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RESEARCH ARTICLE

In Silico Molecular Docking Study on *Soothagathai Udaika Kudineer:* A *Siddha* Medicine with the Target CYP-17-α Hydroxylase against Poly Cystic Ovarian Syndrome

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Abstract

Background: Poly Cystic Ovarian Syndrome (PCOS), in *Siddha* terms known as *Sinaipaineer Katti*, is the most common hormonal and reproductive disorder affecting women. It is marked by hyperandrogenism, hyperinsulinemia, and menstrual irregularities and also significantly contributes to mental health issues. The genes CYP11A, CYP17, and CYP19 encode enzymes involved in the steroidogenic pathway and are considered potential contributors to PCOS, leading to hyperandrogenism. Persistent hyperandrogenism causes menstrual irregularities such as amenorrhea (*Soothaga Thadai*), chronic anovulation, hirsutism, and infertility. **Aim:** To evaluate the interaction between phytoconstituents of the *Siddha* formulation *Soothagathai Udaika Kudineer* (SUK) and the target enzyme CYP-17α-hydroxylase through molecular docking analysis. **Methods:** A docking study was carried out to assess the interaction of nine phytocompounds found in SUK with CYP-17α-hydroxylase. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added using Auto Dock tools. **Results:** The bioactive compounds present in SUK- Chlorogenic acid, gingerenone-A, kaempferitrin, diosgenin, and oleic acid- showed interaction with the active amino acids of CYP-17α-hydroxylase and exhibited strong binding affinity toward the enzyme. **Conclusion:** The inhibition of the target enzyme by these compounds suggests that they may act as therapeutic agents in the management of PCOS. SUK could serve as a safer alternative to existing treatments, which are often less effective or associated with adverse effects.

Major Findings: Key phytoconstituents in SUK showed strong binding with CYP-17α-hydroxylase, indicating their potential to inhibit androgen synthesis and help manage PCOS symptoms.

Keywords: CYP-17-α Hydroxylase, Docking Study, PCOS, *Soothagathai Udaika Kudineer (SUK)*, *Siddha* Polyherbal Formulation

Abbreviations: ALA- Alanine; Å- A unit of length equal to 10^{-10} meters; ARG- Arginine; ASN- Asparagine; ASP- Aspartic acid; CYP11A- Cytochrome P450 side-chain cleavage enzyme; CYP17- Cytochrome P450 17α-hydroxylase/17,20-lyase; CYP19- Aromatase; GLU- Glutamic acid; GLY- Glycine; g/mol- Grams per mole; ID- Identifier; ILE- Isoleucine; Ki- Inhibition Constant; LEU- Leucine; LH- Luteinizing Hormone; LYS- Lysine; PDB- Protein Data Bank; PDBQT- Protein Data Bank, with charges and torsions; PHE- Phenylalanine; PCOS- Polycystic Ovary Syndrome; SDF- Structure Data File; SUK- *Soothagathai Udaika Kudineer*; THR- Threonine; TYR- Tyrosine; VAL- Valine; 2D/3D- Two-dimensional or Three-dimensional.

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1. Introduction

Polycystic ovary syndrome (*Sinaipaineerkatti)*, driven primarily by the greater activity of cytochrome P450 17alpha-hydroxylase (CYP17), is essential in the synthesis of androgens. In women with PCOS, enhanced expression of the cell surface-gene CYP17 has been reported in Theca interna cells, partly due to different transcriptional regulation of the CYP17 gene. Recent research has explored whether this upregulation is also influenced by posttranscriptional mechanisms affecting mRNA stability. These genes encode enzymes implicated in the steroidogenic pathway and therefore are very likely to contribute to PCOS. Of these, the most studied include CYP11A (side-chain cleavage enzyme), CYP17 (cytochrome P450 17-hydroxylase/17, 20-desmolase), and CYP19 (aromatase). The studies show that ovarian theca cells in women with PCOS have an overexpression of enzymes involved in androgen biosynthesis [1,2]. Therefore, alterations in their synthesis lead to hyperandrogenism, which is one of the principal pathophysiological features of PCOS.

It causes chronic anovulation, irregular menstrual cycle, hirsutism, and infertility. By inhibiting this enzyme activity, the synthesis of androgens can be blocked in the ovary. The present treatments available for PCOS are either side effects or not effective. It leads to symptoms such as chronic anovulation, irregular menstrual cycle, hirsutism, and infertility. Androgen synthesis in the ovary can be prevented by inhibiting this enzyme activity. Current treatments for PCOS are either ineffective or have adverse effects. Siddha medicine is one of the oldest traditional healing systems in India, and it provides holistic treatments for various diseases, including PCOS. *Soothagathai Udaika Kudineer*[3] is one of the most well-known polyherbal formulations in the Siddha system. It has been used traditionally to manage symptoms like menstrual irregularities and hyperandrogenism, but the pharmacological basis of its efficacy is not well explored.

The bioactive compounds present in the ingredients of *Soothagathai Udaikka Kudineer* were evaluated with respect to their interaction with the target enzyme CYP-17α-hydroxylase, which is an important enzyme involved in androgen biosynthesis.

Inhibition of this enzyme may reduce hyperandrogenism, which is primarily responsible for symptoms caused by PCOS. Herbal ingredient composition of *Soothagathai Udaikka Kudineer* includes *Trachyspermum ammi* (L.) (Omam), *Anethum graveolens* (L.) *(Sathakuppai), Bambusa vulgaris* (L.) *(Moongil), Crataeva magna* Lour DC *(Maavilangu), Smilax china* (L.) *(Parangipattai), Zingiber officinale* Roscoe *(Chukku), Piper longum* (L.) *(Thippli), Plumbago zeylanica* (L.) *(Kodiveli), Nigella sativa* (L.) *(Karunjeeragam).* These herbs have been recorded for their medicinal properties in the classical text book of *Siddha*, *Gunapadam Mooligaivaguppu.* In an attempt to estimate the therapeutic utility of *Soothagathai Udaikka Kudineer*, this research involves the estimation of the major binding affinities of the primary phytoconstituents through molecular docking towards CYP-17α-hydroxylase in relation to PCOS. This shall provide scientific grounds for *Siddha* formulations used to treat PCOS and would gain further insights regarding their mechanism of action.

2. Materials and Methods

This molecular screening, docking analysis was done through AutoDock Version 4.2.6, a well- known technique which was used for calculating the potential energy of binding between the 3D structure of each ligand and the target protein. The *in silico* molecular docking was performed with all the active compounds from the herbal formulation of *Soothagathai Udaikka Kudineer*, which will target CYP-17α-hydroxylase [PDB – 3RUK] - the major enzyme involved in PCOS.

2.1 Selection and Preparation of Ligands

Nine bioactive compounds from the crude drugs of *Soothagathai Udaikka Kudineer* (SUK)—namely β-pinene, anethole, chlorogenic acid, gingerenone-A, kaempferitrin, diosgenin, piperine, oleic acid, and plumbagin—were selected based on published research papers and standard databases. These ligands were retrieved in SDF format from a public chemical compound database. Subsequently, they were converted into PDB format using the Open Babel program. The torsional requirements necessary for proper binding were defined using the AutoDock 4.2.6 application.

Table 1 lists the vernacular and botanical names of the selected ligands from the raw drugs used in

Soothagathai Udaikka Kudineer, along with their PubChem ID. Table 2 provides further details on the selected ligands, including their molar weight (g/mol), molecular formula, hydrogen bond donors and acceptors, and the number of rotatable bonds.

2.2 Preparation, Dosage, and Indication of *Soothagathai Udaikka Kudineer*

To prepare *Soothagathai Udaikka Kudineer*, 4 grams of each raw ingredient is coarsely pounded and added to 650 ml of water. The mixture is boiled until it reduces to 165 ml, resulting in a decoction. For human consumption, the recommended dosage is 165 ml for three consecutive days. This traditional *Siddha* medicine is primarily indicated for treating *Soothagathadai* (Amenorrhea).

2.3. Protein Preparation

Figure 1: Three-dimensional crystal structure of CYP-17α-hydroxylase (PDB-3RUK) obtained from the Protein Data Bank (PDB), energy-minimized, and prepared in the appropriate PDBQT format. Ligand molecules were placed, oriented, and randomly torsioned. All rotatable torsions were free during docking. 2 independent runs per docking experiment were set up to terminate after a maximum of 250,000 energy evaluations. The population size was set at 150 with a translational step of 0.2 Å and quaternion and torsion steps of 5 applied during the search.

The protein structures were cleaned by removing the lead compounds that were present, breaking the water molecules, and including polar hydrogens in the computation of the charges of Kollman, and then defining the combination of non-polar interactions and flexible rotatable bonds by utilizing AutoDock 4.2.6 software[13,14].

The molecular docking studies were performed against the CYP-17α-hydroxylase [PDB ID: 3RUK] that targeted selected ligands including β-pinene, anethole, chlorogenic

Table 1. Selected ligands for docking from the raw drugs of the selected herbs

Vernacular Name (Tamil)	Botanical Name of the Herbs	Selected Phytochemicals	pubchem ID
Omam	*T. ammi*(L.)[4]	β-pinene	14896
Sathakuppai	*A. graveolens* (L.)[5]	Anethole	637563
Moongil	*B. vulgaris* (L.)[6]	Chlorogenic acid	1794427
Maavilangu	*C. magna* (Lour.) DC[7]	Diosgenin	99474
Parangipattai	*S. china* (L.)[8]	Kaempferitrin	5486199
Chukku	*Z. officinale* Roscoe[9]	Gingerenone-A	5281775
Thippli	*P. longum* (L.)[10]	Piperine	638024
Kodiveli	*P. zeylanica* (L.)[11]	Plumbagin	10205
Karunjeeragam	*N. sativa* (L.)[12]	Oleic acid	445639

Table 2. Properties of ligands selected for docking analysis

Compound	Molar Weight (g/mol)	Molecular Formula	H Bond Donor	H Bond Acceptor	Rotatable Bonds
β-pinene	136.23	$C_{10}H_{16}$	0	0	0
Anethole	148.20	C10H12O	0	1	2
Chlorogenic acid	354.31	C16H18O9	6	9	5
Diosgenin	414.6	$C_{27}H_{42}O_3$	1	3	0
Kaempferitrin	578.5	$C_{27}H_{30}O_{14}$	8	14	5
Gingerenone-A	356.4	$C_{21}H_{24}O_5$	2	5	9
Piperine	285.34	$C_{17}H_{19}NO_3$	0	3	3
Plumbagin	188.182	$C_{11}H_8O_3$	1	3	0
Oleic acid	282.5	$C_{18}H_{34}O_2$	1	2	15

acid, gingerenone-A, kaempferitrin, diosgenin, piperine, oleic acid, and plumbagin. These compounds are each unique in terms of their 2-dimensional and 3-dimensional structural characteristics, as presented in Figure 2.

For example, β-Pinene is a bicyclic monoterpene that demonstrates alternating single and double bonds in its 2D structure, while the 3D model displays its rigid chirality. Anethole is a phenylpropene that has a planar 2D structure, but its 3D arrangement shows the extended propenyl group. Chlorogenic acid, the result of caffeic and quinic acids combining, has a complex 3D configuration with ester bonds and hydrogen interactions, while gingerenone-A, a chalcone derivative, presents a planar configuration favourable to stacking interactions. Kaempferitrin, flavonoid glycoside, presents bulky sugar units protruding out from the core flavonoid, thus affecting solubility. Diosgenin, a steroidal saponin that contains a spirostane skeleton, displays a rigid, much less flexible 3D structure for steroids. Piperine, an alkaloid, exhibits semi-flexibility in its 3D conformation, influenced by its piperidine ring. Oleic Acid's 3D structure reveals a "kinked" conformation due to its cis double bond, affecting its interaction with membranes. Finally, plumbagin, a naphthoquinone, displays a rigid planar naphthalene structure with projecting functional groups.

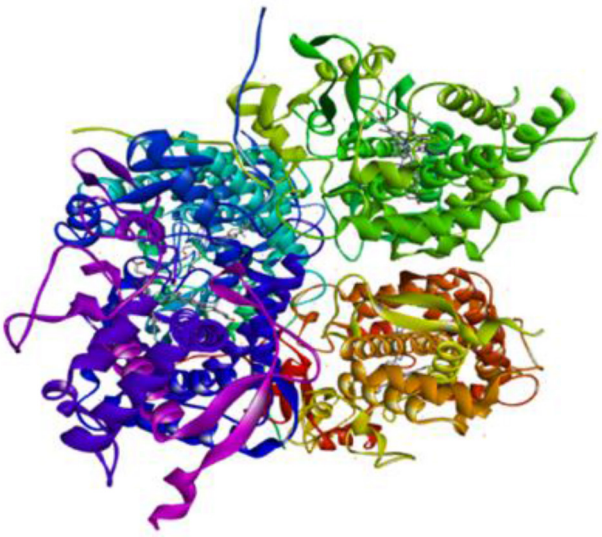


Figure 1. 3D- structure of CYP- 17α-hydroxylase [PDB ID - 3RUK].

2.4 Molecular Docking Methodology[15,16]

Immediately after preparing the target protein and choosing the ligand, molecular docking was attained using Autodock 4.2.6. The values for x, y, and z were set to be 60×60×60 with a resolution of 0.375 Å for generating binding. To get the best docking configurations, the grid box was centred. After preparing the (.gpf) file from the grid file, autogrid should be launched. A preset run count of 10 was used by applying the Lamarckian genetic technique to the computation of docking. After carrying out auto-dock, a PDF file containing the dock results was saved. A (.dlg) file with the final findings on the inhibition constant, the binding energy and residues, indicated the docked findings. Interactions between the ligands and proteins were also seen in structures through Discovery studio.

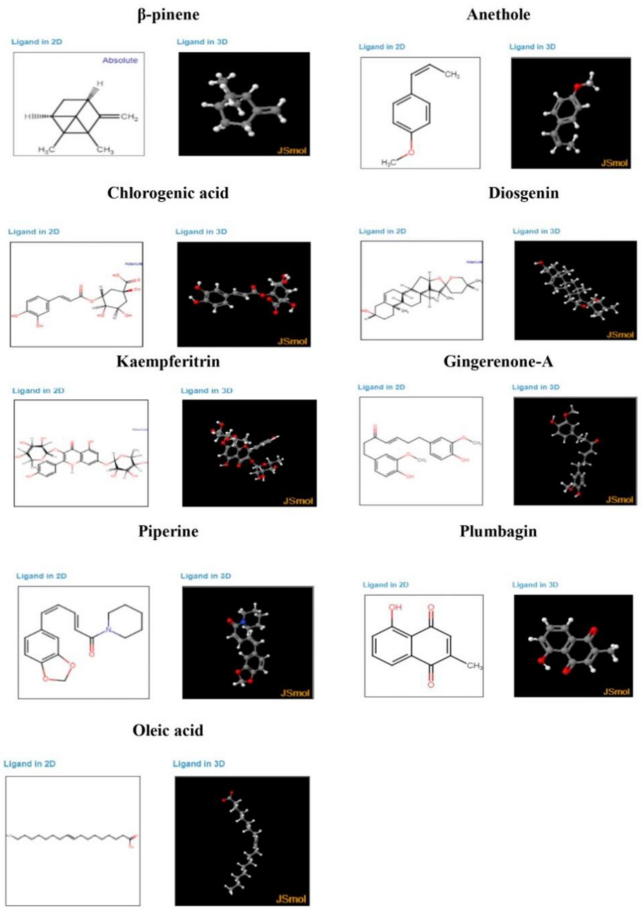


Figure 2. 2D and 3D structure of selected bioactive molecules.

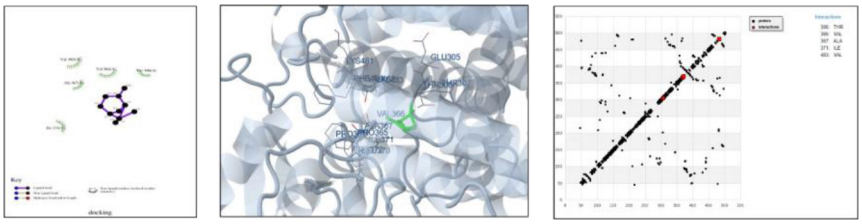


Figure 3. Docking pose of the selected bio-active molecules of the ingredients of the *Soothagathai Udaikka Kudineer.* **(a).** Interactions in 2D and 3D, Hydrogen bond plotting of β-pinene with CYP-17α- hydroxylase [PDB ID 3RUK].

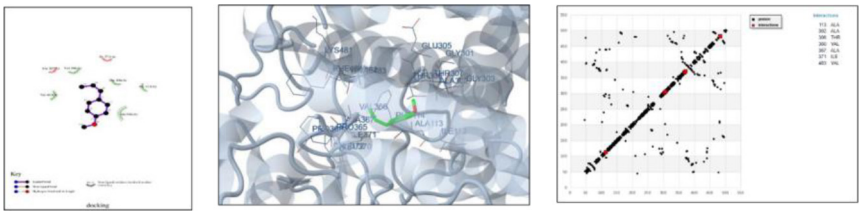


Figure 3(b). Interactions in 2D and 3D, hydrogen bond plotting of anethole with CYP-17α- hydroxylase [PDB ID 3RUK].

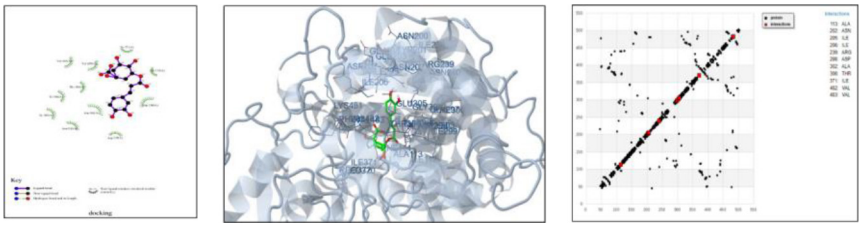


Figure 3(c). Interactions in 2D and 3D, hydrogen bond plotting of chlorogenic acid with CYP-17α- hydroxylase [PDB ID 3RUK].

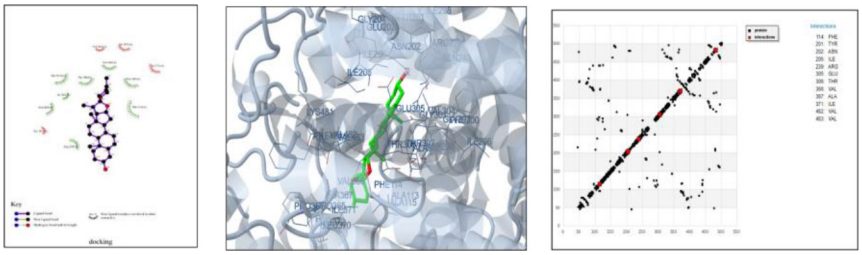


Figure 3(d). Interactions in 2D and 3D, hydrogen bond plotting of diosgenin with CYP-17α- hydroxylase [PDB ID 3RUK].

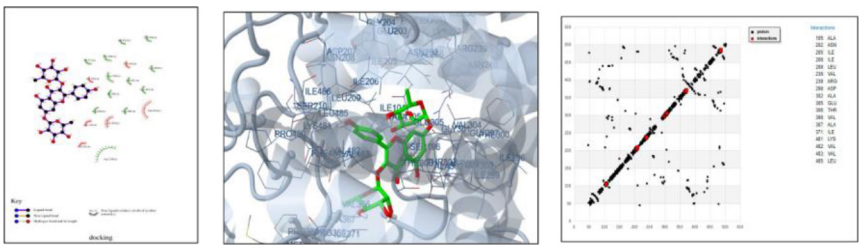


Figure 3(e). Interactions in 2D and 3D, hydrogen bond plotting of kaempferitin with CYP-17α-hydroxylase [PDB ID 3RUK].

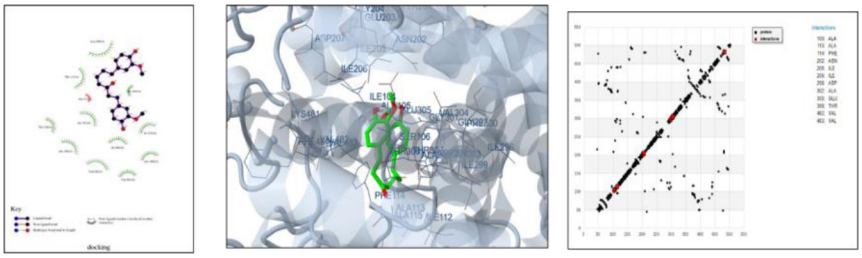


Figure 3(f). Interactions in 2D and 3D, hydrogen bond plotting of gingerenone-A with CYP-17α-hydroxylase [PDB ID 3RUK].

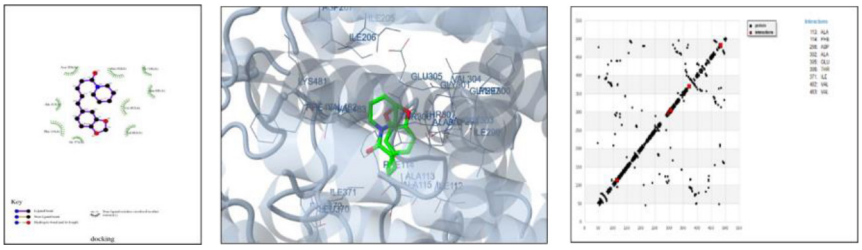


Figure 3(g). Interactions in 2D and 3D, hydrogen bond plotting of piperine with CYP-17α-hydroxylase [PDB ID 3RUK].

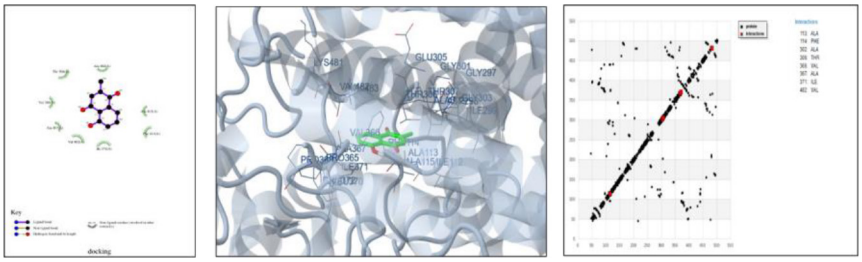


Figure 3(h). Interactions in 2D and 3D, hydrogen bond plotting of plumbagin with CYP-17α-hydroxylase [PDB ID 3RUK].

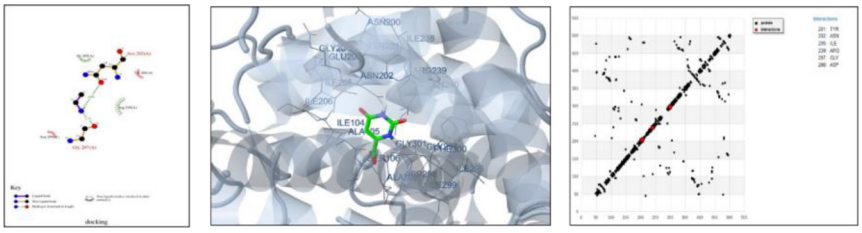


Figure 3 (i). Interactions in 2D and 3D, hydrogen bond plotting of oleic acid with CYP-17α-hydroxylase [PDB ID 3RUK].

3. Results and Discussion[13-16]

Figure 3 represents the docking poses, 2D interaction plot analysis, and hydrogen bond interactions of all ligands together with an analysis of the core amino acids that were involved in the interaction with the target receptor, CYP-17α-hydroxylase [PDB ID: 3RUK]. Table 3 summarizes the molecular docking results of the bioactive molecules against CYP-17α-hydroxylase [PDB ID: 3RUK] in terms of estimated binding free energy, inhibition constant (Ki), electrostatic energy, total intermolecular energy, and interaction surface. Table 4 shows the amino acid residues involved in the interactions of the bioactive molecules with CYP-17α-hydroxylase [PDB ID: 3RUK].

Table 3. Summary of the molecular docking studies of the selected bioactive molecules against CYP-17α-hydroxylase [PDB ID: 3RUK]

Compounds	Est. Free Energy of Binding (kcal/mol)	Est. Inhibition Constant, Ki	Electrostatic Energy (kcal/mol)	Total Intermolec. Energy (kcal/mol)	Interact. Surface
Diosgenin	-11.08	7.51 nM	-11.30	-0.08	668.205
Gingerenone-A	-9.49	110.89 nM	-8.52	-0.04	553.725
Piperine	-8.61	486.59 nM	-8.18	-0.01	467.409
Chlorogenic acid	-8.12	1.13 uM	-7.54	-0.12	530.094
β-pinene	-6.25	26.21 uM	-6.24	-0.01	342.791
Plumbagin	-6.14	31.66 uM	-6.46	-0.03	369.409
Anethole	-5.29	132.44 uM	-5.89	-0.02	377.378
Oleic acid	-3.91	1.35 mM	-4.20	-0.01	303.979
Kaempferitrin	-2.86	8.05 mM	-6.12	-0.10	779.396

Table 4. Amino acid residue interaction of selected bioactive molecules against CYP-17α-hydroxylase [PDB ID: 3RUK]

Ligands	Inter actions	Amino Acid Residues																	
β-pinene	0	306 THR	366 VAL	367 ALA	371 ILE	483 VAL													
Anethole	0	113 ALA	302 ALA	306 THR	366 VAL	367 ALA	371 ILE	483 VAL											
Chlorogenic acid	2	113 ALA	202 ASN	205 ILE	206 ILE	239 ARG	298 ASP	302 ALA	306 THR	371 ILE	482 VAL	483 VAL							
Gingerenone-A	2	105 ALA	113 ALA	114 PHE	202 ASN	205 ILE	206 ILE	298 ASP	302 ALA	305 GLU	306 THR	482 VAL	483 VAL						
Kaempferitrin	3	105 ALA	202 ASN	205 ILE	206 ILE	209 ILE	236 VAL	239 ARG	298 ASP	302 ALA	305 GLU	306 THR	366 VAL	367 ALA	371 IL	481 LYS	482 VAL	483 VAL	485 LEU
Diosgenin	2	114 PHE	201 TYR	202 ASN	205 ILE	239 ARG	305 GLU	306 THR	366 VAL	367 ALA	371 ILE	482 VAL	483 VAL						
Piperine	0	113 ALA	114 PHE	298 ASP	302 ALA	305 GLU	306 THR	371 ILE	482 VAL	483 VAL									
Plumbagin	0	113 ALA	114 PHE	302 ALA	306 THR	366 VAL	367 ALA	371 ILE	482 VAL										
Oleic acid	2	201 TYR	202 ASN	205 ILE	239 ARG	297 GLY	298 ASP												

From Table 3, diosgenin was found to be the most promising candidate, which exhibits strong binding characteristics with low estimated free energy and an exceptionally low inhibition constant, indicating robust affinity for the enzymes active site. Gingerenone-A is less potent but still has some inhibition potential in the nanomolar range. In contrast, the binding energies in piperine and chlorogenic acid are moderate with higher inhibition constants, which shows that they will have to be used at much higher concentrations in order to prove effective inhibition. Compounds like β-pinene and plumbagin depict considerably weaker affinities as binders; anethole and oleic acid show minimal effectiveness, limiting their potential to act as an inhibitor (Table 4). Overall, the findings point out diosgenin as a promising lead candidate that should be pursued further, creating a good platform for future experimental studies and very useful information in the development of new inhibitors of CYP-17α-hydroxylase, with a view of improving therapeutic intervention for conditions like PCOS which is regulated by this enzyme.

Table 5. ADMET profiling of selected bioactive molecules

Sl. No.	Compound	Absorption	Distribution	Metabolism	Excretion	Toxicity
1	β-pinene	High	Moderate BBB permeability.	Low CYP interaction.	Rapid	Non-toxic
2	Anethole	High	Low BBB permeability.	CYP1A2 inhibitor.	Moderate	Non-toxic
3	Chlorogenic acid	Moderate	Low distribution.	Low CYP interaction.	Slow	Low toxicity
4	Diosgenin	Low	High plasma protein binding.	CYP3A4 inhibitor.	Slow	Non-toxic
5	Kaempferitrin	Low	Poor distribution.	Low CYP interaction.	Slow	Possible hepatotoxicity
6	Gingerenone-A	Moderate	Moderate distribution.	CYP2D6 inhibitor.	Moderate	Non-toxic
7	Piperine	High	Moderate distribution.	CYP3A4 and 2D6 inhibitor.	Moderate	Non-toxic
8	Plumbagin	Moderate	Moderate distribution.	Low CYP interaction.	Moderate	Cytotoxic potential
9	Oleic acid	High	Wide distribution.	Low CYP interaction.	Slow	Non-toxic

The interactions of the target enzyme CYP-17α-hydroxylase with various bioactive compounds in the *Siddha* formulation *Soothagathai Udaikka Kudineer* have shown different binding patterns. Interestingly, β-pinene, anethole, piperine, and plumbagin did not show any interaction with the active site of the target, indicating low affinity or an inability to bind. Contrasting these, chlorogenic acid, diosgenin, and oleic acid all showed some interaction with ASN 202 and ARG 239, suggesting a strong penchant for hydrogen bonding and stability of association. Gingerenone-A and kaempferitrin also showed significant interaction with ASN 202 and ALA 105, which reflected a dominance of hydrophobic polar interactions. Kaempferitrin revealed the strongest possible interaction profile, as it interacts with three residues. It signifies a strong binding potential. Key highlights in this regard indicate ASN 202 and ARG 239 are the critical residues involved in compound binding, while ALA 105 is mainly engaged in hydrophobic interactions. Generally, this analysis emphasizes the structural dynamics of CYP-17α-hydroxylase. Thus, an understanding of its structure-activity relationship may eventually guide how to manipulate biochemical processes through inhibition of the target enzyme, CYP-17α-hydroxylase.

From the Table 5, the ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) analysis of the selected phytocompounds from *Soothagathai Udaikka Kudineer* revealed varied pharmacokinetic profiles that support their potential as drug candidates. Most compounds, such as β-pinene, anethole, and piperine, demonstrated high absorption rates and non-toxic profiles, indicating good oral bioavailability. Diosgenin and kaempferitrin showed low absorption and poor distribution, with Kaempferitrin also presenting possible hepatotoxicity, highlighting the need for caution in further evaluation. Several compounds, including diosgenin and piperine, exhibited inhibitory effects on key cytochrome P450 enzymes like CYP3A4 and CYP2D6, suggesting potential drug interaction concerns. While the *in silico* ADMET findings provide preliminary insights into pharmacokinetics and safety, experimental validation is necessary to confirm their *in vivo* behaviour and therapeutic suitability.

4. Conclusion

In conclusion, from the comprehensive interaction analysis between CYP-17α-hydroxylase and various bioactive compounds, crucial insights for their potential use as therapeutic agents are revealed. Diosgenin is the obvious candidate, as it has strong binding affinity and inhibition potential. The varying degrees of effectiveness among other compounds, such as gingerenone-A, piperine, and chlorogenic acid, highlight the significance of understanding the binding dynamics in developing effective inhibitors. The study emphasizes specific amino acid interactions, especially with ASN 202 and ARG 239, as critical for the stability of binding. In addition, the results on β-pinene, anethole, and others indicate that not all of

the compounds present in traditional preparations have inhibitory activity, so their mechanisms require further study. Therefore, these bioactive compounds may be good candidates for therapy by inhibiting the target enzyme CYP-17α-hydroxylase for PCOS treatment.

This study, therefore, lays a foundation for the investigation of structure-activity relationships and opens avenues for the improvement of therapeutic strategies in conditions such as PCOS where CYP-17α-hydroxylase plays a critical regulatory role. Refining our understanding of these interactions can help advance the development of more targeted and effective treatments, contributing to better health outcomes. Preclinical and clinical studies are necessary to assess the safety and efficacy of *Soothagathai Udaikka Kudineer.*

5. Limitations of the Study

The study provides preliminary insights through molecular docking, but lacks experimental validation to confirm therapeutic efficacy and biological relevance of the identified compounds. Though ADMET profiling was performed *in silico*, experimental studies are necessary to verify absorption, metabolism, and potential toxicity of these phytocompounds. The absence of molecular dynamics simulations and bioavailability data limits understanding of compound stability and real-time behaviour in physiological conditions.

6. Authors Contribution

Jayalakshmi J - Conceptualization, methodology, study plan, data collection, writing the original draft. Sivakkumar S, Ethel shiny S and Bharath Christian. C.B.S - Data analysis, validation, formal analysis. Thirunarayanan G - Data interpretation, final article drafting, grammar and spelling correction. All authors have read and agreed to the published version of the manuscript.

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