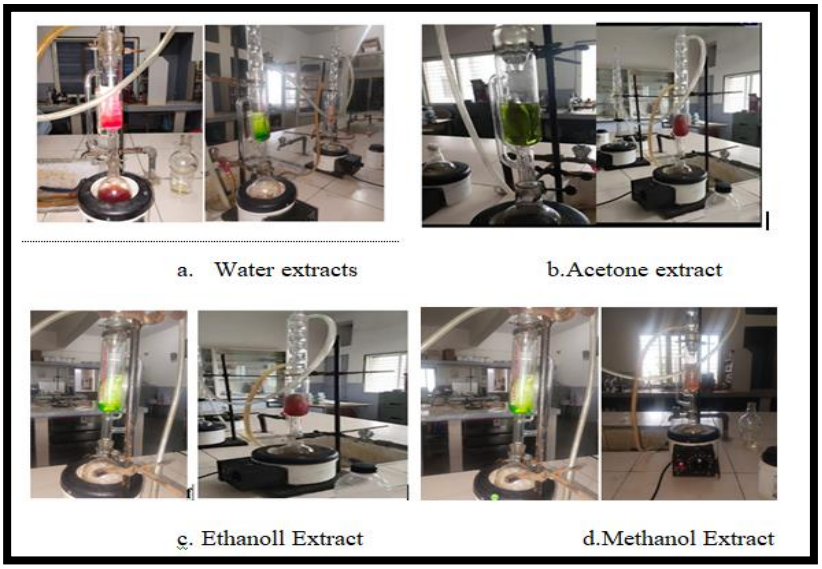


Figure 5.7: Extraction of *Sesbania grandiflora* and *Beta vulgaris* powder.

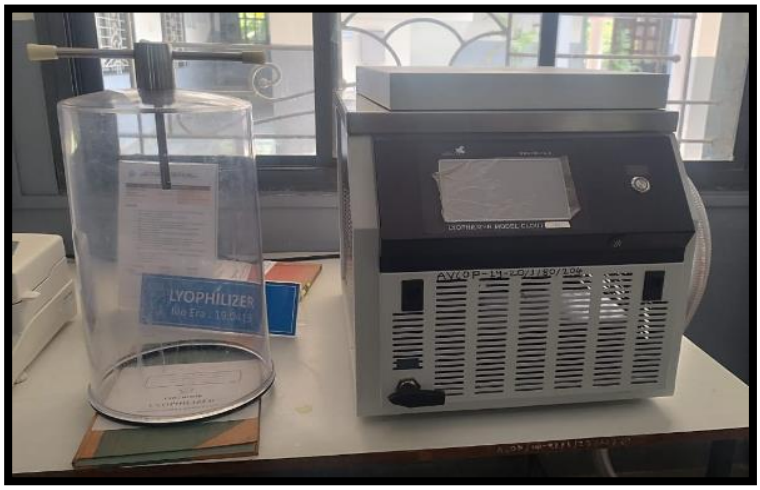


Figure 5.8: Lyophillizer

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.

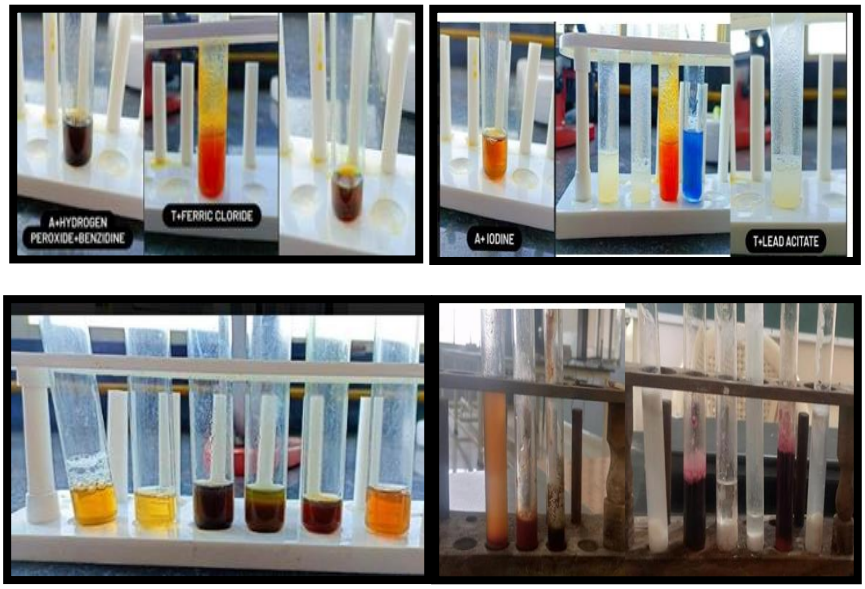


Figure 5.9: Preliminary Phytochemical Screening of Plant Extracts

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.

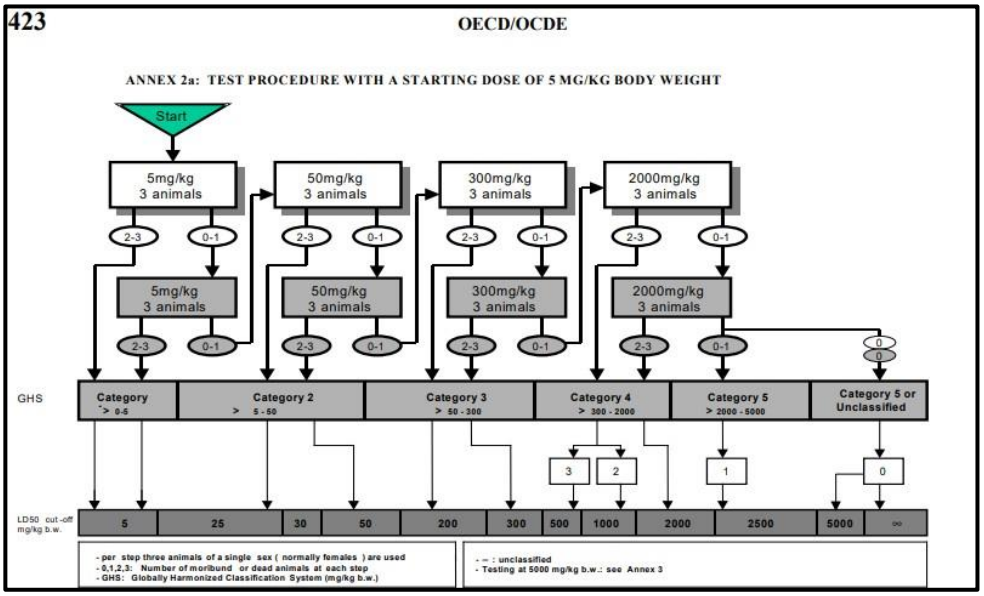


Figure 5.12: OECD guidelines for acute toxicity studies

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 ℃, 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 ($1/10^{th}$ of the dosage of acute toxicity studies.)

Group 4:F400: 45mg/kg Streptozotocin (STZ) & 400mg/kg of PHF 2 (F400) extract ($1/20^{th}$ 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

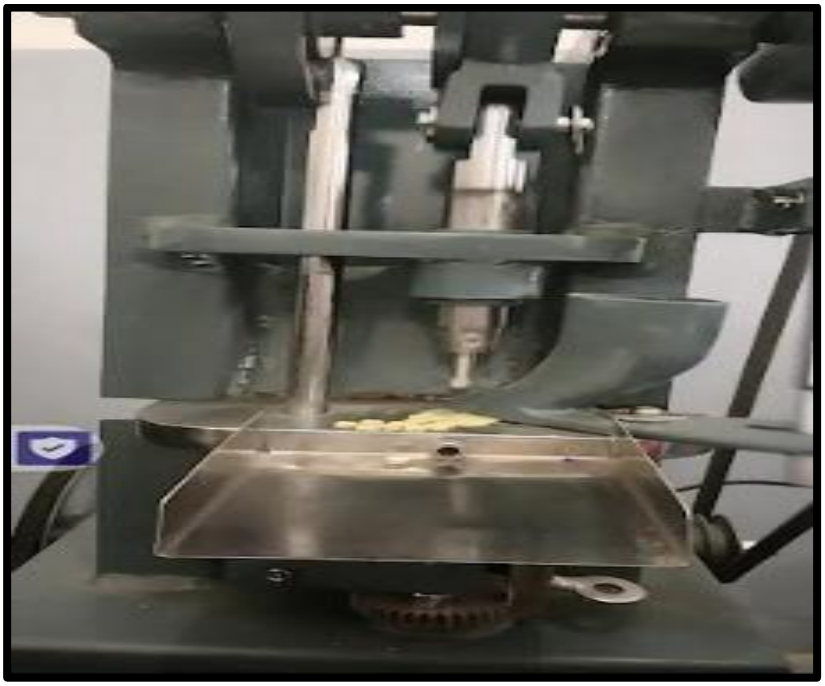


Figure 5.14: Tablet compression machine

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.