**TITLE OF THE PROJECT**

**Multi-Stage Screening of Medicinal Plants for Women’s Lifestyle Disorder Management *via* *in silico* Molecular Mechanics, Bio-Activity Guided Fractionation and *in vivo* Validation**

**1. Introduction**

Women’s lifestyle disorders have become increasingly prevalent due to changing diets, stress, and sedentary lifestyles. The manifestation of such disorders is seen in relation to hormonal, metabolic, and reproductive malfunctions. The resultant symptoms primarily include Polycystic Ovary Syndrome (PCOS), which causes multifactorial endocrine disorders in women.characterized by hyperandrogenism, insulin resistance, menstrual irregularities, and polycystic ovaries. These disorders not only affect reproductive health but also increase the risk of long-term complications like diabetes, cardiovascular diseases, and infertility. While modern pharmacological treatments exist individually for addressing each aspect of the syndrome, they are often associated with side effects and limited long-term efficacy, highlighting the need for safer, natural alternatives that provide holistic management of the discrepancies caused by such lifestyle-related disorders.

Herbal medicines have contributed in a crucial way to traditional healthcare systems. The wide range of available plant wealth are a rich source of bioactive compounds with therapeutic potentials. .Nevertheless, systematic scientific validation that can translate these herbal remedies into evidence-based interventions is not so commonly found. This project proposes a multi-stage screening approach for identifying and validating plant-derived compounds effective in managing women’s lifestyle disorders. By leveraging the strengths of computational biology, phytochemistry, and experimental pharmacology, the comprehensive workflow schedule outlined in this proposal aims to bridge traditional knowledge with modern science, facilitating the discovery of novel therapeutic agents tailored to women’s health needs.

The Kalyani University Biodiversity Educational and Conservation Park features a Herbal Garden established with initial financial assistance from the National Medicinal Plant Board under the Ministry of AYUSH. This medicinal sanctuary currently hosts more than 100 varieties of therapeutic plants and trees. The collection encompasses both widely recognized medicinal species used for diverse human ailments and an abundant yet underexploited array of plant resources that show promise for addressing persistent conditions such as PCOS.. This research will use the Biodiversity Park to study medicinal plants that might help with PCOS. The multidisciplinary team will predict how phytochemicals interact with biological targets through computer modeling, then separate and identify the active ingredients.Promising candidates will then be tested in living animals (*in vivo*) to assess efficacy and safety. Finally, they will be used to create plant-based treatments that target the pathways associated with PCOS.The project will culminate in the development of a standardized herbal formulation. This will demonstrate the park's value for turning research into useful products, ensuring a rational, targeted, and evidence-based study of medicinal plants to develop possible treatments for women's health.**2. Study Design & Objectives**

1. To identify biologically active compounds from the medicinal plant repository of the Kalyani University Herbal Garden, initially funded by the Ministry of AYUSH, that can target proteins related to lifestyle disorders like PCOS (Polycystic Ovary Syndrome), diabetes, and obesity.

2. To identify key proteins involved in women’s lifestyle disorders like PCOS, diabetes and obesity.

3. To perform *in silico* screening and study the binding affinity of the listed phyto-compounds with the key proteins and study the stability of their interactions by Molecular Dynamics simulations.

4. To extract, fractionate, and isolate bioactive compounds using bio-activity guided fractionation.

5. To study the cytotoxicity of the screened/ effective bioactive compounds in vitro in normal cell lines to determine their toxicities (if any).

6. Validate the therapeutic potential of the bioactive compounds effective against key proteins (as per docking and molecular dynamics simulation) in animal models of PCOS, diabetes, and obesity.

**3. Strategic Use of the Herbal Garden within the Kalyani University Biodiversity Park**

The Herbal Garden in Kalyani University's Biodiversity Park was started with help from The Government of India's Ministry of AYUSH. It was created to be a resource hub for education, research, and conservation.The park now maintains, along with other sections like Spice Garden, Dye Garden, Underground Tuber Crops Garden, Fruits and Nuts Garden, a thriving Herbal Garden with:

• Over **100 medicinal plant** species (including both herbs and trees);

• Plants with proven applications in hormonal regulation, metabolic modulation, and gynaecological health and also plants less or never bio-prospected for the above purposes;

• A sustainable in-house supply of plant materials for research and academic training.

This project represents a **direct outcome of the initial investment**, turning conservation into innovation. It will also enhance the **interdisciplinary visibility and utility of the Kalyani University Biodiversity Educational and Conservation Park in general, as well as the Herbal Garden in particular** in multiple research fields ranging from pharmacognosy and drug discovery towomen's health research.

**a. Selection of Medicinal Plants**

Plants will be selected based on prior scientific studies and ethnomedicinal evidence.

**b. In Silico Screening**

This step shall involve computational methods to predict the interactions between plant-derived compounds and proteins involved in the pathophysiology of the disorders.

* **Compound Database:** Phytoconstituents will be selected based on their reported ability to influence hormonal balance, reduce insulin resistance, and regulate menstrual cycles, and also on the basis of phytoconstituents that have no such reported properties but possess similar characteristics in terms of chemical structure and bonding nature.
* **Target Proteins:** In the next step, key proteins involved in the diseases shall be identified. For PCOS, this might include proteins related to hormonal regulation (e.g., androgen receptor, insulin receptor). For diabetes, consider proteins related to glucose metabolism (e.g., GLUT4, insulin receptor). For obesity, proteins related to adipogenesis or fat metabolism (e.g., PPAR-γ, leptin, AMPK).
* **Molecular Docking:** Subsequently, molecular batch-docking simulations shall be employed to evaluate the binding affinity of each compound with the target proteins. Traditional docking software, such as AutoDock Vina, may be augmented with machine-learning paradigms such as convolutional neural networks and reinforcement learning and then used for this purpose.**ADMET Prediction:** Assessment of the absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of the identified compounds will be performed using computational tools like SwissADME or pkCSM.
* **Molecular Dynamics:** Finally, High-Performance Computing (HPC) systems capable of processing several hundred teraflops will be leveraged to conduct computational simulations of the dynamics of phytocompounds interacting with the protein of interest. The advanced HPC hardware, combined with MD software tools such as GROMACS, allow for detailed simulations over extended time periods (of the order of 500 nanoseconds of sim time) to effectively study the kinetics and energetics of all biochemical interactions between the molecules.

**c. Extraction and Bio-Activity Guided Fractionation**

* Collection of appropriate plant components (leaves, stems, bark, seeds, etc.) will proceed from the Herbal Garden following our aforementioned comprehensive screening process. The gathered materials will undergo bio-activity guided fractionation according to the following protocol:Shade drying and pulverization using a mechanical grinder to obtain a coarse powder.
* Cold maceration/ Soxhlet extraction using different solvents (n-Hexane/ Chloroform/ Ethyl acetate/ Methanol/ Water) of plant extracts depending on thermal sensitivity of targeted phytoconstituents and concentration of the same using rotary evaporator.
* Preliminary Phytochemical Screening via qualitative tests for Alkaloids, Flavonoids, Saponins, Tannins, Terpenoids, Steroids, Glycosides.
* TLC profiling to identify fractions of interest.
* Bioactivity Screening employing *in vitro* assays to identify the most active extract for further fractionation
* Bio-Activity Guided Fractionation using the following techniques and pool similar fractions and test bio-activity
  + Liquid-Liquid Partitioning to separate active extracts and sequentially partition with Petroleum ether, Chloroform, Ethyl acetate, and n-Butanol
  + Column Chromatography
* Identify and characterize active compounds *via* analytical tools, viz., HPLC, FTIR, LC-MS, NMR
* Compound characterization *via* spectroscopic analysis

**d. In Vitro Studies**

In vitro studies will test the cytotoxicity of the selected compounds on normal cell lines

* **Cell Line Selection:** Appropriate cell lines for testing will be selected. For example, for PCOS, ovarian cell lines could be used; for diabetes, adipose or muscle cell lines may be suitable. Also, toxicities of the phyto compounds on normal cells are to be studied.
* **Cytotoxicity Testing:** Assays like MTT, XTT, or trypan blue exclusion to evaluate the cytotoxicity of the plant extracts and their compounds will be done.
* **Dose-Response Curve:** Determination of the effective concentration range of the compounds that is non-toxic and biologically active will be determined.

**e. In Vivo Studies**

* **Animal Models:**
  + **PCOS:** Rodent models (e.g., rats) induced with PCOS using hormonal treatments (e.g., with letrozole or dihydrotestosterone) and high-fat diet will be used for PCOS.
  + **Diabetes:** Diabetic animal models (e.g., streptozotocin-induced diabetic rats or db/db mice) will be used for the study.
  + **Obesity:** Obesity in animals through a high-fat diet or genetic models will be used for the study.
* **Endpoints and Measurements:** Measure key disease markers:
  + **For PCOS:** Hormonal profile (testosterone, LH/FSH ratio), ovarian histology, insulin levels, OGTT, lipid profile will be measured.
  + **For Diabetes:** Blood glucose, insulin sensitivity (using OGTT, ITT), HbA1c levels along with the Insulin signalling will be measured.
  + **For Obesity:** Body weight, adiposity index, blood lipid profiles, and glucose tolerance will be measured.
* **Histopathology and Biochemical Assays:** Analyze tissue samples for changes in the morphology and expression of key proteins related to the disease.

**2. Key Proteins to Target**

**a. For PCOS:**

* **Insulin receptor**: Targeting insulin resistance, which is common in PCOS.
* **Androgen receptor**: PCOS often involves elevated androgen levels, leading to symptoms like hirsutism.
* **CYP17A1**: An enzyme involved in androgen biosynthesis.
* **AMH (Anti-Müllerian Hormone)**: Elevated in PCOS, influencing ovarian function.

**b. For Diabetes:**

* **Insulin receptor (IR)**: Central to glucose uptake and regulation of insulin sensitivity.
* **GLUT4**: The glucose transporter responsible for insulin-mediated glucose uptake.
* **AMPK (AMP-activated protein kinase)**: A key regulator of energy balance, involved in glucose and lipid metabolism.
* **PPAR-γ (Peroxisome proliferator-activated receptor-gamma)**: A regulator of adipogenesis and glucose metabolism.
* **GSK-3β (Glycogen synthase kinase-3 beta)**: A kinase involved in insulin signaling.
* **P**-ERK: MAPK involved in insulin signaling

**c. For Obesity:**

* **Leptin receptor**: Involved in regulating appetite and energy balance.
* **PPAR-γ**: A major regulator of fat cell differentiation and metabolism.
* **CPT-1 (Carnitine palmitoyltransferase 1)**: Involved in fatty acid oxidation.
* **Adiponectin**: A protein that modulates glucose regulation and fatty acid breakdown.

**3. Expected Outcomes**

* Identification of novel bioactive compounds from available less/ unutilized medicinal plant resources of the Kalyani University Medicinal Garden that can modulate disease-related proteins.
* Understanding of the molecular mechanisms by which these compounds exert their effects.
* Validation of the efficacy and safety of the identified compounds in preclinical models of PCOS, diabetes, and obesity.
* Potential development of these compounds into therapeutic agents for lifestyle disorders.

**4. Timeline of the Study : 3 years**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Phase** | **Activity** | **Duration** | | | | | |
| **1st Year** | | **2nd Year** | | **3rd Year** | |
| **1st half** | **2nd half** | **1st half** | **2nd half** | **1st half** | **2nd half** |
| Phase 1 | Literature review & molecular docking |  |  |  |  |  |  |
| Phase 2 | Extraction & bioassay screening |  |  |  |  |  |  |
| Phase 3 | Fractionation & compound isolation |  |  |  |  |  |  |
| Phase 4 | In vivo animal model testing |  |  |  |  |  |  |
| Phase 5 | Herbal formulation & safety testing |  |  |  |  |  |  |
| Phase 6 | Data analysis & documentation |  |  |  |  |  |  |

**5. Budget Estimate (₹ INR)**

**Ai. Recurring (Manpower)**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Sl. No.** | **Position (3 Positions)** | **Monthly Consolidated Emolument #** | **Year 1** | **Year 2** | **Year 3** | **Total** |
| 1 | **Junior Research Fellow (2)** | @ Rs. 25,000/- | 6,00,000/- | 6,00,000/- | 6,00,000/- | **18,00,000/-** |
| 2 | **Laboratory-cum-Field Assistant (1)** | @ Rs. 10,000/- | 1,20,000/- | 1,20,000/- | 1,20,000/- | **3,60,000/-** |
| **Sub-total of Ai (1 + 2)** | | | **7,20,000/-** | **7,20,000/-** | **7,20,000/-** | **21,60,000/-** |

**Aii. Recurring (Consumables, Analysis, Contingency & Report Preparation)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sl. No.** | **Other Expenditure Heads** | **Year 1** | **Year 2** | **Year 3** | **Total** |
| 1 | **Consumables** |  |  |  |  |
| 2 | **Analytical Charges (outsourced)** |  |  |  |  |
| 3 | **Contingencies** |  |  |  |  |
| 4 | **Formulation & Prototype Development** | -- | -- | 1,50,000/- | **1,50,000/-** |
| 5 | **Institutional Overhead charge** |  |  |  |  |
| **Sub-total of Aii (1 + 2 + 3 + 4 + 5)** | |  |  |  |  |
| **Sub-total of A (Ai + Aii)** | |  |  |  |  |

**B. Non-Recurring (Equipment)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sl. No.** | **Equipment Name (No. of units required)** | **Unit Price** | **Year 1** |  |
| 1 | **Software & Docking Resources (1 HPC setup)** | **50,00,000/-** | **50,00,000/-** |  |
| **Grand Total (A + B)** | | |  |  |
| **Rupees** | | | | |

**6. Significance**

This interdisciplinary approach offers a comprehensive and rational framework to develop natural therapeutics for management of PCOS. Combining traditional knowledge with modern tools like molecular docking and animal model testing/ cell line evaluation ensures a scientifically validated path from plant to product. The resulting formulation could serve as a lead for clinical trials and potential commercialization as a safe, plant-based formulation for PCOS management.

This project builds directly on the prior investment in the University Biodiversity Educational and Conservation Park by the funding authority. It aims to transform preserved biodiversity into applied therapeutic research through an interdisciplinary strategy spanning computational chemistry, pharmacology, and herbal technology. The outcomes will validate the park’s potential as a national model for biodiversity-driven drug discovery and innovations in disease management.

**7. Proof of Concept**

**a. Selection of Medicinal Plants**

Based on preceding scientific research and ethnomedicinal evidence, an initial selection of plants has been made. A thorough literature review was conducted examining the botanical resources found in the Herbal Garden of Kalyani University. Table 1 presents a summary featuring 20 plants from this garden, highlighting their applications for specific health conditions.

**Table – 1. Excerpts from the comprehensive study on phytocompounds & pharmaceutical properties of plant resources of the Kalyani University Herbal Garden**

| **Plant Name**  **Family**  **Reference** | **Part(s) used** | **Major Phytocompounds** | **Pharmaceutical properties** |
| --- | --- | --- | --- |
| *Aloe vera* (L.) Burm. f.  **[Asphodelaceae]**  (Hęś et al., 2019; Kar & Bera, 2018, Nalimu et al., 2021; Kim et al., 2023) | Leaves  Flowers | **Anthraquinones:** Aloeresin E, Isoaloeresin D, and 2'-O-Feruloylaloesin  **Flavonoids:**  Orientin, Vicenin II, and Lucenin II  **Chromones:** Aloesin, 8-C-glucosyl-7-O-methyl-(S)-aloesol and Isoaloeresin D.  **Phenyl pyrones:** Aloenin & Aloenin B  **Anthrones:**  Aloe emodin, Aloin A and B, 8-O-methyl-7-hydroxyaloin A and B, and 10-hydroxyaloin A, Sinapic acid, Chlorogenic acid, Aloin, Aloe-emodin 8-O-beta-D-glucopyranoside, Catechin, and Epicatechin  **Acids:** Lauric acid, Palmitic acid,  **Phenolic compounds:**  Quercitrin, Gentisic acid, and Epicatechin, Coumarin, Gallic acid, Caffeic acid, D-catechin, Vanillic acid, Naringenin, Resveratrol, Cinnamic acid, Thymol, Luteolin, Apigenin, Isoorientin, Isovitexin, Kaempferol, Saponarin, and Lutonarin  **Anthranoids:** Aloe emodin | Wound Healing,  Anti-inflammatory, Antibacterial, Antifungal, Antioxidant,  Anti-cancerous, Immunomodulatory, **Antidiabetic** |
| *Barleria cristata* (L.)  **[Acanthaceae]**  (Snehal et al., 2024; Harish et al., 2018; Hemalatha et al., 2012) | Leaves | **Triterpene:** Oleanolic acid,  flavonoids: Luetoline and 7-methoxy luetoline, Quercetin  **Iridoidal glycosides:** Barlerin and schanshiside methyl ester | Antioxidant, Hepatoprotective, Anti-cancerous  Anti-inflammatory, Antibacterial,  **Anti-diabetic** |
| *Costus speciosus* (Koening) Sm.  **[Costaceae]**  (Ali et al., 2018; Shaikh et al., 2021; Sohrab et al., 2021 ) | Leaves and rhizomes | **Saponin:** Diosgenin, Gracillin, Dioscin, Prosapogenins A and B of dioscin, β-D-glucoside  **Terpenes:** Eremanthin, Costunolide  **Sterols**: β-sitosterol  **Carotenoid:** β-carotene  **Tocopherol:** α-tocopherol | Effective against *Salmonella typhi* and *Staphylococcus aureus*, Antioxidant, Anti-cancerous, Anti-inflammatory, Hepatoprotective,  **Antidiabetic**, **Hypolipidemic**, **Steroidogenic** |
| *Hedychium coronarium* J. Koenig  **[Zingiberaceae]**  (Cruz et al., 2023; Kumar et al., 2022; , Ray et al., 2019) | Whole plant | **Terpenoids:** **1**,8-Cineole, β-Pinene and (E)-Caryophyllene, Eucalyptol, Linalool, Coronarin-E. | Anti-cancerous, Antioxidant, Antimicrobial, **Antidiabetic** |
| *Stevia rebaudiana* Bert.  **[Asteraceae]**  (Nasrullah et al., 2023; Lremizi et al., 2023; Lremizi et al., 2023; Verma 2024; Stefaniuk et al., 2024 ; Orellana-Paucar, 2023; Faramayuda et al., 2022.) | Leaves | **Glycoside:** Rebaudioside A, Stevioside, Rebaudioside B, C, D, E, F, and Rubusoside,  **Terpenes:** Caryophyllene oxide, Spathulenol, Nerolidol, and Manool oxide | Antihypertensive, Aids in BP regulation,  Dental and Immuno-modulatory issues Anti-inflammatory, Antioxidant,  Anti-cancerous.  **Antiobesity** |
| *Sphagneticola calendulacea* (L.) Pruski  **[Asteraceae]**  (Ritesh & Gopalkrishnan, 2022) | Leaves | **Phenolics:** Chlorogenic acid, Gallic acid, Caffeic acid, Ferulic acid  **Flavonoids:** Jaceosidin | Antioxidant,  Anti-inflammatory **Antidiabetic** |
| *Sansevieria cylindrica* Bojer ex Hook.  **[Asparagaceae]**  (Buyun et al., 2018; Aung et al., 2020; Shewale et al., 2023) | Leaves &  Rhizomes | **Dicarboxylic acid:** Phthalic acid  **Fatty Acid:** Hexadecanoic acid  **Flavonoids:** 3-benzyl chroman-4-one | Anti-inflammatory, Antioxidant Antimicrobial  Antioxidant, **Antidiabetic** |
| *Cissus quadrangularis* L.  **[Vitaceae]**  (Hamid & Patil, 2023; TP & Nisha, 2023; Pratap et al., 2024) | Stem | **Flavonoids:** Quercetin, Myricetin  **Phenolics:** Resveratrol  **Sterol:** β-sitosterol,  **Alkaloids:** Hydrastine, Berberine | Antimicrobial, Antidiabetic,  Anti-inflammatory,  Bone healing, Cardiovascular protectant,  **Anti-obesity** |
| *Bryonia laciniosa* L.  **[Cucurbitaceae]**  (Sivakumar et al., 2004; Shah, 2022; Kadam et al., 2023) | Leaves | **Saponins:** Bryonin  **Triterpenoids:** Bryonolic acid | Analgesic and Antipyretic,  Anti-inflammatory, Fertility enhancement,  **Anti-diabetic** |
| *Euphorbia tithymaloides* L.  **[Euphorbiaceae]**  (Foda et al., 2022; Bhardwaj et al., 2024; Srivastava & Soni, 2019) | Stems leaves and roots | **Triterpenes:** Friedelane-3β-ol, 3-oxo-Friedelane, Euphane derivatives.  **Diterpene:** 1α, 13β, 14α-trihydroxy-3β, 7β-dibenzenzoyloxyjatropha  **Phytosterol:** β-Sitosterol.  **Flavonoids:** Rutin and Luteolin | Anthelmintic,  Anti-cancerous,  Antifilarial, Analgesic, Antioxidant,  Anti-inflammatory, Antifungal, Antiviral, Antitumor,  Hepatoprotective,  **Antidiabetic** |
| *Aerva lanata* (L.) Juss.  **[Amaranthaceae]**  (Shanmuganathan et al., 2024; Pieczykolan et al., 2022; Appapalam et al., 2017; Riya et al., 2014; Gertruda et al., 1992) | Whole plant | **Alkaloids:** Quinine, Canthin-6-one and β-Carboline derivatives.  **Phenolics:** Tiliroside  **Flavonoids:** Rutin, Quercetin, Kaempferol, Astragalin.  **Micronutrients:** Potassium, Magnesium, Calcium, and Zinc | Antimicrobial,  Anti-inflammatory, Antifungal activity against *Cryptococcus neoformans* and *Candida albicans*Inhibit α-glucosidase and α-amylase.  **Antidiabetic** |
| *Aegle marmalos* (L.) Correa  **[Rutaceae]**  (Alam, 2023; Baliga et al., 2013; Sharma & Sharma, 1981, Dhalwal et al., 2008) | Leaves, Stem Bark, Roots and Fruits | **Glycosides:** Cardenolide  **Phenolic ether:** Marmeline  **Terpenes:** Lupeol, Limonene  **Phenolics:** Eugenol  **Flavonoid**s: Rutin  **Coumarins:** Marmesin, Psoralen, Xanthotoxol, Marmelosin**,** Umbelliferone, Scopoletin,  **Glycosides:** Sitosterol Glucoside | Anticancer, Antibacterial,  Anti-inflammatory, Antioxidant, **Cytoprotective against oxidative stress in diabetic conditions** |
| *Hygrophila auriculata* (Schumach.) Heine  **[Acanthaceae]**  (Sethiya et al., 2018; Govindarajan et al., 2006; Hussain et al., 2016; Shanmugasundaram & Venkataraman, 2006) | Aerial parts | **Flavonoids:** Apigenin, Luteolin, Ellagic Acid, Gallic Acid, and Quercetin.  **Alkaloids:** Asteracanthine and Asteracanthicine.  **Triterpenes:** Lupeol, Lupenone, Hentricontane, and Betulin  **Sterols:** Stigmasterol and Asterol | Hepatoprotective, Antioxidant, **Antidiabetic** |
| *Cinnamomum verum* J. Presl  **[Lauraceae]**  (Sharifi-Rad et al., 2021; Farag et al., 2022,; Narayanankutty et al., 2021; Aggarwal et al., 2022) | Leaves, Stem, Friuts | **Essential oil-** Cinnamaldehyde  **Flavonoid-** Eugenol  **Phenolic compounds-** Coumarin, Cinnamic acid | Antimicrobial, Antioxidant,  Anti-inflammatory, Neuroprotective, **Antidiabetic** |
| *Pterocarpus santalinus* L.f.  **[Fabaceae]**  (Dahat et al., 2021; Bulle et al., 2016; Kodithuwakku et al., 2011; Pagadala et al., 2021; Akhouri et al., 2021; Kim et al., 2008; Gopinath et al., 2018) | Bark  and Heartwood | **Flavonoid:** Pterocarpin, **Luteolin,** Dihydroquercetin (Taxifolin), Kaempferol, **Quercetin**  **Phytosterol: Beta-Sitosterol**  **Tannin: Alpha-cedrene**  **Polyphenolic compound:** Resveratrol, Pterocarpin, Curcumin | Antioxidant,  Anti-inflammatory, Antimicrobial, **Astringent Neuroprotective Cardiovascular Protection,**  **Anti-cancerous Antidepressant, Antidiabetic** |
| *Diospyros malabarica* (Desr.) Kostel.  **[Ebenaceae]**  (Ribeiro et al., 2023; Polash et al., 2022; Zareen et al., 2022; uddin et al., 2023) | Leaves, Bark, Fruit, Stem, Seeds | **Flavonoids:** Betulinic Acid, Oleanolic Acid, Lupeol, Furano-(2",3",7,8)-3',5'-dimethoxy-5-hydroxyflavone, 4’-hydroxy-3,6,3’,5’-tetramethoxy-7,8-pyranoflavone, Tetrahydroxy-3,5,3-methoxyflavanone-4,O-L-rhamnopyranoside, 5,7,3,4-Tetrahydroxyflavanone-3,O-D-glucopyranosyl-1,4-L-rhamnopyranoside  **Terpenoids- M**yricylalcohol, Betulin, β-Sitosterol, Oleanolic Acid | Antimicrobial, Antioxidant,  Anti-inflammatory, Antinociceptive Antidiarrheal,  Anti-cancerous, **Antidiabetic** |
| *Haldina cordifolia* (Roxb.) Ridsdale or *Adina cordifolia* (Roxb.) Brandis  **[Rubiaceae]**  (Sharma et al., 2019; Patil et al., 2021; Hossain et al., 2015; Raypa et al., 2018; Rao et al., 2021; Desai and Tarikere, 2023) | Leaves, Bark | **Alkaloids-** Tinosporine, Magnoflorine, Berberine, Jatrorrhizine, Choline, Palmatine  **Terpenoids-** Tinosporide, Furanolactone diterpene, Ecdysterone, Cordifoliosides A, B, C, D, E  **Steroids-** Giloinsterol, β-Sitosterol, 20α-Hydroxy ecdysone  **Flavonoids-** Quercetin, Naringenin | Antimicrobial Antioxidant, Hepatoprotective, Anti-ulcer, Antimalarial,  Anti-cancerous, **Antidiabetic** |
| *Piper chaba* W. Hunter  **[Piperaceae]**  (Islam et al., 2020; Naz et al., 2012; Panthong et al., 2020; Rahman et al., 2023) | Leaves, Stems, Roots, Seeds, Flower | **Alkaloids:** Piperine, Piplartine, Chabamides, Retrofractamides A/B, Piperlonguminine, Pipernonaline, Dehydropipernonaline  **Flavonoid-** Quercetin, Rutin, Catechin, Syringic acid | Antimicrobial,  Anti-inflammatory, Cytotoxicity Against Cancer Cells, Gastroprotective, **Antidiabetic** |
| *Eryngium foetidum* L.  **[Apiaceae]**  (Leitão et al., 2023; Cárdenas-Valdovinos et al., 2023; Rodrigues et al., 2022; Zhang et al., 2022; Daimari & Deka 2024) | Leaves, Stems, Roots, Flower | **Phenolic Compounds-** Ferulic acid, Syringic acid, Gallic acid, p-Coumaric acid, Protocatechuic acid, Sinapic acid  **Flavonoids-** Quercetin, Rutin, Kaempferol  **Essential Oils-** β-sitosterol | Antioxidant, Antimicrobial,  Anti-inflammatory, Anthelmintic,  Anti-cancerous,  **Antidiabetic** |
| *Curcuma longa* L.  **[Zingiberaceae]**  (Kulyal et al., 2021; Lee at al., 2014; Noori et al., 2022; Zhang et al., 2021; Li et al., 2011; Amalraj et al., Koo et al., 2012) | Rhizomes, Leaves, Flower | **Phenolics-** Curcumin, Demethoxy-curcumin, Bisdemethoxy-curcumin  **Essential Oils (Terpenoids)-** α-Phellandrene, Zingiberene, Sabinene, Cineol (1,8-cineole), α-Turmerone, β-Turmerone, Epi-α-Bisabolol, α-Bisabolol | Antioxidant,  Anti-inflammatory, Antimicrobial, Neuroprotective, Cardioprotective Anti-coagulant,  Anti-cancerous, **Antidiabetic,**  **Anti-hyperlipidemic** |

**b. In Silico Screening**

This step involved computational methods to predict the interaction between plant-derived compounds and proteins involved in the pathophysiology of the disorders.

* **Compound Database:** Based on the above preliminary study, phytoconstituents were selected in terms of their reported ability to influence hormonal balance, reduce insulin resistance, and regulate menstrual cycles, and also on the basis of phytoconstituents that have no such reported properties but possess similar characteristics in terms of chemical structure and bonding nature. A few of the phytoconstituents thus selected are presented in Table – 2. A more detailed and comprehensive approach shall be taken up during the course of execution of this proposed project.

**Table – 2. Documented Phytoconstituents with Significant Therapeutic Effect against the targeted disease and their source plants in the KU Herbal Garden**

| **Name of phytoconstituent** | **Source plants in the Kalyani University Herbal Garden** | **Molecular formula** | **Chemical structure** |
| --- | --- | --- | --- |
| Apigenin | *Aloe vera, Andrographis paniculata*, *Kalanchoe pinnata, Hygrophila auriculata, Cymbopogon citratus, Ocimum sanctum, Mentha piperita* | C15H10O5 |  |
| Catechin | *Aloe vera, Centella asiatica, Phoenix dactylifera, Flacourtia indica, Cinnamomum verum, Pandanus amaryllifolius, Piper chaba, Aegle marmalos, Terminalia arjuna* | C15H14O6 |  |
| Berberine | *Haldina cordifolia,*  *Tinospora cordifolia* | C20H18NO4+ |  |
| Glycyrrhizin | *Abrus precatorius* | C42H62O16 |  |
| Mangiferin | *Mangifera indica*  *Belamcanda chinensis*  *Bombax ceiba* | C19H18O11 |  |
| Baicalin | *Oroxylum indicum*  *Thymus vulgaris* | C21H18O11 |  |
| Curcumin | *Curcuma longa*  *Curcuma aromatica*  *Curcuma caesia*  *Curcuma amada*  *Curcuma zedoaria*  *Pterocarpus santalinus* | C21H20O6 |  |
| Silibinin | *Phyllanthus amarus* | C25H22O10 |  |
| Resveratrol | *Aloe vera*  *Vitis vinifera*  *Arachis hypogaea*  *Pterocarpus santalinus*  *Pterocarpus marsupium* | C14H12O3 |  |
| Naringin | *Pandanus amaryllifolius*  *Citrus limon*  *Citrus aurantium*  *Citrus aurantiifolia*  *Citrus reticulata*  *Saraca asoca* | C27H32O14 |  |
| Vitexin | *Justicia adhatoda* | C21H20O10 |  |
| Rutin | *Pandanus amaryllifolius Euphorbia tithymaloides Aerva lanata*, *Acalypha hispida, Aegle marmalos*, *Fraxinus floribunda*, *Tabebuia aurea,* *Piper chaba, Eryngium foetidum, Desmodium gangeticum*, *Saraca asoca, Tamarindus indica* | C27H30O16 |  |
| Rhamnocitrin | *Syzygium aromaticum* | C16H12O6 |  |
| Gallic acid | *Abrus precatorius, Aloe vera Pandanus amaryllifolius, Abutilon indicum, Acalypha hispida, Hygrophila auriculata, Terminalia chebula*, *Terminalia arjuna*, *Syzygium aromaticum*, *Abroma augustum, Saraca asoca*, *Moringa oleifera*, *Eryngium foetidum* | C7H6O5 |  |

* **Target Proteins:** Key proteins involved in the diseases shall be identified. For PCOS, this might include proteins related to hormonal regulation (e.g., androgen receptor, insulin receptor). For diabetes, consider proteins related to glucose metabolism (e.g., GLUT4, insulin receptor). For obesity, proteins related to adipogenesis or fat metabolism (e.g., PPAR-γ, leptin, AMPK).
* **Molecular Docking:** Subsequently molecular docking simulations were employed on the selected phytoconstituents mentioned in Table - 2 to evaluate the binding affinity of each compound with the target proteins (Table - 3). Docking software such as AutoDock 4 and AutoDock Vina, along with AutoDock Tools were used for this purpose.

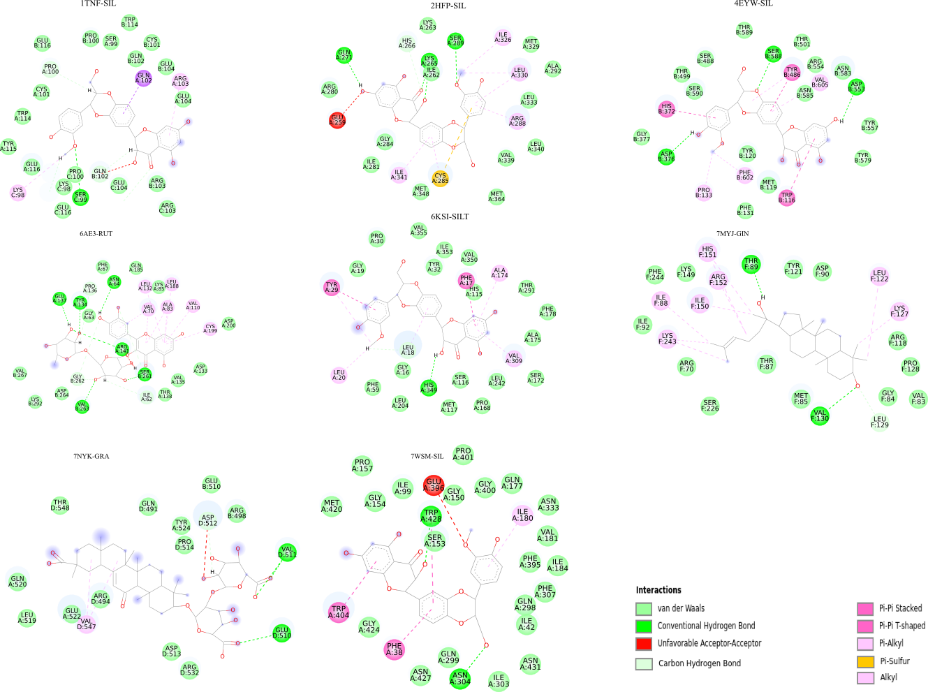
**Table – 3. Docking scores (in kcal/ mol) of selected phytoconstituent ligands with target proteins**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **PDB ID\* /LIG\*\*** | **1TNF** | **2HFP** | **4EYW** | **6AE3** | **6KSI** | **7MYJ** | **7NYK** | **7WSM** |
| API | -8.717 | -7.797 | -8.908 | -8.758 | -8.921 | -9.088 | -6.749 | -8.583 |
| BAI | -10.538 | -9.794 | -9.385 | -9.546 | -9.369 | -9.548 | -7.896 | -9.517 |
| BER | -9.385 | -9.115 | -8.384 | -9.313 | -9.585 | -9.110 | -7.323 | -9.646 |
| CAT | -9.085 | -7.884 | -8.749 | -8.162 | -8.986 | -8.270 | -7.090 | -8.316 |
| CUR | -8.950 | -8.206 | -9.925 | -8.565 | -8.539 | -8.278 | -6.032 | -9.300 |
| GAL | -7.160 | -5.598 | -6.652 | -5.636 | -6.211 | -6.633 | -6.687 | -5.820 |
| GIN | -8.869 | -8.748 | -9.424 | -10.243 | -9.549 | **-9.848** | -7.804 | -10.722 |
| GRA | -7.941 | -7.933 | -10.073 | -10.461 | -8.555 | -9.768 | **-8.223** | -10.837 |
| MAN | -10.390 | -8.500 | -8.399 | -10.146 | -9.093 | -8.560 | -7.516 | -9.102 |
| NAR | -10.487 | -9.349 | -10.409 | -10.302 | -9.456 | -9.608 | -8.206 | -10.251 |
| RES | -7.551 | -8.529 | -8.202 | -7.492 | -7.919 | -8.003 | -6.970 | -7.647 |
| RHA | -8.904 | -8.049 | -8.108 | -8.238 | -9.108 | -8.506 | -6.680 | -8.873 |
| RUT | -9.566 | -10.002 | -9.433 | **-11.514** | -8.731 | -9.138 | -7.598 | -9.942 |
| SIL | **-10.607** | **-10.082** | **-10.587** | -9.816 | **-9.802** | -8.646 | -7.412 | **-11.110** |
| VIT | -9.223 | -8.563 | -9.147 | -8.769 | -9.075 | -9.563 | -7.101 | -9.359 |
| **BEST** | **-10.607** | **-10.082** | **-10.587** | **-11.514** | **-9.802** | **-9.848** | **-8.223** | **-11.110** |

|  |  |
| --- | --- |
| **\*Protein PDB Codes** | **Description** |
| 7WSM | GLUT 4 BOUND TO CYTOCHALASIN |
| 7MYJ | AMPK WITH ACTIVATOR MOLECULE |
| 2HFP | PPAR GAMMA LIGAND BINDING DOMAIN |
| 2M76 | REGULATORY DOMAIN OF HUMAN CPT |
| 4EYW | 4EYW CPT IN COMPLEX |
| 6KS1 | Adiponectin 2 receptor |
| 7NYK | sh3 DOMAIN OF jnk |
| 1TNF | TNF ALPHA |

| **Sl. No.** | **\*\*Ligand Residue name** | **Name of phytoconstituents** |
| --- | --- | --- |
| 1 | API | Apigenin |
| 2 | CAT | Catechin |
| 3 | BER | Berberine |
| 4 | GRA | Glycyrrhizin |
| 5 | MAN | Mangiferin |
| 6 | BAI | Baicalin |
| 7 | CUR | Curcumin |
| 8 | RES | Resveratrol |
| **9** | **SIL** | **Silibinin** |
| 10 | NAR | Naringin |
| 11 | VIT | Vitexin |
| **12** | **RUT** | **Rutin** |
| 13 | RHA | Rhamnocitrin |
| 14 | GIN | Ginsenosides |
| 15 | GAL | Gallic acid |

The best docking scores were obtained for the phytoconstituents Rutin and Silibinin. The best docked ligands are highlighted in Table – 3 and presented as Figure – 1.



**Figure – 1. Protein-Ligand interaction profiles of the phytoconstituent molecules with the best-docked target proteins (see Table-3). Each complex is labeled according to the 3-letter codes of their constituent protein and ligand (see table-3). The participating protein residues (labeled by their residue name, chain id and residue number) are color-coded by the physio-chemical nature of their interaction with the ligand.**This preliminary investigation into the medicinal flora at Kalyani University's Herbal Garden has revealed encouraging pathways for additional exploration of the botanical collection, which was initially funded by the Ministry of AYUSH. The garden represents a successful transformation of conservation efforts into innovative research opportunities. The initiative aims to serve as a cornerstone for advancing studies in medicinal plant preservation, bio-prospecting, pharmacognosy, pharmaceutical development, and women's health, while also promoting the broader value of institutional Herbal Gardens.**Justification for purchase of dedicated HPC system:**

Kalyani University's Herbal Garden is a treasure trove of biodiversity, offering a unique opportunity to explore the potential of medicinal plants in treating Polycystic Ovary Syndrome (PCOS) and related women's health disorders. Our project aims to systematically screen selected plants using advanced computational methods, such as molecular docking and dynamics simulations, to predict the interactions between plant-derived compounds and key biological targets associated with these disorders.

However, the success of this project hinges on the availability of dedicated high-performance computing (HPC) resources. With a strict 3-year timeline and the University's existing HPC resources already stretched to their limits by other research groups, our project faces a significant hurdle in completing the necessary simulations within the given timeframe. To overcome this challenge, we require a dedicated HPC system that can operate continuously without interruption from other projects.

The combination of advanced HPC hardware and powerful molecular dynamics software tools, such as GROMACS, will enable us to conduct detailed simulations spanning extended time periods (up to 500 nanoseconds of simulation time). This will allow us to effectively study the kinetics and energetics of biochemical interactions between the molecules, which is crucial for identifying promising plant-derived compounds for further experimental validation.

Without access to dedicated high-performance computing resources, we would be unable to perform these critical *in silico* screening and molecular dynamics studies within the 3-year project timeline. These studies form the bedrock of our research, and their successful completion is vital to the overall success of the project. Moreover, a dedicated HPC setup will not only benefit this specific project but will also serve as a valuable resource for future computational drug discovery and molecular modeling research at the University, fostering innovation and scientific advancement in the field.