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Original Research Article

Exploring the Pharmacognosy and Anti-Diabetic Potential of Valeriana Wallichii Using Ultra Sonication Extraction Method

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Abstract: Valeriana wallichii has been traditionally used for various therapeutic applications, yet its anti-diabetic potential remains underexplored. This study investigates the pharmacognostic characteristics and anti-diabetic activity of V. wallichii extracts obtained through ultrasound-assisted extraction (UAE). The extraction process was optimized using UAE, and the phytochemical composition was analyzed. The anti-diabetic potential was evaluated through in vitro α-amylase and α-glucosidase inhibition assays. Additionally, in silico molecular docking and network pharmacology studies were conducted to elucidate the interactions of bioactive compounds with key diabetic targets and metabolic pathways. The optimized UAE method significantly enhanced the yield of total phenolic and flavonoid content. The extract exhibited potent α-amylase (IC_{50} = 60.36 µg/mL) and α-glucosidase inhibition (IC_{50} = 54.64 µg/mL), demonstrating activity comparable to standard acarbose. Network pharmacology analysis revealed strong interactions of key phytoconstituents, including Beta-Sitosterol, Linoleic acid, and Hesperidin, with crucial metabolic targets such as PPARG, INSR, AKT1, PIK3R1, MTOR, and IGF1R. These targets are central to insulin signaling, lipid metabolism, and inflammation. Key pathways, including PPAR signaling, AMPK signaling, insulin resistance, and adipocytokine signaling, exhibited high connectivity (>400 edges), indicating their crucial role in metabolic homeostasis. The study underscores the polypharmacological potential of V. wallichii, where multiple bioactive compounds act synergistically to regulate metabolic pathways, a characteristic feature of natural product based drug discovery. V. wallichii demonstrates strong anti-diabetic potential through enzyme inhibition and modulation of key metabolic pathways. The findings highlight its multi-targeted therapeutic approach, supporting its potential use in diabetes management. Further in vivo and clinical studies are warranted to validate these results.

Keywords: Anti-Diabetic, Anti-Oxidant Activity Valeriana Wallichii.

INTRODUCTION

Herbal plants have played a crucial role in human health and well-being for centuries, serving as a foundation for natural remedies across cultures [1]. The medicinal properties of these plants stem from their diverse array of bioactive compounds, which often provide effective alternatives to synthetic drugs [2]. These alternatives are increasingly appealing due to their potential to offer therapeutic benefits with fewer side effects. For instance, widely recognized herbs such as turmeric, ginger, and aloe Vera are celebrated not only for their flavor but also for their significant anti-inflammatory, antioxidant, and healing properties [3]. These qualities contribute to a holistic approach to health, promoting the idea that natural remedies can support overall well-being. Recent scientific research has further validated the efficacy of many herbal plants, highlighting their ongoing relevance in both preventative and therapeutic healthcare contexts. This resurgence of interest underscores a broader movement towards integrating traditional herbal knowledge with modern medical practices, fostering a more comprehensive understanding of health and healing [4].

Among the notable herbal plants is Valeriana wallichii, commonly known as Indian Valerian. This Himalayan herb has long been utilized in Ayurvedic medicine for its sedative, anxiolytic, and neuroprotective effects [5]. The roots of Valeriana wallichii are rich in valerenic acids, which are known to promote relaxation and support the nervous system, making the herb particularly valuable for treating conditions such as anxiety, insomnia, and stress [6]. Additionally, emerging research suggests that Valeriana wallichii may have therapeutic potential for managing epilepsy, depression, and other neurological disorders, reinforcing its significance in both traditional and modern medicinal contexts [7]. This dual applicability highlights the herb's importance not only in historical healing practices but also in contemporary medicine, where it can provide a natural adjunct to pharmacological treatments for mental health issues. The tradition of herbal medicine dates back thousands of years, with ancient civilizations in Egypt, China, India, and Greece utilizing plant-based remedies to address a wide array of health concerns [8].

These practices have been passed down through generations, relying on the medicinal properties of various plant parts, including roots, leaves, seeds, and flowers. Systems of traditional medicine, such as Ayurveda and Traditional Chinese Medicine (TCM), continue to emphasize the value of herbal remedies, promoting a holistic view of health that encompasses physical, mental, and spiritual well-being [9, 10]. In recent years, modern scientific exploration has begun to scrutinize these age-old remedies, recognizing their potential therapeutic benefits. This integration of traditional knowledge and contemporary research is vital, as it positions herbal medicine as an essential component of complementary medicine, offering patients holistic and natural treatment options [11]. One of the techniques facilitating the extraction of bioactive compounds from herbal plants is ultrasonication extraction [12]. This innovative method employs high frequency sound waves (ultrasound) to enhance the extraction process.

During ultrasonication, ultrasound waves generate cavitation bubbles within the solvent, which collapse to produce localized high temperatures and pressures [13]. This phenomenon disrupts the cell walls of plant materials, allowing for a more efficient release of target compounds into the solvent [14]. The advantages of ultrasonication include shorter extraction times and the ability to operate at lower temperatures, which helps to preserve heat-sensitive compounds. This method has gained popularity in the field of herbal medicine, as it maximizes the yield of bioactive substances while maintaining their integrity, ultimately leading to more potent extracts for therapeutic use [15-16]. Diabetes, a chronic condition affecting blood sugar regulation, represents a significant public health challenge worldwide [17].

The disease is categorized into two primary types: Type 1 and Type 2 diabetes. Type 1 diabetes arises when the immune system mistakenly attacks the insulin-producing cells in the pancreas, resulting in little or no insulin production [18]. This type typically develops during childhood or adolescence and necessitates lifelong insulin therapy for management. In contrast, Type 2 diabetes is more common and often develops in adulthood. It occurs when the body becomes resistant to insulin or fails to produce enough insulin to maintain normal blood sugar levels [19]. Lifestyle factors, including poor diet and physical inactivity, play a substantial role in the onset and progression of Type 2 diabetes. If not managed effectively, both types of diabetes can lead to serious complications, such as cardiovascular disease, kidney failure, and nerve damage, significantly impacting the quality of life and increasing mortality risk [20, 21]. Management of diabetes primarily involves blood glucose-lowering medications, lifestyle modifications, and regular monitoring of blood sugar levels [22]. While there is currently no cure for diabetes, early detection and proper treatment can prevent or delay the onset of complications, enhancing the quality of life for individuals with the condition [23]. Interventions aimed at lifestyle changes—such as improved diet, increased physical activity, and weight management—are crucial in the management strategy [24-25]. These approaches not only help control blood sugar levels but also contribute to overall health improvements, reducing the risk of comorbidities associated with diabetes.

1. Plant Profile Taxonomy and Classification [26]

- Kingdom: Plantae
- Phylum: Angiosperms
- Class: Dicotyledonae
- Order: Dipsacales
- Family: Caprifoliaceae (formerly Valerianaceae)
- Genus: Valeriana
- Species: Valeriana wallichii DC

2. Common Names

- English: Indian Valerian, Tagar
- Hindi: Sugandhbala, Tagar-Ganthoda
- Sanskrit: Tagara, Nat, Bhutajata
- Nepali: Sugandhawal
- Bengali: Tagar

- Punjabi: Sugandhabala

3. Botanical Description

Growth Habit: Valeriana wallichii is a perennial herb or small shrub growing up to 45–60 cm in height. It has aromatic rhizomes and erect stems with compound leaves [27].

Key Morphological Features

- Roots & Rhizomes: Thick, fibrous, aromatic, and dark brown with a characteristic strong odor.
- Stems: Erect, branched, and pubescent.
- Leaves: Opposite, compound or simple, ovate to lanceolate, hairy on both surfaces, with serrated margins.
- Flowers: Small, pale pink or white, in terminal cymose inflorescences; blooms in June-August.
- Fruits: Small achene, light brown, with feathery pappus aiding in seed dispersal.

4. Geographical Distribution

- Native to the Himalayan region, growing at altitudes 1200–3000 m.
- Found in India, Nepal, Bhutan, China, and Pakistan.
- In India, it is commonly found in Uttarakhand, Himachal Pradesh, Jammu & Kashmir, Arunachal Pradesh, and Sikkim.

5. Habitat and Ecology

- Prefers cool, moist, and shady habitats with rich organic soil.
- Grows in temperate forests, grasslands, and along stream banks.
- Tolerates partial to full sunlight but thrives in well-drained, loamy soil.

6. Phytochemical Constituents [28]

- Alkaloids: Actinidine, Valepotriates
- Sesquiterpenes: Valerenic acid, Hydroxyvalerenic acid
- Flavonoids: Luteolin, Kaempferol
- Essential Oils: Bornyl acetate, Camphene, Isovaleric acid
- Other Compounds: Terpenoids, Iridoids (Valtrate, Isovaltrate), β-sitosterol

MATERIALS AND METHODS

SECTION A – MACROSCOPICAL STUDIES

Macroscopical investigations cover the form and structure of the interior components, such as cells, as well as the external appearance (shape, structure, colour, and pattern). Drug identification characteristics include some of these gross morphological characteristics, including form, size, edge, apex, and venation. These characteristics provide significant information about the drugs.

Collection of Plant Material

Valeriana wallichii samples were procured from the local market in Kolhapur and authenticated by the Agharkar Research Institute, Pune, Maharashtra. The collected plant materials underwent a thorough preparation process, including washing to remove impurities, draining to eliminate excess moisture, and slicing for uniform drying. The sliced samples were then dried under controlled conditions to preserve their bioactive compounds. Once dried, the material was finely blended to obtain a uniform powder and subsequently sieved to ensure consistency. This prepared plant material was then utilized for further pharmacognostic and anti-diabetic studies, ensuring quality and reliability in experimental evaluations.

STANDARDIZATION PARAMETERS

The standardization of crude drugs involves assessing various parameters such as foreign organic matter, loss on drying, ash values, and extractive values. These evaluations provide insight into the specific characteristics of the crude drug, including its macro-morphological, cyto-morphological, and microscopical properties in both whole and powdered forms.

Foreign Organic Matter

Foreign organic matter refers to any plant organ or component not described as part of the crude drug. The presence of such materials can indicate contamination, adulteration, or improper processing.

Procedure

A sample of 500 g of Valerian wallichii was evenly spread in a thin layer and examined using a 6X magnification lens or the naked eye. Any visible foreign organic matter was manually separated and removed to ensure purity. After separation, the remaining drug material was weighed, and the percentage of foreign organic matter was calculated [29].

Ash Value Determination

Ash value analysis is essential for detecting low-quality crude drugs, exhausted plant material, and contamination with extraneous inorganic substances such as sand, dirt, calcium oxalate, or chalk powder, which may be added to enhance the appearance of the sample.

Determination of Total Ash

Total ash estimation helps assess the presence of inorganic components in crude drugs. It is a crucial parameter for identifying adulteration and ensuring purity.

Procedure: A silica crucible was used to weigh 2 to 3 g of air-dried crude drug powder. The sample was then subjected to controlled incineration at 450°C until all organic matter was completely burnt off, leaving only ash. The crucible was then cooled, and the residual ash was weighed [30].

This standardization process ensures the quality and authenticity of Valerian wallichii, preventing adulteration and maintaining pharmacognostic integrity for further studies.

$$\text{Total ash} = \frac{\text{weight of total ash}}{\text{weight of crud drug}} \times 100$$

Determination of Acid Insoluble Ash

Acid insoluble ash is the residue left over after boiling the whole ash with dil hydrochloric acid and heating the residual insoluble material. This measures the presence of silica, particularly in the form of sand and siliceous earth.

Procedure

The insoluble material remains after boiling total ash with 25ml of 2M Hydrochloric acid. The solution was then filtered using ashless filter paper and the residue was washed twice in hot water. Put the filter paper and residue in a crucible and maintain it in a muffle furnace set to 450℃ cooled and weighed the percentage of acid insoluble ash was calculated [31].

$$\% \text{ Acid insoluble ash} = \frac{\text{weight of acid insoluble ash}}{\text{weight of crud drug}} \times 100$$

Determination of Water-Soluble Ash

The ash was rinsed with 25ml of water and then placed in a 100ml beaker and boiled for 5 mints. The solution was then filtered using ashless filter paper and the residue was washed twice in hot water. Put the ashless filter paper and residue in a crucible and maintain it in a muffle furnace set to 450℃ cooled and weighed. Calculate the amount of water-soluble ash [32].

$$\% \text{ Water soluble ash} = \frac{\text{Weight of total ash} - \text{weight of water soluble ash} \times 100}{\text{weight of crud drug}}$$

Determination of Sulphated Ash

Sulphated ash is the result of treating the drug with sulfuric acid before burning, converting all oxides and carbonates into sulphates.

Procedure

Silica crucible was heated to redness for 10 min and allowed to cool in a desiccator and weighed. 1g of the substance being examined was placed in the dish, moistened with sulphuric acid, ignited gently, moistened again with sulphuric acid, and ignited at about 800°C. It was then cooled and weighed. The percentage of sulphated ash was calculated with reference to the air-dried drug [33].

EXTRACTIVE VALUES

When extracting plant materials with the proper solvents, the extractive values are a key component in determining the chemical constituents present in a crude drug. When a crude drug is extracted by using a specific solvent, a solution

comprises several phytocompounds. Depending on the type of drug and solvent employed, the composition of these phytoconstituents in that solvent varies. And table 1 shows the results of all extractive values. Determination of water-soluble extractive.

Procedure

A 250 ml dry conical flask should be filled with approximately 4g of drug that has been coarsely powdered. Pour 100ml of water into the flask, cork it, and shake it frequently for 24 hours. Filter the solvent quickly while taking steps to prevent water loss, then pour 25ml of the filtrate into a petri plate. Dry the product completely by evaporating it on a water bath. Water soluble extractive is calculated by following formula [34].

$$\% \text{ Water soluble extractive} = \frac{weight\ of\ extract}{weight\ of\ plant\ material} \times 100$$

Determination of Ethanol Soluble Extractive

5g of the air-dried drug, which is coarsely powdered must be macerated with 100ml of ethanol for 24hours in a flask, stirring frequently for the first 6 hours and leaving it stand for the remaining 18hr. thereafter, filter the filtrate quickly while taking steps to prevent loss of. Solvent, collect 25ml of filtrate in shallow dish with a flat bottom, evaporate the solvent and weighing it. Calculate the ethanol soluble extractive value by the following formula [35].

$$\% \text{ Ethanol soluble extractive} = \frac{weight\ of\ extract}{weight\ of\ plant\ material} \times 100$$

Petroleum Ether Soluble Extractive

Petroleum ether was used as a solvent, and the same process as with the ethanol soluble extractive was used.

Determination of Hexane Soluble Extractive

Hexane was used as a solvent, and the same process as with the ethanol soluble extractive was used.

Determination of Chloroform, Ethyl Acetate Soluble Extractive

In the same manner as with the ethanol-soluble extractive, chloroform and ethyl acetate were utilised as solvents.

Preparation of Extract

The powdered plant material was first cleaned and chopped into smaller pieces before being subjected to shade drying. The extraction process was optimized using a Quality by Design (QbD) approach, employing an ultrasonic bath apparatus (LABMAN, Chennai, India). Hydroalcoholic solvent was used for the extraction process, and various experimental parameters were investigated. The independent variables included a solid-to-solvent ratio of 1:20, 1:25, and 1:30 g/mL, extraction temperatures of 40, 50, and 60 °C, and extraction durations of 15, 30, and 45 minutes. After extraction, the resultant mixture was cooled to room temperature and stored for subsequent analyses [36].

PRELIMINARYPHYTOCHEMICAL SCREENING

Initial phytochemical screening helps to identify the types of secondary metabolites that are present in plants. There is discussion of the numerous chemical tests that were performed [37, 38].

- **Test for Carbohydrates:**
  a) Molisch's test: Few drops of alcoholicalpha naphthol solution, "few drops of concentrated sulphurics acid were added "given sample 'was taken .in a test tube. The test result gave a purple or violet-coloured.
  b) Benedict's test: Added Benedict's reagent to the given sample in a tests tube, heated the test tube in a boiling water bath. Red precipitation was formed.
  c) Fehling's Test: Filtrates were heated with Fehling's A & B solutions; formation of red precipitate indicates the presence of reducing sugars.

- **Detection of alkaloids**
- **Alkaloids Test**
  a) Mayer's Test "Mayer's reagent added to sample yellow ppt was formed.

b) Dragendroff's Test "Dragendroff's reagent added to sample orange ppt was formed.
c) Wagner's Test "Wagner's reagent added to sample reddish brown ppt was formed.
d) Hager's Test "Hager's reagent added to sample yellow ppt was formed.

- **Test for Steroids and Sterols**
  a) Libermann-Burchad test "Added 2ml chloroform, 2 drops of concentrated, sulphuric acid, 10 drops of acetic anhydride to the extract. The chloroform layer showed a Bluish red color.
  b) Salwoski test "Added 1-2 drops of concentrated sulphuric acid & chloroform to extract. The bluish red color was formed.

- **Glycosides Test**
  a) Legal test "Added sodium nitroprusside, pyridine to the extract, pinkish-red color was formed.
  b) Baljet test "Added picric acid to the given sample. The orange color showed a positive result.

- **Flavonoids Test**
  a) Shinoda test "1-2 drops of concentrated hydrochloric acid, magnesium turnings to extract. The red color showed a positive result.
  b) Ferric chloride test "Added ferric chloride to the sample greenish-black color was formed.

- **Test for Tannin**

Gelatin Test

Added 10% sodium chloride in 1% gelatin solution to the extract gave white precipitation. Detection of phenols Ferric chloride test: 1ml of sample 3-4 drops of ferric chloride added in clean test tube formation of blue colour indicates the presence of phenols.

QUANTITATIVE ESTIMATION OF PHYTOCONSTITUENTS

Tannins, flavonoids, phenols, alkaloids, and several otheraromatic compounds or secondary metabolites present in plants that serve as a defence. Mechanism against a variety of bacteria, insects, and herbivores. These secondary metabolites may contribute to the therapeutic capabilities of plants. Some common and other different ailments may be treated using medicinal herbs.

1. Estimation of Total Phenolic Content

Plants include phenolic compounds, well-known phytochemicals. They are composed of benzoic acid, cinnamic acid, coumarins, tannins and lignin's. Plant phenolic compounds are divided into simplephenols, and polyphenols based on the total amount of phenol units in the molecule. Total phenolics are detectedchemically, and phenolic components are recognised and quantified using spectrophotometric and chromatographic methods. An aromatic ring with one or more hydroxyl groups functions as the fundamental structural component of phenolic compounds. The biological features of these chemicals include antioxidant, anti-aging, anti-carcinogen, protection against autoimmune, immunological, and cardiovascular illnesses, as well as protection against brain disorders including Parkinson's, Alzheimer's, and Huntington's diseases [39-40]. Instrument: Shimadzu UV Visible spectrophotometer, Model 1800 Reagents a) Folin Ciocalteu reagent (1N) An equivalent volume of distilled water was used to dilute the readily accessible Folin Ciocalteu Reagent (2N). The finished solution has been placed in a brown bottle and maintained in a at 4°C refrigerator. b) Sodium carbonate solution (10%)

Procedure

Gallic acid was weighed and diluted in water to create a solution with a concentration of 1 mg/ml. This solution was further diluted to produce concentrations ranging from 2 to 10 µg/ml. Folin Ciocalteu reagent (0.5 ml) was added to the solutions and allowed to stand for 15 minutes. Then, 1 ml of 10% sodium carbonate solution was added and the mixtures were made up to 10 ml with distilled water. They were allowed to stand for 30 minutes at room temperature, and the total phenols were determined by spectrophotometrically measuring the absorbance at 760 nm with the reagent as the blank. The extract of Valerian wallichii was also weighed and diluted to get a solution of 1 mg/ml. Different concentrations of the solution were taken in separate test tubes and the same procedure was repeated. A calibration curve was generated by plotting the concentration of gallic acid versus absorbance, and a linear regression equation was determined using regression analysis. The total phenol content was calculated using the linear regression equation and expressed as mg of gallic acid equivalent per gm of extract (mg GAE/g).

2. Estimation of Total Flavonoid Content (TFC)

The total flavonoid content (TFC) is typically determined using the Aluminum Chloride ($AlCl_3$) Colorimetric Assay, which measures the flavonoids' ability to formstable complexes with aluminum chloride, resulting in a color change measurable at 415 nm [51, 52].

Materials Required:

- Plant extract/sample
- Aluminum chloride ($AlCl_3$, 10%)
- Sodium nitrite ($NaNO_2$, 5%)
- Sodium hydroxide (NaOH, 1M)
- Methanol (80%)
- Quercetin or Rutin (Standard flavonoid compound)
- UV-Visible Spectrophotometer
- Distilled water

Procedure:

1. **Preparation of Standard Solution:** A stock solution of quercetin/rutin (standard flavonoid) is prepared in methanol at a concentration of 1 mg/mL. o A calibration curve is generated by preparing standard dilutions (e.g., 10, 20, 40, 60, 80, and 100 µg/mL).
2. **Sample Preparation:** The plant extract is dissolved in 80% methanol to obtain a suitable concentration for analysis.
3. **Reaction Setup:** 0.5 mL of sample or standard solution is taken in a test tube. 2 mL of distilled water is added. 0.15 mL of 5% $NaNO_2$ is mixed and incubated for 5 minutes at room temperature. 0.15 mL of 10% $AlCl_3$ is then added, followed by another incubation for 5 minutes. 1 mL of 1M NaOH is added to the reaction mixture, and the total volume is adjusted to 5 mL with distilled water. The solution is mixed thoroughly and allowed to stand for 15 minutes.
4. **Absorbance Measurement**: The absorbance of the reaction mixture is measured at 415 nm using a UV-Visible spectrophotometer.
5. **Calculation of Total Flavonoid Content:** A standard calibration curve is used to determine the flavonoid content in the plant extract.

TFC is expressed as mg quercetin equivalent (QE) per gram of dry weight (mg QE/g DW) using the formula:
$TFC=C\times V/M$

Where:

- C = Concentration from the standard curve (mg/mL)
- V = Volume of extract (mL)
- M = Mass of plant extract used (g)

***IN VITRO* ANTIOXIDANT ACTIVITY**

Since antioxidants neutralize or eliminate "reactiveoxygen species" (ROS) or free radicals before theycause cell harm, they have becomean essentialcomponent of our life today. The oxidation caused by ROS leads to DNA mutations, membrane protein damage, and cell membrane disintegration, which accelerates the ageing process and the progression of a number of diseases, including arteriosclerosis, cancer, diabetes mellitus,liver damage, inflammation, skin damage,coronary heart disease, and arthritis.Antioxidants are chemical substances that slow down the lipid oxidation process in food systems [41]. Antioxidants are defined as compounds that fight oxidation or suppress processes induced by oxygen or peroxides; many of these substances are preservatives used in a variety of goods. Antioxidants are compounds that are added to products for the purpose of preventing degradation caused by the action of oxygen in the air [42]. They can be synthetic or natural. Examples include enzymes or other chemical compounds like vitamin E or -carotene. A chemical entity known as a free radical possesses one or more unpaired electrons and is capable of independent life. Free radicals can react with molecules without regard to their structure since they are less stable than non-radicals [43]. Once generated, radicals can either react with other radicals or with molecules that are not radicals through a variety of interactions. Covalent bonds are created when two radicals meet and share one electron. Non-radical compounds are the most prevalent found in in vivo [44]. A radical change from being a radical to being a non-radical by giving or taking an unpaired electron from other molecules. A new radical form at the same moment [45].

Method 1. Free Radicalscavenging Activity Using Diphenylpicrylhydrazyl (DPPH) Free Radical

According to Brand Williams, the capacity of the extracts decreases the colour of DPPH. Serves as a measure of their free radical scavenging capability. Principle The DPPH stable free radical technique is a simple, quick and9accurate way to9evaluate a compound's or plant extract's level of antioxidant activity. The stableyd2,2-diphenyl-1-picrylhydrazyl (DPPH)4radical is used in a straightforward approach that has9been devised to assess the antioxidant activity99of plants [46]. The odd electron of the DPPH free radical causes an important absorption maximum with a purple colour at 517 nm. Odd electron of DPPH-H reacts with hydrogen atom of sample purple colour of DPPH turns into yellow. Regarding the quantity of electrons caught, the ensuing decolorization is stoichiometric [47]. DPPH + AH= DPPH -H + A.

Procedure:

Valerian wallichii sample stock solution with a 10 mg/ml concentration was prepared. 4ml of DPPH was added to 1ml of test samples at varying doses. Control was made without the sample. In the case of a blank, ethanol was used instead of DPPH. Incubate the sample for 30 mints. Readings were then determined at 517 nm. Using9the formula, the percentage of scavenging was determined. Ascorbic acid used as reference.

% Inhibition = [(Control-Test)/Control] x 100. Using linear regression analysis, the sample concentration needed to achieve a reduction of 50% in absorbance (IC50) was determined. The results obtained are presented in table 5 and fig 4. Method 2: Nitric Oxide (NO) Scavenging Assay The nitric oxide (NO) scavenging activity of the test samples was evaluated using the sodium nitroprusside (SNP) assay method, following the standard protocol with slight modifications.

Chemicals and Reagents

- Sodium nitroprusside (SNP)
- Griess reagent (Sulfanilamide, Phosphoric acid, Naphthyl ethylenediamine dihydrochloride)
- Phosphate-buffered saline (PBS, pH 7.4)
- Ascorbic acid or gallic acid (Standard)
- Dimethyl sulfoxide (DMSO) (if required for sample solubilization)

Preparation of Solutions

1. Sodium Nitroprusside Solution: A 10 mM solution of SNP was prepared in phosphate-buffered saline (PBS, pH 7.4).
2. Griess Reagent: A freshly prepared mixture of equal volumes of 1% sulfanilamide in 5% phosphoric acid and 0.1% Naphthyl ethylenediamine dihydrochloride (NED) was used
3. Standard Solution: Ascorbic acid or gallic acid was prepared in different concentrations (e.g., 10–100 µg/mL) as a reference antioxidant.
4. Test Sample Preparation: The plant extract or phytosome formulation was prepared in PBS or DMSO to obtain different concentrations (10–100 µg/mL).

Assay Procedure

1. Reaction Mixture Preparation: o A total of 1 mL of different concentrations of the test sample (or standard) was mixed with 1 mL of 10 mM SNP solution in PBS. o The mixture was incubated at 25°C for 150 minutes under light to allow the production of nitric oxide.
2. Nitric Oxide Detection: After incubation, 0.5 mL of the reaction mixture was mixed with 0.5 mL of freshly prepared Griess reagent. The mixture was incubated in the dark for 10 minutes at room temperature.
3. Absorbance Measurement: o The absorbance of the pink-coloured complex was measured at 546 nm using a UV-Visible spectrophotometer.

A control (without test sample) and blank (without SNP) were also prepared. Calculation of % Inhibition the percentage inhibition of nitric oxide production was calculated using the following equation:
% Inhibition= [(Control-Test)/Control] x 100.

Where:

- Ac = Absorbance of the control (without extract)
- As = Absorbance of the sample or standard

In vitro antidiabetic activity α-amylase Inhibition Assay The α-amylase inhibition assay was conducted using acarbose as a positive control. To each test tube, 1 mL of α-amylase solution and 1 mL of extract (20 100 µg/mL) were added and incubated at 37 °C for 10 minutes. Subsequently, 1 mL of 1% (v/v) starch solution was added, and the mixture was incubated at 37 °C for an additional 15 minutes. The reaction was terminated by adding 2 mL of dinitro salicylic acid (DNSA) reagent.

RESULTS & DISCUSSION

SECTION A – MACROSCOPICAL EVALUATION

Macroscopical Features of Valerian wallichii. Morphology of Valeriana wallichii Valeriana wallichii, commonly known as Indian Valerian or Tagar, is a perennial herbaceous plant belonging to the Valerianaceae family. It is widely found in temperate and alpine regions of the Himalayas.

Morphological Characteristics

- Roots and Rhizomes Thick, creeping, and aromatic rhizomes with fibrous secondary roots.
- Brownish to yellow in color, with a strong, pungent odor due to the presence of volatile oils.

1. Stem

- Erect, hollow, and slightly hairy, reaching a height of 30–100 cm.
- Unbranched or sparsely branched, with nodes and internodes.

2. Leaves

- Opposite, pinnately compound, or deeply lobed.
- Dark green, lanceolate, with serrated or entire margins.
- Basal leaves have petioles, while upper leaves are sessile.

3. Flowers

- Small, pinkish-white to pale purple, arranged in terminal corymbose inflorescences.
- Bisexual, funnel-shaped corolla, with five lobes.
- Stamens are epipetalous and exerted.

4. Fruits and Seeds

- The fruit is an achene with feathery pappus-like structures that aid in wind dispersal.

Figure 1: Valerian wallichii Standardization Parameters

Table 1 shows that the amount of foreign organic materials in the crude material was extremely low. The amount of total ash was determined to be 12± 0.63percent, the amount of water-soluble ash to be 5.05± 0.25percent, the amount of acid-insoluble ash to be 4.05± 0.33percent, and the amount of sulfated ash to be 2.1± 0.47percent. By evaluating the ash levels, it is easy to determine where the powdered material was contaminated with sand and other inorganic material. The quantity of inorganic material contained in the crude medication may be determined using the water-soluble ash, whereas the amount of sand and other debris can be determined using the acid insoluble ash. The varying extraction values using various solvents have been identified. Alcohol has the highest extractive value (6.44± 0.42), followed by water (3.24± 0.53), Hexane, ethyl acetate, pet ether and chloroform. The extractive values play a role in determining the best solvent to utilise for extracting the most active principle.

Table 1: Standardization parameters of *Valerian wallichi*

Parameters	Values (%)
Total ash	7.0± 0.50
Acid Insoluble ash	1.5± 0.26
Water soluble ash	3.5± 0.30
Sulphated ash	2.5± 0.47
Alcohol soluble extractive	8.0± 0.35

Parameters	Values (%)
Water soluble extractive	5.05± 0.59
Pet ether extractive	3.35± 0.95
Hexane extractive	4.31± 0.12
Ethyl acetate extractive	3.5± 0.32
chloroform soluble extractive	2.35± 0.83

Values are mean ± SD (n = 3)

Phytochemical Screening

Phytochemical screening is a qualitative analysis used to detect the presence of bioactive compounds in plant extracts. These compounds, known as secondary metabolites, contribute to the plant's medicinal properties and biological activities. The Hydroalcoholic extract of *Valeriana wallichii* (V.W.) was subjected to different chemical tests to identify the presence of Steroids, Alkaloids, Flavonoids, Tannins, Triterpenoids, and Carbohydrates and the results are mentioned in Table 2.

Phytochemical investigations of *V.wallichii* have revealed the presence of sesquiterpenoids, alkaloids, volatile oils, flavonoids, monoterpenes and Sesquiterpenes and commonly selected phytochemicals which essential for anticancer activity like Dihydrovaltrate, 6-methylapigenin, hesperidin, Beta-D-Glucose, camphene, terpineol, valerianine, Linarin, iridoids, Valerenic acid, acetic acid, arachidonic acid, beta-sitosterol, butyric acid, formic acid, linoleic acid, oleic acid and phytochemistry of common phytocompounds were described (figure 2).

Table 2: Phytochemistry of *V. wallichii* phytocompound

Metabolites	V W extract	Metabolites	V W extract
Carbohydrates	+	Flavonoids	+
Gums	-	Alkaloids	+
Mucilage	-	Phenols	-
Amino acids	+	Tannins	+
Steroids	+	Sesquiterpenoids	+
Glycosides	+	Resins	-
Saponins	+	Volatile oils	+

(+): the presence; (-) the absence

Phytochemistry

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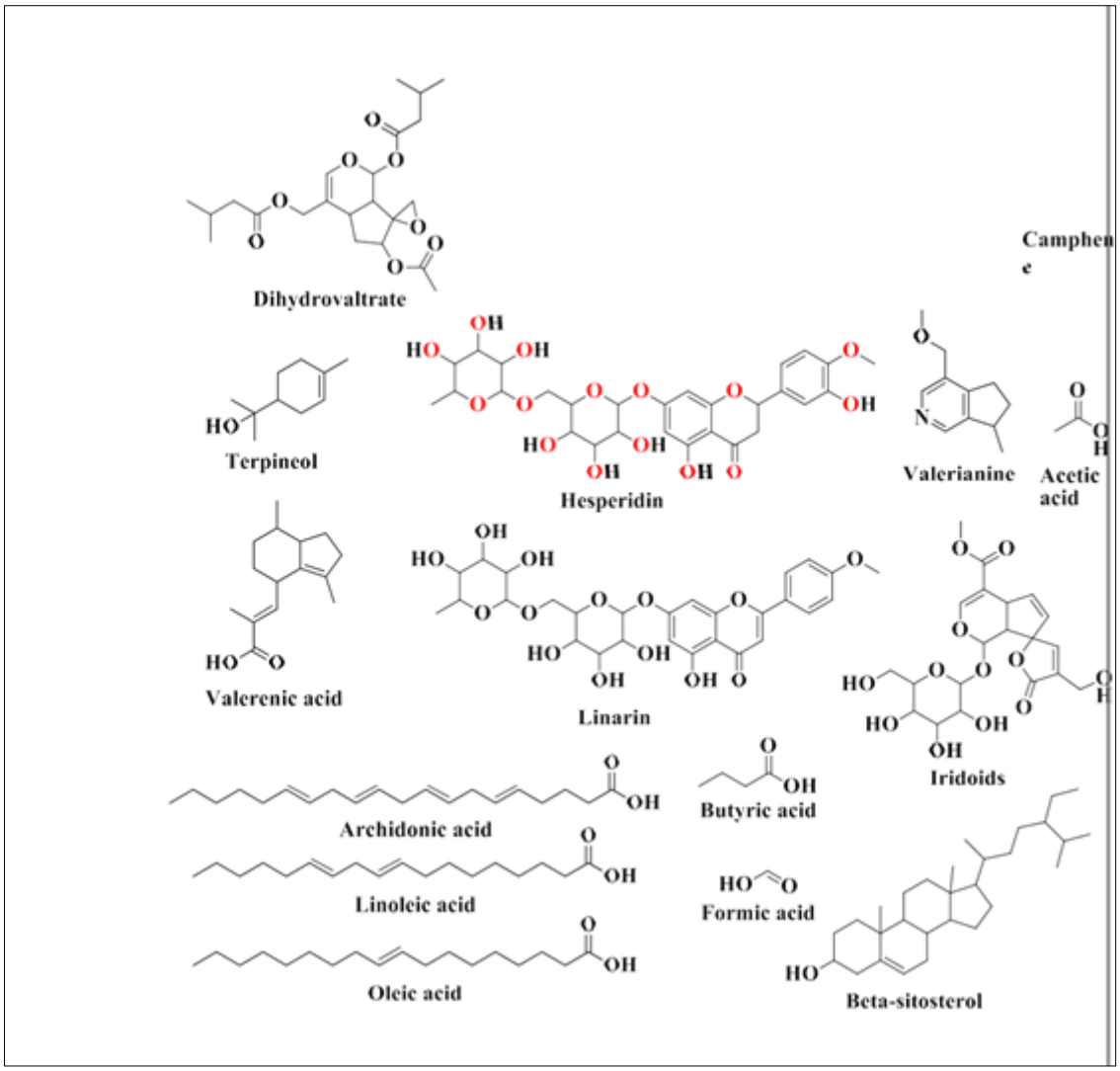

Figure 2: Phytochemistry of *V. wallichii* phytocompound

SECTION A -*IN VITRO* ANTIOXIDANT ACTIVITY

The *in-vitro* antioxidant activity of Valerian wallichii was studies by different methods. The results obtained by different methods are presented in table 3.

Method I: The outcome of free radical scavenging activity against DPPH radical is presented in table 3.

Table 3: Percentage inhibition of Valerian wallichii extract and std ascorbic acid against DPPH at 517nm

Sl.no	Conc (µg/ml)	Ascorbic acid	Hydroalcoholic extract
1	20	20.266	10.030
2	40	37.529	24.155
3	60	55.817	37.393
4	80	68.474	50.597
5	100	88.161	68.065
IC50		13.937	38.386

The DPPH free radical scavenging activity of a hydroalcoholic extract in comparison to ascorbic acid, a standard antioxidant, at different concentrations (20–100 µg/ml). The percentage inhibition of DPPH increases with concentration for both samples, indicating a dose-dependent antioxidant effect.

Method 2: Nitric Oxide Method

Mechanism of Nitric Oxide Scavenging Activity

Nitric oxide (NO) is a free radical generated in biological systems, primarily by nitric oxide synthases (NOS). It plays a crucial role in physiological functions but, in excess, contributes to oxidative stress, inflammation, and tissue

damage by reacting with superoxide anions to form peroxynitrite ($ONOO^-$), a highly reactive oxidant. In the nitric oxide scavenging assay, sodium nitroprusside (SNP) is used as a NO donor, which, in an aqueous medium at physiological pH, spontaneously releases NO.

The results obtained for the Nitric oxide method of *Valerian wallichii* extract and std ascorbic acid are presented in table 4.

Table 4: Results of different extract of *Valerian wallichii* and std ascorbic acid in Nitric oxide method

S.no	Conc(µg/ml)	Ascorbic acid	Hydro Alcohol
1	20	31.37019	20.3726
2	40	39.1226	31.43029
3	60	51.80288	42.1875
4	80	61.17788	55.40865
5	100	69.71154	63.76202
IC50		29.35386	73.30264

IN VITRO ANTIDIABETIC ACTIVITY

1. Alpha-Amylase Inhibitory Activity

The alpha-amylase inhibitory activity of the plant extract was evaluated using both the standard method and the ultrasound-assisted extraction (UAE) method, with the results indicating a concentration-dependent inhibition of the enzyme. The standard method exhibited a slightly higher inhibitory effect at all tested concentrations compared to the UAE method. At 100 µg/mL, the inhibition was 86.83% for the standard method and 82.51% for the UAE method, reflecting a minor reduction in activity with ultrasound extraction.

Table 5: Percentage inhibition alpha-amylase inhibitory activity

Conc.	Standard	Ultra sonication method
20	21.4800577	16.6746756
40	38.1547333	33.1090822
60	56.0307544	45.8433445
80	69.1975012	65.497357
100	86.8332532	82.5084094
IC 50 value	54.6376901	60.3558454

Alpha-Glucosidase Inhibitory Activity

The alpha-glucosidase inhibitory activity of the plant extract was assessed using both the standard method and ultrasound-assisted extraction (UAE) method, showing a concentration-dependent inhibition of the enzyme. The standard method exhibited a stronger inhibitory effect, with an IC_{50} of 39.00 µg/mL, compared to 50.21 µg/mL for the UAE method. This suggests that the standard method yields a more potent extract for alpha-glucosidase inhibition.

Network Pharmacology

Mining of Phytoconstituents and Targets

The components of Valerian wallichii were obtained from Dr Duke, IMPPAT and Published articles, a total of 17 bioactive components were obtained. The chemical composition of Valerian wallichii was confirmed using the PubChem. The detailed information given in table 6. The targets of identified compounds were screen out by using digep pred software.

Table 6: Main active ingredients in *valerian wallichii*

S.NO	Phytocompounds	PubChem CID	MOL WT	MOL FORMULA
1	Dihydrovaltrate	65689	424.5	C22H32O8
2	6-methylapigenin,	9965615	284.26	C16H12O5
3	Hesperidin	10621	610.6	C28H34O15
4	Beta-D-Glucose	64689	180.16	C6H12O6
5	Camphene	6616	136.23	C10H16
6	Terpineol	17100	154.25	$C10H_{18}O$
7	Valerianine	442555	177.24	$C_{11}H_{15}NO$
8	Lunarian	5317025	592.5	C28H32O14
9	Iridoids	453214	456.4	C20H24O12
10	valerenic acids	6440940	234.33	C15H22O2
11	acetic acid	176	60.05	C_2H4O_2

S.NO	Phytocompounds	PubChem CID	MOL WT	MOL FORMULA
12	arachidonic acid	444899	304.5	C20H32O2
13	beta-sitosterol	222284	414.7	C29H50O
14	butyric acid	264	88.11	$C_4H_8O_2$
15	formic acid	284	46.025	CH_2O_2
16	linoleic acid	5280450	280.4	C18H32O2
17	oleic acid	445639	282.5	C18H34O2

Identification of Diabetes Mellitus Targets

A total of 10253 genes were obtained from the Gene Card databases using "type 2 diabetes mellitus," as keyword; These were compared with the target genes of the active ingredients to obtain potential diabetes mellitus target genes for each active ingredient with the help of venny 2.0. After comprehensive acquisition of the potential diabetes mellitus target genes of *Valerian wallichii*, a total 66 potential targets related to the treatment of diabetes mellitus were selected as candidate targets.

Protein-Protein Interaction and Cluster Analysis

Protein-Protein Interaction Network of *Valerian Wallichii* - Diabetes Mellitus Regulated Genes

Protein-Protein Interaction (PPI) networks are essential tools for understanding how bioactive compounds from *Valeriana wallichii* influence key proteins involved in diabetes mellitus. These networks help decipher the molecular mechanisms underlying the plant's anti-diabetic effects, identify potential drug targets, and reveal critical pathways associated with glucose metabolism, insulin sensitivity, and pancreatic function. A significant observation in the PPI network is the presence of key hub proteins, which act as major regulators in diabetes management. AKT1, a central protein in the insulin signaling pathway, plays a pivotal role in glucose metabolism and cell survival by promoting glucose uptake and glycogen synthesis.

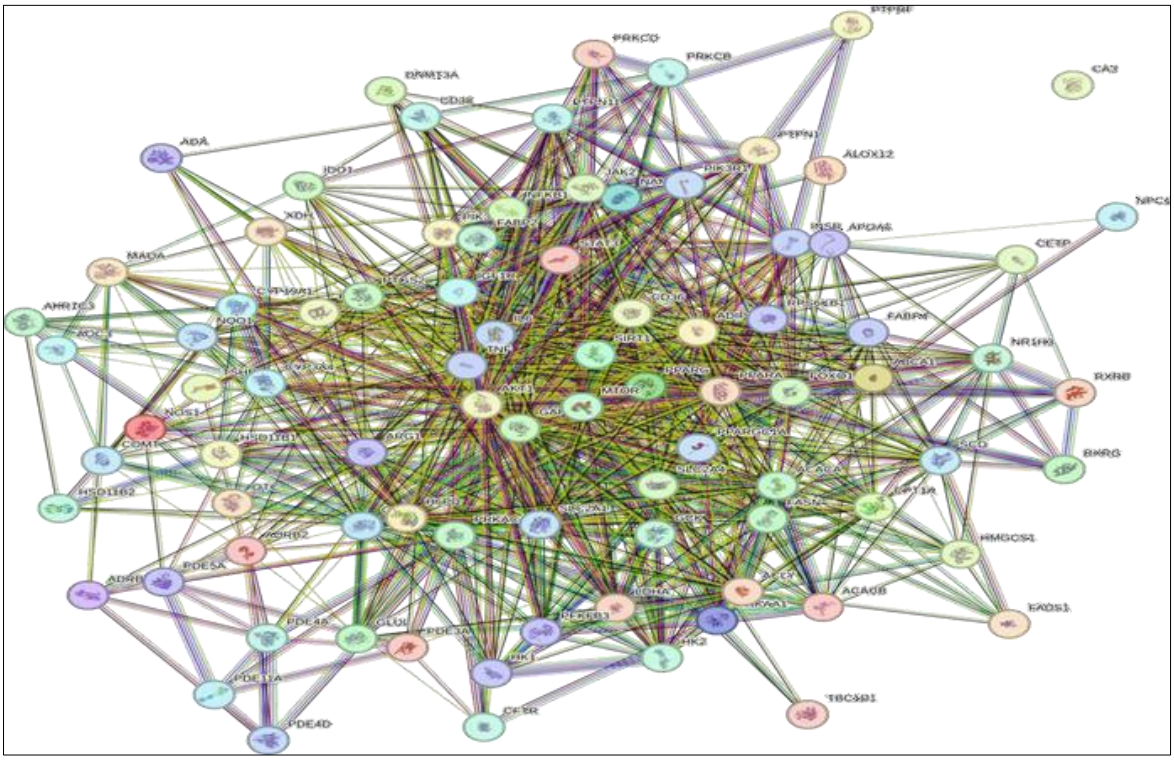

Figure 3: The Protein protein interaction of common targets between phytoconstituents and diabetes mellitus. (A)Valerian wallichii - diabetes mellitus PPI network

GO and KEGG pathway enrichment analyses of cluster 1 for diabetes mellitus Gene Ontology (GO) analysis (Biological process)

GO and KEGG Pathway Enrichment Analyses of Cluster 1 for Diabetes Mellitus Gene Ontology (GO) analysis (Biological process)

The biological process Gene Ontology (GO) analysis of *Valeriana wallichii* in diabetes mellitus treatment highlights key pathways influenced by its bioactive compounds.

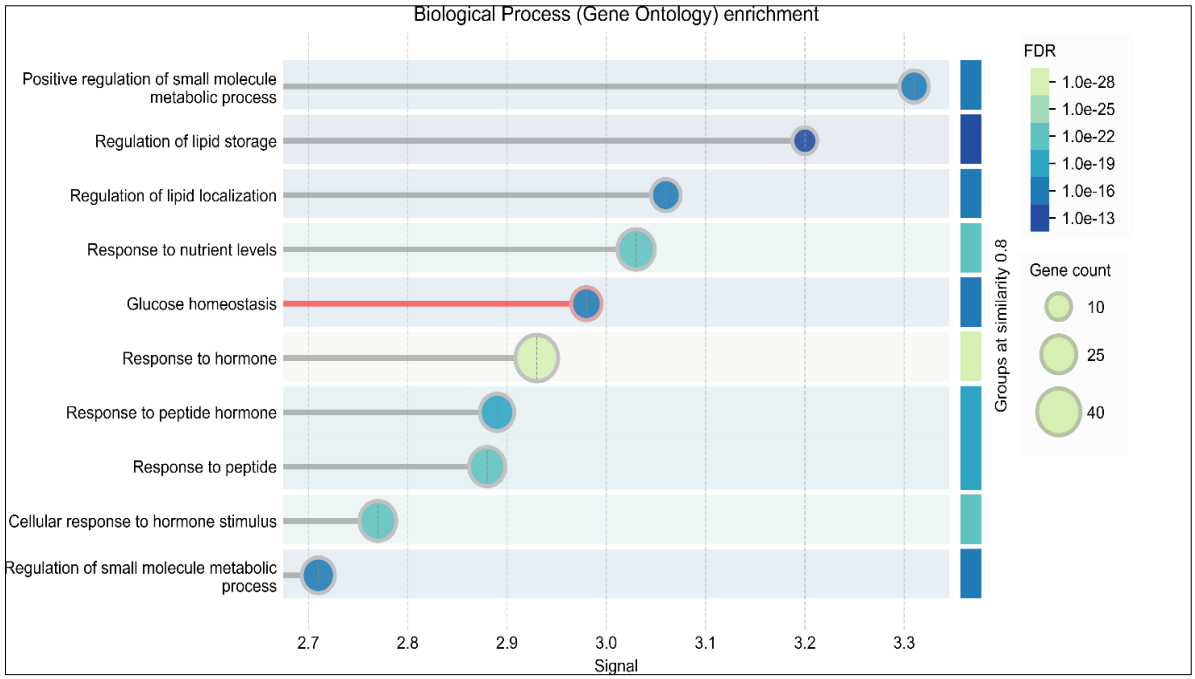

Figure 4: Gene Ontology (GO) analysis (Biological process)

Gene Ontology (GO) Analysis (Molecular Function)

The molecular function Gene Ontology (GO) analysis of *Valeriana wallichii* in the treatment of diabetes mellitus highlights key functional roles of its bioactive compounds in modulating diabetes-related molecular mechanisms. The enrichment analysis suggests significant involvement in enzyme binding, receptor interaction, and signal transduction. Several genes are associated with ATP and kinase activity, particularly those involved in insulin receptor binding and phosphorylation events critical for glucose homeostasis.

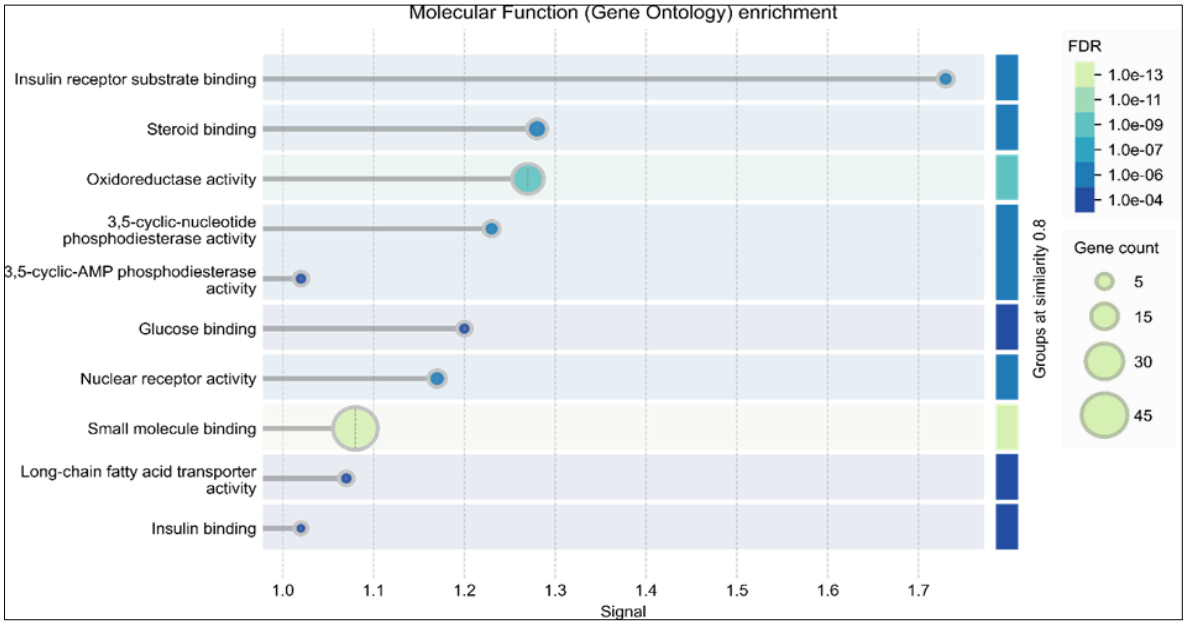

Figure 5: Gene Ontology (GO) analysis (molecular function)

Gene Ontology (GO) Analysis (Cellular Component)

The cellular component Gene Ontology (GO) analysis of *Valeriana wallichii* in the treatment of diabetes mellitus identifies key subcellular locations where its bioactive compounds exert their effects. The enrichment analysis highlights the involvement of membrane-associated components such as the plasma membrane, which plays a crucial role in insulin receptor signaling and glucose transporter activity. The cytoplasm is another significant component, where key metabolic enzymes and signaling proteins regulate glucose metabolism, lipid homeostasis, and oxidative stress responses. Exerts its

antidiabetic effects by targeting critical cellular structures involved in insulin sensitivity, energy regulation, and metabolic homeostasis.

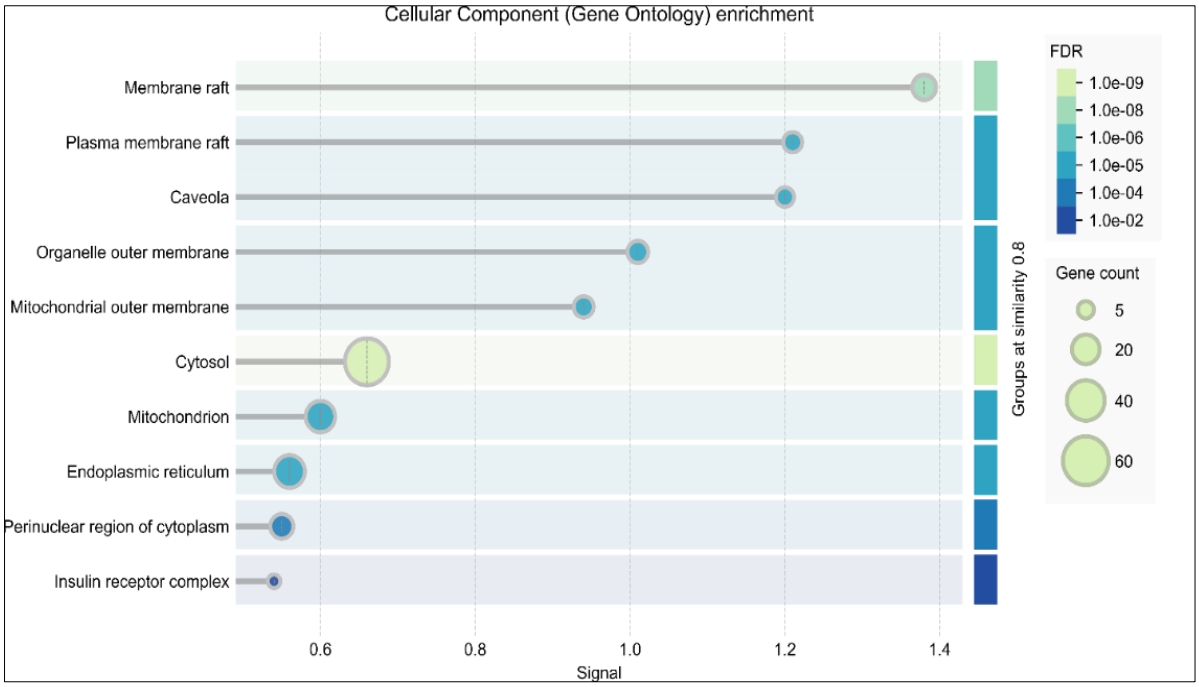

Figure 6: Gene Ontology (GO) analysis (cellular component) KEGG pathway enrichment analyses

The KEGG pathway analysis of *Valeriana wallichii* in diabetes mellitus treatment highlights its regulatory influence on multiple metabolic and signaling pathways that play a crucial role in glucose homeostasis, insulin signaling, lipid metabolism, and energy balance. Through its modulation of key metabolic pathways involving genes such as *SIRT1*, *GCK*, *PIK3CA*, *HK2*, *FASN*, and *GLUL*, *Valeriana wallichii* enhances glucose metabolism and lipid synthesis, which are fundamental processes in managing diabetes.

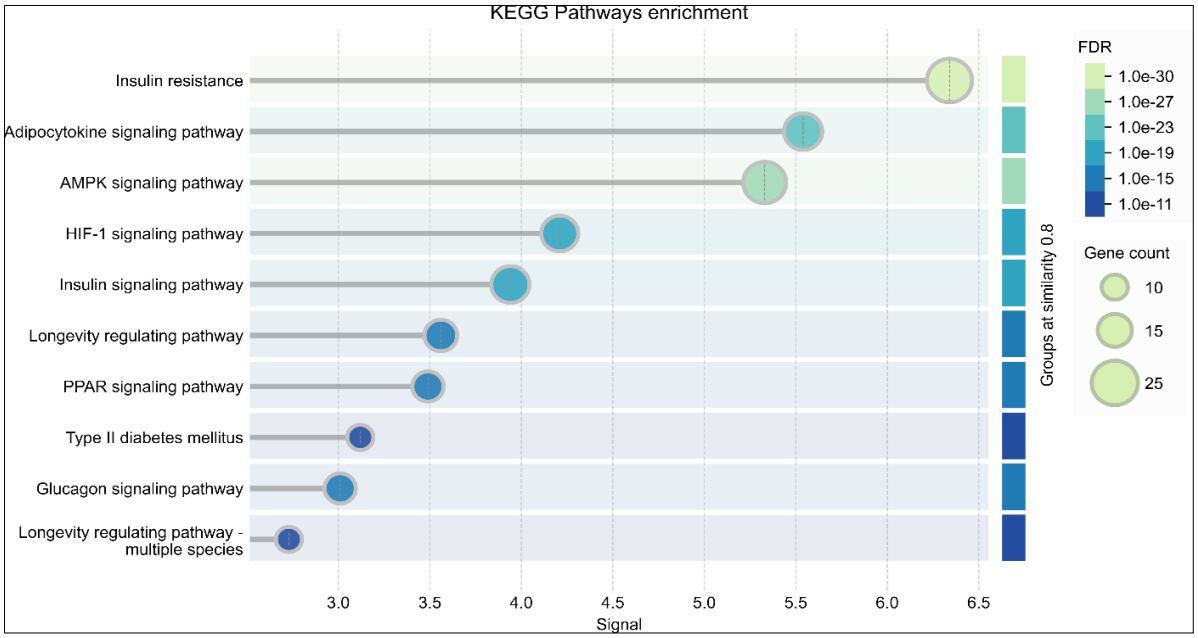

Figure 7: KEGG pathway enrichment analyses Network construction and analysis

Network pharmacology visualization highlights the interactions between bioactive compounds, target genes/proteins, and metabolic pathways involved in metabolic disorders such as obesity and diabetes. The network consists of three major node types: bioactive compounds (diamond-shaped nodes on the left), target genes/proteins (circular nodes on the right), and metabolic pathways (triangular or hexagonal nodes in the center/bottom). The edges (lines) represent the

relationships between these elements, illustrating how bioactive compounds influence target genes and how those targets are involved in key metabolic pathways. Some of the most connected compounds, such as Beta-Sitosterol, Linoleic acid, and Hesperidin, exhibit extensive interactions with multiple targets, with edge counts exceeding 400, indicating their broad pharmacological action. Highly connected target genes include PPARG, INSR, AKT1, PIK3R1, MTOR, and IGF1R, which are crucial in metabolic regulation, insulin signaling, lipid metabolism, and inflammation.

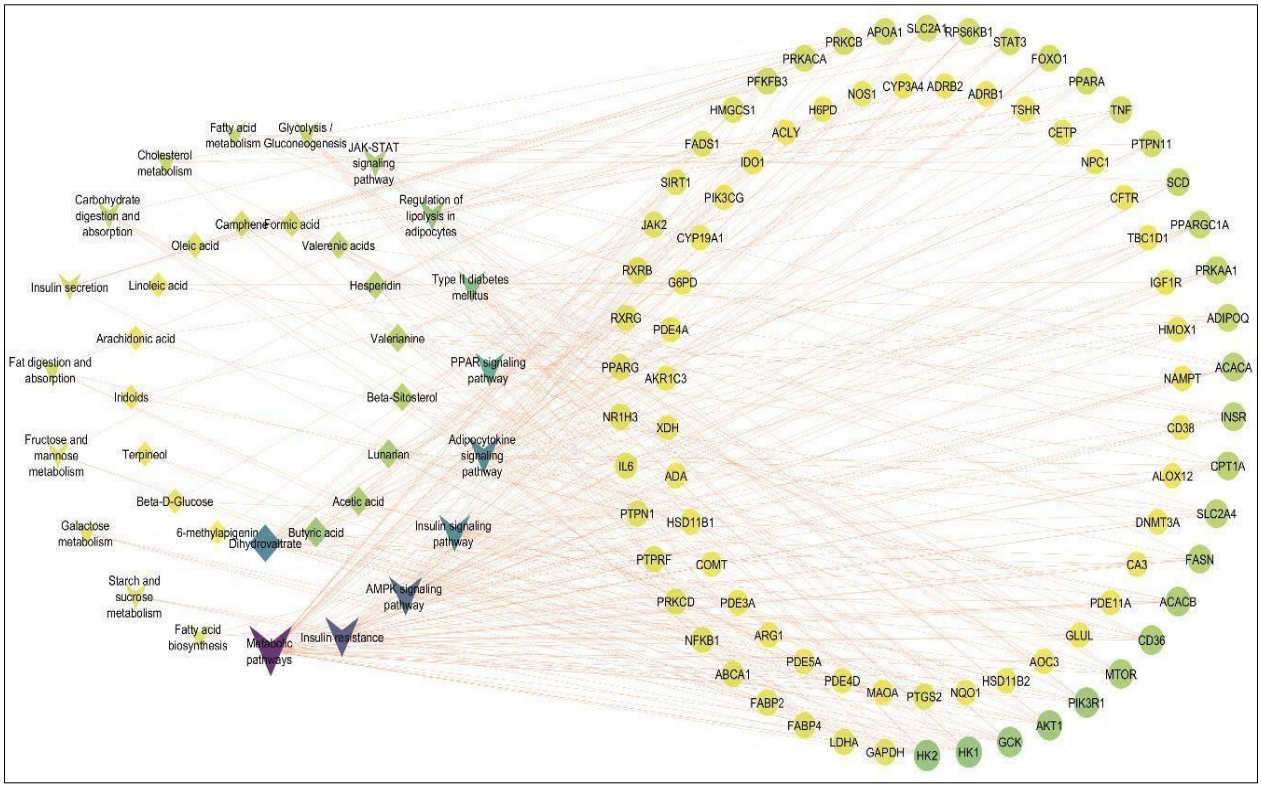

Figure 8: Network construction between Phytoconstituents, Targets and Pathways in diabetes mellitus

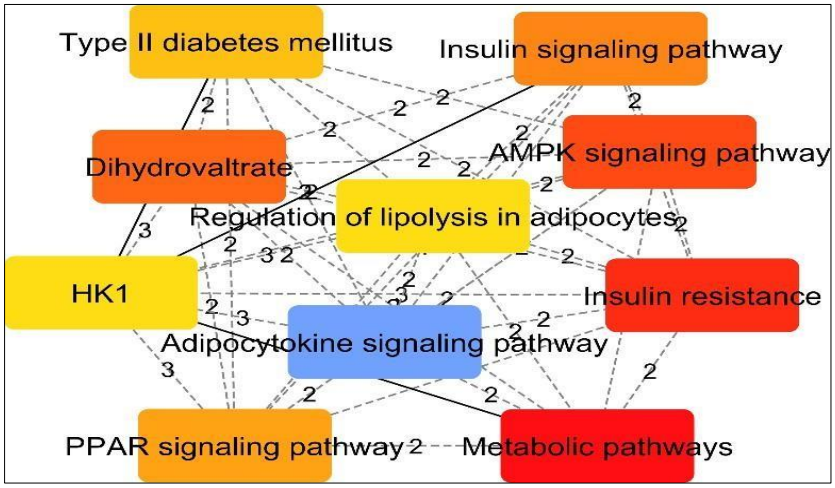

Figure 9: Cytohubba analysis of merged network by selecting top 10 Nodes of diabetes mellitus.

CONCLUSION

The present study explored the pharmacognosy and anti-diabetic potential of *Valeriana wallichii* utilizing an optimized ultrasound-assisted extraction (UAE) method. The extraction parameters were carefully optimized to maximize the yield of bioactive constituents, particularly total phenolic and flavonoid content, which are known for their therapeutic properties. The *in silico* analysis provided insights into the anti-diabetic mechanisms, highlighting the interaction of *Valeriana wallichii* phytoconstituents with key molecular targets involved in glucose metabolism. The findings support the potential of *Valeriana wallichii* as a promising natural candidate for diabetes management. Further *in vitro* and in vivo investigations are warranted to validate its efficacy and safety for potential pharmacological applications.

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