**TITLE OF THE PROJECT**

**Integrated Multi-Stage Screening of Bioactive Compounds from Medicinal Plants for treating Women's Lifestyle Disorders**

**Arunima Biswas**

**1. Study Design & Objectives**

1. To identify biologically active compounds from medicinal plants of Herbal and Medicinal PlantGarden, University of Kalyani funded by Ministry of Ayush, Govt. of India. that can target proteins related to lifestyle disorders like PCOS (Polycystic Ovary Syndrome), diabetes, and obesity.

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

3. In silico screening to study the binding affinity of the listed phyto-compounds with the key proteins and study the stability of their interactions by Molecular Dynamics.

4. Study the cytotoxicity of the screened/ effectivebioactive compounds in vitro in normal cell lines to determine their toxicities (if any).

5. 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.

**a. In Silico Screening**

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

* **Selection of Medicinal Plants:** Choose rare plants from your garden that have a history of medicinal use in treating conditions like PCOS, diabetes, and obesity.
* **Compound Database:** Extract or gather data for bioactive compounds from these plants, either by literature mining or phytochemical analysis.
* **Target Proteins:** Identify key proteins involved in the diseases. 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:** Use molecular docking simulations to screen the binding affinity of each compound to the selected proteins. Tools like AutoDock, DOCK, or PyRx can be used for this purpose.
* **ADMET Prediction:** Assess the absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of the identified compounds using computational tools like SwissADME or pkCSM.
* **Molecular Dynamics:**Study the interactions of the phytocompounds with the screened protein of interest over a time scale of 300 ns to observe any interaction by H-bonding.

**b. 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.

**c. 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 signaling 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 receptor**: A protein that modulates glucose regulation and fatty acid breakdown.

**3. Expected Outcomes**

* Identification of novel bioactive compounds from rare medicinal plants 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.

**5. Conclusion**

By combining in silico, in vitro, and in vivo methods, this approach can provide robust data on the therapeutic potential of plant-derived compounds for managing lifestyle disorders in women. Additionally, it can contribute to the identification of new leads for drug development, emphasizing the significance of natural compounds in modern medicine.

PDB FILES

[7WSM](https://www.rcsb.org/structure/7WSM) GLUT 4 BOUND TO CYTOCHALASIN

[7MYJ](https://www.rcsb.org/structure/7MYJ)  AMPK WITH ACTIVATOR MOLECULE

### [6AE3](https://www.rcsb.org/structure/6AE3) GSK 3B WITH MORIN

[2HFP](https://www.rcsb.org/structure/2HFP)  PPAR GAMMA LIGAND BINDING DOMAIN

### [2M76](https://www.rcsb.org/structure/2M76) REGULATORY DOMAIN OF HUMAN CPT

### [4EYW](https://www.rcsb.org/structure/4EYW)  CPT IN COMPLEX

### [6KS1](https://www.rcsb.org/structure/6KS1)  Adiponectin 2 receptor

### [7NYK](https://www.rcsb.org/structure/7NYK)  sh3 DOMAIN OF jnk

# 1TNF TNF ALPHA