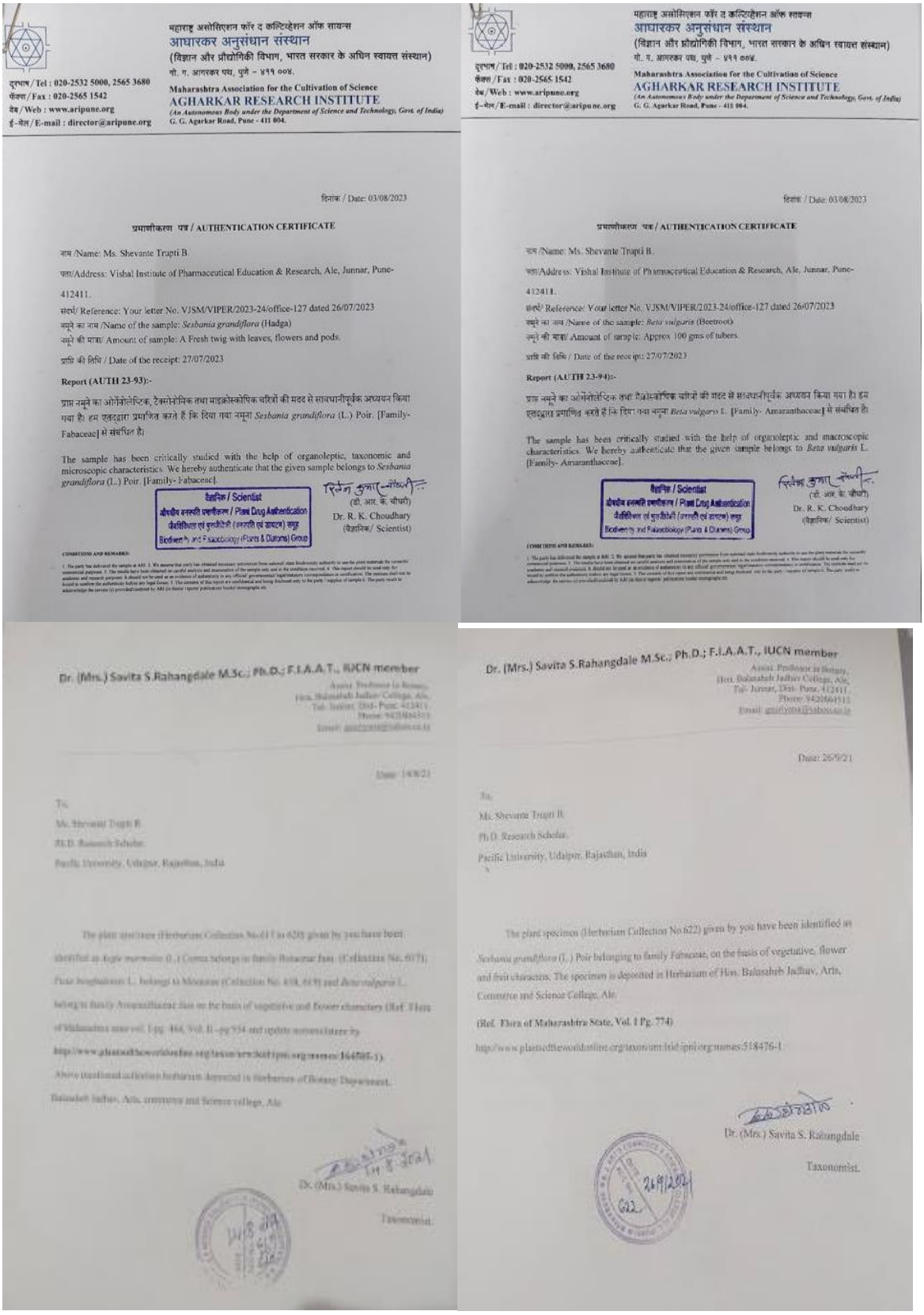
## 6.1 COLLECTION AND AUTHENTICATION OF PLANTS:

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.
दूरभाष / Tel : 020-2532 5000, 2565 3680
फॅक्स / Fax : 020-2565 1542
वेब / Web : www.aripune.org
ई-मेल / E-mail : director@aripune.org

Maharashtra Association for the Cultivation of Science
AGHARKAR RESEARCH INSTITUTE
(An Autonomous Body under the Department of Science and Technology, Govt. of India)
G. G. Agarkar Road, Pune - 411 004.

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 ऑर्गेनोलेप्टिक, 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 वनस्पती प्रमाणीकरण / Plant Drug Authentication
जैवविविधता एवं मृदाजैवकी (वनस्पती एवं प्राणीज) समूह
Biodiversity and Palaeobiology (Plants & Diatoms) Group

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

CONDITIONS AND REMARKS:

1. The party has delivered the sample at ARI. 2. We assume that party has obtained necessary permission from national/ state biodiversity authority to use the plant materials for the...

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 ऑर्गेनोलेप्टिक तथा टैक्सोनोमिक 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 वनस्पती प्रमाणीकरण / Plant Drug Authentication
जैवविविधता एवं मृदाजैवकी (वनस्पती एवं प्राणीज) समूह
Biodiversity and Palaeobiology (Plants & Diatoms) Group

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

CONDITIONS AND REMARKS:

1. The party has delivered the sample at ARI. 2. We assume that party has obtained necessary permission from national/ state biodiversity authority to use the plant materials for the...

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

Assist. Professor in Botany,
Hon. Balasaheb Jadhav College, Ale,
Tal- Junnar, Dist- Pune, 412411.
Phone: 9420864513
Email: gsrl1980@yahoo.co.in

Date: 14/8/23

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

The plant specimen (Herbarium Collection No.611 to 620) given by you have been identified as *Argyreia nervosa* (L.) Cuntz belongs to family Boraginaceae (Collection No. 617), *Ficus benghalensis* L. belongs to Moraceae (Collection No. 618, 619) and *Beta vulgaris* L. belongs to family Amaranthaceae (Collection No. 620) on the basis of vegetative and flower characters (Ref. Flora of Maharashtra state vol. I pg. 464, Vol. II –pg 934 and update nomenclature by http://www.plantsoftheworldonline.org/taxon/urn:lsid:ipni.org:names:166705-1).

Above mentioned collection herbarium deposited in Herbarium of Botany Department, Balasaheb Jadhav, Arts, commerce and Science college, Ale.

Dr. (Mrs.) Savita S. Rahangdale

Taxonomist.

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

Assist. Professor in Botany,
Hon. Balasaheb Jadhav College, Ale,
Tal- Junnar, Dist- Pune, 412411.
Phone: 9420864513
Email: gsrl1980@yahoo.co.in

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.

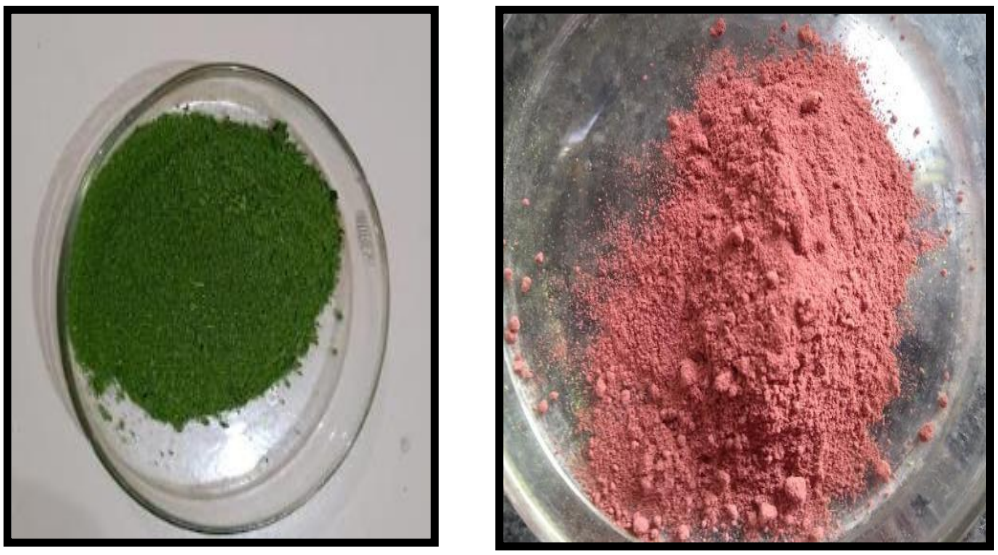


**Figure 6.2: *Sesbania Grandiflora* leaves powder and *Beta Vulgaris* root powder**

## 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. |

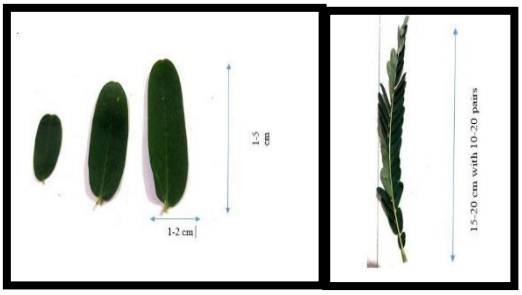



**Figure 6.3: *Sesbania Grandiflora*** linn leaves

***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<br>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.

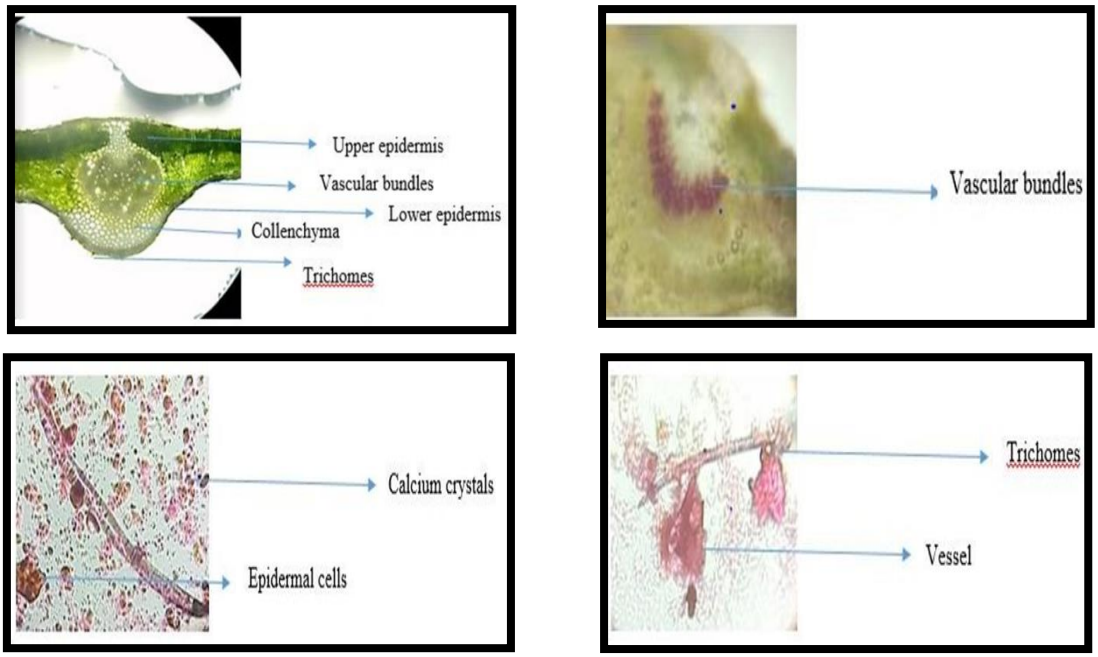



**Figure 6.5: Microscopic Evaluation of *Sesbania Grandiflora Linn***

***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.

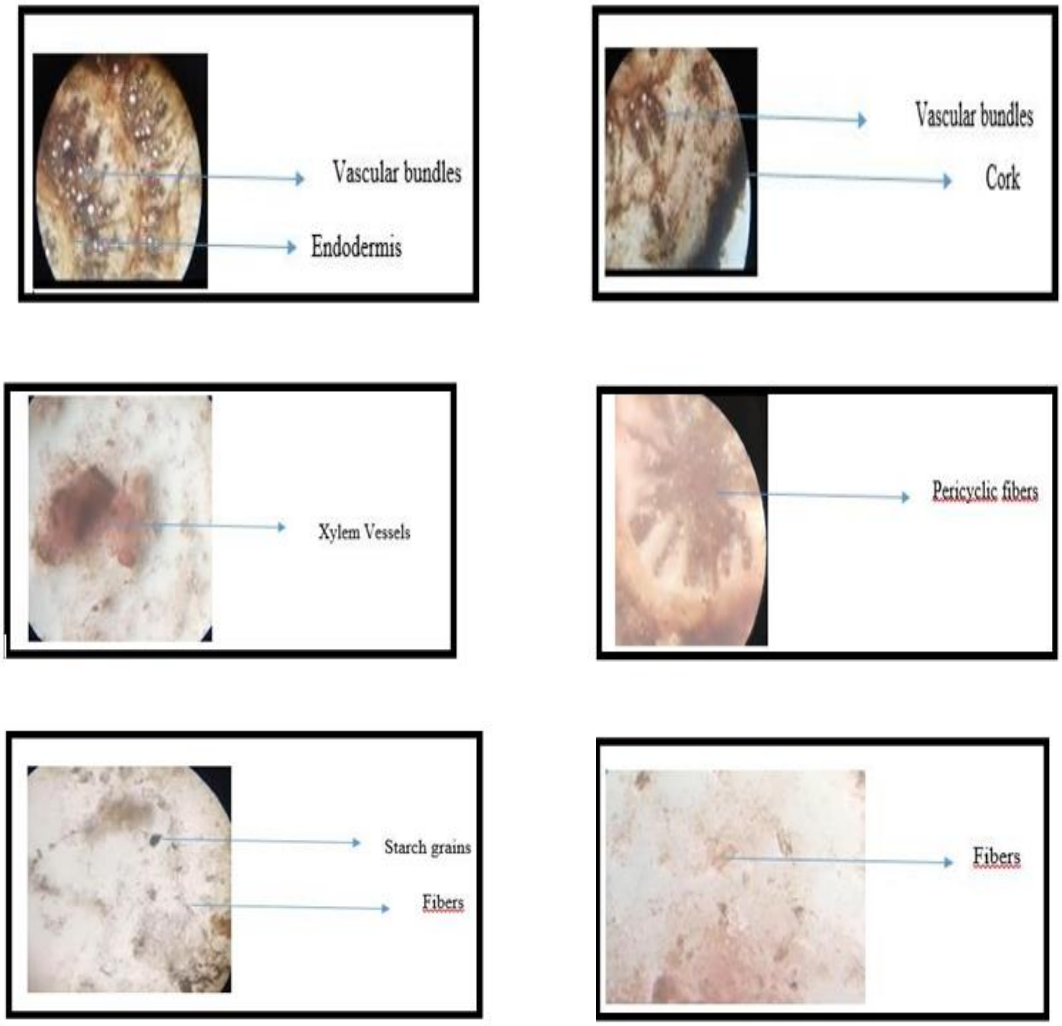



**Figure 6.6: Microscopic Evaluation of *Beta Vulgaris* Root**

### 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:

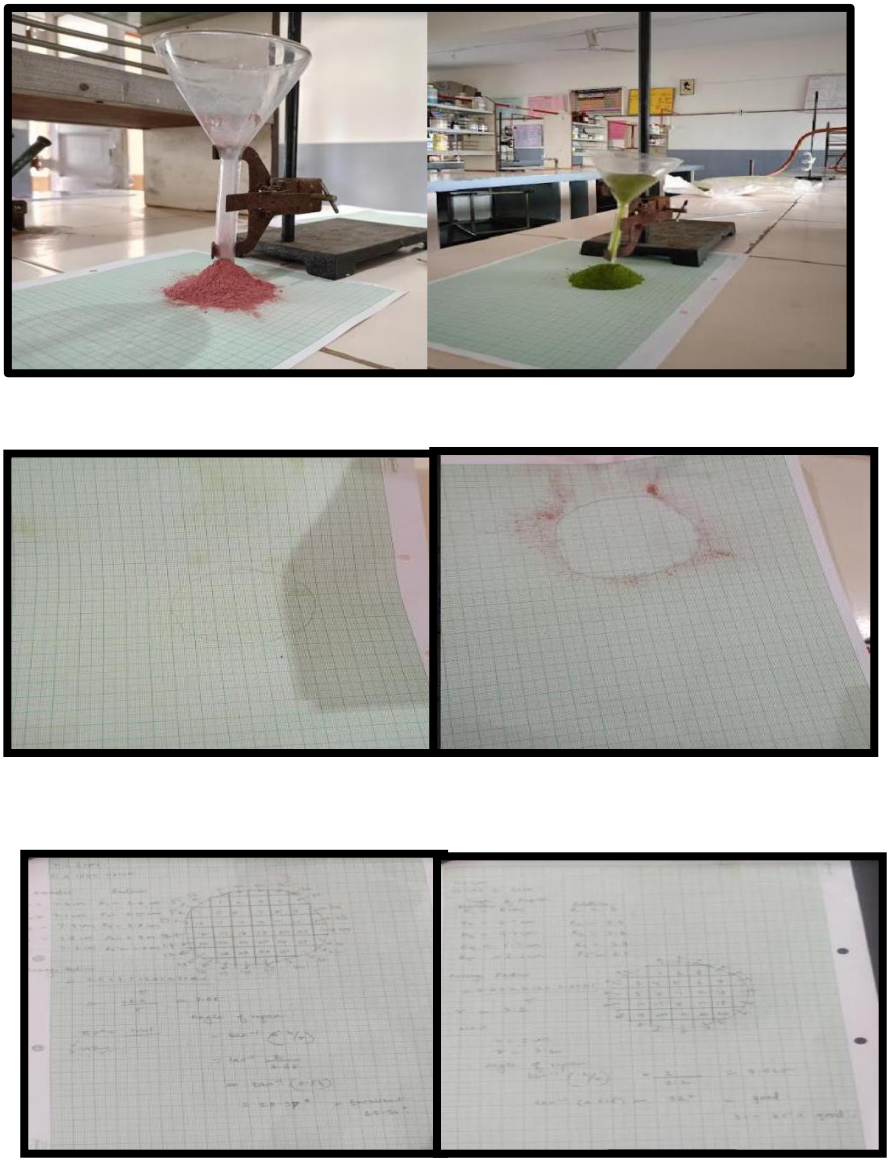


**Figure 6.7: Angle of Repose of *Sesbania Grandiflora* leaves powder and *Beta Vulgaris* Root powder**

**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**

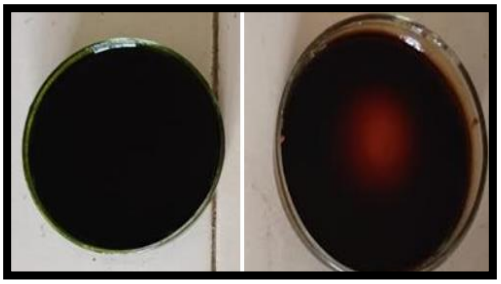


**Figure 6.12: Methanolic Extract of leaves of Sesbania Grandiflora and root of Beta vulgaris L**

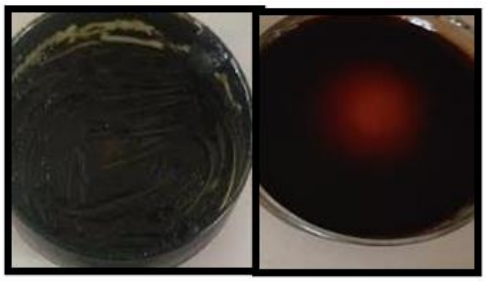


**Figure 6.13: Acetone Extract of leaves of Sesbania Grandiflora and root of Beta vulgaris L**

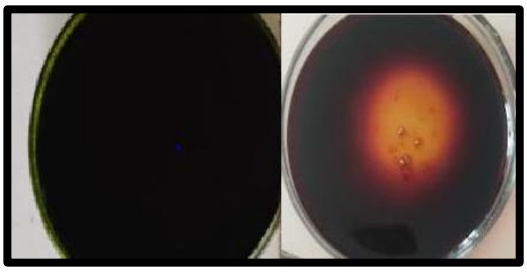


**Figure 6.14: Ethanolic extract of leaves of Sesbania Grandiflora and root of Beta vulgaris** L

### 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 |

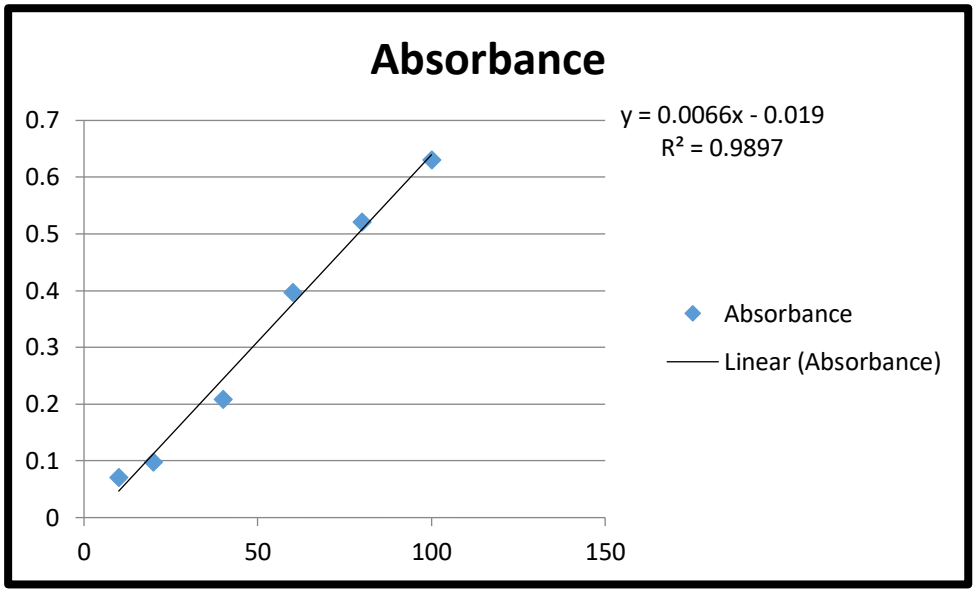



**Figure 6.15: Standard curve of Gallic acid at 760 nm**

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 |

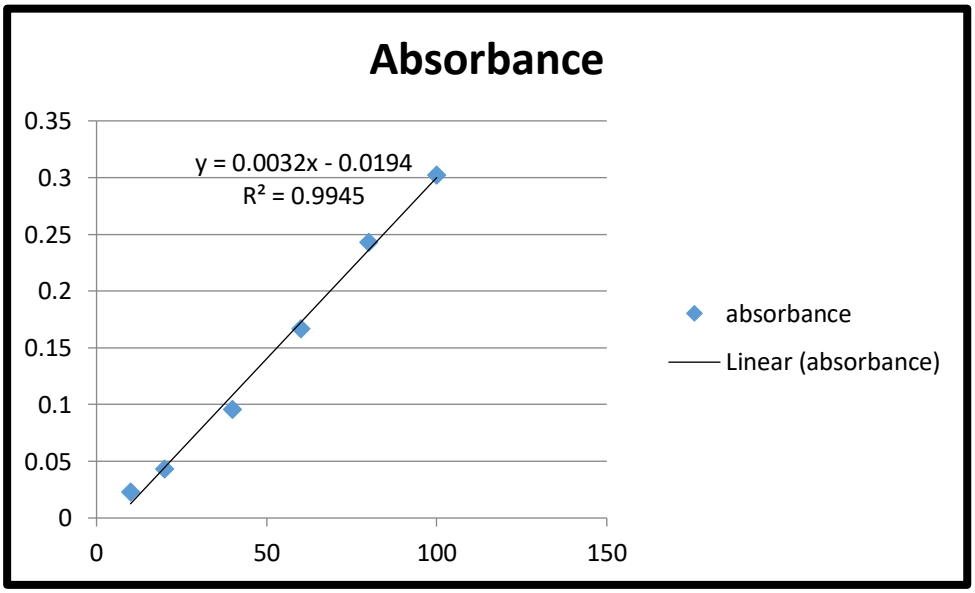



**Figure 6.16: Standard curve of Quercetin at 510nm**

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.

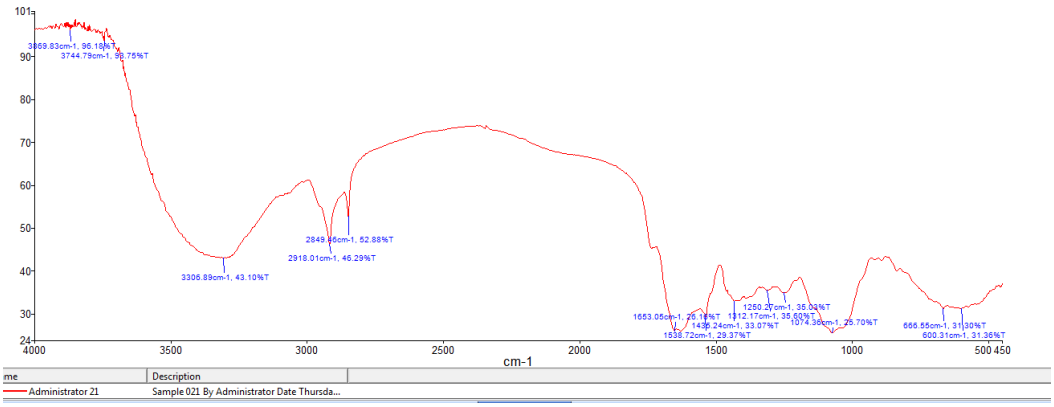



**Figure 6.17: I.R Spectra of methanolic extract of *Sesbania Grandiflora* leaves**

**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$^{-1}$ |

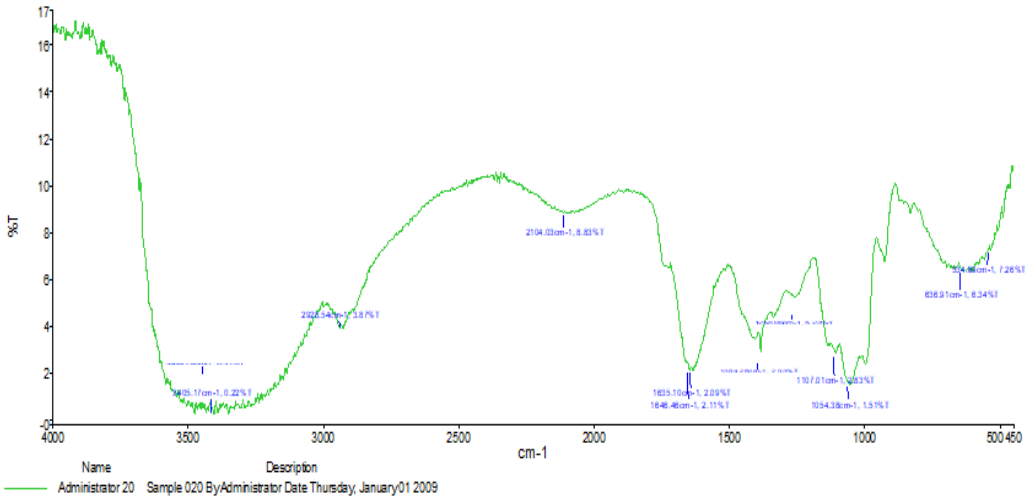



**Figure 6.18:I.R Spectra of methanolic extract of *Beta Vulgaris* root.**

**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.

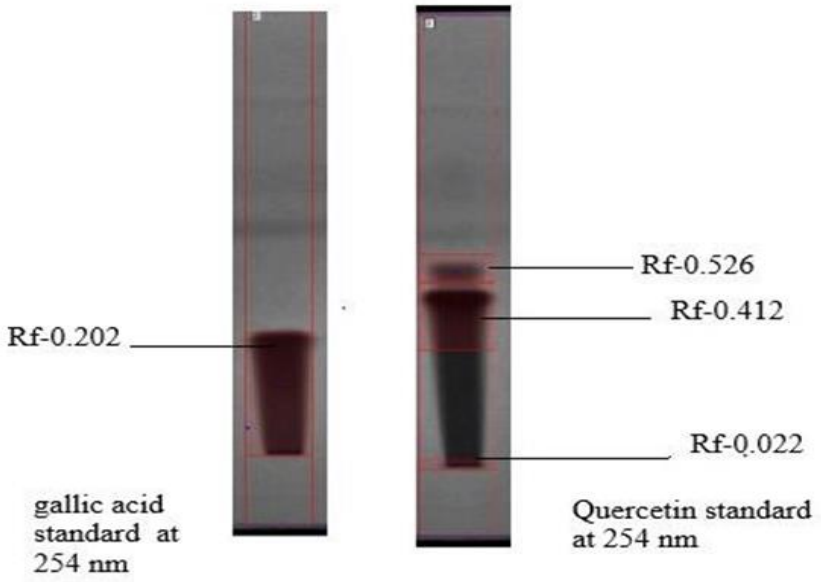



**Figure 6.19: HPTLC finger printing of Gallic acid and Quercetine at 254 nm and 366 nm**

### 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: $R_f$ Value 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 |

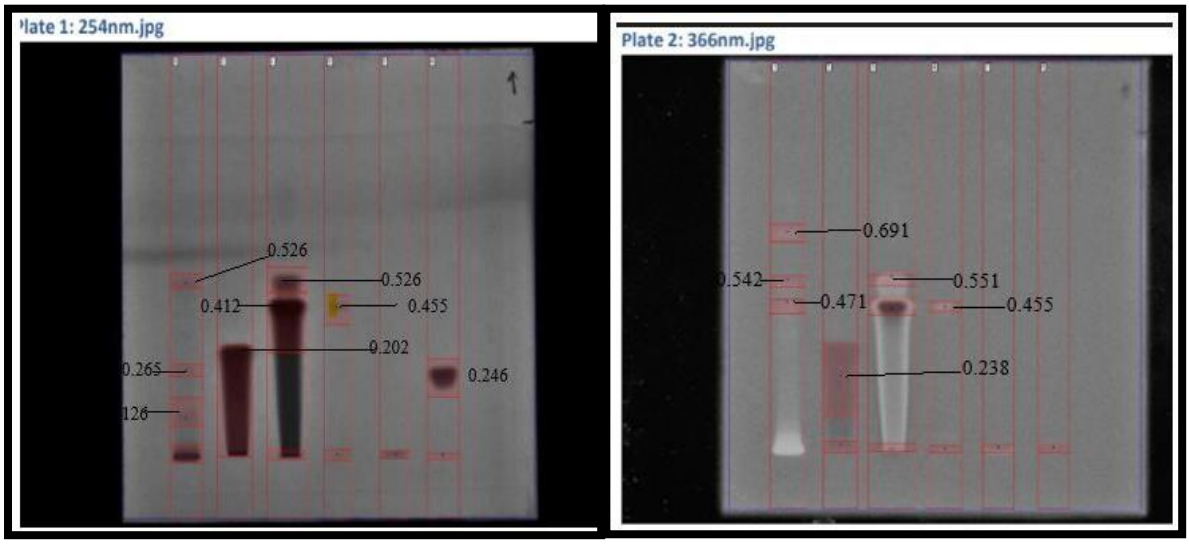



**Figure 6.20: HPTLC finger printing of Methanolic extract of root of *Beta Vulgaris* at 254 nm and 366 nm**

**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 |

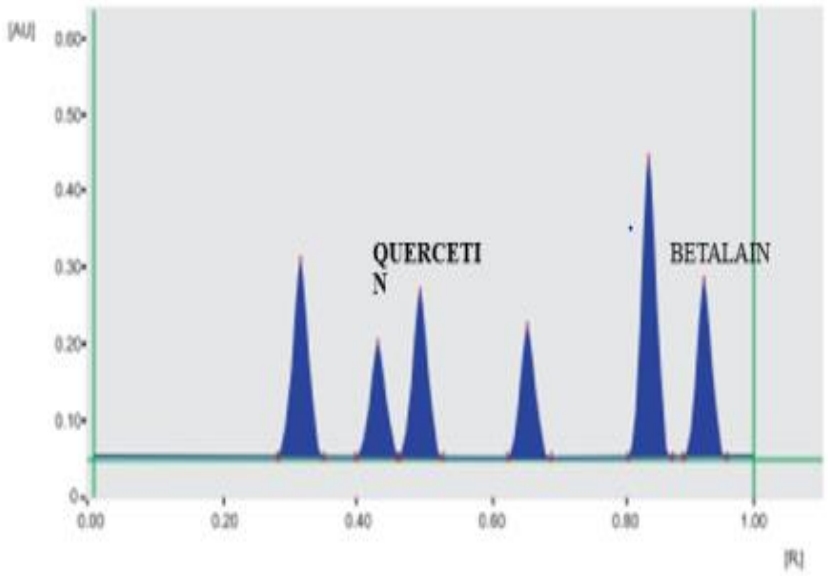



**Figure 6.21: HPTLC Densitogram of Methanolic root Extract of *Beta Vulgaris***

**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;<br>Ethyl acetate;<br>Formic acid<br>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 |

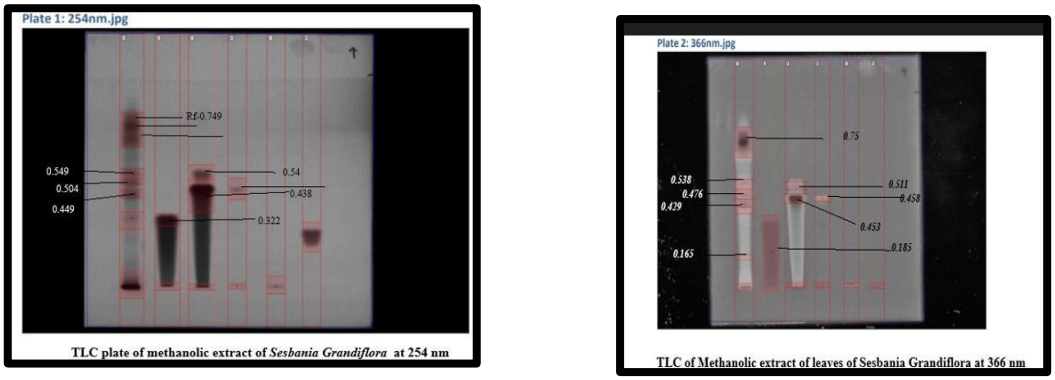



**Figure 6.22: HPTLC densitogram of Methanolic extract of leaves of *Sesbania Grandiflora* at 254nm and 366nm**

**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 |

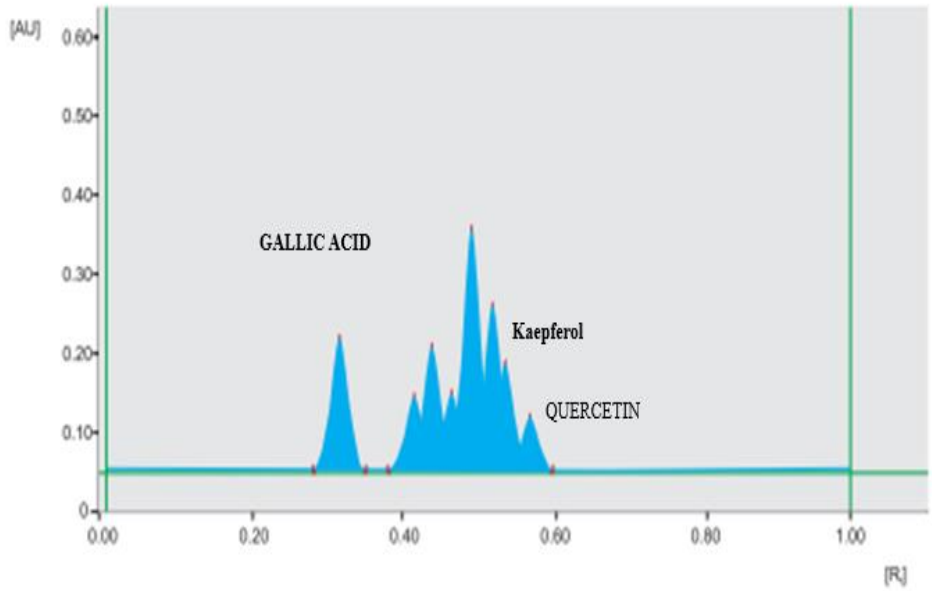



**Figure 6.23: Densitogram of Methanolic Leaves Extract of *Sesbania Grandiflora***

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

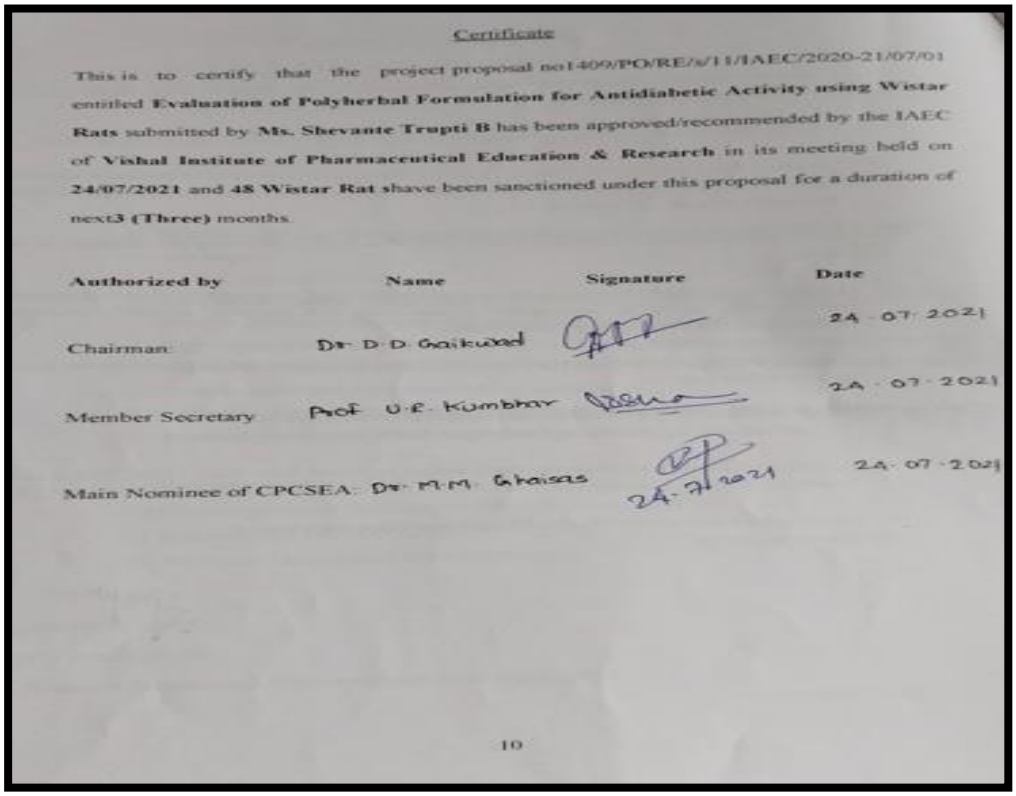
| Track | Peak Start | Peak End | Area in % |
|---|---|---|---|
| 1 | 0.336 | 0.342 | 100 |
| 2 | 0.429 | 0.434 | 100 |
| 3 | 0.449 | 0.454 | 100 |
| 4 | 0.476 | 0.481 | 100 |
| 5 | 0.504 | 0.513 | 100 |
| 6 | 0.538 | 0.545 | 100 |
| 7 (Kaepferol) | 0.583 | 0.587 | 100 |
| 8 (Quercetin) | 0.511 | 0.523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

### 1.10.1 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.P. Kumbhar | | 24.07.2021 |
| Main Nominee of CPCSEA | Dr. M.M. Ghaisas | 24.7.2021 | 24.07.2021 |

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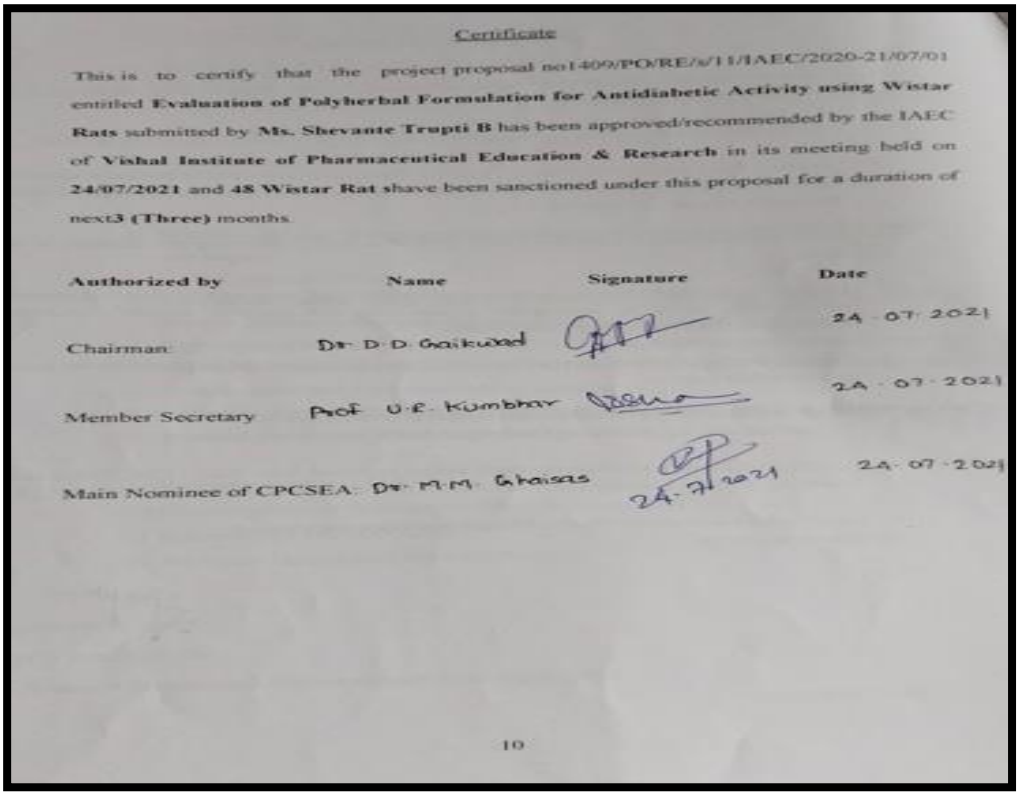

Certificate

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| Chairman | Dr. D.D. Gaikwad | | 24.07.2021 |
| Member Secretary | Prof U.P. 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.

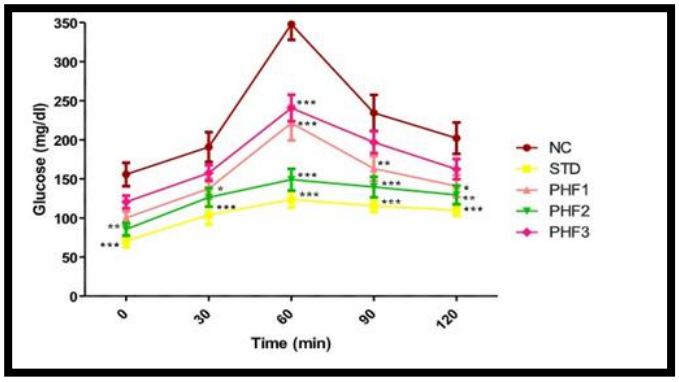



**Figure 6.25: Effect of PHF 1, PHF 2, and PHF 3 extracts on blood glucose levels (mg/dl) in a group of experimental rats subjected to an oral glucose load.**

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.

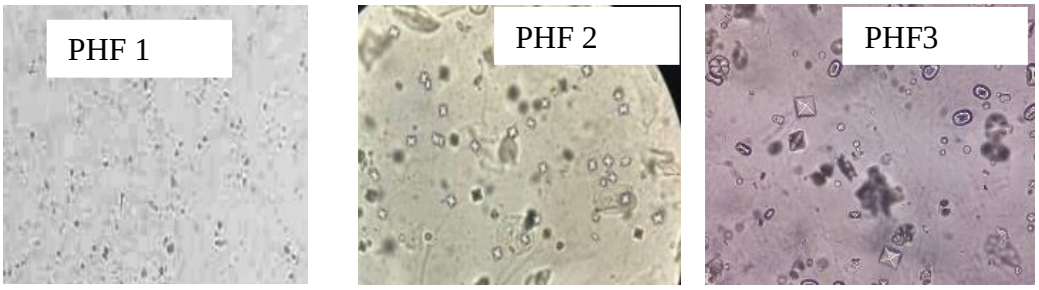



**Figure 6.27: Microscopical examination of Urine collected after treatment of PHF1, PHF2, PHF3**

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

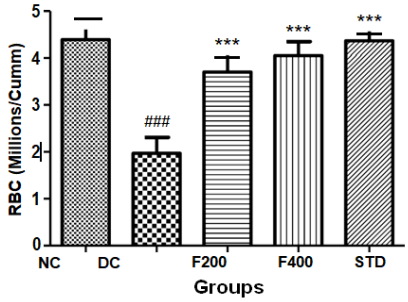



**Figure 6.28 presents the Effect of PHF2 doses (F200 and F400) on the red blood cell (RBC) count (Million/Cu mm) in STZ-induced diabetes in rats.**

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

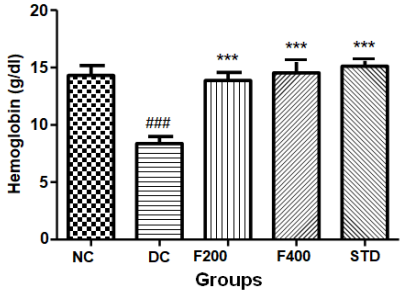



**Figure 6.29 shows the effect of PHF2 at doses of F200 and F400 on haemoglobin levels (g/dl) in rats with streptozotocin-induced diabetes.**

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 ($p < 0.001$).

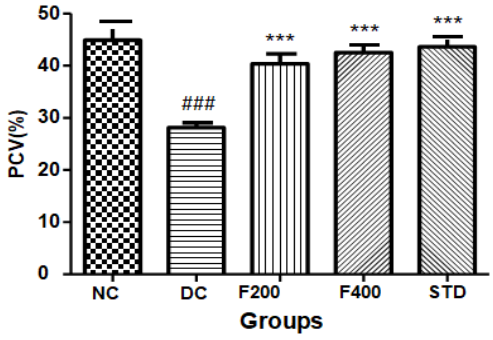



**Figure 6.30 illustrates the effect of PHF2 at doses of 200 mg/kg (F200) and 400 mg/kg (F400) on packed cell volume (PCV) in rats with streptozotocin-induced diabetes.**

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.

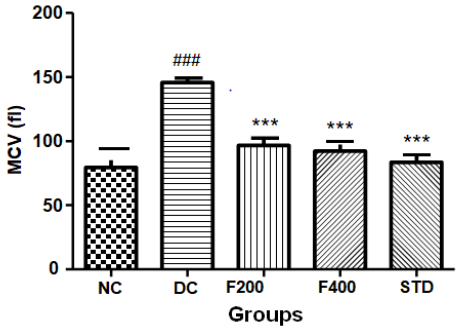



**Figure 6.31 illustrates the effect of PHF2 at dosages of F200 and F400 on mean corpuscular volume (MCV, fl) in rats with streptozotocin-induced diabetes.**

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.

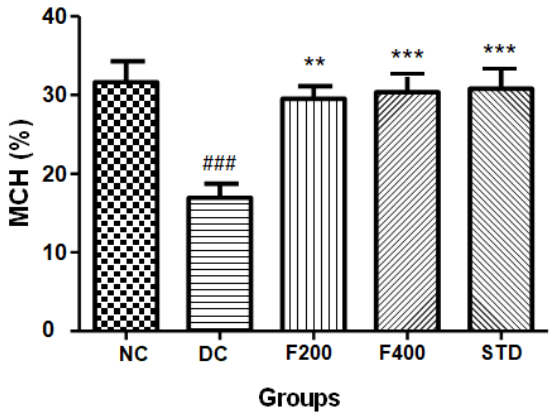



**Figure 6.32 presents the effects of PHF2 at doses of 200 mg/kg (F200) and 400 mg/kg (F400) on mean corpuscular hemoglobin (MCH) in rats with streptozotocin-induced diabetes.**

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.

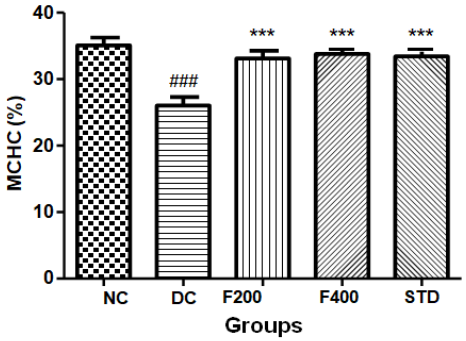



**Figure 6.33 illustrates the effect of PHF2 on mean corpuscular hemoglobin concentration (MCHC) in rats with diabetes induced by streptozotocin.**

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 ($p < 0.001$).

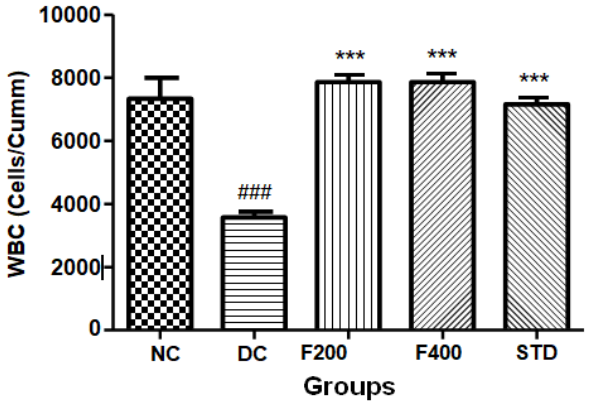



**Figure 6.34 illustrates the effect of PHF2 at doses of 200 mg/kg (F200) and 400 mg/kg (F400) on white blood cell (WBC) count in rats with streptozotocin-induced diabetes.**

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 ($p < 0.001$).

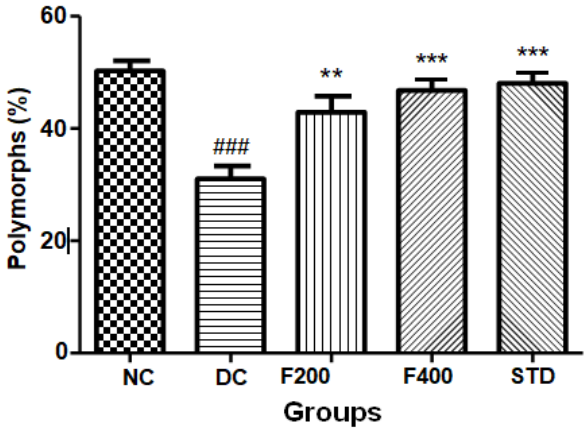



**Figure 6.35 illustrates the effect of PHF2 at doses of 200 mg/kg (F200) and 400 mg/kg (F400) on polymorphonuclear leukocytes (polymorphs) in rats with streptozotocin- induced diabetes.**

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.

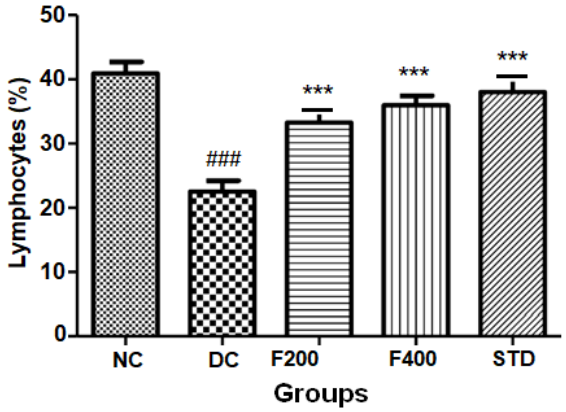



**Figure 6.36 illustrates the effect of PHF2 at doses of 200 mg/kg (F200) and 400 mg/kg (F400) on lymphocyte levels in streptozotocin-induced diabetic rats**.

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

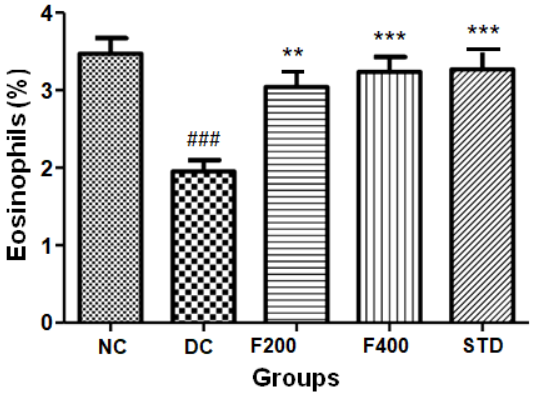


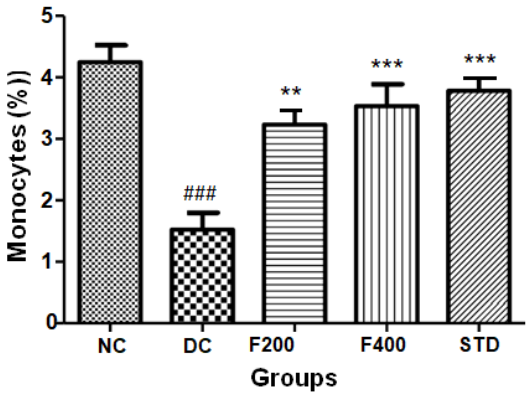

**Figure 6.37 depicts the effect of PHF2 administered at doses of 200 mg/kg (F200) and 400 mg/kg (F400) on eosinophil levels in rats with streptozotocin-induced diabetes.**

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.

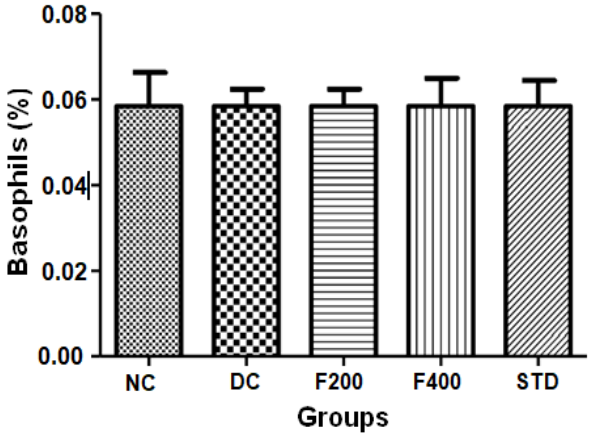



**Figure 6.39 illustrates the effects of PHF2 administered at doses of 200 mg/kg (F200) and 400 mg/kg (F400) on basophil counts in streptozotocin-induced diabetic rats.**

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.

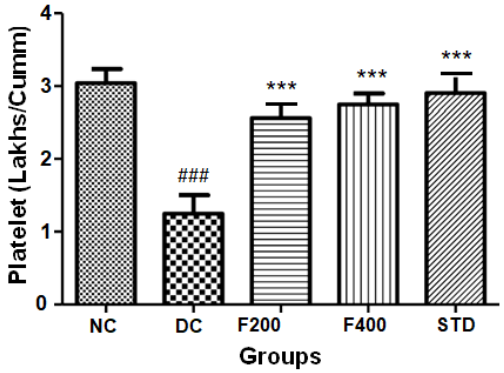



**Figure 6.40 illustrates the effect of PHF2 at doses of F200 and F400 on platelet count (Lakhs/Cumm) in rats with streptozotocin-induced diabetes.**

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 ($p < 0.001$).

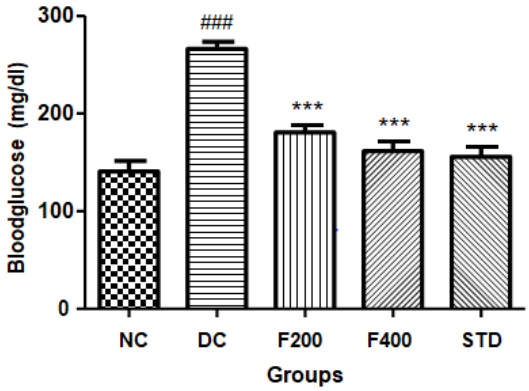



**Figure 6.41.The effect of PHF2 administration on blood glucose levels in rats with streptozotocin-induced diabetes.**

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 ($p < 0.001$).

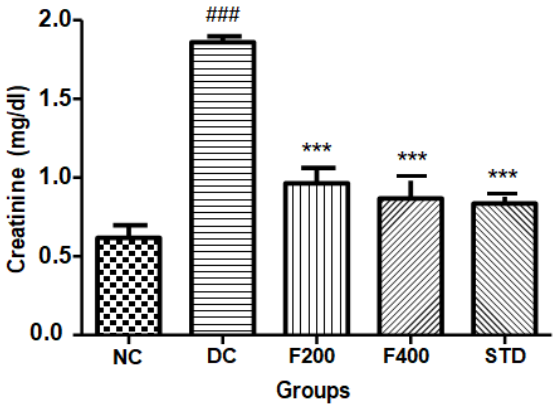



**Figure 6.42 illustrates the effect of PHF2 administered at doses of 200 mg/kg (F200) and 400 mg/kg (F400) on creatinine levels (mg/dl) in rats with streptozotocin-induced diabetes.**

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 ($p < 0.001$).

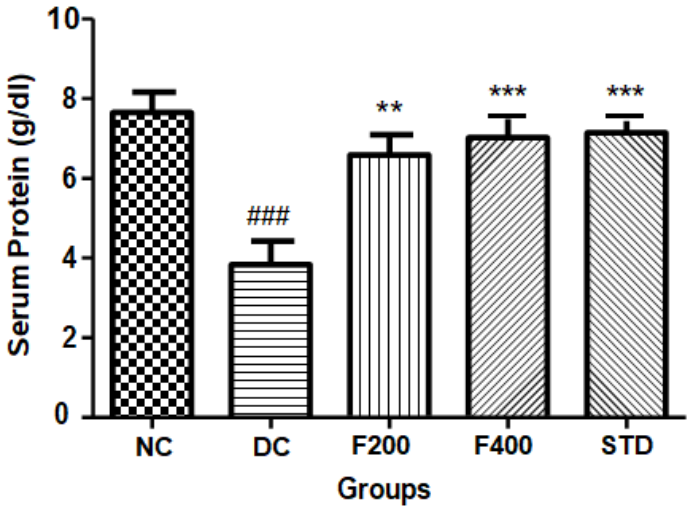



**Figure 6.43: illustrates the effect of PHF2 at doses of 200 mg/kg (F200) and 400 mg/kg (F400) on serum protein levels (mg/dl) in rats with streptozotocin-induced diabetes.**

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 ($p<0.001$).

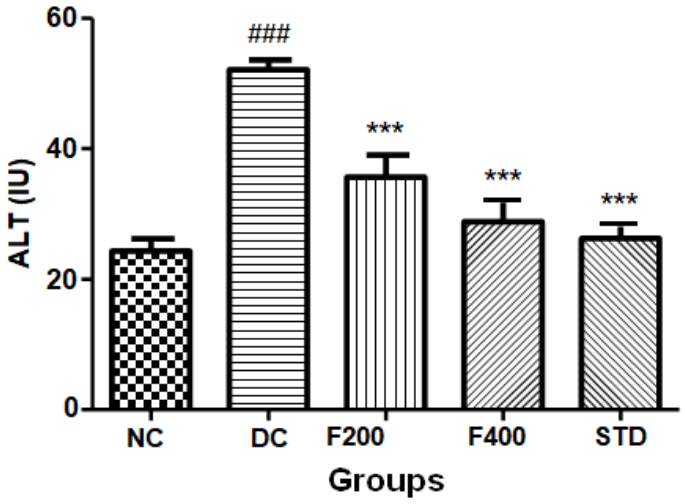
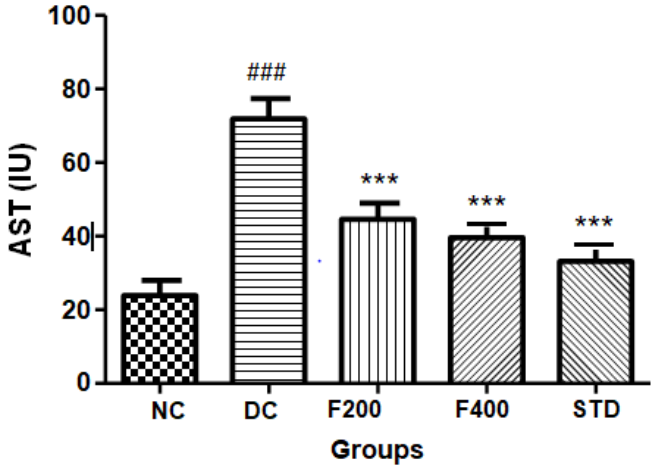



**Figure 6.44: illustrates the effects of PHF2 at doses of 200 mg/kg (F200) and 400 mg/kg (F400) on alanine transaminase (ALT) levels (IU/L) in rats with streptozotocin-induced diabetes.**

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.

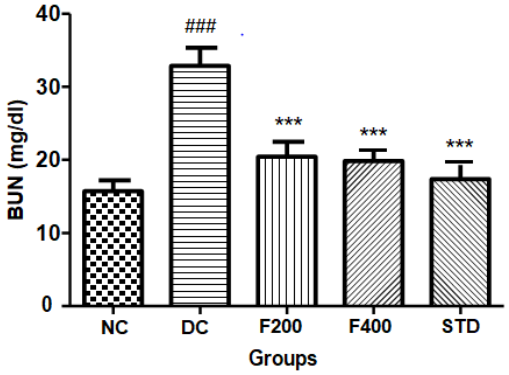



**Figure 6.46 depicts the effect of PHF2 at doses F200 and F400 on blood urea nitrogen (BUN) levels (mg/dl) in rats with streptozotocin-induced diabetes.**

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 ($p < 0.001$).

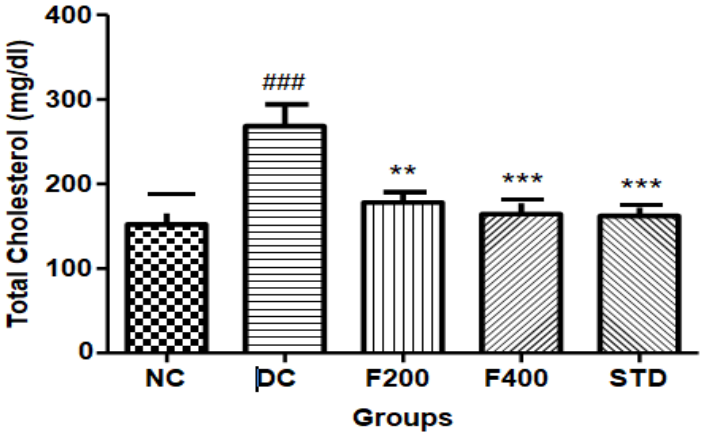
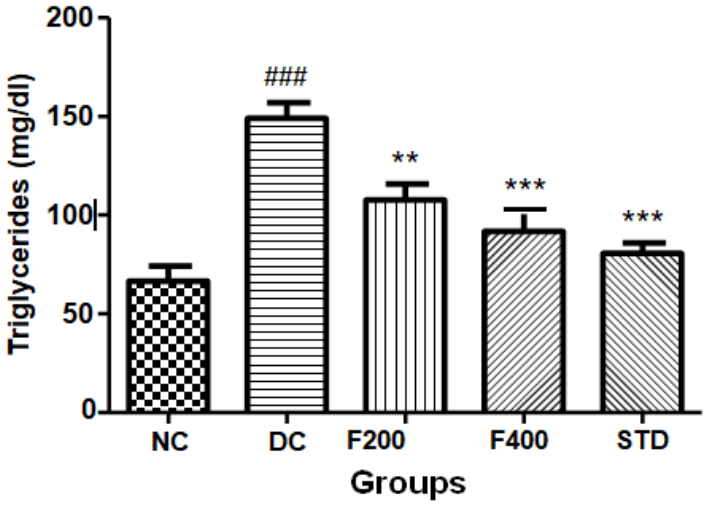



**Figure 6.47 illustrates the effects of PHF2 administered at doses of 200 mg/kg (F200) and 400 mg/kg (F400) on total cholesterol levels (mg/dl) in rats with streptozotocin-induced diabetes.**

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**

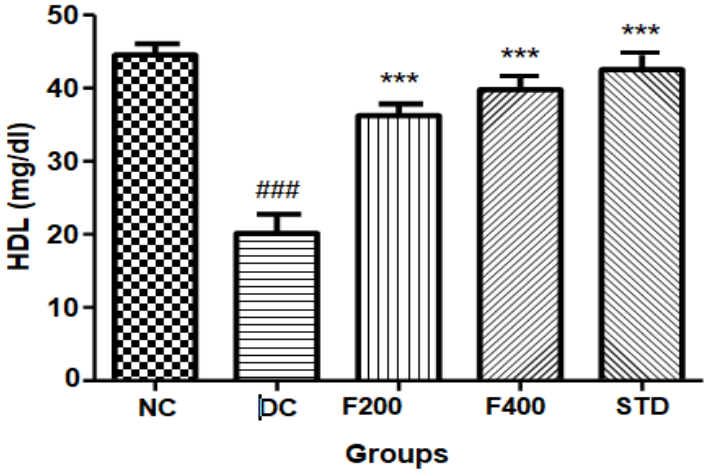



**Figure 6.49 shows the effect of PHF2 at doses of F200 and F400 on high-density lipoprotein (HDL) levels (mg/dl) in rats with streptozotocin-induced diabetes.**

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 ($p < 0.001$).

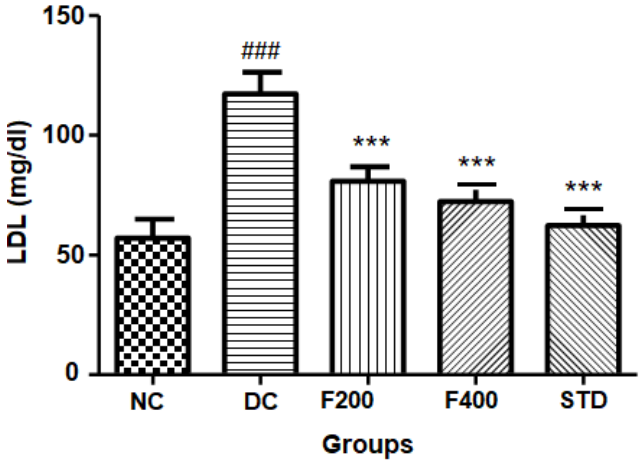



**Figure 6.50 depicts the effect of PHF2 given at doses of 200 mg/kg (F200) and 400 mg/kg (F400) on low-density lipoprotein (LDL) levels (mg/dl) in rats with streptozotocin-induced diabetes.**

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**

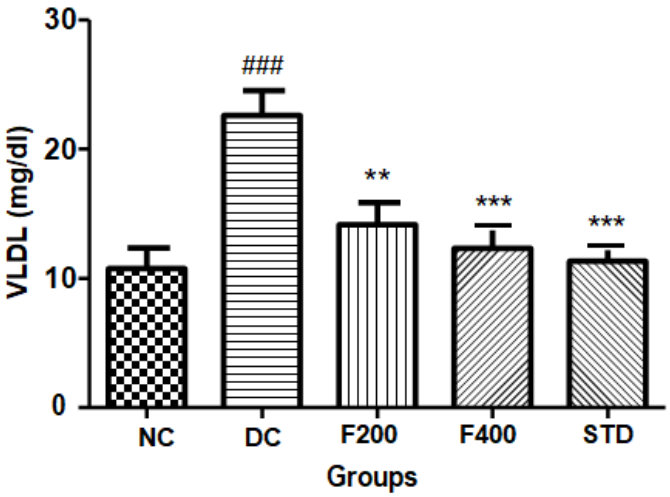



**Figure 6.51 presents the effects of PHF2 administered at doses of 200 mg/kg (F200) and 400 mg/kg (F400) on very low-density lipoprotein (VLDL) levels (mg/dl) in rats with streptozotocin (STZ)-induced diabetes.**

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.

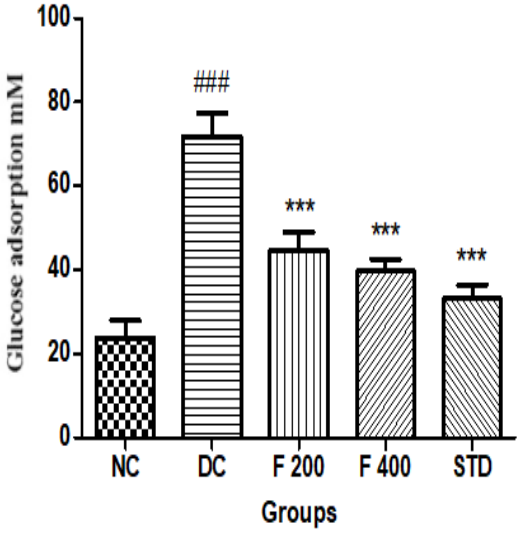



**Figure 6.52 presents the effects of PHF2 administered at doses of 200 mg/kg (F200) and 400 mg/kg (F400) on Glucose absorption levels mM in rats with streptozotocin (STZ)-induced diabetes.**

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.

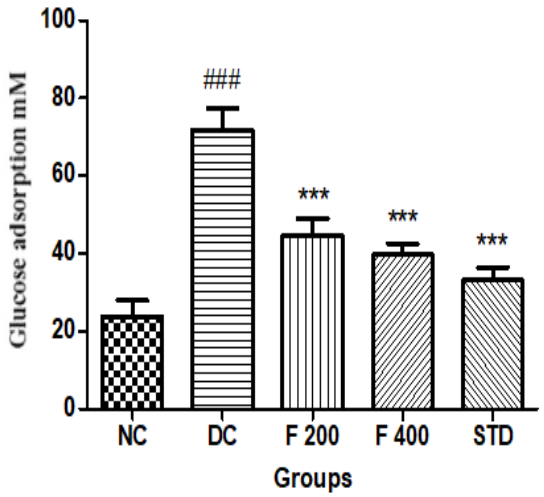


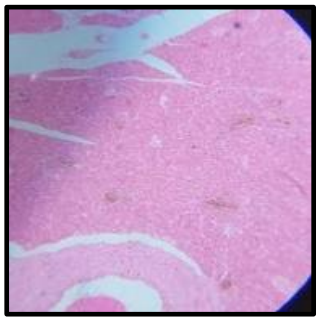
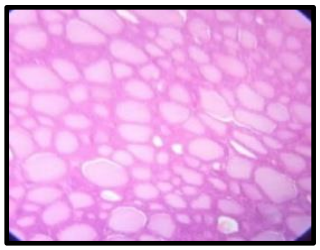
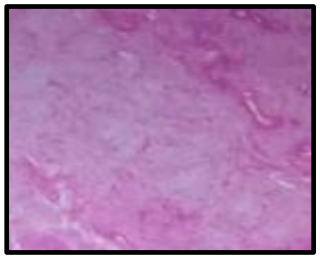
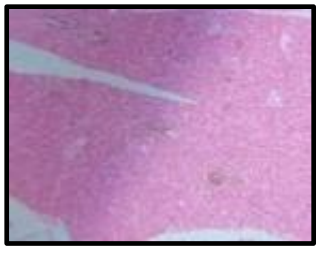
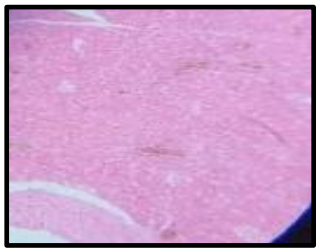

**Figure 6.53 presents the effects of PHF2 administered at doses of 200 mg/kg (F200) and 400 mg/kg (F400) on HbA1c level in rats with streptozotocin (STZ)-induced diabetes.**

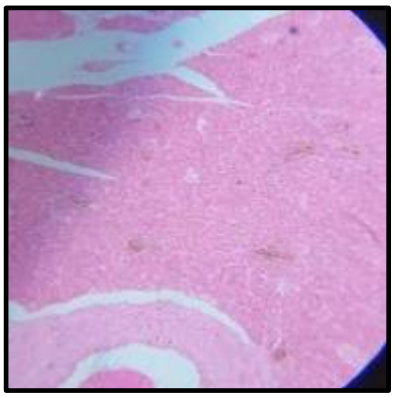
The treatment of rats with STZ (45 mg/kg, i.p.) induced significant ($p<0.001$) increment in HbA1c levels when compared with DC rats. However, the treatment of rats with PHF (200 and 400 mg/kg, p.o.) and Glibenclamide (5 mg/kg, p.o.) exhibited significant ($p<0.01$; $p<0.001$) decrement in HbA1c levels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

**1.10.4.2 Histopathological analysis**

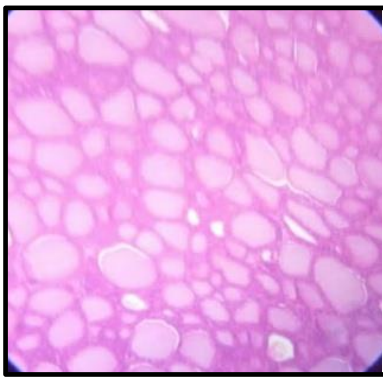
**6.10.4.3.1 Histopathology of Pancreas**: Pancreas histology revealed normal cells and acini in the islets of Langerhans in the normal control pancreas (A). In diabetic animals receiving prolonged in diabetic rats (B), damage to the islets of langerhans and reduced islet dimensions were noted. Treatment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

**Table 6.26: Histopathology of Pancresae in groups**

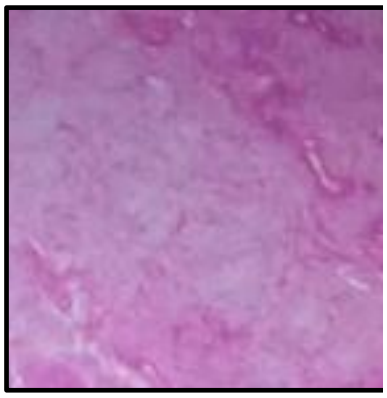
| ORGAN | GROUP | HISTOLOGY | OBSERVATION |
|---|---|---|---|
| PANCREASE | NORMAL |  | No fibrosis or inflammation was present, and the pancreas displayed normal histoarchitecture with wells of acini containing healthy islet cells. |
| | DISESED |  | Islet cell shrinkage and atrophy associated with 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 |



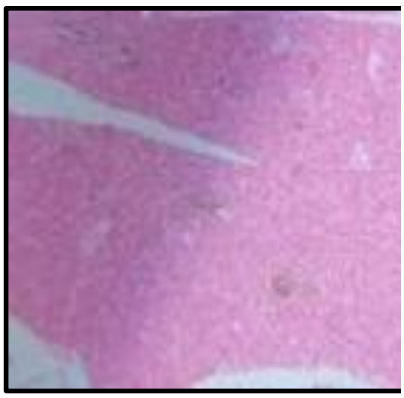
**A. NORMAL**



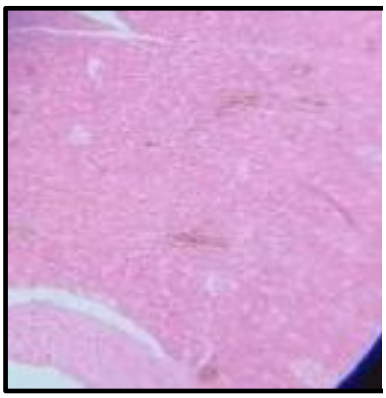
**B. DISESED**



**C.PHF2 F200MG**



**D. PHF2 F400MG**



**E. STANDARD**

**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.

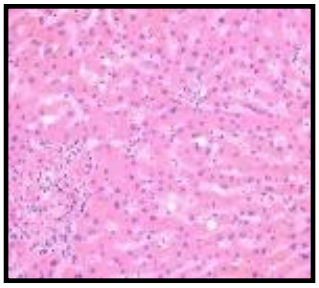
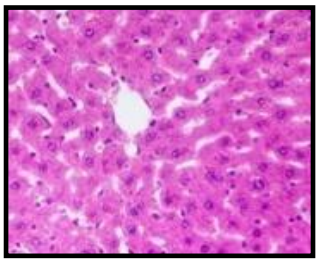
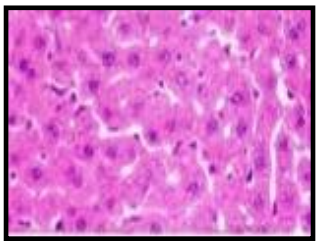
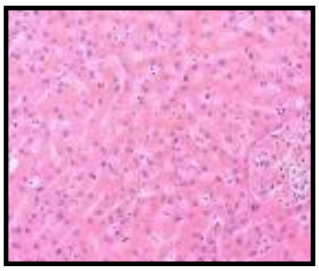
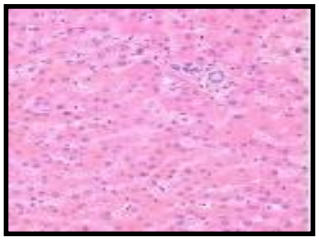
Group V: STZ treated with glibenclamide (45mg/kg)–arrow indicates recovered normal arrangement pancreatic cells

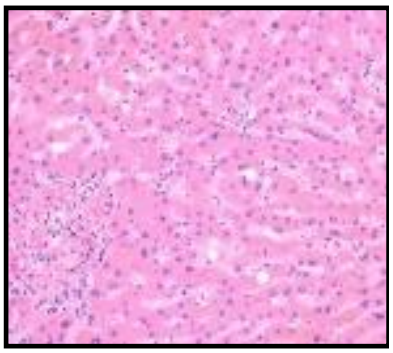
Pathohistological changes were employed in the pancreas of normal and diabetic rats to examine PHF2 and its role in diabetes. The pancreas of diabetic rats showed a decrease in islets cells and an increase in adipose tissue. Less fatty acid infiltration was seen in the pancreas of diabetic rats. PHF2 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**

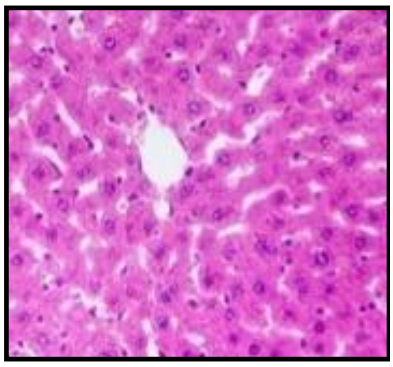
Result revealed typical hepatic cells with the central vein (A), nucleus, and cytoplasm well intact. The typical lobular organization of the group II diabetic rats was maintained. The major vein was clearly visible and notably clogged. There were also noticeable focal foci of bleeding. It was obvious 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**

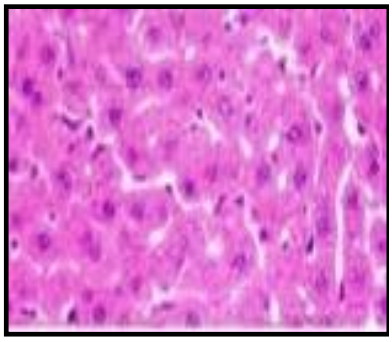
| ORGAN | GROUP | HISTOLOGY | OBSERVATION |
|---|---|---|---|
| LIVER | NORMAL |  | Hepatic cells in normal condition, with the central vein, cytoplasm, and nucleus all intact. |
| | DISEASED |  | **Necrosis and Inflammation** |
| | PHF2 200MG |  | **Regeneration** |
| | PHF2 400MG |  | **No Necrosis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



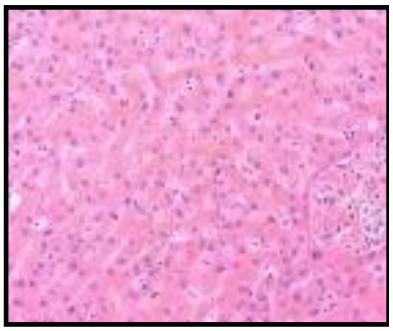
**A. NORMAL**



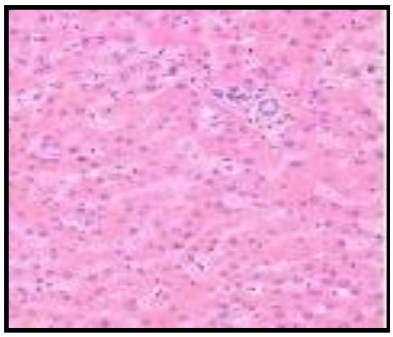
**B. DISESED**



**C.PHF2 F200MG**



**D. PHF2 F400MG**



**E. STANDARD**

**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**

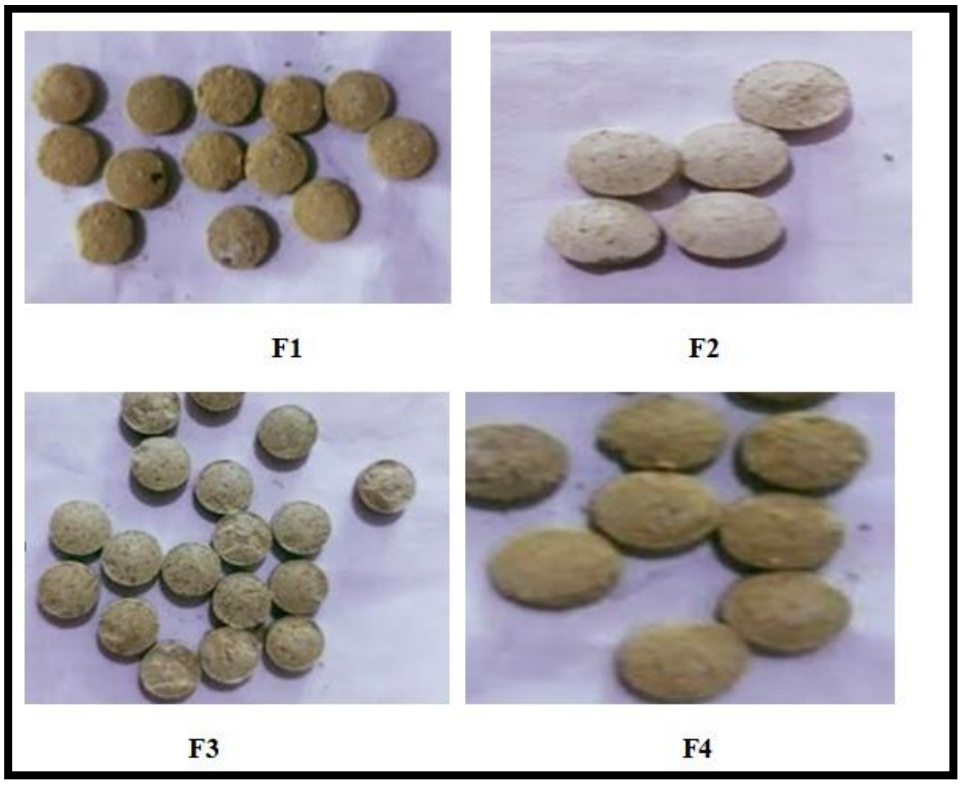



**Figure 6.59: F1 TO F4 TABLETS**

**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.

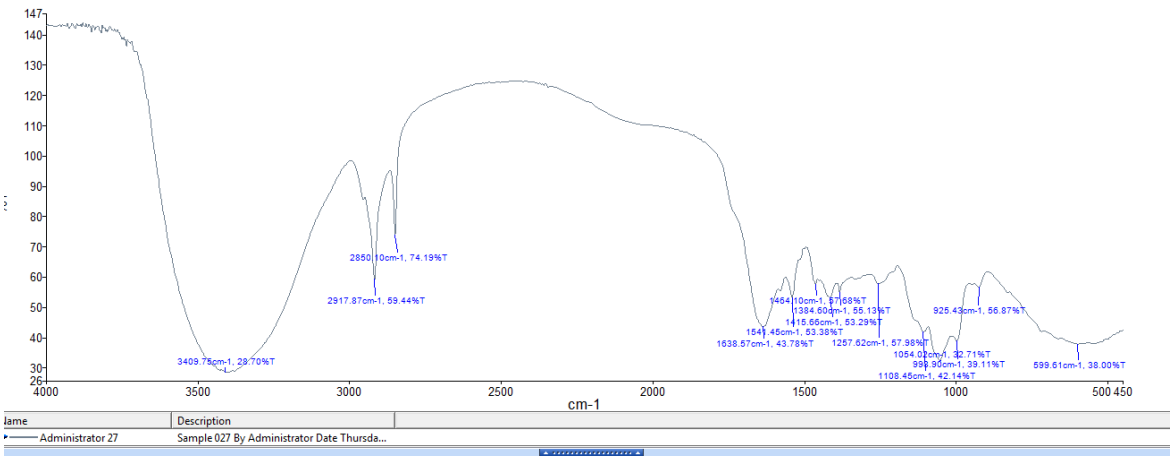



**Figure 6.60: FTIR of Polyherbal formulation**

**Table 6.30: Functional group in Polyherbal Formulation**

| Functional group | Vibrations | Peak |
|---|---|---|
| Alkane | Streching | 2917.87$cm^{-1}$, 2850.10$cm^{-1}$ |
| Amine N-H | Bending | 1653.05$cm^{-1}$ |
| Sulfoxide S=O | Stretching | 1074.38$cm^{-1}$ |
| Alkene C=C | Bending | 925.43$cm^{-1}$ |
| 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

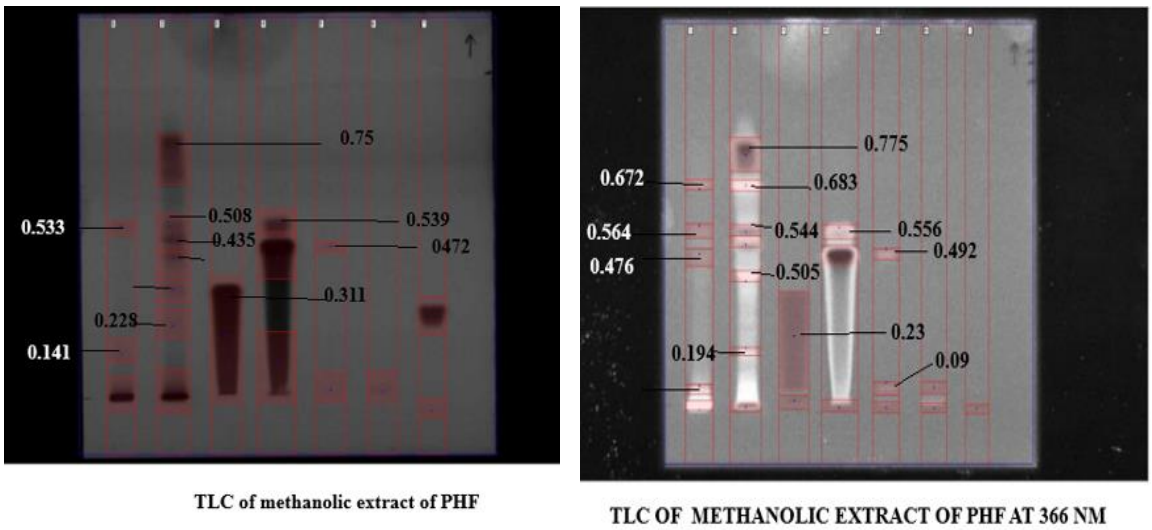



**Figure 6.61: HPTLC finger printing of Methanolic extract of root of *PHF 2* at 254**

**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: $R_f$ Value 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**

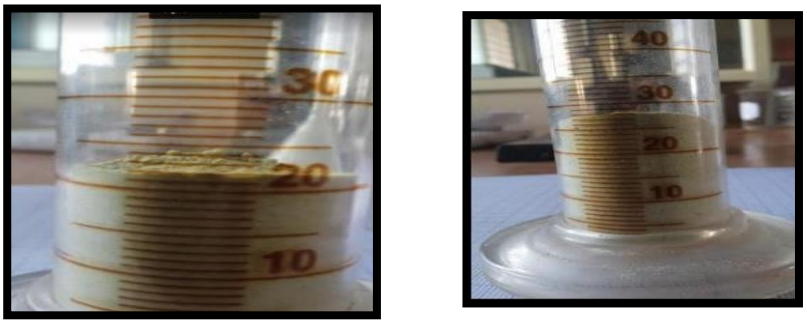



**Figure 6.63: Bulk and Tapped Density**

### 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 |

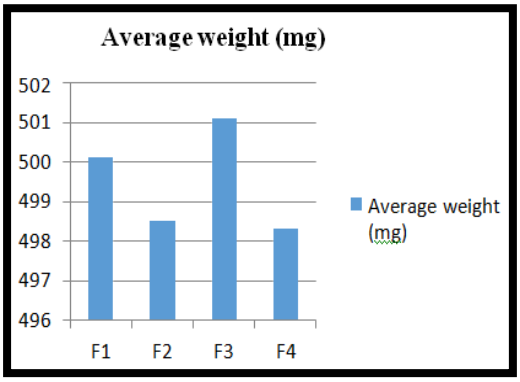



**Figure 6.64: Average Weight**

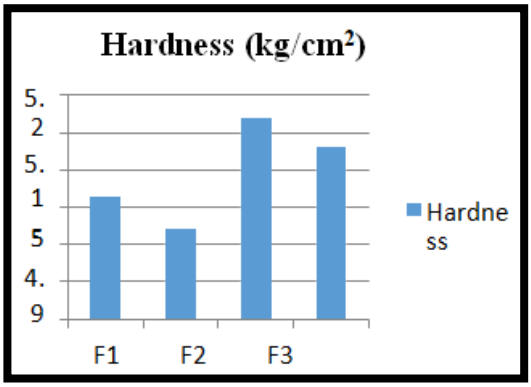



**Figure 6.65: Hardness**

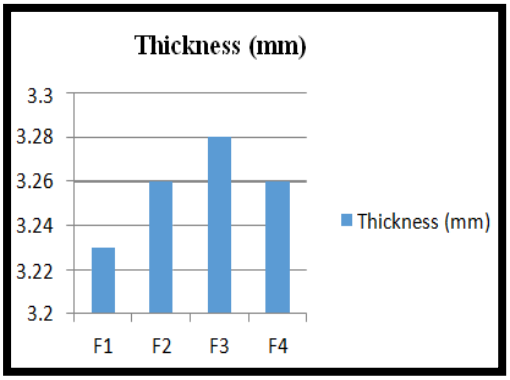



**Figure 6.66: Thickness**

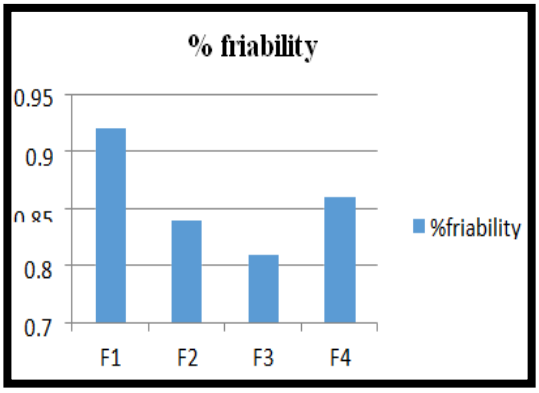



**Figure 6.67: Friability**

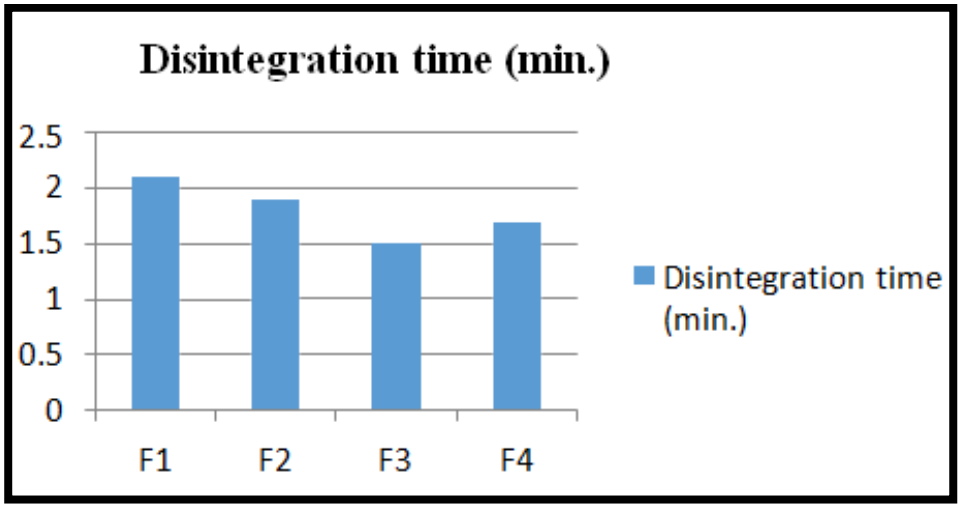



**Figure 6.68Disintegration Time**

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%** | | |
| | **$0^{th}$** | **$7^{th}$** | **$15^{th}$** | **$30^{th}$** |
| **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% | | |
| | $0^{th}$ | $7^{th}$ | $15^{th}$ | $30^{th}$ |
| **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 |