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Research Article

In-silico Analysis of *Trillium govanianum* as a Potential Therapeutic for Polycycstic Ovarian Syndrome through Molecular Docking and Network Pharmacology

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Abstract

Polycystic Ovarian Syndrome (PCOS) can be characterized as a complex syndrome with reproductive, endocrine, and metabolic manifestations. It is an issue of major concern owing to its increasing predominance among young and middle aged women. This has led to a surge in requirement of discovering new therapeutics or in development of the available therapeutics at an advanced level to combat this disease with a multidimensional approach. *Trillium govanianum* commonly known as Nag Chhatri has been used as folk medicine to facilitate the labor of child birth as well as to treat menstrual and sexual disorders. In the current work an *in-silico* analysis of phytoconstituents of Trillium plant as potential therapeutics was done for PCOS. Receptors responsible for regulating hormone levels [Peroxisome proliferator-activated receptor-gamma, Progesterone receptor, Estrogen receptor (alpha), Androgen receptor, Follicle Stimulating Hormone Receptor], insulin levels [Insulin receptor] and obesity [Leptin receptor, and Melanocortin-4 Receptor (MC4R)] were selected as targets for in-silico studies representing the multi-facet dimension of the disease. Steroidal saponin constituents of *Trillium govanianum* like Borassoside D, Diosgenin, Pennogenin, Pennogenin-3-O-beta-D-glucopyranosidewere found to have better binding affinity and interactions with key residues of the active sites of various target proteins. Network pharmacology study was done to understand the probable mechanism of action of the phytoconstituents of *Trillium govanianum*.

The results showed that phytoconstituents of Thuja exerted their effect against PCOS through modulation of hormonal pathways, Trillium through modulation of insulin resistance, adipocyte cells and lipolysis. Thus *Trillium govanianum* could emerge as potential therapeutic for PCOS.

Keywords: PCOS; *Trillium govanianum*; Molecular Docking; Gene Ontology; KEGG Pathway

Abbreviations

PCOS: Polycystic Ovarian Syndrome; CC: Cellular Component; GO: Gene Ontology; MF: Molecular Function; BP: Biological Process; PPAR-γ: Peroxisome Proliferator-Activated Receptor-Gamma; FSH: Follicle Stimulating Hormone; MC4R: Melanocortin-4 Receptor

Introduction

Polycystic Ovarian Syndrome (PCOS) has emerged as a significant health concern over the past two decades, with its prevalence steadily rising among adolescent and adult women. Epidemiological data suggest that approximately one in five women is affected by this multifaceted disorder. Traditionally classified as a reproduc-

tive endocrinopathy, PCOS is characterized by anovulation, menstrual irregularities, acne, hirsutism, and infertility. However, its clinical manifestations extend beyond reproductive dysfunction, encompassing a broad spectrum of metabolic disturbances [1-5].

Central to PCOS pathophysiology is insulin resistance, which contributes to a cascade of hormonal imbalances. These include impaired glucose tolerance, compensatory hyperinsulinemia, early-onset type 2 diabetes, obesity, metabolic syndrome, non-alcoholic fatty liver disease, and an elevated risk of cardiovascular complications. Insulin resistance not only amplifies insulin's biological effects but also stimulates androgen production by ovarian theca cells—an action typically mediated by Luteinizing Hormone (LH). The synergistic stimulation by insulin and elevated LH levels results in excessive androgen synthesis. Concurrently, insulin suppresses the synthesis of Sex Hormone Binding Globulin (SHBG), thereby increasing the bioavailability of circulating androgens [6-8].

Women with PCOS often exhibit a reduced Follicle Stimulating Hormone (FSH) level, leading to a heightened LH/FSH ratio and a state of hyperandrogenism. Moreover, the activity of the Aromatase P-450 enzyme—expressed in granulosa cells and responsible for converting androgens into estradiol—is diminished, further disrupting estrogen biosynthesis. Current pharmacological interventions primarily target hyperandrogenic symptoms, yet they are frequently associated with adverse effects. This underscores the need for therapeutic strategies that address both the endocrine and metabolic dimensions of PCOS. Consequently, the development of multi-targeted treatments with improved safety profiles remains a critical priority in PCOS research [9-12].

Trillium govanianum also known as Nag Chhatri, is found in north eastern parts of India and has been used as folk medicine to facilitate the labor of child birth as well as to treat menstrual and sexual disorders. In the current work an *in-silico* analysis of phytoconstituents of Trillium plant as potential therapeutic was done for PCOS. Receptors responsible for regulating hormone levels, insulin levels and obesity were selected as targets for *in-silico* studies representing the multi-facet dimension of the disease. Network pharmacology study was done to understand the probable mechanism of action of the phytoconstituents of all three extracts. GO and KEGG pathway enrichment analysis was done to identify the set of overexpressed genes and pathways and their interaction with the phytoconstituents.

Material and Method

Retrival of phytoconstituents of all three plants

The list of chemical constituents of plants was obtained through thorough literature survey. Majority of phytoconstituents were obtained from IMPPAT [13].

Molecular docking study

Preparation of ligands

Smiles format of all ligands were obtained from PubCHEM, Drug bank and ZINC database. These ligands were subjected to Frog: a FRee Online druG 3D conformation generator. Many of the steps are optional and can be controlled by choosing options in the panel or by specifying command-line options. The process includes the following steps: structure format conversion, structure selection, hydrogen atom addition, removal of unwanted molecules, neutralization of charged groups, generation of ionization states, tautomer generation, filtering of structures, generation of alternative chiralities, low-energy ring conformations, and optimization of the geometry of the ligands. Ligand structure with minimum potential energy and similar chirality to that of the selected ligand IUPAC name was chosen for the docking experiment.

Retrieval of target receptors

The PDB format of key targets involved in PCOS were retrieved from the RCSB Protein Data Bank. Table 1 lists the specifics of their resolution and accession numbers. The files with the lowest resolution and Homo sapiens as the source were prioritized among the several PDB IDs accessible on the protein databank website. Additionally, the X-ray diffraction method was chosen as the means of obtaining the structure. The pre-made receptor was saved in PDB format after the complexes attached to it were eliminated using SPDBV, the non-essential water molecule was eliminated, and polar hydrogen was supplied.

Table 1: Details of target receptors used for molecular docking.

Protein (Target)	PDB ID	Source	Resolution	The process used to obtain the structure
Progesterone Receptor	1A28	Homo sapiens	1.80	X-ray diffraction
Peroxisome Proliferator Activated Receptor -γ	1FM6-X	Homo sapiens	2.10	X-ray diffraction
Estrogen Receptor	1GWR	Homo sapiens	2.40	X-ray diffraction
Androgen Receptor	2AX6	Homo sapiens	1.50	X-ray diffraction
Follicle Stimulating Hormone Receptor	4MQW	Homo sapiens	2.90	X-ray diffraction
Melanocortin-4 Receptor	6W25	Homo sapiens	2.75	X-ray diffraction
Insulin Receptor	1GAG	Homo sapiens	2.70	X-ray diffraction

Molecular docking

Molecular docking was done using PyRx and AutoDock. PyRx is a Virtual Screening software for Computational Drug Discovery that can be used to screen libraries of compounds against potential drug targets. Autodock vina is an in-built feature of Pyrx. All ligands are loaded in sdf format with the help of OpenBable. These ligands are then minimized and converted into pdbqt format as a part of preparation for docking. Macromolecule (target protein) is loaded in pyrx in pdb format. This protein was prepared for docking by eliminating water, unwanted chain and a bound ligand. After checking for missing amino acids, polar hydrogen and Kollman charges were added to the protein structure. The Lamarckian genetic algorithm was used to carry out process of docking. Docking of natural products as well as drugs was carried out using AutoDock vina as an in-built feature of Pyrx on windows platforms with machine configuration of 8 GB RAM and Intel i5 processor.

Result analysis and Complex interaction visualization

Results are obtained in the form of binding affinity of ligands towards the receptors. Ligands having highest binding affinity are sought to be most interacting ligand. Complex of ligand-receptor was visualized using PyMoL and residual interactions were obtained from LigPLOT.

Network pharmacology

A search of PCOS-related targets and genes

PCOS-related genes were obtained by retrieving data from the Polycystic Ovary Syndrome database (PCOSKB) (http://pcoskb.bicnirrh.res.in/). Information on associated genes, SNPs, diseases, gene ontologies, and pathways was collated and integrated into PCOSKB. Various tools were embedded in the database, such as comorbidity analysis, network analysis, and Venn analysis [25]. Target matching analysis of PCOS-related genes and phytoconstituents of THF, TRS and CAA Extracts was conducted to select the targets for respective extracts acting on PCOS. The top targets were selected as the main therapeutic targets for all three extracts individually.

Target prediction and validation

Target prediction was used to gain a better understanding of PCOS. DrugBank database was used to confirm relevant targets for identified phytoconstituents. The target protein name of the active component was converted to the standard target gene name using the UniProt Knowledge base. The official symbol was obtained by entering the target protein name into UniProtKB with the organism set to "Homo sapiens" [26,27].

Construction of target network and analysis

Target analysis was performed to determine the interaction between the phytoconstituents of extracts. The relevant targets of PCOS were uploaded against polycystic ovaries to the STRING database (https://string-db.org/). Cytoscape software was used to visually display and analyze the network structure. Two networks were created: a component-component target network formed by connecting the active components of Extracts and their corresponding components, and a core network identified based on the degree of value derived from the component-component target network.

Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis

The DAVID database (https://david.ncifcrf.gov), Cytoscape 3.2 software, and R 3.6.3 software were used to perform the GO function and KEGG pathway enrichment analysis on the targets at *P*

<0.5. The database for annotation, visualization, and integrated discovery (DAVID) was used to identify enriched biological themes, particularly GO terms that involved biological processes (BP), cell components (CC), and molecular functions (MFs), and visualize genes on KEGG pathway maps. The target of phytoconstituents of extracts acting on PCOS was inputted into the DAVID database with the organism limited to "Homo sapiens" [28].

Results

Molecular docking

Binding energy is the key parameter formed as a result of molecular docking. It offers information on the strength and affinity of the ligand-receptor interaction. The binding energy increases with weaker contact and decreases with stronger contact. As a result, throughout the docking process, we looked for the ligand with the lowest binding energy, which conferred strong affinity to the target among the test compounds. The phytocompounds that displayed binding affinity towards more than one target, but only those with the lowest docking score and binding energy, were considered suitable ligand-target interactions. In this investigation, phytocompounds with high quantifiable binding affinities for the target residues were evaluated. The binding affinities indicated the ligand's contribution to and flexibility towards the target.

Peroxisome proliferator activated receptor -γ

Human Peroxisome Proliferator Activated Receptor -γ structure co-crystallized with Rosiglitazone was utilized for molecular docking. Identification of active sites is a pre-requisite requirement for appropriate molecular docking. Active sites were identified through Pymol visualization. During molecular docking a grid is to be selected which represents the area where ligands will try to interact with the protein. Center Grid box was selected using x: -3.322, y: 68.496, z: 36.461, and the number of points in all dimensions x, y, z were considered 60x60x60 Å, and the grid spacing was selected as 0. 375 Å. The binding affinities of all The number of phytoconstituents interacting with PPAR-γ receptor were low for Trillium. Borassoside D of Trillium with highest binding affinity of -8.97 kcal/mol had the maximum interaction with PPAR-γ receptor among the other constituents. Rosiglitazone was used as the standard with the binding affinity of -8.5 kcal/mol. It can be seen that binding affinity of phytoconstituents is higher than the standard. The respective interactions of PPAR-γ receptor with Borassoside D are shown in figure 1.

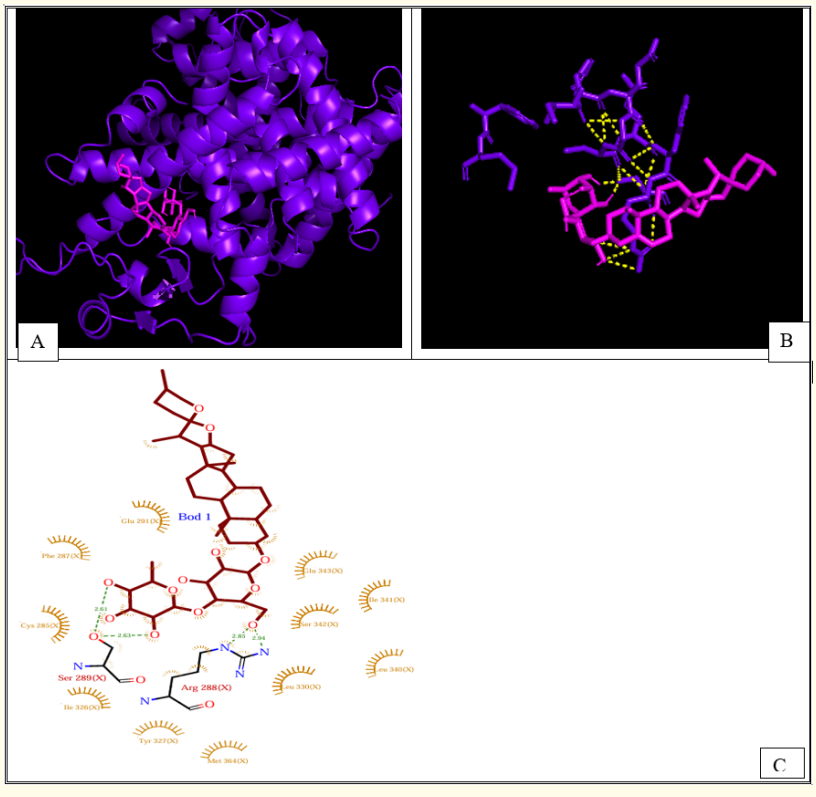


Figure 1: 2D and 3D interaction of Borassoside D with PPAR-γ receptor (A: Full view of interaction in 3D, B: Zoomed view of Ligand-receptor interaction in 3D, C: Zoomed view of Ligand- receptor interaction in 2D).

Borassoside D showed a large number of non-bonded interactions leading to the highest binding affinity towards PPAR-γ receptor receptor. Apart from large number of non-bonded interactions, it formed five hydrogen bond interactions with ARG 288 and SER 289 residues of PPAR-γ receptor receptor. Various residues of PPAR-γ receptor interacting with ligands and the type of interactions formed are shown in table 3.

Follicle stimulating hormone receptor

Structure of follicle-stimulating hormone in complex with the entire ectodomain of its receptor (P31) (PDB ID: 4MQW) was used for molecular docking. Identification of active sites is a pre-requisite requirement for appropriate molecular docking. Active sites were identified through Metapocket, since co-crystallized ligand was not available with this structure. During molecular docking a grid is to be selected which represents the area where ligands will try to interact with the protein. Center Grid box was selected using x: -14.097, y: 3.821, z: -2.956, and the number of points in all dimensions x, y, z were considered 110x110x110 Å, and the grid spacing was selected as 0. 957 Å. The binding affinities of all the phytoconstituents is given in table 2.

Table 2: Binding affinity of constituents towards various Receptor.

Binding affinity of ligands towards different receptor				
Phytoconstituents	**PPAR-γ receptor (1FM6)**	**FSH receptor (4MQW)**	**MC4R receptor (6W25)**	**Insulin receptor (IGAG)**
Borassoside D	-8.97	-	-	-
Diosgenin	-8.82	-7.84	-7.72	-6.96
Pennogenin	-8.39	-7.49	-6.09	-6.65
Pennogenin-3-O-beta-D-glucopyranoside	-	-6.28	-	-

The phytoconstituents of Trillium showed good binding affinity towards Follicle Stimulating Hormone Receptor. Diosgenin and Pennogenin got highest binding affinity of -7.84 and -7.49 kcal/mol respectively. Figure 2 shows the interaction of Diosgenin with FSH receptor.

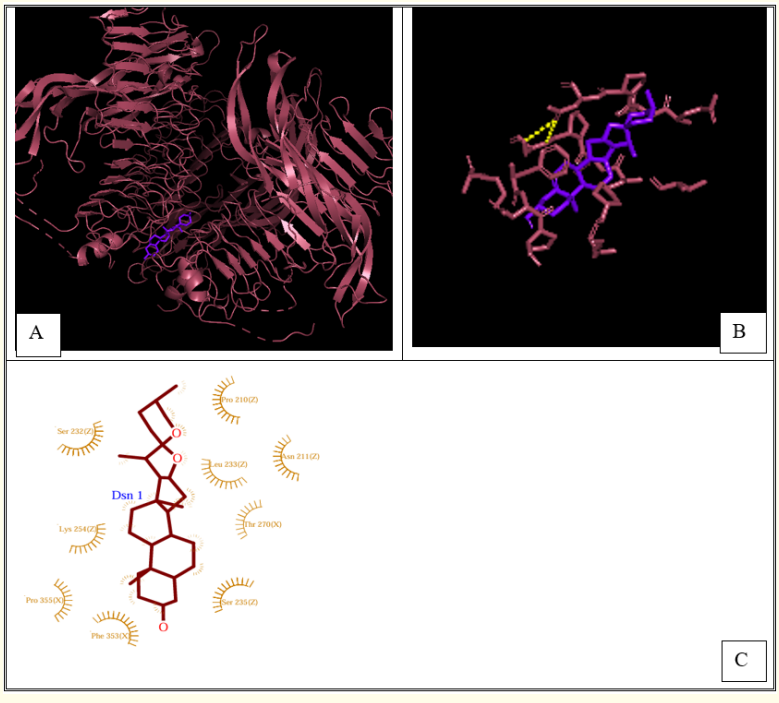


Figure 2: 2D and 3D interaction of Diosgenin with FSH Receptor (A: Full view of interaction in 3D, B: Zoomed view of Ligand- receptor interaction in 3D, C: Zoomed view of Ligand- receptor interaction in 2D).

From figure 2 it can be seen that Diosgenin had the highest number of interactions with FSH receptor. Various hydrophobic interactions, including π – π stacking, π – cation, and π – σ interactions were responsible for the highest binding affinity of Diosgenin. Various residues of FSH receptor interacting with ligands and the type of interactions formed are shown in table 3.

Table 3: Binding interactions between various receptor and ligands.

Residues of various receptor interacting with ligands				
	PPAR-γ receptor	**FSH receptor**	**MC4R receptor**	**Insulin receptor**
Types of Interaction	Borassoside D	Diosgenin	Diosgenin	Diosgenin
H- bond	LEU 340, SER 289	-	-	SER 1006
Vander Waals	LYS 265, VAL 290, GLU 291, SER 342, GLU 343, LEU 333, ILE 326, TYR 327, LYS 367	THR 270, PRO 272, SER 232, PHE 353, LYS 254, PRO 355, ASN 211, LEU 233, PRO 234, SER 235, LYS 260.	ILE 66, PHE 81, CYS 84, VAL 88, LEU 92, SER 85, GLY 167, ILE 170	GLU 1047
Alkyl	LEU 330, MET 364	-		-
Pi- Alkyl	ARG 288	-	TRP 174	-

Melanocortin-4 receptor

Crystal structure of the Melanocortin-4 Receptor (MC4R) in complex with SHU9119 (PDB ID: 6W25) was utilized for molecular docking. Identification of active sites is a pre-requisite requirement for appropriate molecular docking. Active sites were identified through Metapocket, since co-crystallized ligand was not available with this structure. During molecular docking a grid is to be selected which represents the area where ligands will try to interact with the protein. Center Grid box was selected using x: 167.380, y: -2.889, z: 129.061, and the number of points in all dimensions x, y, z were considered 82x66x100 Å, and the grid spacing was selected as 0. 972 Å. The binding affinities of all the phytoconstituents is given in table 2.

The Melanocortin-4 Receptor was able to interact moderately to only handful of phytoconstituents. Majority of phytoconstituents showed zero interaction due to the complex structure of the receptor and the non-compatibility and inability of ligand to fit in the active site pocket and bond formation. It is evident from the table above that Diosgenin of Trillium had highest binding affinity of -7.72 kcal/mol.

The interactions of Diosgenin with MC4R Receptor shown in figure 3 depicts major involvement of non-bonded interactions responsible for binding affinity. From the above figure it can be seen that Diosgenin had the highest number of interactions with MC4R receptor. Various hydrophobic interactions, including π – π stacking, π – cation, and π – σ interactions were responsible for the highest binding affinity of Diosgenin. Various residues of MC4R receptor interacting with ligands and the type of interactions formed are shown in table 3.

Insulin receptor

Crystal structure of the Insulin Receptor Kinase in complex with a bisubstrate inhibitor (PDB ID: 1GAG) was utilized for molecular docking. Identification of active sites is a pre-requisite requirement for appropriate molecular docking. Active sites were identified through Metapocket, since co-crystallized ligand was not available with this structure. During molecular docking a grid is to be selected which represents the area where ligands will try to interact with the protein. Center Grid box was selected using x: -26.518, y: 39.022, z: 12.690, and the number of points in all dimensions x, y, z were considered 70x56x72 Å, and the grid spacing was selected as 0. 952 Å. The binding affinities of all the phytoconstituents is given in table no: 2.

Insulin receptor was also not receptable to the chemical constituents of all three plants and showed poor binding affinity results. Diosgenin of Trillium was only chemical constituent that showed good binding affinity as compared to other constituents. It is evident from the table above that Diosgenin of Trillium had highest binding affinity of -6.96 kcal/mol followed by Pennogenin with -6.65 kcal/mol.

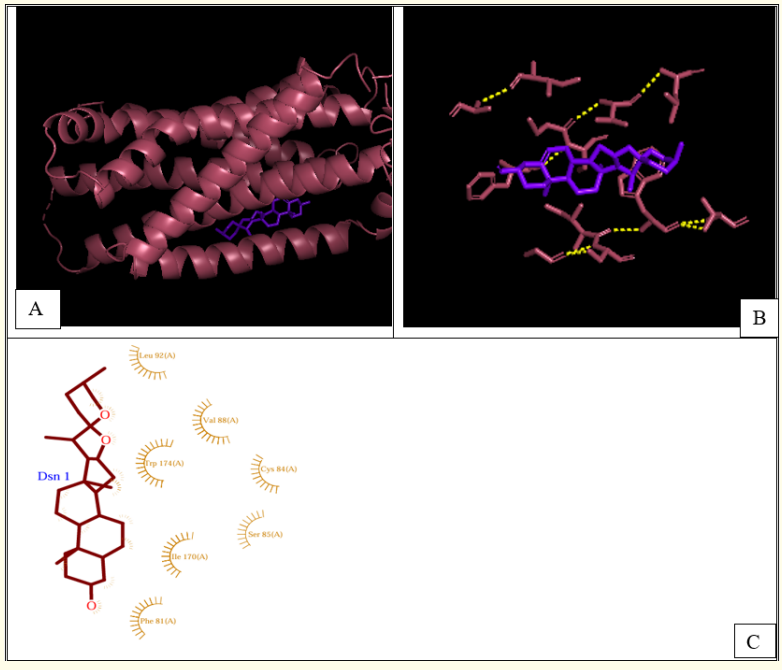


Figure 3: 2D and 3D interaction of Diosgenin with MC4R receptor (A: Full view of interaction in 3D, B: Zoomed view of Ligand- receptor interaction in 3D, C: Zoomed view of Ligand- receptor interaction in 2D).

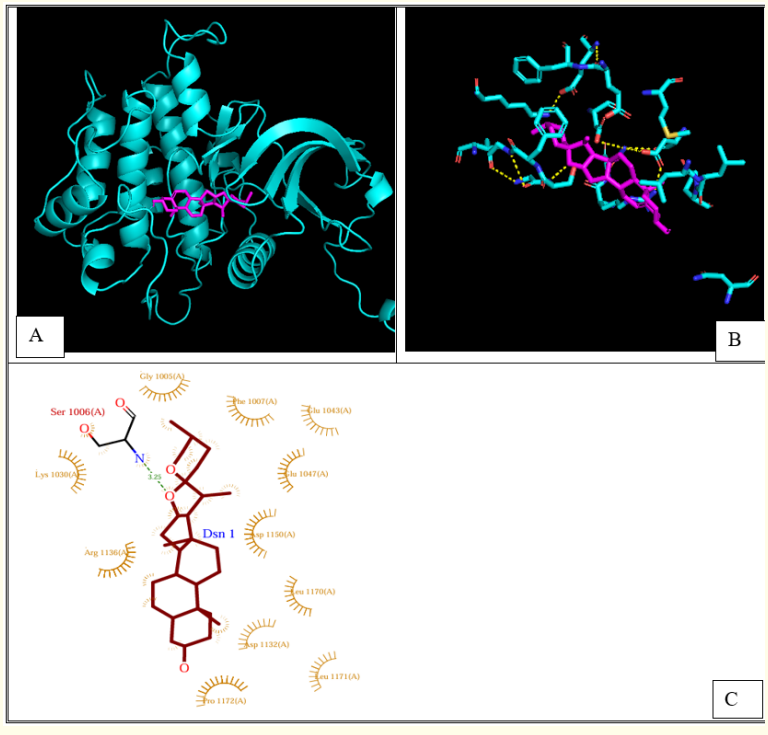


Figure 4: 2D and 3D interaction of Diosgenin with Insulin receptor (A: Full view of interaction in 3D, B: Zoomed view of Ligand-receptor interaction in 3D, C: Zoomed view of Ligand- receptor interaction in 2D).

From the above figure 4, it can be seen that Diosgenin had the highest number of interactions with Insulin receptor. It formed a hydrogen bond interaction SER 1006 residue of the receptor along with various hydrophobic interactions, including π – π stacking, π – cation, and π – σ interactions led the highest binding affinity of Diosgenin.

Network pharmacology study

Network construction and analysis

The network pharmacology analysis of *Trillium govanianum* reveals:

- Number of Nodes: 47
- Number of Edges: 59
- Average Number of Neighbors: 2.511
- Network Diameter: 3
- Network Radius: 1
- Characteristic Path Length: 1.290
- Clustering Coefficient: 0.000
- Network Density: 0.027
- Connected Components: 2

These statistics indicate a complex interaction network that may underlie the therapeutic potential of Trillium Govanianum in treating PCOS and related disorders.

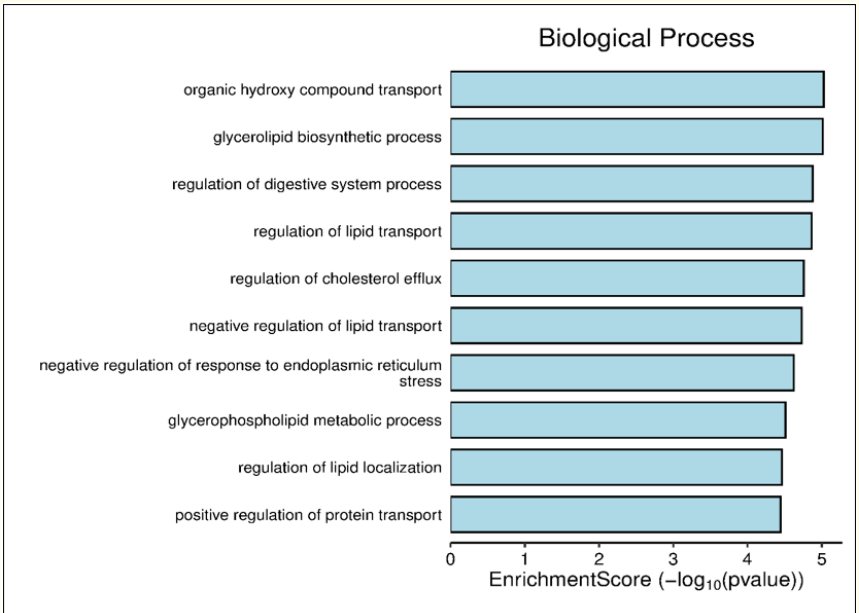
GO function enrichment analysis

The Cytoscape 3.7.2 software and R 3.6.3 software were used to analyze the potential targets of Trillium against PCOS using GO annotation analysis.

Biological processes (BP)

The biological processes are represented through various plots like bar plot, dot plot that highlights different aspects of enrichment analysis. These plots are mentioned in Figure 5.

The biological processes were enriched in organic hydroxy compound transport, glycerolipid biosynthetic process, regulation of digestive system process, regulation of lipid transport, regulation of cholesterol efflux, negative regulation of lipid transport, stress, negative regulation of response to endoplasmic reticulum, glycerophospholipid metabolic process, regulation of lipid localization and positive regulation of protein transport.



The horizontal axis of the bar plot represents the number of genes enriched in each biological process mentioned on vertical axis.

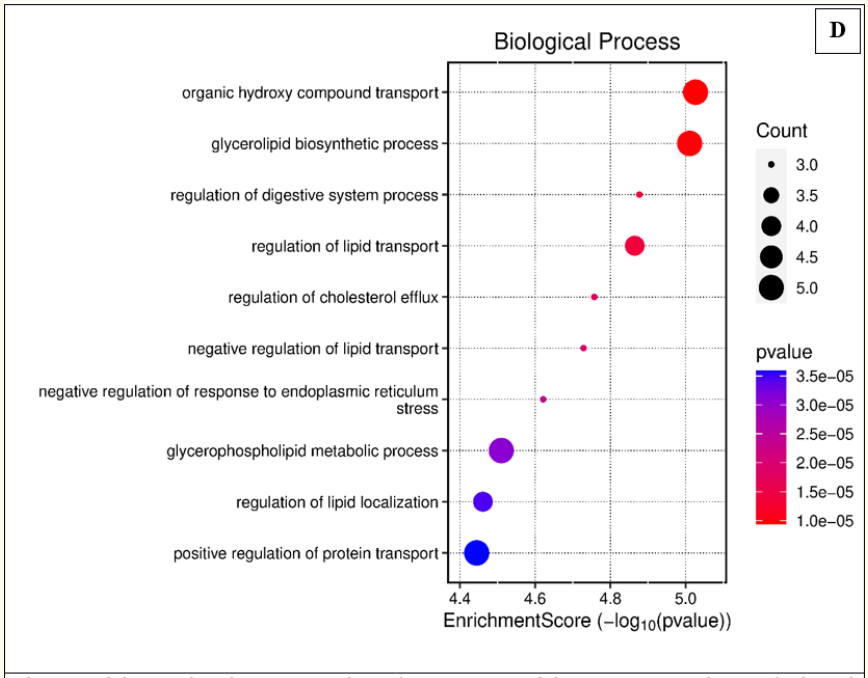
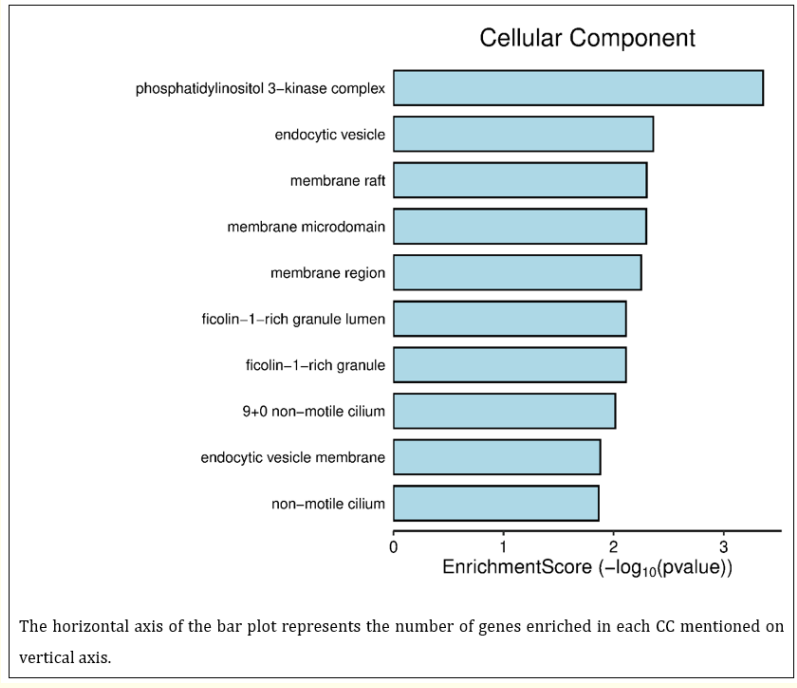


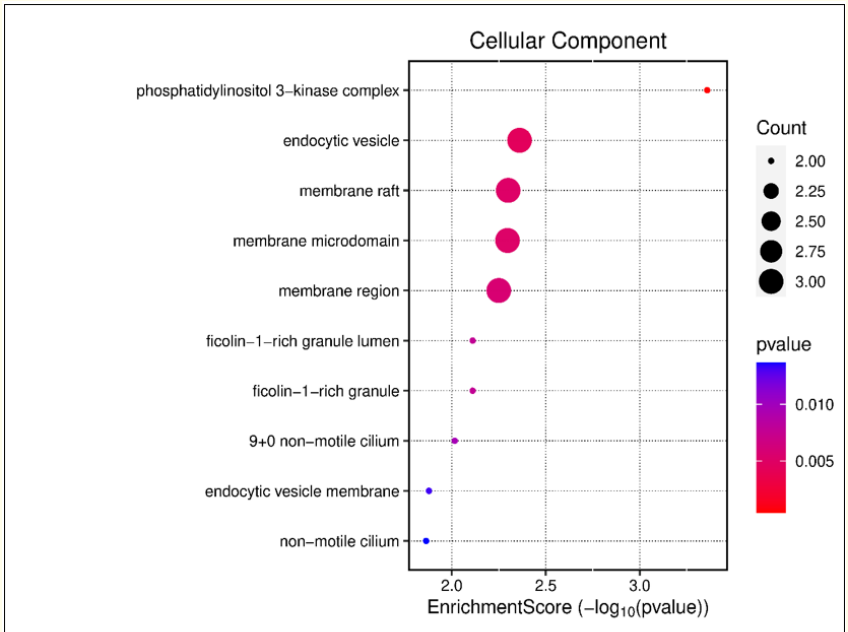
Figure 5: Different types of plots showing enriched BP for Trillium (Top to bottom: Bar plot and dot plot).

Cellular components (CC)

The Cellular Components are represented through various plots like bar plot, dot plot that highlights different aspects of enrichment analysis. These plots are mentioned in Figure 6.

The Cellular Components were enriched in phosphatidylinositol 3-kinase complex, endocytic vesicle, membrane raft, membrane microdomain, membrane region, ficolin–1–rich granule lumen, ficolin–1–rich granule, 9+0 non–motile cilium, endocytic vesicle membrane, non–motile cilium.





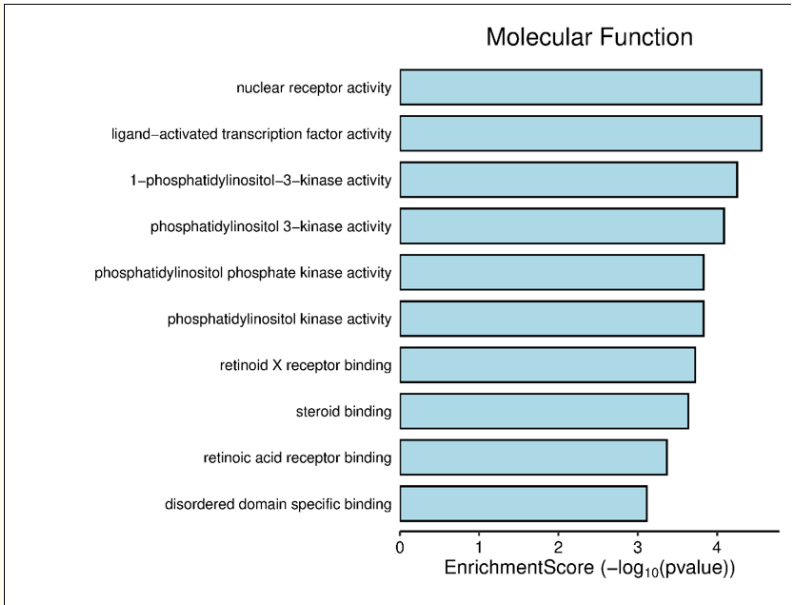
The size of dots in dot plot corresponds to the percentage of dataset genes overlapping cellular components versus all genes in CC and the colour represents adjusted p-value.

Figure 6: Different types of plots showing enriched CC for Trillium (Top to bottom: Bar plot and dot plot).

Molecular functions (MF)

The Molecular Functions are represented through various plots like bar plot, dot plot that highlights different aspects of enrichment analysis. These plots are mentioned in Figure 7.

The Molecular Functions were enriched in nuclear receptor activity, ligand−activated transcription factor activity, 1−phosphatidylinositol−3−kinase activity, phosphatidylinositol 3−kinase



The horizontal axis of the bar plot represents the number of genes enriched in each MF mentioned on vertical axis.

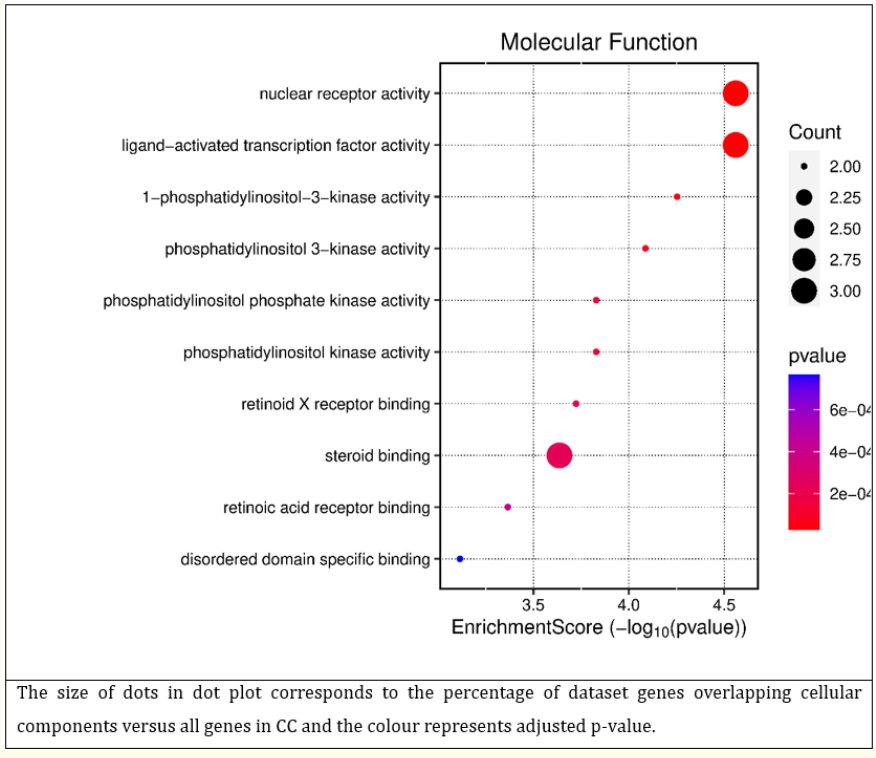


Figure 7: Different types of plots showing enriched MF for Trillium (Top to bottom: Bar plot and dot plot).

activity, phosphatidylinositol phosphate kinase activity, phosphatidylinositol kinase activity, retinoid X receptor binding, steroid binding, retinoic acid receptor binding and disordered domain specific binding.

Genes involved in BP, CC and MF enrichment analysis are mentioned in table 4.

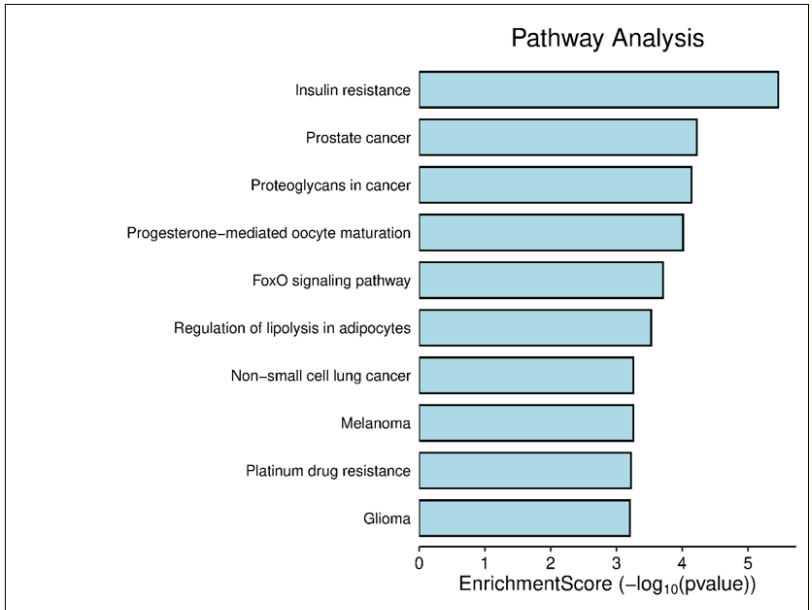
Table 4: Genes involved in GO enrichment analysis for Trillium.

Biological Processes	SMO, MDM2, PTPN1, TRPV1, PIK3CD, PIK3CB, CRHR1, SHH, NR1H2, NR1H4, NR1H3
Cellular Component	PTGS1, CFD, SHH, KCNA3, HSP90AA1, SMO, MDM2, PIK3CD, PIK3CB
Molecular Function	HSP90AA1, MDM2, HSD11B1, PIK3CD, PIK3CB, NR1H2, NR1H4, NR1H3.

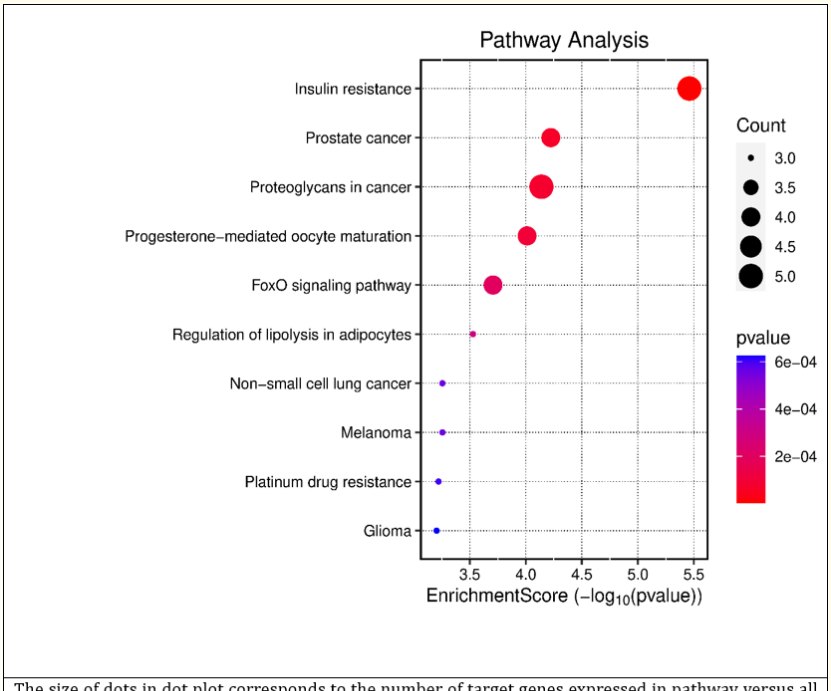
KEGG pathway enrichment analysis

KEGG pathway enrichment are represented through various plots like bar plot and dot plot that highlights different aspects of enrichment analysis. These plots are mentioned in Figure 8.

The related pathway of Trillium was obtained through KEGG enrichment analysis. 136 signaling pathways were discovered, and the top 10 along with genes involved in specific pathway enrichment are shown in table 5.



The Y-axis represents the significant pathway of the target genes, and the X-axis shows the enrichment factor. The enrichment factor represents the ratio of the number of target genes belonging to a pathway to the total number of all annotated genes located in the pathway. A higher enrichment factor represents a higher enrichment level.



The size of dots in dot plot corresponds to the number of target genes expressed in pathway versus all annotated genes located in pathway and the colour represents adjusted p-value.

Figure 8: Different types of plots showing enriched KEGG pathways for Trillium (Top to bottom: Bar plot and dot plot).

Table 5: Genes involved and enriched in KEGG pathways for Trillium.

ID	Pathway	Genes involved in pathway
hsa04931	Insulin resistance	NR1H3/PTPN1/NR1H2/PIK3CB/PIK3CD
hsa05215	Prostate cancer	MDM2/PIK3CB/PIK3CD/HSP90AA1
hsa05205	Proteoglycans in cancer	MDM2/SMO/SHH/PIK3CB/PIK3CD
hsa04914	Progesterone-mediated oocyte maturation	PLK1/PIK3CB/PIK3CD/HSP90AA1
hsa04068	FoxO signaling pathway	MDM2/PLK1/PIK3CB/PIK3CD
hsa04923	Regulation of lipolysis in adipocytes	PIK3CB/PIK3CD/PTGS1
hsa05218	Melanoma	MDM2/PIK3CB/PIK3CD
hsa05223	Non-small cell lung cancer	ALK/PIK3CB/PIK3CD
hsa01524	Platinum drug resistance	MDM2/PIK3CB/PIK3CD
hsa05214	Glioma	MDM2/PIK3CB/PIK3CD

Active ingredient-target-disease network

An active ingredient target-disease network was constructed for the phytoconstituents of Trillium as shown in figure 9. Network analysis was done for six steroidal saponins namely Diosgenin, Pennogenin and Pennogenin-3-O-beta-D-glucopyranoside (P3D). The network was constructed and analyzed to elucidate the possible interaction of above mentioned phytoconstituents and target proteins of PCOS.

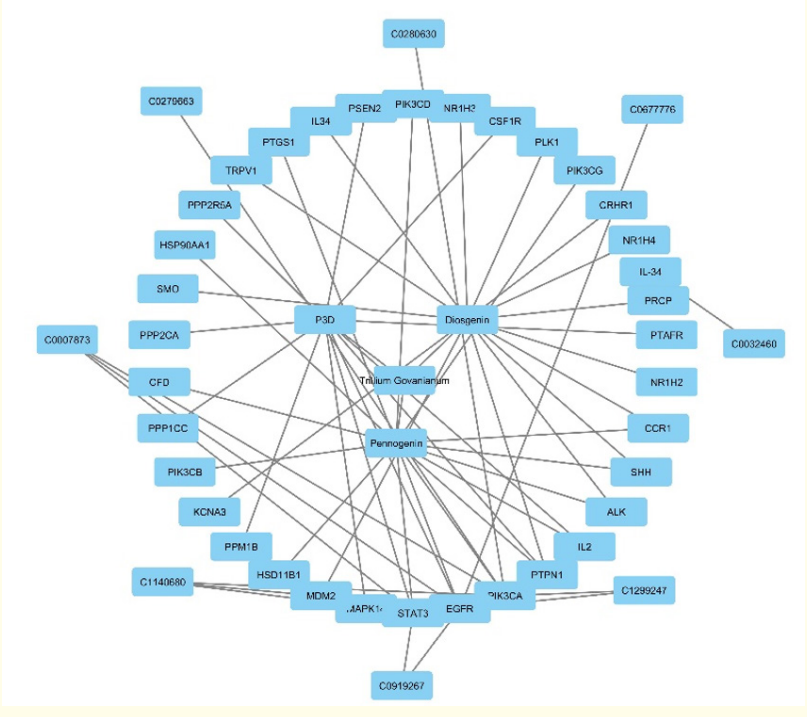


Figure 9: Active Ingredient – Target disease network for phytoconstituents of Trillium.

Diosgenin

Diosgenin is associated with several targets, including:

- IL-34: Linked to Polycystic Ovary Syndrome (PCOS).
- NR1H3, ALK, PTPN1, MDM2, KCNA3, CCR1, SMO, TRPV1, SHH, PLK1, CRHR1, PRCP: These targets are involved in various cellular processes and signaling pathways that may influence reproductive health and hormonal balance.

Pennogenin

Pennogenin has a broader range of interactions, including:

- IL-2: Associated with immune response.
- STAT3: Linked to ovarian neoplasms and other cancers.
- SHH, HSD11B1, PIK3CB, ALK, CCR1, PTPN1, CFD, HSP90AA1, PTGS1, PIK3CD, PIK3CG, PIK3CA, EGFR: These targets are implicated in cancer development, hormonal regulation, and metabolic processes.

Pennogenin and Pennogenin-3-O-beta-D-glucopyranoside (P3D)

P3D interacts with several important targets, such as:

- IL-2, STAT3, PTPN1, PTAFR, EGFR, MAPK14, PPM1B, PPP1CC, PPP2CA, PPP2R5A, PSEN2, CSF1R: These targets are involved in critical signaling pathways related to cell proliferation, apoptosis, and inflammation.

Discussion

In the current work an *in-silico* analysis of phytoconstituents of Trillium as potential therapeutic was done for PCOS. Receptors responsible for regulating hormone levels [Peroxisome proliferator-activated receptor-gamma, Progesterone receptor, Estrogen receptor (alpha), Androgen receptor, Follicle Stimulating Hormone Receptor], insulin levels [Insulin receptor] and obesity [Leptin receptor, and Melanocortin-4 Receptor (MC4R)] were selected as targets for *in-silico* studies representing the multi-facet dimension of the disease.

There are different types of interactions between the ligands and the residues of the protein receptor within the binding pocket. Organic compounds can link to proteins via creating chemical bonds and non-covalent interactions between the ligand and the protein [19]. Hydrogen bond interaction and hydrophobic interactions are significant factors considered during the assessment of binding affinity and capability of new drug molecule to exhibit certain pharmacological properties. Hydrogen bond interactions are not only common linking bonds but also play a crucial role in ligand-receptor interaction while maintaining the integrity of the receptor and protein and also in enhancing various biological functions [19-21]. The bond is assumed to be hydrogen if a valid hydrogen bond acceptor–donor pair is within the correct distance. The stronger the hydrogen bond, the closer it gets to perfect geometry [22].

The close contact of the lipophilic group of the ligand with the non-polar amino acid side chain of the receptor results in formation of hydrophobic interactions. The binding affinity of ligand towards the substantial lipophilic groups is due to hydrophobic interactions. Hydrophobicity impacts a variety of biological processes, including biological molecule transport, distribution, and metabolism; molecular recognition; and protein folding. Higher the hydrophobic interactions distance, higher the bioavailability of ligand [23,24].

In case of phytoconstituents of Trillium, it was observed that interactions were predominantly non-covalent in nature that specifically caters to the function of maintaining 3D structures of large molecules. These interactions indicate that both ligand and receptor are large molecules and are transiently bound to each other to produce pharmacological or biological response. These are relatively weak electric forces that attract neutral molecules. These forces can be seen in the complex formed between Borassoside D with PPRA-γ and Pennogenin-3-O-beta-D-glucopyranoside with FSHR. The other hydrophobic interactions majorly contributed to the moderate binding affinity of Diosgenin and Pennogenin with receptors like MC4R and Insulin receptor. It was observed that phytoconstituents of Trillium were not receptive towards hormonal receptors except FSHR and also the number of phytoconstituents interacted were quite few.

It was also noted during the studies, phytoconstituents belonging to same class of constituents exhibited best binding affinity towards receptors. Similarly phytoconstituents of Trillium were found to be from category of Steroidal Saponins.

The concept of network pharmacology enables researchers to decode the probable underlying mechanism of action of drug or phy-

toconstituents against a disease. Network pharmacology primarily involves mining all set of genes involved directly or indirectly in the pathogenesis of a particular disease. It then proceed with the network construction and its analysis, where by interaction of all the phytoconstituents of a plant with the set of genes is studied and the phytoconstituents relaying maximum and significant interaction are screened.

GO enrichment analysis and KEGG pathway enrichment anlaysis are the next crucial elements of network pharmacology that primarily aids in deciphering the probable mechanism of action of phytoconstituent against the disease. GO enrichment analysis is a technique used to identify which biological processes, cellular components, or molecular functions (based on Gene Ontology - GO) are significantly overrepresented within a set of genes of interest. KEGG pathway enrichment primarily focuses at the identified pathways with significantly low p-values, which indicate that a group of genes from disease relevant dataset are over expressed in that pathway compared to the whole genome, suggesting a potential biological process or mechanism is enriched in concerned data.

KEGG pathway enrichment analysis for Trillium throws light on three major pathways enriched with set of genes, making them the favourable for modulation via phytoconstituents of Trillium to exert activity against PCOS. The major affected pathways involve Insulin resistance, FOXO signaling pathway and Progesterone-mediated Oocyte Maturation. Genes like ALK, PTGS1, HSP90AA1, PTPN1, PIK3CB are overexpressed in Insulin resistance pathway. Insulin resistance is a critical factor in PCOS. Overexpression of genes like ALK and PIK3CB can amplify insulin signaling, leading to hyperinsulinemia. This, in turn, exacerbates androgen production in ovaries and contributes to irregular menstrual cycles and other PCOS symptoms. FOXO Signaling pathway is affected by overexpression of PIK3CD, PIK3CB, HSP90AA1, MDM2 genes. The FoxO pathway is crucial for cellular homeostasis and metabolism. Overexpression in this pathway can disrupt the balance, leading to metabolic issues, including insulin resistance, often seen in PCOS patients. Progesterone-mediated Oocyte Maturation is affected by overexpression of PLK1, SMO, SHH genes, there by disrupting the balance, leading to metabolic issues, including insulin resistance, often seen in PCOS patients. Genes like PIK3CD, PIK3CB and PTPN1 are overexpressed in lipolysis regulating pathway in adipocytes directly affecting the lipid profile and weight fluctuation in PCOS patients. The phytoconstituents of Trillium may exert their therapeutic effect by modulating these enriched genes and pathways. These probable mechanisms and pathways align with the observations during *in-silico* molecular docking experiment, where by it phytoconstituents showed highest binding affinity towards Insulin receptor and MC4R receptor representing the obesity factor. During *in-vivo* experiment it was observed that TRS extract was able to restore glucose level and body weight to normal level and significantly reduce the lipid levels as compared to PCOS group.

Conclusion

In-silico studies were done by docking the phytoconstituents of all three plants on different types of receptors. Receptors responsible for regulating hormone levels [Peroxisome proliferator-activated receptor-gamma, Progesterone receptor, Estrogen receptor (alpha), Androgen receptor, Follicle Stimulating Hormone Receptor], insulin levels [Insulin receptor] and obesity [Leptin receptor, and Melanocortin-4 Receptor (MC4R)] were selected as targets for *in-silico* studies representing the multi-facet dimension of the disease. The phytoconstituents of Trillium were found to have highest binding affinity towards receptors regulating insulin and obesity. Network pharmacology studies showed the possible interaction and modulation of phytoconstituents of Trillium on overexpressed genes and pathways relevant to PCOS.

The studies concluded that the phytoconstituents of Trillium prominently modulate overexpressed genes in insulin resistance, lipolysis and adipocytes there by providing relief from insulin resistance, elevated body weight and lipid levels. Molecular docking studies showed the stronger interaction of phytoconstituents of Trillium with insulin and obesity related receptors whereas network pharmacology supported the findings obtained from molecular docking by throwing light on the possible mechanism of action by modulating genes involved in insulin resistance and obesity. Hence it can be concluded that phytoconstituents of Trillium can act as a potential therapeutic for PCOS by majorly controlling insulin resistance and obesity along with minor modulation of hormones.

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Conflict of Interest

None.

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