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# Anti-Diabetic Activity And Phytochemical Estimation Of Extracts Of Withania Coagulans, Picrorrhiza Kurroa, And Gymnema Sylvestre

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Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia, often leading to severe complications involving multiple organ systems. With the increasing limitations and side effects of conventional antidiabetic drugs, the focus has shifted toward safer, plant-based alternatives with traditional medicinal relevance. This study investigates the antidiabetic efficacy and phytochemical composition of extracts derived from Withania coagulans, Picrorhiza kurroa, and Gymnema sylvestre, which are traditionally known for their therapeutic roles in Ayurveda. The study involved collection, authentication, drying, and successive extraction of plant materials using ethanol and petroleum ether. The extracts underwent both qualitative and quantitative phytochemical screenings to detect key bioactive compounds such as alkaloids, flavonoids, phenols, glycosides, tannins, and saponins. Their antidiabetic potential was evaluated through in vitro enzymatic assays including α-amylase and α-glucosidase inhibition, and the results were compared with standard drug acarbose. Among the three, Gymnema sylvestre exhibited the most potent inhibitory activity on both enzymes, indicating enhanced glucose uptake and reduced carbohydrate breakdown. Withania coagulans and Picrorhiza kurroa also demonstrated considerable hypoglycemic activity and contained high phenolic and flavonoid content, contributing to their antioxidant properties. The findings validate the traditional use of these plants in managing diabetes and emphasize their potential in formulating effective, low-toxicity herbal therapeutics. Future in vivo and clinical studies are recommended to confirm efficacy and ensure safety for long-term use in diabetic patients.

**Keywords:** Withania coagulans, Picrorhiza kurroa, and Gymnema sylvestre.

## 1. INTRODUCTION:

### 1.1. DIABETES

There's an ever-adding interest in reciprocal and indispensable drug to combat habitual ails. The motivation to exploit similar indispensable curatives lies in the intent to identify new motes that may potentially be more effective than being medicines, with possibilities of

reduced side goods. Also, the advantage of using the traditional system of drug lies in the fact that numerous of these naturally attained excerpts offer an early intervention and health conservation approach in easing conditions. Similar naturally attained excerpts could also serve as leads to the chemical conflation of new medicines[1].
According to the World Health Organization (WHO, 2008), medicinal shops are defined as establishments that contain substances or compounds which can be used for therapeutic purposes, or that synthesize metabolites to produce beneficial medicines. The significance of traditional medicinal shops is increasing nowadays due to various advantages they offer over synthetic medicines [2].

### 1.1.1 Types of Diabetes

There are several types of diabetes, each with its own characteristics and causes. The main types of diabetes include

- **Type 1 Diabetes**

This type of diabetes is an autoimmune condition wherethe vulnerable system inaptly attacks and destroys the insulin- producing cells in the pancreas. As a result, the body produces little to no insulin. Type 1 diabetes generally develops in nonage or nonage, and people with this type of diabetes bear insulin injections or the use of an insulin pump to manage their blood sugar situation.

- **Type 2 Diabetes**

Type 2 diabetes is the most common form of diabetes, counting for the maturity of cases. It occurs when the body becomes resistant to the goods of insulin or when the pancreas does n't produce enough insulin to meet the body's requirements.

- **Other Types:** There are also other rarer forms of diabetes, including monogenic diabetes (caused by mutations in a single gene), cystic fibrosis – related diabetes, and drug-induced diabetes. It's important to note that pre diabetes is a condition that occurs when blood sugar levels are Higher than normal but not highenoughtobeclassifiedastype2diabetes.Prediabetesisoftena precursor to type 2 diabetes and can be managed with lifestyle changes to prevent or delay the onset of diabetes[2,3].

## 1.2. Medicinal Plants Under Study

Due to the side effects of synthetic anti-diabetic drugs, there is growing interest in herbal remedies that are safer and more affordable[4].
This study focuses on three medicinal plants with proven traditional uses in managing diabetes:

A. **Withania coagulans (Indian Rennet)** Family: Solanaceae
- Known for its anti-inflammatory and antioxidant properties.
- **Pharmacological Properties:**
  - **Hypoglycemic Activity:** Helps reduce elevated blood glucose levels.
  - **Antioxidant Activity:** Protects pancreatic β-cells from oxidative damage.
  - **Anti-inflammatory:** Reduces inflammation associated with metabolic disorders[5,6].

B. **Picrorhiza kurroa (Kutki)** Family: Plantaginaceae
- Traditionally used for liver conditions.
- Also possesses blood sugar-lowering effects.
- **Pharmacological Properties:**
- **Hepatoprotective:** Supports liver function, which is crucial in glucose metabolism.
- **Hypoglycemic:** Enhances insulin sensitivity and glucose utilization.
- **Antioxidant and Anti-inflammatory:** Reduces systemic oxidative stress in diabetics[7,8].

C. **Gymnema sylvestre (Gurmar)** Family: Apocynaceae
- Renowned for its ability to suppress sugar cravings.
- Enhances insulin secretion and supports pancreatic function[9].
- **Pharmacological Properties:**
- **Anti-Diabetic:** Gymnemic acids suppress the taste of sugar and reduce sugar absorption in the intestine.
- **Pancreatic Regeneration:** Stimulates regeneration of β-cells.
- **Insulin Mimetic:** Increases insulin production and activity[11].

## 2. MATERIAL AND METHOD

**2.1 MATERIALS:-** Numerous relevant materials, were used. This chapter deals with the material and methods used for withania coagulans, picrorrhiza kurroa, and gymnema sylvestre.

2.1.1 **Chemicals:**

Chemicals/Herbs used in the investigation are listed in Table 1.

**Table 1: chemicals used for study**

| Sr. No. | Chemicals/Herbs | Purpose/Use | Supplier |
|---|---|---|---|
| 1. | **Ethanol** | **Extraction of plant material** | **Analytical** |
| 2. | **Petroleum Ether** | **Defatting of plant material** | **Analytical** |
| 3. | **Chloroform** | **Phytochemical analysis** | **Analytical** |
| 4. | **Methanol** | **Extraction and preparation of standard solutions** | **Analytical** |
| 5. | **Dragendorff's Reagent** | **Detection of alkaloids** | **Laboratory** |
| 6. | **Mayer's Reagent** | **Detection of alkaloids** | **Laboratory** |
| 7. | **Wagner's Reagent** | **Detection of alkaloids** | **Laboratory** |
| 8. | **Hager's Reagent** | **Detection of alkaloids** | **Laboratory** |

| | | | |
|---|---|---|---|
| 9. | **Fehling's Solution A & B** | **Detection of reducing sugars** | **Laboratory** |
| 10. | **Benedict's Reagent** | **Detection of carbohydrates** | **Laboratory** |
| 11. | **Barfoed's Reagent** | **Detection of monosaccharides** | **Laboratory** |
| 12. | **Sulphuric Acid ($H_2SO_4$)** | **Phytochemical tests (Salkowski, Borntrager, etc.)** | **Concentrated** |
| 13. | **Hydrochloric Acid (HCl)** | **Microscopy staining and testing** | **Concentrated** |
| 14. | **Ferric Chloride** | **Detection of phenols and tannins** | **Laboratory** |
| 15. | **Folin-Ciocalteu Reagent** | **Estimation of total phenol content** | **Laboratory** |
| 16. | **Sodium Carbonate** | **Reagent in phenolic estimation** | **Laboratory** |
| 17. | **Gallic Acid** | **Standard for phenol estimation** | **Analytical** |
| 18. | **Quercetin** | **Standard for flavonoid estimation** | **Analytical** |
| 19. | **α-Amylase Enzyme** | **α-amylase inhibition assay** | **Enzymatic** |
| 20. | **α-Glucosidase Enzyme** | **α-glucosidase inhibition assay** | **Enzymatic** |
| 21. | **ρ-Nitrophenyl-α-D-glucopyranoside (pNPG)** | **Substrate for α-glucosidase assay** | **Laboratory** |
| 22. | **Acarbose** | **Standard α-amylase/α-glucosidase inhibitor** | **Analytical** |
| 23. | **Acetic Anhydride** | **Detection of resins/steroids** | **Laboratory** |
| 24. | **Sodium Bicarbonate** | **Detection of saponins** | **Laboratory** |
| 25. | **Gelatin Solution** | **Detection of phenolic compounds** | **Laboratory** |

2.1.2 **Instruments:**

Instruments used in the investigation are listed Table 2.

**Table 2: instruments used for study**

| Sr. No. | Instruments | Purpose |
|---|---|---|
| 1. | **Rotary Vacuum Evaporator** | **For concentration of extracts post maceration** |
| 2. | **Soxhlet Apparatus** | **For extraction of plant constituents** |

| | | |
|---|---|---|
| 3. | **UV-Visible Spectrophotometer** | **For estimation of phenolic and flavonoid content** |
| 4. | **Digital Weighing Balance** | **For accurate weighing of crude drugs and chemicals** |
| 5. | **Hot Air Oven** | **For drying plant material and samples** |
| 6. | **Muffle Furnace** | **For determination of ash values** |
| 7. | **Microscope (Compound and Fluorescence)** | **For powder microscopy and fluorescence analysis** |
| 8. | **Water Bath** | **For maintaining constant temperature during reactions** |
| 9. | **pH Meter** | **To measure pH of solutions and buffers** |
| 10. | **Clevenger Apparatus** | **For moisture content determination** |
| 11. | **Glassware (beakers, test tubes, pipettes etc.)** | **General experimental procedures** |

## 2.2 METHODS:

Materials Selected for Extraction:- The study utilized the following herbs for extraction: It consist softhree herbsviz., Withaniacoagulans, Picrorrhizakurroa, Gymnema sylvestre

### 2.2.1 Selection, Collection, and Identification of Herbs

Herbs Withaniacoagulans, Picrorhizakurroa, Gymnemasylvestreusedfor evaluation were procured from the authentic suppliers of Indore and after cleaning, plant parts were dried under shade at room temperature for 3 days and then in oven at 45°C till complete dryness. Dried plant parts were stored in air tight glass containers in dry and cool place to avoid contamination and deterioration[11].

### 2.2.2 Authentification of Following Herbs By Botanist

Authentication of selected traditional plant - The leaves of Withania coagulans, Picrorhiza kurroa, Gymnemasylvestre medicinalplant was authenticated by a plant taxonomist in order to confirm its identity and purity. Authentication of herbs were done by SS.IN Botany & Environment Management, Vikram University, Ujjain M.P[11].

### 2.2.3 Processing of Raw Materials

The procured plant materials were cleaned thoroughly. They were then dried under shade for a weekorso. Once they were completely dried, they were ground into coarse powder and stored in air tight containers and preserved for the further processing[12].

### 2.2.4 Pharmacognostic Studies

Morphological characters: The leaves were examined macroscopically with reference to its shape, size, colour, and odour. Shade dried powdered plant materials of the plants, Withaniacoagulans, Picrorrhizakurroa, Gymnema sylvestre used for the standardization of raw materials[12].

**a) Macroscopic/Organoleptic Evaluation**

Organoleptic evaluation defines the majority of information on the identity, purity of the material which are of primary importance for the establishment of degree of quality done by sensory organs for the evaluation of drugs colour, odour, taste and specific characters. In this

study the following organoleptic characters like physical appearance, taste and odour of plant materials were evaluated and confirmed with reference samples[13].

**b) Microscopical Evaluation**

**Powder Characteristic**

Powder characters show the detailed examinationofa drug which is mainly used to identify the organised drugs by their known structural characters. The structural characters are distinguished with various reagents and stains. The powder characters of the crude drug powder were studied using microscope[14].

**Methodology**

A pinch of the powdered sample was mountedona microscopic slide with a drop of Fluoroglucinol and conc. HCl. Characters were observed under microscope[14].

2.2.5 **Physico Chemical Evaluation**

Out of the numerous practical applications of pharmacognosy, the great importance for the pharmaceutical industry is in the evaluation of the crude drugs. The evaluation of these parameters shows the clear idea about the specific characteristic of crude drugs. It is virtually impossible to avoid some naturally occurring inorganic and organic contaminants while collection from soil[15].

**A. Determination of Moisture Content**

Ten grams of chopped leaves from each plant species were immersed in 125 mL of toluene. The mixtures were then transferred to 500 mL distillation flasks connected to a modified Clevenger apparatus. Using heating mantles, the flasks were heated to a temperature of 100 ±5°C. The distillates, which separated into aqueous and organic phases, were collected in graduated glass tubes, and the volume of the aqueous phase was recorded. Moisture content was determined based on a 100 g sample. The experiment followed a completely randomized design with three repetitions, utilizing a 3×4 factorial arrangement. The factors included distillation time (1, 2, and 3 hours) and the use of toluene as the solvent[15].

**B. Loss On Drying**

The loss on drying (LOD) test measures both water content and volatile substances in crude drugs. This test is crucial, especially for hygroscopic herbal materials, as excessive moisture can promote microbial growth, fungal contamination, insect infestation, and overall deterioration. In modern pharmaceutical applications, moisture content plays a key role in determining drug quality and shelf life. Loss on drying is expressed as a percentage of weight loss (% w/w). To conduct the test, approximately 10 g of the drug sample was weighed into a pre-dried, tarred flat weighing bottle. The sample was then dried at 105°C for five hours, cooled in a suitable desiccators, and reweighed. Drying continued at one-hour intervals until a constant weight was achieved[16].

**Loss on drying % = Final weight of the sample/ Initial weight of the sample×100**

**C. Determination of Ash Values**

The ash content of crude drug is generally taken as the residue remaining after incineration. It usually represents the non-volatile inorganic salts like metallic salts and silica naturally occurring in the drug and adhering to it, but it may include inorganic matter added for the purpose of adulteration, contamination and substitution. This is important parameter for the evaluation of crude drugs. The

ash value can be determined by three different methods like total ash, acid insoluble ash and water soluble ash. Sulphated ash is also ash value to find out the sulphated residue[16].

- **Total Ash**

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

**%Total ash =Weight of residue obtained/Weight of sample taken×100**

- **Acid in soluble Ash**

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

**% Ash insoluble Ash = Weight of Residue obtained/ Weight of sample taken ×100**

- **Water-Soluble Ash Determination**

Water-soluble ash is the difference in weight between the total ash and the residue remaining after treating the total ash with water.

To determine water-soluble ash, 25 mL of water is added to the crucible containing the total ash, and the mixture is boiled for 5 minutes. The insoluble matter is then collected using a sintered-glass crucible or an ashless filter paper, washed with hot water, and ignited in a crucible for 15 minutes at a temperature not exceeding 450°C. The weight of this residue (in mg) is subtracted from the total ash weight, and the water-soluble ash content is calculated in mg per gram of air-dried material.

**% Water soluble ash = Weight of residue obtained/Weight of sample taken × 100**

**D. Determination of Extractive values**

The method determines the amount of active constituents in a given amount of crude drugs when extracted with the solvents. The extraction process of crude drug with a particular solvent yields a solution containing different Phyto-constituents. The composition of these Phyto-constituents provides the preliminary information on the quality of a particular drug sample[15,16].

- **Water Soluble Extractive**

To determine the water-soluble extractive value, 5 g of air-dried, coarsely powdered drug was macerated with 100 mL of chloroform water in a closed flask for 24 hours. The mixture was shaken frequently for the first 6 hours and then allowed to stand undisturbed for the remaining 18 hours.

After maceration, the solution was filtered, and 25 mL of the filtrate was evaporated to dryness in a pre-weighed, flat-bottomed shallow dish. The residue was dried at 105°C until a constant weight was achieved, then weighed. The percentage of water-soluble extractive was determined by calculating the weight of the dried residue obtained from the filtrate and expressing it as a percentage of the initial weight of the air-dried drug sample.

**% Water soluble extractive value = Weight of residue obtained after drying / Weight of sample taken × 100**

- **Alcohol Soluble Extractive**

Macerated 5 g of the air dried, coarsely powdered drug, with 100 ml of ethanol (95%) in a closed flask for twenty-four hours, shaking frequently during six hours and allowed to stand for eighteen hours. Filtered and evaporated 25 ml of the filtrate to dryness in a tarred flat bottomed shallow dish, dried at 105ºCto constant weight and weighed. The percentage of water-soluble extractive value was calculated based on the weight of the dried extract obtained from the filtrate in relation to the initial weight of the air-dried drug sample.

**Alcohol soluble extractive value = Weight of residue obtained after drying/Weight of sample taken × 100**

- **Ether-Soluble Extractive Determination**

Ether-soluble extractive values are used to evaluate crude drugs by measuring both volatile and non-volatile ether-soluble components.

**Volatile ether-soluble** extractives represent the volatile oil content of the drug.

**Non-volatile ether-soluble** extractives include resins, fixed oils, or coloring matter present in the drug[15,16].

**Ether soluble extractive value = Weight of residue obtained after drying/Weight of sample taken × 100**

**E. Determination Of Pesticide Residue**

**F. Determination Of Foreign Organic Matter**

**G. Determination Of Swelling Index**

**H. Determination Of Foaming Index**

### 2.2.6 Preparation of Extract (Extraction)

- **Extraction Methods:**

Three primary methods are commonly used for extracting plant materials:

I. **Maceration** A simple extraction method where plant material is soaked in a solvent at room temperature for a specific period, allowing the active constituents to dissolve. This method is suitable for extracting thermolabile (heat-sensitive) compounds.

II. **Percolation** A continuous extraction process where the solvent passes through a column of plant material, ensuring efficient extraction of active constituents. Like maceration, it is preferred for thermo-labile compounds.

III. **Successive Soxhlet Extraction** A rapid and continuous process where the solvent is repeatedly heated, evaporated, and condensed, continuously extracting the plant material. This method is particularly useful when maceration or percolation is insufficient for complete extraction.

Due to its efficiency and various advantages, Soxhlet extraction was chosen for the present study[17].

Crude drug leaves
↓
Defatted with petroleum ether
↓
Extract was collected and dried
↓
Extraction with Methanol
↓
Extract was collected and dried
↓
Calculated % yield

Extraction is the preliminary step involved in the phytochemical studies. Ethanol proves to the universal solvent. As the present study utilizes a combination of herbs, ethanol was opted for

the extraction of the active constituents from the individual plants separately. The method of extraction is Hot Percolation method[17].

**Actual yield Theoretical yield x 100**

- **Hot Percolation Method**

Approximately 200 g of coarsely powdered plant material was subjected to extraction using ethanol at a temperature of 60–70°C. The extracts obtained from individual plants were then concentrated using a rotary vacuum evaporator.

The percentage yield, color, and consistency of all extracts were recorded. These extracts were further analyzed for detailed phytochemical and pharmacological screening to assess their bioactive components and therapeutic potential[18].

**Fig 1: Hot Percolation Method**


2.2.7 **Phytochemical Studies**

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

compoundsandsubsequentlymayleadtothedrugdiscoveryanddevelopment.All the plant raw materials were subjected to preliminary phytochemical screening for the detection of various plant constituents[19].

**1. Preliminary Phytochemical Screening**

Experiment was performed to identify presence or absence of different phyto constituents by detailed qualitative phyto chemical analysis. The colour intensity or the precipitate formation was used as medical responses to tests. Following standard procedures were used.

a) **Triterpenoids and Steroids**

**Salkowski Test:** The extract was dissolved in chloroform, and an equal volume of concentrated sulphuric acid was added. The presence of steroids was indicated by the formation of a bluish-red to cherry-red colour in the chloroform layer and green fluorescence in the acid layer[20].

b) **Flavonoids**

**Shinoda's Test:** A few magnesium turnings and a little drop of concentrated hydrochloric acid were added to 1 ml of extract in alcohol. It was heated on a water bath. The formation of red to pink colour indicated the presence of flavonoids[21].

c) **Alkaloids**

- **Dragendorff's Test:** 1 ml of extract was taken. Alcohol was mixed and shaken well with a few drops of acetic acid and Dragendorff's reagent. The presence of alkaloids was indicated by the formation of an orange-red precipitate[22].
- **Mayer's Test:** 1 ml of extract was dissolved in acetic acid, and a few drops of Mayer's reagent were added. The presence of alkaloids was indicated by the formation of a dull white precipitate.
- **Wagner's Test:** In acetic acid, 1 ml of extract was dissolved. A few drops of Wagner's reagent were added. The presence of alkaloids was indicated by a reddish-brown precipitate.
- **Hager's Test:** 1–2 ml of extract was dissolved in acetic acid. To it, 3 ml of Hager's reagent was added. The formation of a yellow precipitate indicated the presence of alkaloids.

d) **Carbohydrates**

- **Molisch's Test:** The aqueous solution of the extract (1 ml) was mixed with a few drops of Molisch reagent (naphthol), and concentrated $H_2SO_4$ (sulphuric acid) was added dropwise along the wall of the test tube. The formation of a purple-coloured ring at the junction indicated the presence of carbohydrates[23].
- **Fehling's Test:** Equal amounts of Fehling A and Fehling B solutions (1 ml each) were mixed, and 2 ml of the aqueous extract solution was added. It was boiled for 5–10 minutes in a water bath. The formation of a reddish-brown precipitate due to cuprous oxide formation showed the presence of reducing sugars[24].
- **Benedict's Test:** In a test tube, equal amounts of Benedict's reagent and extract were mixed and heated for 5–10 minutes in a water bath. Depending on the amount of reducing sugar present in the test solution, a green, yellow, or red colour appeared, indicating the presence of reducing sugars[25].
- **Barfoed's Test:** In the aqueous solution of the extract, 1 ml of Benedict's solution was added and heated to boiling. In the presence of monosaccharides, a red colour appeared due to the formation of cupric oxide.

e) **Glycosides**

- **Borntrager's Test**

**Procedure:** 3 mL of the test solution was treated with dilute sulfuric acid, boiled for 5 minutes, and then filtered. The cold filtrate was mixed with an equal amount of benzene or chloroform and shaken well. The organic solvent layer was separated, and ammonia was added.
**Observation:** The formation of a pink to red color in the ammoniacal layer confirmed the presence of anthraquinone glycosides[26].

- **Keller-Killiani Test**

**Procedure:** 2 mL of the test solution was mixed with 3 mL of glacial acetic acid and one drop of 5% ferric chloride solution. Then, 0.5 mL of concentrated sulfuric acid was carefully added.
**Observation**: The presence of cardiac glycosides was indicated by the formation of a blue color in the acetic acid layer[27].

f) **Phenols**

- **Ferric Chloride Test**
  **Procedure:** A small quantity of the drug was dissolved in 2 mL of distilled water, and a few drops of 10% aqueous ferric chloride solution were added.
  **Observation:** The formation of a blue or green color indicated the presence of phenols[28].

**g) Proteins (Biuret Test)**
**Procedure:** To 1 mL of ethanolic extract, 5 to 8 drops of 10% copper sulfate solution were added.
**Observation:** The formation of a violet color confirmed the presence of proteins[29].

**h) Resins**

**Procedure:** A small quantity of the ethanolic extract was dissolved in 5–10 mL of acetic anhydride and gently heated. The solution was cooled, and 0.05 mL of concentrated sulfuric acid was added.
**Observation:** A bright purplish-red color that rapidly changed to violet indicated the presence of resins[29].

**i) Saponins**

- **Froth Test:** 1 ml of extract was added to distilled water and shaken well. The presence of saponins was indicated by stable froth formation.
- To 5 ml of an extract of the drug, a drop of sodium bicarbonate solution was added. The mixture was shaken vigorously and left for 3 minutes. The development of honeycomb-like froth indicated the presence of saponins.
  J. Tannins and Phenolic Compounds
- **Ferric Chloride Test:** An amount of extract was dissolved in distilled water. A few drops of a dilute solution of ferric chloride were added. The formation of a dark blue colour indicated the presence of tannins.
- **Gelatin Test:** An amount of extract was dissolved in distilled water. Then, 2 ml of a 1% gelatin solution containing 10% sodium chloride was added. The presence of phenolic compounds was indicated by the development of a white precipitate[29,30].

**j) Steroids**

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

turned green, indicating the presence of steroids.

2. **Fluorescence Analysis**

This fluorescence technique is useful for detecting adulteration in crude drugs.
Raw materials used for polyherbal capsule preparation were tested for any colour changes under UV light. Samples were tested as such and after treatment with organic solvents, alkali, and acidic solutions. They were then viewed under ordinary light, short UV (254 nm), and long UV (365 nm) to study their fluorescence properties[31].

**3. Quantitative Studies of Phytoconstituents**

**A. Estimation of Total Phenolic Content**

The total phenolic content of each excerpt was determined by the modified folin-ciocalteu system. 10 mg Gallic acid was dissolved in 10 ml methanolto make 1mg/ ml result, colorful aliquotsof10- 50µg/ mlwasprepared inmethanol. 10mgofdriedextractwasdissolved in10 ml methanoland sludge. Two ml( 1mg/ ml) of this excerpt was for the estimation of phenol. 2 mlof excerpt and each standard was mixed with 1 ml of Folin- Ciocalteu reagent( preliminarily adulterated with distilled water 110 v/ v) and 1 ml( 7.5 g/ l) of sodiumcarbonate. The admixture was vortexed for15sandallowedto standfor10min forcolourdevelopment.Theabsorbancewasmeasuredat 765 nm using a spectrophotometer[31].

B. **Estimation of total flavonoids content**

Determination of total flavonoids content was grounded on aluminium chloride system. 10 mg quercetinwasdissolved in10 mlmethanol, and variousaliquotsof5- 25µg/ mlwere prepared in methanol.10 mgofdriedextract wasdissolved in10mlmethanoland filter.Three ml( 1mg/ ml) ofthisextract wasforthe estimationofflavonoids.1 mlof2 AlCl3solutionwasadded to 3 ml ofextractoreachstandardandallowedto standfor 15minat roomtemperature; absorbancewas measured at 420 nm[31].

2.2.8 **CHARACTERIZATION OF EXTRACT:**

I. TLC
II. UV VISIBLE SPECTROSCOPY
III. CHROMATOGRAPHY

**I. TLC**

**Thin Layer Chromatography (TLC)**

Pre-coated silica gel 60 $F_{254}$ plates served as the stationary phase. Extracts were spotted onto the plates using capillary tubes, maintaining a margin of about 1 cm from the base. Several solvent systems were tested and optimized based on the polarity of the targeted compounds, serving as the mobile phase. After the plates were developed, they were air-dried and examined under UV light at wavelengths of 254 nm and 365 nm to identify fluorescent spots. Further visualization was achieved by spraying the plates with specific reagents such as iodine vapor and anisaldehyde-sulphuric acid, which helped highlight different classes of phytochemicals. The retention factor (Rf) for each observed spot was calculated using the formula:
**Rf = Distance travelled by the compound / Distance travelled by the solvent front**

The resulting TLC profiles displayed multiple distinct bands, indicating the presence of various phytochemicals. These findings contributed to the preliminary standardization of the extracts and confirmed the presence of flavonoids, alkaloids, phenolics, and steroids. The generated chromatographic fingerprints can be used as reference standards for future quality assessment and authentication of these plant extracts[32].

**Table 3: TLC Profile of Plant Extract of Withania coagulans (Ethanolic Extract)**

| Sr. No | Solvent System (v/v) | No. of Spots | Rf Values | Spot Color (UV 254 nm) | Spot Color (Anisaldehyde-$H_2SO_4$) |
|---|---|---|---|---|---|
| 1 | Toluene : Ethyl Acetate (7:3) | 3 | 0.25, 0.54, 0.76 | Blue | Purple, green, pink |
| 2 | Chloroform : Methanol (9:1) | 2 | 0.42, 0.67 | Light blue | Pink, violet |

**Table 4: TLC Profile of Plant Extract of Picrorhiza kurroa (Ethanolic Extract)**

| Sr. No. | Solvent System (v/v) | No. of Spots | Rf Values | Spot Color (UV 254 nm) | Spot Color (Iodine Vapour) |
|---|---|---|---|---|---|
| 1 | Chloroform : Methanol (8:2) | 3 | 0.30, 0.59, 0.81 | Fluorescent blue | Yellow brown |
| 2 | Ethyl Acetate : Methanol : Water (10:1.35:1) | 2 | 0.48, 0.71 | Greenish spots | Light yellow |

**Table 5: TLC Profile of Plant Extract of Gymnema sylvestre (Ethanolic Extract)**

| Sr. No | Solvent System (v/v) | No. of Spots | Rf Values | Spot Color (UV 365 nm) | Spot Color (Anisaldehyde-$H_2SO_4$) |
|---|---|---|---|---|---|
| 1 | Toluene : Ethyl Acetate (6:4) | 3 | 0.22, 0.49, 0.78 | Blue green | Violet, brown |
| 2 | Chloroform : Methanol (9:1) | 2 | 0.41, 0.63 | Greenish blue | Pink, orange |

TLC profiling of the extracts provided preliminary phytochemical fingerprints that confirmed the presence of multiple bioactive compounds in each plant extract. These profiles are essential for standardization and can be used in future quality assurance protocols for identification and authentication of herbal materials[32].

**Table 6: TLC Profile of Plant Extract**

| **Solvent system (v/v)** | **No. of Spots** | **Spot color (UV)** | **Spot color (UV)** | | | **Spot color Spray)** | |
|---|---|---|---|---|---|---|---|
| | | **Rf Values** | **Withania coagulans** | | | **Gymnema sylvestre** | |
| **Withania coagulans (Ethanolic Extract)** | | | **Blue** | | | | **P** |
| **Toluene:Ethyl Acetate** | **3** | **0.25**<br>**0.54**<br>**0.76** | **0** | **-** | **Prife** | **Purple** | **Green Pink** |
| **Chloroform : Methanol (9:1)** | **2** | **0.42**<br>**0.67** | **0** | **Light Blue** | **Pink** | **Violate** | **Violate** |
| **Picrorhiza kurroa (Ethanolic Extract)** | | | | | | | |
| Chloroform : Methanol (8:2) | **3** | **0.30**<br>**0.59**<br>**0.81** | **3** | **Fluorescent Blue** | **Yellow-Brown** | **Blue-Green** | **Violate-Brown** |
| **Ethyl Acetate: Methanol : Water** | **2** | **0.48**<br>**0.71** | **2** | **Greenish** | **Light Yellow** | **Flu-green** | **Brown** |
| **Gymnema sylvestre (Ethanolic Extract)** | | | | | | | |
| Toluene : Ethyl Acetate (6:4) | 3 | 0.22, 0.49, 0.78 | **3** | **Blue-green** | **Violate-Brown** | **Violate** | **Brown** |
| Chloroform : Methanol (9:1) | 2 | 0.41, 0.63 | **2** | **Greenish-blue** | **Pink** | **Pink** | **Orange** |

2.2.9 **Antidiabetic Activity of Extract**

**In-vitro Studies of Antidiabetic Activity**

**I. α-Amylase Inhibition Assay**

**II. α-Glucosidase Inhibition Assay**

**I. α-Amylase Inhibition Assay**

Plants have been used for treating diabetes for a long time, and recent studies have confirmed their effectiveness. They can act through various pathways, such as:

- Increasing insulin secretion
- Enhancing glucose uptake
- Inhibiting glucose production and absorption
- Preventing oxidation and inflammation, which are associated with diabetes

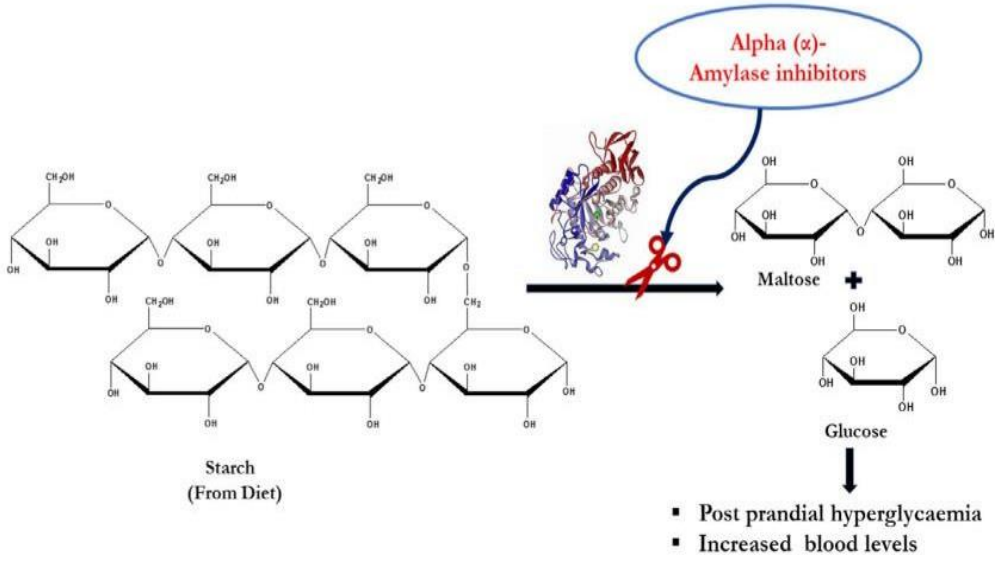
Inhibiting glucose absorption is an important approach for diabetes treatment. By blocking the enzymes responsible for breaking down carbohydrates, we can prevent high blood glucose levels after meals. One such enzyme is **α-amylase**, and its inhibition is a potential therapeutic strategy for managing diabetes. The potential inhibitory effect of plant extracts from **Withania coagulans, Picrorhiza kurroa, and Gymnema sylvestre** will be tested against this enzyme to uncover its mode(s) of action[32].

i. **Alpha-Amylase Enzyme** Alpha-amylase is an enzyme that plays a vital role in the digestion of carbohydrates. It is produced in various parts of the body, including the **salivary glands** and the **pancreas**.

- Alpha-amylase catalyzes the hydrolysis of complex starch molecules into smaller polysaccharides and, ultimately, into simpler sugars like maltose and glucose.
- This enzyme initiates the digestion of starches in the **mouth (salivary amylase)** and continues its action in the **small intestine (pancreatic amylase)**.
- The breakdown of starch by alpha-amylase is an essential step in converting dietary carbohydrates into a form that can be absorbed and utilized by the body for energy.

However, this process contributes to **elevated blood glucose levels** and the development of **hyperglycemia**. Therefore, targeting **α-amylase** has emerged as an important therapeutic approach to **manage and control** the increase in blood glucose levels after meals[33].

**Fig 2: Role of α-Amylase Inhibitors in the Diabetes Mellitus**



ii. **Role of α-Amylase Inhibitors in the Treatment of Diabetes Mellitus** This conversion is necessary because glucose is essential for proper brain function. However, excessive starch breakdown leads to increased blood glucose levels, contributing to hyperglycemia**.** α-Amylase inhibitors help suppress this enzyme's activity, thereby reducing postprandial (after-meal) glucose levels. One well-known α-amylase inhibitor is Acarbose**,** an antidiabetic agent that slows carbohydrate digestion and absorption, making it valuable in managing diabetes and obesity[34].

iii. **Procedure**

**Preparation of Standard**

10 mg of acarbose was dissolved in 10 ml of methanol**,** and different aliquots (100–500 µg/ml) were prepared in methanol.

**Preparation of Sample**

10 mg of plant extracts were dissolved in 10 ml of methanol**.**

500 µl of this solution was used for enzyme inhibition estimation.

iv. **Method**

- 500 µl of test samples and standard drug (100–500 µg/ml) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5 mg/ml).
- The mixture was incubated at 25°C for 10 minutes.
- After incubation, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube.
- The reaction mixtures were further incubated at 25°C for 10 minutes.
- The reaction was stopped by adding 1.0 ml of 3,5-dinitrosalicylic acid (DNS) reagent.
- The test tubes were incubated in a boiling water bath for 5 minutes, then cooled to room temperature.
- The reaction mixture was diluted with 10 ml of distilled water, and absorbance was measured at 540 nm[34].

v. **Control**

A control test (100% enzyme activity) was performed in a similar manner, but the plant extract was replaced with a **vehicle (solvent)**.

**II. α-Glucosidase Inhibitory Activity**

The **α-glucosidase inhibition assay** was performed to evaluate the ability of plant extracts to inhibit **α-glucosidase**, an enzyme responsible for breaking down carbohydrates into simple sugars. By inhibiting this enzyme, glucose absorption in the intestines is reduced, thereby preventing rapid spikes in blood sugar levels after meals.

**a) Procedure for α-Glucosidase Inhibition Assay**

1. Preparation of Reaction Mixture
   - Each reaction mixture contained 0.01 mg/mL concentrations of plant extracts.
   - 5 µL of 0.1 M sodium phosphate buffer (pH 6.9) containing α-glucosidase solution (0.25 U) was added.
2. Pre-Incubation
   - The mixture was incubated at 25°C for 30 minutes (pre-incubation step).
3. Substrate Addition

- 25 µL of ρ-nitrophenyl-α-D-glucopyranoside (ρ-NPG) solution (5 mM) containing 0.1 M sodium phosphate buffer (pH 6.9) was added to each tube.

4. Absorbance Measurement
- The absorbance of the mixture was measured at 415 nm at 60-second intervals.
- The results were compared against a control reaction (without any extract).
- A positive control was also set up using Acarbose, a standard α-glucosidase inhibitor.

5. Calculation of α-Glucosidase Inhibition.

The percentage inhibition of α-glucosidase activity by each extract was calculated using the following formula:

**%Inhibition = [(A415 Control – A415 Extract)] x 100 A415 Control**

**b) α-glucosidase inhibition assay**

After being screened for α-amylase inhibition, the extracts were also examined for α-glucosidase inhibition. The monitoring of α-glucosidase inhibition using corresponding plant extract was conducte data 60-second interval and the results were compared with control and standard inhibitor a carbose responses[35].

## 3. RESULTS AND DISCUSSION

### 3.1 Pharmacognostic Studies

The leaves of Withania coagulans Dunal The leaves are simple, ovate, with entire margins. The leaves of the Picrorhiza kurroa were flat, oval and sharply serrated. The leaves of the Gymnema sylvestre were opposite, usually elliptic or ovate.

**Table 7: Physico-chemical Evaluation of Powdered Material:**

| S.No. | Withaniacoagulans | Picrorhizakurroa | Gymnemasylvestre |
|---|---|---|---|
| Moisture content(%) | **45** | **55** | **50** |
| Swelling index(ml) | **1.56** | **1.78** | **2.25** |
| Loss on Drying (%) | **11** | **14** | **16** |
| Total Ash Value(%) | **18** | **15** | **20** |
| Acid Insoluble Ash(%) | **15** | **14** | **17** |
| Water Soluble Ash Value(%) | **1.7** | **1.8** | **2.0** |

| | | | |
|---|---|---|---|
| Water soluble Extractive value (%) | **20.53** | **22.74** | **23.84** |
| Alcohol soluble Extractive value (%) | **10.46** | **12.86** | **15.48** |

### 3.2 Preparation and Extraction of Plant Materials

- **Extraction of Withania coagulans, Picrorhiza kurroa, Gymnema sylvestre Leaves**
  Shade-dried powdered material was extracted in petroleum ether and ethanol by using the maceration method for 48 hours, which was then filtered and dried using a vacuum evaporator at 40°C. The percentage yield of the plant material was then determined.
- **Percentage of Yield of Crude Extract by Maceration Process**
  In phytochemical extraction, the percentage yield is crucial to determine the standard efficiency of extraction for a specific plant, various sections of the same plant, or different solvents used. The yield of extracts received from Withania coagulans was 32.18% in ethanol, Picrorhiza kurroa was 25.72% in ethanol, and Gymnema sylvestre was 30.78%.

**Table 8: Showing %yield of the crude extract of Withaniacoagulans Extracts**

| S.No. | Solvent | Weight of the powdered material(gm) | Volume of solvent(ml) | Weight of the extract (gm) | %Yield* (W/W) |
|---|---|---|---|---|---|
| 1. | Pet.Ether | 150 | 500 | 4.025 | 2.68% |
| 2. | Ethanol | 150 | 500 | 48.276 | 32.18% |

**Table 9: Showing % yield of the crude extract of Picrorhizakurroa Extracts**

| S.No. | Solvent | Weight of the powdered material(gm) | Volume of solvent(ml) | Weight of the extract (gm) | %Yield* (W/W) |
|---|---|---|---|---|---|
| 1. | Pet.Ether | 150 | 500 | 2.479 | 1.65% |
| 2. | Ethanol | 150 | 500 | 38.582 | 25.72% |

**Table 10: Showing % yield of the crude extract of Gymnemasylvestr Eextracts**

| S.No. | Solvent | Weightofthe powdered material(gm) | Volume of solvent(ml) | Weightofthe extract (gm) | %Yield* (W/W) |
|---|---|---|---|---|---|
| 1. | Pet.Ether | 150 | 500 | 2.479 | 1.65% |
| 2. | Ethanol | 150 | 500 | 38.582 | 30.78% |

### 3.3 Preliminary Phytochemical Screening (Qualitative)

In the case of Withania coagulans, Picrorhiza kurroa, and Gymnema sylvestre extracts, as described by Kokate, a series of qualitative chemical tests were conducted. These tests aimed to explore and characterize the chemical composition of the extracts, providing valuable insights into their specific constituents and properties.

#### 3.3.1 Qualitative Phytochemical Screening of the leaf extracts of Withania coagulans

The qualitative phytochemical screening of the leaf extracts of Withania coagulans revealed the presence of carbohydrates, flavonoids, phenols, saponins, terpenoids, and tannins in the ethanolic extracts.

**Table 11: Phytochemical testing of Withaniacoagulans Extract**

| S.No. | Experiment | Presence or absence of phytochemical test | |
|---|---|---|---|
| | | Pet. Ether extract | Methanolic extract |
| **1. Alkaloids** | | | |
| 1.1. | Dragendroff's test | + | + |
| 1.2. | Mayer'sreagent test | + | + |
| 1.3. | Wagner'sreagent test | + | + |
| 1.4. | Hager'sreagent test | + | + |
| **2. Glycoside** | | | |
| 2.1 | Borntrager test | - | - |
| 2.2 | Killer-Killiani test | - | - |
| **3. Carbohydrates** | | | |
| 3.1 | Molish's test | - | + |
| 3.2 | Fehling's test | - | + |

| | | | |
|---|---|---|---|
| 3.3 | Benedict's test | - | + |
| 3.4 | Barfoed's test | - | + |
| **4. Flavonoids** | | | |
| 4.1. | Shinoda's Test | - | + |
| **5. Tanninand Phenolic Compounds** | | | |
| 5.1. | FerricChloride test | - | + |
| 5.2. | Gelatin test | - | + |
| **6. Saponin** | | | |
| 6.1. | Froth test | - | + |
| **7. Test for Triterpenoids and Steroids** | | | |
| 7.1. | Salkowski's test | + | + |

3.3.2 **Qualitative Phytochemical Screening of the leaf extracts of Picrorhiza kurroa**
Qualitative phytochemical screening of the leaves extracts of Picrorhiza kurroa revealed that glycosides,carbohydrates,flavonoids,phenols,saponins,terpenoidsandtanninswerepresentin ethanolic extracts.

**Table 12 : Phytochemical testing of Picrorhizakurroa Extracts**

| S.No. | Experiment | Presenceorabsenceofphytochemicaltest | |
|---|---|---|---|
| | | Pet.Etherextract | Ethanolicextract |
| **1. Alkaloids** | | | |
| 1.1 | Dragendroff's test | - | - |
| 1.2 | Mayer'sreagent test | - | - |
| 1.3 | Wagner'sreagent test | - | - |
| 1.4 | Hager'sreagent test | - | - |
| **2. Glycoside** | | | |
| 2.1 | Borntrager test | - | + |
| 2.2 | Killer-Killiani test | - | + |

| | | | |
|---|---|---|---|
| **3. Carbohydrates** | | | |
| 3.1 | Molish's test | - | + |
| 3.2 | Fehling's test | - | + |
| 3.3 | Benedict's test | - | + |
| 3.4 | Barfoed's test | - | + |
| **4. Flavonoids** | | | |
| 4.1. | Shinoda's Test | - | + |
| **5. TanninandPhenolicCompounds** | | | |
| 5.1. | FerricChloride test | - | + |
| 5.2. | Gelatin test | - | + |
| **6. Saponin** | | | |
| 6.1. | Froth test | - | + |
| **7. TestforTriterpenoidsandSteroids** | | | |
| 7.1. | Salkowski's test | + | + |

3.3.3 **Qualitative Phytochemical Screening of the leaf extracts of Gymnema sylvestre**
The qualitative phytochemical screening of the fruit extracts of Gymnema sylvestre revealed the presence of alkaloids, glycosides, carbohydrates, flavonoids, phenols, saponins, terpenoids, and tannins in the ethanolic extracts.

**Table 13 : Phytochemical testing of Gymnema sylvestre Extracts**

| **S.No.** | **Experiment** | **Presenceorabsenceofphytochemicaltest** | |
|---|---|---|---|
| | | **Pet.Etherextract** | **Ethanolicextract** |
| **7. Alkaloids** | | | |
| 1.1 | Dragendroff's test | + | + |
| 1.2 | Mayer'sreagent test | + | + |
| 1.3 | Wagner'sreagent test | + | + |
| 1.4 | Hager'sreagent test | + | + |

| | | | |
|---|---|---|---|
| **8. Glycoside** | | | |
| 2.1 | Borntrager test | - | + |
| 2.2 | Killer-Killiani test | - | + |
| **9. Carbohydrates** | | | |
| 3.1 | Molish's test | - | + |
| 3.2 | Fehling's test | - | + |
| 3.3 | Benedict's test | - | + |
| 3.4 | Barfoed's test | - | + |
| **10. Flavonoids** | | | |
| 4.1. | Shinoda's Test | - | + |
| **11. TanninandPhenolicCompounds** | | | |
| 5.1. | Ferric Chloride test | - | + |
| 5.2. | Gelatin test | - | + |
| **12. Saponin** | | | |
| 6.1. | Froth test | - | + |
| **8. Testfor Triterpenoids and Steroids** | | | |
| 7.1. | Salk owski's test | + | + |

### 3.4 Quantitative Analysis

The quantitative analysis conducted in this thesis reveals that the extracts exhibit a notable concentration of phenolics and flavonoids.

3.4.1 **Determination of Total Phenolic Content (TPC) & Total Flavonoid Content (TFC)**

The primary contributors to the antioxidant and medicinal properties of plants are phenolics, with flavonoids being the most prominent among them. In this study, the **Folin-Ciocalteu assay** was employed to determine the total phenolic content, while the **Aluminum chloride colorimetric assay** was used to estimate the total flavonoid content.

Phenolic compounds found in plant extracts play a crucial role in their overall structure. These compounds consist of aromatic rings with one or multiple hydroxyl groups, enabling them to effectively neutralize free radicals by forming stable phenoxyl radicals through resonance stabilization. The quantification of total phenolics was achieved through the electron transfer process from phenolic compounds to the Folin-Ciocalteu reagent under alkaline conditions.

This method has been identified as a straightforward and efficient approach due to its simplicity and rapidity (Evans, 2009; C.K. Kokate, 1994).
Flavonoids, on the other hand, are polyphenolic compounds that are widely abundant in nature and are categorized based on their chemical structure. Flavonoids are particularly renowned for their antioxidant activity (C.K. Kokate, 1994).

3.4.2 **Estimation of Total Phenolic Content (TPC)**

Total phenolic compounds (TPC) were expressed as **mg/100 mg of gallic acid equivalent (GAE)** of the dry extract sample using the equation obtained from the calibration curve: y=0.014x+0.004,R2=0.999y = 0.014x + 0.004, \quad R^2 = 0.999y=0.014x+0.004,R2=0.999 where **X** represents the gallic acid equivalent (GAE) and **Y** denotes the absorbance.

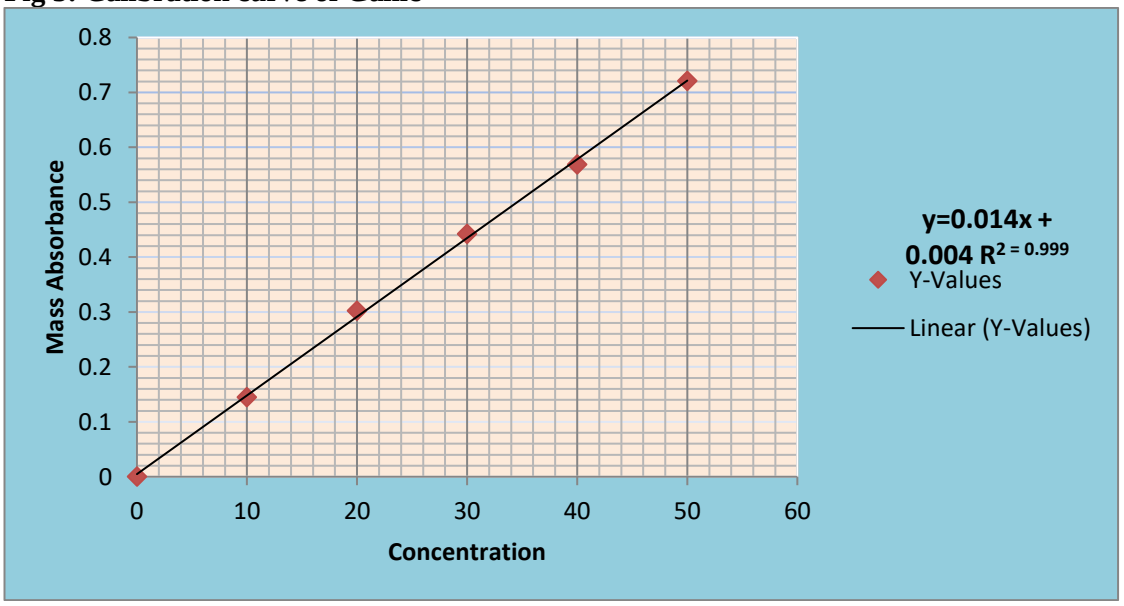
- **Calibration Curve of Gallic acid**

**Table 14 : Preparation of Calibration curve of Gallic acid**

| S. No. | Concentration (µg/ml) | Mean Absorbance |
|---|---|---|
| 1 | 10 | 0.145 |
| 2 | 20 | 0.302 |
| 3 | 30 | 0.442 |
| 4 | 40 | 0.569 |
| 5 | 50 | 0.721 |

(n=3,Mean±SD)

**Fig 3: Calibration curve of Gallic**



### 3.4.3 **Estimation of Total Flavonoid Content (TFC)**

Total flavonoid content (TFC) was calculated as **quercetin equivalent (mg/100 mg)** using the equation based on the calibration curve:
y=0.021x+0.008,R2=0.999y = 0.021x + 0.008, \quad R^2 = 0.999y=0.021x+0.008,R2=0.999
where **X** represents the quercetin equivalent (QE) and **Y** denotes the absorbance.

- **Calibration curve of Quercetin**

**Table 15: Preparation of calibration curve of Quercetin**

| S.No. | Concentration(µg/ml) | MeanAbsorbance |
|---|---|---|
| 1 | 5 | 0.123 |
| 2 | 10 | 0.225 |
| 3 | 15 | 0.337 |
| 4 | 20 | 0.44 |
| 5 | 25 | 0.541 |

(n=3,Mean±SD)

**Fig 4 : Graph of calibration curve of Quercetin**

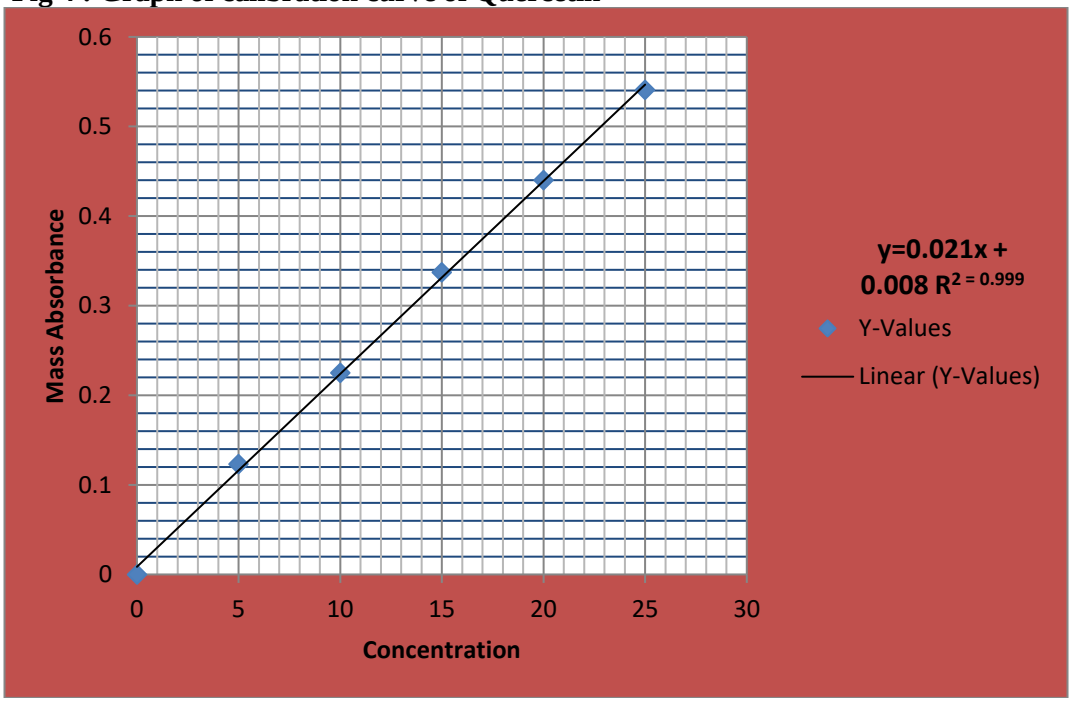


**Table 16 : Estimation of total phenol and flavonoids content of Withania coagulans extract**

| S.No. | Extract | Totalphenolcontent | Totalflavonoids Content |
|---|---|---|---|
| 1. | Hydroalcoholic | 0.935mg/100mg | 0.900mg/100mg |

**Table 17: Estimation of total phenol and flavonoids content of Picrorhiza kurroa extract**

| S.No. | Extract | Totalphenolcontent | Totalflavonoids Content |
|---|---|---|---|
| 1. | Hydroalcoholic | 0.745mg/100mg | 0.647mg/100mg |

**Table 18 : Estimation of total phenol and flavonoids content of Gymnemasylvestre extract**

| S.No. | Extract | Totalphenolcontent | Totalflavonoids Content |
|---|---|---|---|
| 1. | Hydroalcoholic | 0.815mg/100mg | 0.736mg/100mg |

### 3.5 In vitro anti-diabetic activity using inhibition of alpha amylase enzyme

**Table 19: Absorbance of acarbose and all the extracts**

| S. No. | Concentration (µg/ml) | Absorbance | | | |
|---|---|---|---|---|---|
| | | **Acarbose** | Withania**extract** | Picrorhizaextract | Gymnemaextract |
| | Control | 0.256 | | | |
| 1 | 25 | 0.149 | 0.215 | 0.286 | 0.258 |
| 2 | 50 | 0.127 | 0.185 | 0.195 | 0.185 |
| 3 | 75 | 0.119 | 0.165 | 0.154 | 0.168 |

| | | | | | |
|---|---|---|---|---|---|
| 4 | 100 | 0.098 | 0.147 | 0.185 | 0.153 |
| 5 | 125 | 0.065 | 0.132 | 0.148 | 0.158 |
| 6 | 150 | 0.045 | 0.112 | 0.126 | 0.138 |

**Table 20 : %Inhibition of acarbose and all the extracts**

| **S.No.** | **Concentration (µg/ml)** | **%Inhibition** | | | |
|---|---|---|---|---|---|
| | | **Acarbose** | Withania**extract** | Picrorhiza extract | Gymnema extract |
| 1 | 25 | 41.80 | 16.02 | 23.83 | 24.96 |
| 2 | 50 | 50.39 | 27.73 | 33.98 | 29.46 |
| 3 | 75 | 53.52 | 35.55 | 42.58 | 37.94 |
| 4 | 100 | 61.72 | 42.58 | 46.88 | 49.83 |
| 5 | 125 | 74.61 | 48.44 | 52.34 | 55.93 |
| 6 | 150 | 82.42 | 56.25 | 54.69 | 53.95 |
| | $IC_{50}$(µg/ml) | 54.50 | 127.53 | 118.81 | 117.08 |

### 3.6 Assay of α-glucosidase inhibition

The extracts that were screened for α-amylase inhibition were further evaluated for α-glucosidase inhibition. The inhibition of α-glucosidase by the respective plant extracts was monitored at a time interval of 60 seconds and compared against the control and the standard inhibitor acarbose.

**Table 21 : %Inhibition of α-glucosidase and all the extracts**

| **S.No.** | **Concentrati** | **%Inhibition** |
|---|---|---|

| | **on (µg/ml)** | **Acarbose** | Withania**extrac t** | Picrorhi za extract | Gymne ma extract |
|---|---|---|---|---|---|
| 1 | 25 | 41.80 | 25.65 | 30.84 | 32.85 |
| 2 | 50 | 50.39 | 38.47 | 42.64 | 45.65 |
| 3 | 75 | 53.52 | 48.75 | 55.85 | 57.85 |
| 4 | 100 | 61.72 | 55.84 | 67.84 | 63.56 |
| 5 | 125 | 74.61 | 60.74 | 73.56 | 70.85 |
| 6 | 150 | 82.42 | 71.85 | 81.38 | 82.85 |
| IC50(µg/ml) | | 54.50 | 86.86 | 66.24 | 63.90 |

## 4. DISCUSSION

The study focused on the extraction of Withania coagulans, Picrorhiza kurroa, and Gymnema sylvestre for an antidiabetic effect, including improved blood sugar control, improved insulin sensitivity, lower levels of harmful cholesterol, improved lipid profiles, and a reduced risk of developing diabetes-related complications. Additionally, some research suggests that these compounds may help to reduce inflammation, reduce oxidative stress, improve cognitive functioning, and protect against hepato-toxicity. The expected outcomes of using these herbs are that they may help to improve overall health, reduce obesity, increase the absorption of fats, vitamins, and minerals, and enhance digestive system function. They may also help to reduce inflammation, improve immune system function, and support cardiovascular health by aiding in hormone production and promoting healthy cholesterol levels. Furthermore, these herbs have been found to support liver health, act as blood purifiers, and assist in managing insomnia and exhaustion.

## 5. CONCLUSION

This study highlights the potential health benefits of Withania coagulans, Picrorhiza kurroa, and Gymnema sylvestre, particularly in managing diabetes and improving overall well-being. The extracts demonstrated promising antidiabetic properties, including improved blood sugar control, enhanced insulin sensitivity, and better lipid profiles, which may contribute to reducing the risk of diabetes-related complications. Additionally, the presence of bioactive compounds in these plants suggests their potential to reduce inflammation, oxidative stress, and hepatotoxicity, while also supporting cardiovascular health, immune function, and cognitive performance. Their ability to aid digestion, promote the absorption of essential nutrients, and manage obesity further reinforces their therapeutic value. Overall, these medicinal plants offer a natural and holistic approach to health management, with significant implications for diabetes treatment and general wellness. Further research and clinical studies

are recommended to validate these findings and explore their broader applications in modern medicine.

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