JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2024/35226

SHORT COMMUNICATION

Molecular Docking Studies of *Siddha* Herbal Formulations *Keezharnelli Choornam* and *Kalarchi Choornam*on CYP-17α -Hydroxylase for the Treatment of Polycystic Ovarian Syndrome

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Abstract

Background: Molecular docking has several uses in *Siddha* medicine, notably in herbal formulations. For *Kalarchi choornam* and *Keezharnelli choornam*, this molecular docking experiment has been conducted. **Aim:** Through molecular docking tests, the current study seeks to scientifically establish the effectiveness of *Kalarchi* and *Keezharnelli choornam* in treating polycystic ovarian syndrome and to identify the primary component interaction with CYP-17-hydroxylase. **Methods:** Auto dock 4 was used to perform docking calculations. By creating a hydrogen bond with the targets essential amino acids (Ala105, Arg239, and Asn202), phytocomponents block the PDB - 3RUK enzyme CYP-17-hydroxylase activity. These amino acid residues are in charge of substrate and inhibitor binding. Therefore, phytochemicals that block the target enzyme CYP-17-hydroxylase may be helpful in the treatment of PCOS. **Results:** The bioactive compounds discovered in *Kalarchi* and *Keezharnelli choornam*, including piperine, campesterol, beta-sitosterol, linoleic acid, and piperic acid, bind strongly to the target enzyme CYP-17-hydroxylase. **Conclusion:** The study shows that these compounds may alleviate PCOS by inhibiting the enzyme CYP-17-hydroxylase, reducing the pathogenesis of PCOS.

Keywords: CYP- 17α-hydroxylase, *Kalarchi Choornam*, *Keezharnelli Choornam*, Molecular Docking, Polycystic Ovarian Syndrome

1. Introduction

Polycystic Ovarian Syndrome (PCOS), is one of the most prevalent hormonal disorders affecting women who are of reproductive age. Though the onset commonly occurs in adolescence, the symptoms could change. Hormonal abnormalities, irregular periods, high testosterone levels, and ovarian cysts can all be symptoms of PCOS. Unusual menstrual cycles, typically accompanied by insufficient ovulation, can pose challenges to conceiving[1]. PCOS is one major contributor to infertility and a serious public health concern. An estimated 8-13% of women who are of reproductive age have the illness, and up to 70% of instances go untreated[2].

Hyperandrogenism is regarded as the common characteristic of PCOS, accompanied by Luteinizing Hormone (LH) hypersecretion, Follicle Stimulating Hormone (FSH) reduction, and Insulin Resistance (IR). The increased androgen was primarily secreted from theca cells in PCOS[3]. Several studies have reported that the androgen level in PCOS was augmented, compared to normal women, by the isolation and cultivation of theca cells[4]. The excess biosynthesis of androgen in PCOS was attributed to the enhanced expression of steroid-17-α-hydroxylase (CYP17A1 gene) in theca cells. The cytochrome P450 family 17 (CYP17), which encodes the activity of enzymes 17-α-hydroxylase and 17,20 lyase, is characterized as the crucial enzymes in the androgen biosynthesis pathway. In humans, it is coded by CYP17A (CYP17 subfamily) gene located at chromosome 10q24.32[5]. Studies have shown

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Article Received on: 28.09.2023 Revised on: 21.10.2024 Accepted on: 11.12.2024

that Single Nucleotide Polymorphisms (SNPs) in the CYP17 gene, a substitution of Thymine (T) with Cytosine (C) at the 34th bp upstream from the translation initiation point in the promoter region of CYP17 (rs 743572), enhances the expression of CYP17 in PCOS, which indicates the CYP17 T/C gene polymorphism may be potential pharmaceutical targets in fertility treatment and gynecology. In that case, a CYP17 antagonist would be recommended for the prevention of PCOS, as well as for improving symptoms of hyper androgenaemia[6]. Chemical-based medication used for the treatment of disease causes many side effects therefore natural therapies were preferred to re-establish the normal rhythm of the menstrual cycle by balancing the hormones and also, they are found to restore the imbalance of dos has occurring due to ovulation[7].

The *Siddha* system of medicine, an ancient and revered tradition, offers a comprehensive and holistic approach to managing Polycystic Ovary Syndrome (PCOS), a complex endocrine disorder. According to the fundamental principles of *Siddha* medicine, PCOS is attributed to the derangement of *Vatham* and *Kabam*, two vital humors that govern the body's physiological functions. This imbalance triggers a cascade of symptoms, including abdominal discomfort, dysmenorrhoea, low back pain, constipation, amenorrhoea, and heaviness of the thigh, collectively known as *Garpavaayu*, as described in the esteemed *Siddha* textbook, *Pararasasekera*[8].

The *Panchabootham* and *Mukkutram* theories, corner stone concepts in *Siddha* medicine, propose that specific tastes, notably bitter (*Kaippu Suvai*) and astringent (*Thuvarppu Suvai*), exert a profound influence on *Vatham*, thereby neutralizing the imbalance of *Pitham* and *Kabam*. In consonance with this theory, *Siddha* literature emphasizes the therapeutic potential of herbs possessing emmenagogue and deobstruent properties, predominantly characterized by their bitter taste. Hence, bitter herbs are strategically employed to restore balance to the deranged humor[9].

In this context, traditional *Siddha* formulations, renowned for their efficacy, have been investigated for their potential in managing PCOS-like conditions. *Kalarchi* and *Keezharnelli choornam*, a time-tested *Siddha* medicine documented in *Siddha Vaittiyattirattu*[10], has demonstrated exceptional promise in treating orchitis and elephantiasis-like disorders. Its ingredients, *Caesalpinia bonduc*, and *Piper nigrum* boast deobstruent and emmenagogue actions, underscoring their therapeutic potential. Similarly, *Kalarchi* and *Keezharnelli choornam*, a monoherbal formulation detailed in *Gunapadam Mooligai Vaguppu*[11], has shown remarkable efficacy, courtesy of its bitter taste and deobstruent action.

To further elucidate the scientific basis of these traditional medicines, an *in-silico* analysis was conducted to investigate the CYP-17-hydroxylase inhibitor activity of the lead molecules in *Kalarchi* and *Keezharnelli choornam*. This study aims to provide a rational framework for the therapeutic application of these *Siddha* formulations in managing PCOS, paving the way for their broader acceptance and integration into contemporary healthcare practices.

2. Materials and Methods

2.1 Standard Operating Procedure

Kalarchi and *Keezharnelli choornam* is an herbal formulation containing *C. bonduc* and *P. nigrum*. The ingredients are taken in a 4:1 ratio, finely powdered, and stored in air-tight containers. This powder is combined with hot water and consumed twice daily in doses ranging from 300 to 1000 mg for *Kalarchi* and *Keezharnelli choornam*[10], the whole plant is taken, cleaned, dried, powdered, and stored in air-tight containers. 1000 mg of this powder is combined with hot water and must be taken twice daily[11].

2.2 Docking Simulation Software

A conventional docking tool was used to predict the potential of the leads under investigation over specified enzyme targets (Auto Dock version 4). Different orientations of the lead molecules to the target CYP- 17-hydroxylase with PDB - 3RUK were evaluated by the Autodock version 4 program and the best dock pose was selected based on the interaction study analysis[12].

2.3 Ligand Preparation

The herbal ingredients of *Kalarchi* and *Keezharnelli choornam*; *C. bonduc*, and *P. nigrum* have phytochemicals such as linoleic acid, campesterol, sitosterol, piperidine, piperic acid, and piperine[13,14,15].

The bioactive constituents derived from *Kalarchi* and *Keezharnelli choornam* are rutin and ellagic acid[16]. A total of eight phytocomponents (Figure 1) structures were retrieved from the eight herbal ingredients present in the *Siddha* formulations *Kalarchi* and *Keezharnelli choornam* through a systematic literature survey against the target CYP- 17-hydroxylase with PDB - 3RUK. The structure of all the ligands was obtained from PubChem and prepared as a single sdf file, further optimization and minimization of all ligands were done in Cresset flare software with default settings. The ligands file is read in autodetect under full protonation mode.

2.4 Preparation of Protein

The crystalline three-dimensional structure of the target enzyme, CYP-17-hydroxylase (PDB - 3RUK) (Figure 2) was retrieved from the protein data bank, and the protein clean-up process was done and essential missing hydrogen atoms were added. The protein construct was optimized by eliminating the preloaded

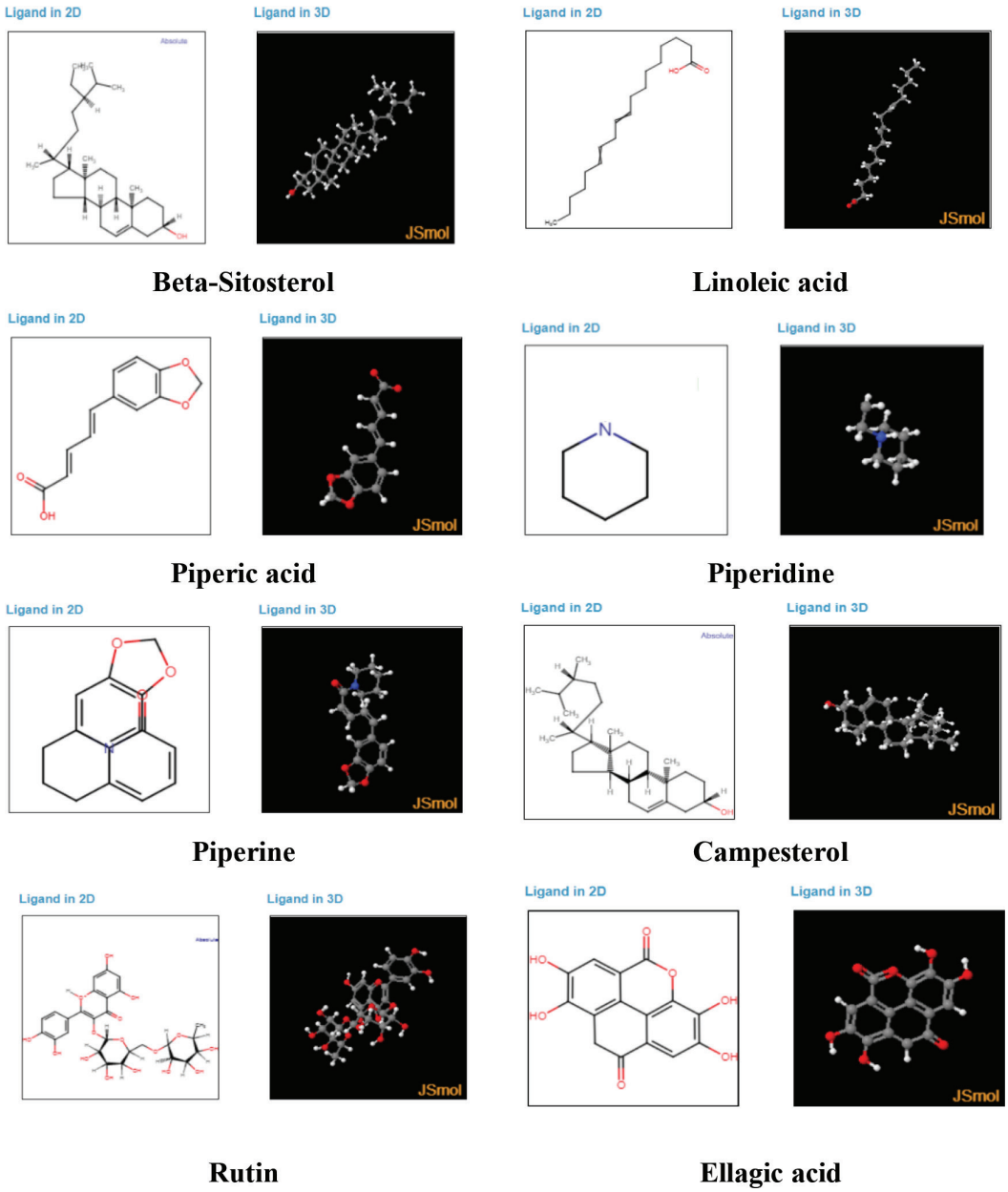


Figure 1. Structures in two and three dimensions of phytocomponents of *Kalarchi* and *Keezharnelli choornam* retrieved from Pubchem database.

lead candidate by cleaving adjoining water molecules. Auto dock 4 calculated with extra polar hydrogen atoms, combining non-polar and rotatable links.

2.4 Methodology of Auto Docking

Docking analyses against the CYP-17-hydroxylase target enzyme were performed for recovered phytocomponents. The ligand characteristics of the selected compounds for docking analysis are given in Table 1. Essential hydrogen atoms, kollman united atom type charges, and solvation parameters were added with the aid of autodock tools.



Receptor Structure

Figure 2. 3D- structure of CYP- 17α-hydroxylase (PDB) - 3RUK.

Affinity (grid) maps of ×× Å grid points and 0.375 Å spacing were generated using the Autogrid program[12]. Autodock parameter set and distance-dependent dielectric functions were used in the calculation of the van der waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian Genetic Algorithm (LGA) and the Solis and Wets local search method[17]. The initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from two different runs that were set to terminate after a maximum of 2,50,000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied.

Table 1. Ligand characteristics of the selected cmpounds for docking analysis

Compound	Molar Weight g/mol	Molecular Formula	H Bond Donor	H Bond Acceptor	Rotatable Bonds
Beta-sitosterol	414.7g/mol	$C_{29}H_{50}O$	1	1	6
Linoleic acid	280.45 g/mol	$C_{18}H_{32}O_2$	1	2	14
Piperic acid	218.2 g/mol	$C_{12}H_{10}O_4$	1	4	3
Piperidine	85.15 g/mol	$C_5H_{11}N$	1	1	0
Piperine	285.34 g/mol	$C_{17}H_{19}NO_3$	0	3	3
Campesterol	400.7 g/mol	$C_{28}H_{48}O$	1	1	5
Rutin	610.5 g/mol	$C_{27}H_{30}O_{16}$	10	16	6
Ellagic acid	302.194 g/mol	$C_{14}H_6O_8$	4	8	0

Table 2. Summary of substances molecular docking investigations against CYP-17-hydroxylase (PDB: 3RUK)

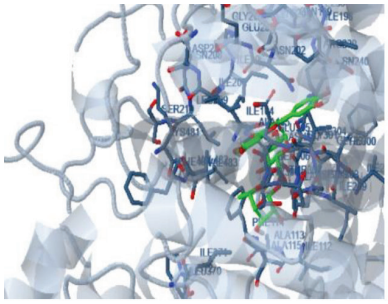
Compound	Estimation of Free Energy of Binding	Estimation of Inhibition Constant, Ki	Electrostatic Energy	Total Intermolecular Energy	Interaction Surface
Beta-sitosterol	-12.82 kcal/mol	400.12 pM	-0.07 kcal/mol	-14.45 kcal/mol	708.861
Linoleic acid	-7.09 kcal/mol	6.30 uM	-0.09 kcal/mol	-9.62 kcal/mol	505.028
Piperic acid	-5.42 kcal/mol	106.43 uM	-0.15 kcal/mol	-6.30 kcal/mol	445.411
Piperidine	-4.59 kcal/mol	430.44 uM	-1.74 kcal/mol	-4.59 kcal/mol	335.36
Piperine	-8.55 kcal/mol	539.97 nM	-0.02 kcal/mol	-8.09 kcal/mol	463.868
Campesterol	-12.26 kcal/mol	1.04 nM	-0.12 kcal/mol	-12.80 kcal/mol	648.247
Rutin	-5.90 kcal/mol	47.47 uM	-0.23 kcal/mol	-7.26 kcal/mol	724.291
Ellagic acid	-7.06 kcal/mol	6.69 uM	-0.07 kcal/mol	-5.94 kcal/mol	452.762

3. Results

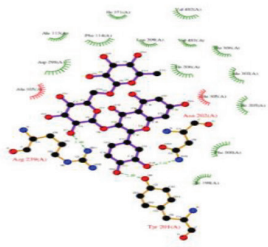
Molecular docking studies were performed over 8 phyto constituents retrieved from the herbs on the binding of target CYP-17-hydroxylase enzyme to find a potential drug candidate for treating polycystic ovarian syndrome. All these 8 molecules were docked against the target CYP-17-hydroxylase. Binding energy determines whether the desired leads tend to occupy the target amino acid residues that mediate the enzyme

Table 3. Lead chemical interactions with amino acid residues to inhibit CYP- 17-hydroxylase (PDB) - 3RUK

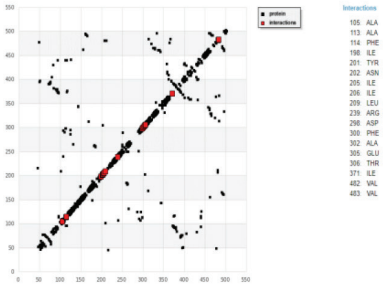
Compounds	Interaction	Amino acid residues														
Beta-sitosterol	2	105 ALA	114 PHE	205 ILE	214 LEU	236 VAL	239 ARG	298 ASP	302 ALA	305 GLU	306 THR	366 VAL	367 ALA	371 ILE	482 VAL	483 VAL
Linoleic acid	3	105 ALA	113 ALA	114 PHE	201 TYR	202 ASN	205 ILE	209 LEU	239 ARG	298 ASP	302 ALA	482 VAL				
Piperic acid	2	113 ALA	202 ASN	205 ILE	209 LEU	239 ARG	298 ASP	302 ALA	306 THR							
Piperidine	1	199 GLN	202 ASN	203 GLU	206 ILE	305 GLU										
Piperine	2	114 PHE	202 ASN	205 ILE	206 ILE	239 ARG	298 ASP	305 GLU	482 VAL							
Campesterol	3	105 ALA	114 PHE	202 ASN	206 ILE	209 LEU	239 ARG	298 ASP	302 ALA	305 GLU	306 THR	371 ILE	442 CYS	482 VAL		
Rutin	3	105 ALA	113 ALA	114 PHE	198 ILE	201 TYR	202 ASN	205 ILE	206 ILE	209 ILE	239 ARG	298 ASP	300 PHE	302 ALA	305 GLU	306 THR
Ellagic acid	2	105 ALA	113 ALA	205 ILE	206 ILE	239 ARG	298 ASP	302 ALA	305 GLU	306 THR	483 VAL					



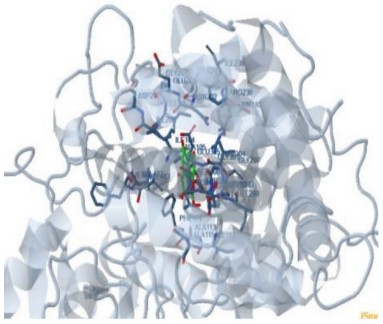
Rutin with CYP- 17α-hydroxylase (PDB) - 3RUK



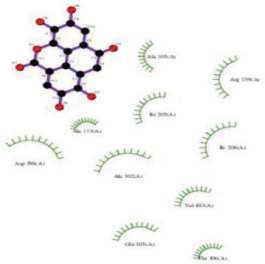
Analysis of 2D Interaction Plots



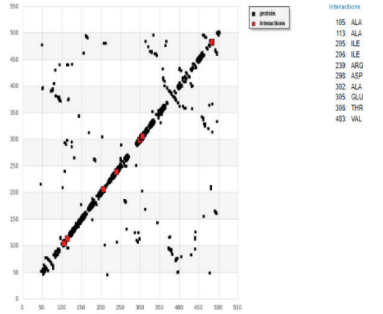
Plotting hydrogen bonds using analysis of key amino acids



Ellagic acid with CYP- 17α-hydroxylase (PDB) - 3RUK

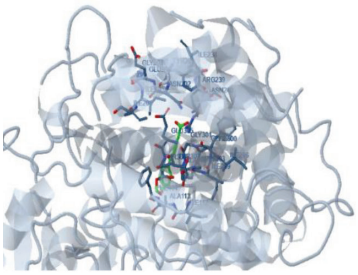


2D Interaction Plot Analysis

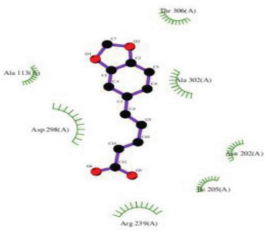


Plotting hydrogen bonds using analysis of key amino acids

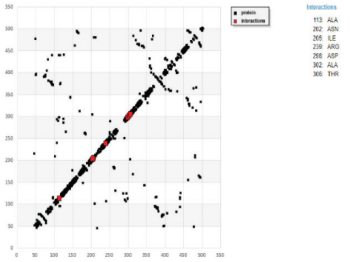
Figure 3. Docking pose of the lead molecules with the target protein CYP-17-hydroxylase.



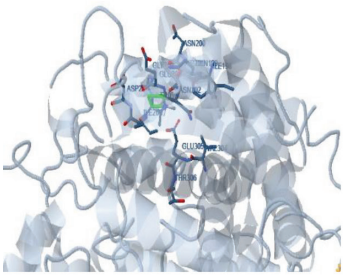
Piperic acid with CYP- 17α-hydroxylase (PDB) - 3RUK



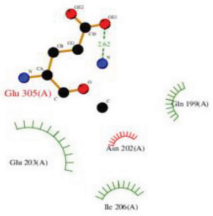
Analysis of 2D Interaction Plots



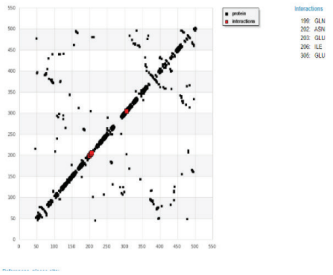
Plotting hydrogen bonds using analysis of key amino acids



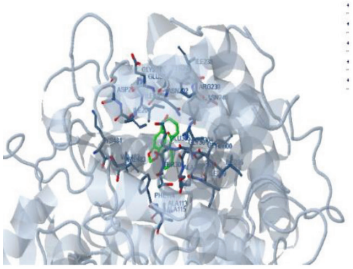
Piperidine with CYP- 17α-hydroxylase (PDB) - 3RUK



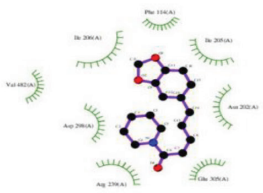
Analysis of 2D Interaction Plots



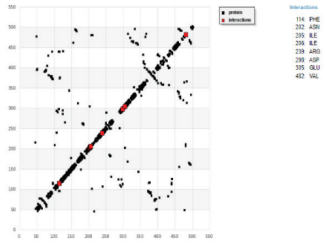
Plotting hydrogen bonds using analysis of key amino acids



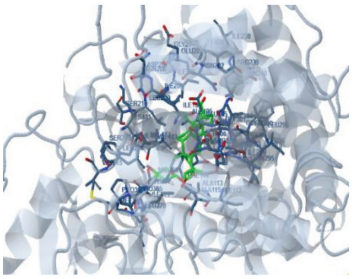
Piperine with CYP- 17α-hydroxylase (PDB) - 3RUK



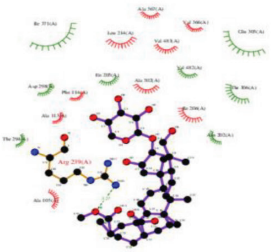
Analysis of 2D Interaction Plots



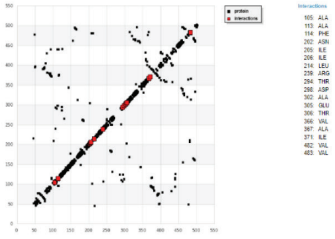
Plotting hydrogen bonds using analysis of key amino acids



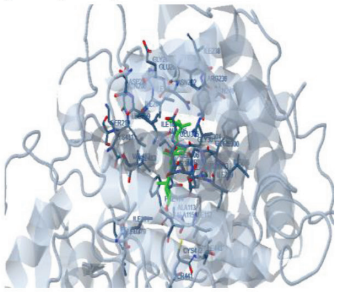
Phyllaciduloid B with CYP- 17α-hydroxylase (PDB) - 3RUK



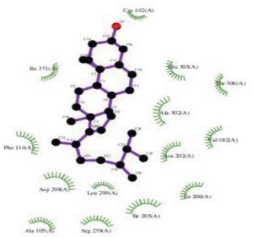
Analysis of 2D Interaction Plots



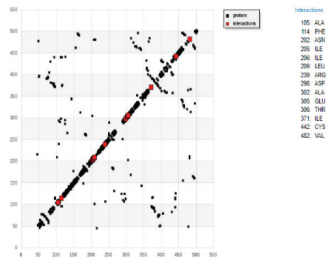
Plotting hydrogen bonds using analysis of key amino acids



Campesterol with CYP- 17α-hydroxylase (PDB) - 3RUK

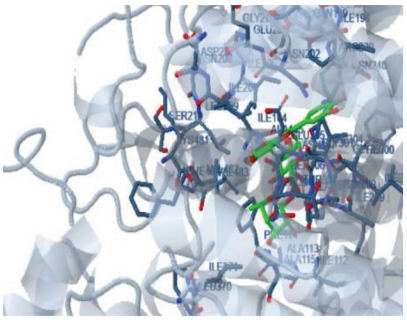


Analysis of 2D Interaction Plots

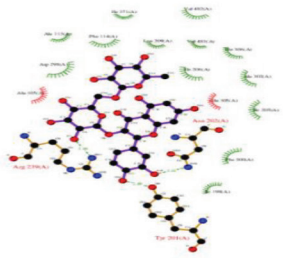


Plotting hydrogen bonds using analysis of key amino acids

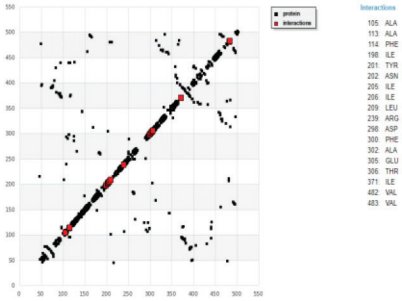
Figure 3. Continued...



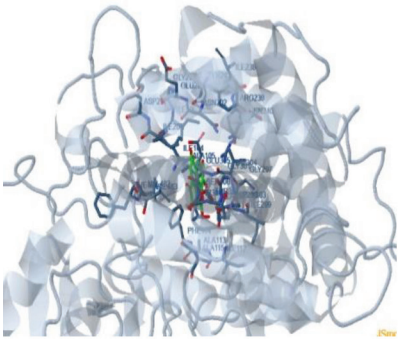
Rutin with CYP- 17α-hydroxylase (PDB) - 3RUK



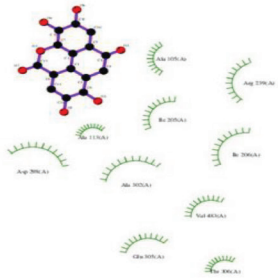
Analysis of 2D Interaction Plots



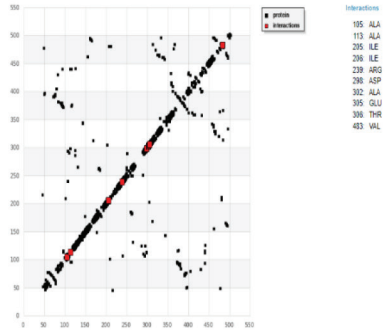
Plotting hydrogen bonds using analysis of key amino acids



Ellagic acid with CYP- 17α-hydroxylase (PDB) - 3RUK



2D Interaction Plot Analysis



Plotting hydrogen bonds using analysis of key amino acids

Figure 3. Continued...

function and its relative biological action[18]. Among the docking studies performed on phyto compounds, all the analogs had effective binding interactions with CYP- 17-hydroxylase (binding energy ranges from -4.59 kcal/mol to -12.82 kcal/mol). The higher the negative docking score, the better the docking affinity, and ligand and protein docking was ordered based on binding affinity. The results reveal that Phytoconstituents with the highest docking score were seen for beta-sitosterol, campesterol, piperine, linoleic acid, and ellagic acid showing -12.82 kcal/mol, -12.26 kcal/mol, -8.55 kcal/mol, -7.09 kcal/mol, and -7.06 kcal/mol respectively (Table 2). Whereas rutin, piperic acid, and piperidine with a binding energy of -5.90, -5.42, and -4.59kcal/mol respectively showed moderate binding affinity against the target protein.

From the reported data of the herbs, the phytochemicals such as piperine, campesterol, beta-sitosterol, linoleic acid, rutin, ellagic acid, and piperic acid reveala maximum of two to three interactions with the core active amino acid residues present on the target enzyme CYP 17 hydroxylase (Table 3, Figure 3).

4. Discussion

The primary cause for symptoms of PCOS is deranged *vatham* and *kabam*. According to *panchabootham* and *mukkutram* theory, bitter taste (*kaippusuvai*) and astringent taste (*thuvarppusuvai*) influence *vatham* and neutralize *pitham* and *kabam*[9]. In *Siddha* literature, herbs possessing emmenagogue and deobstruent actions are mainly bitter[11]. Hence, bitter herbs are used to normalize the deranged humor. *K. choornam*, a traditional *Siddha* formulation recorded in the *Siddha* text *Siddha Vaittiyattirattu*, primarily treats orchit is and elephantiasis like disorders. Ingredients in *Kalarchi* and *Keezharnelli choornam* are *C. bonduc* and *P. nigrum*[10], which have deobstruent and emmenagogue actions and are incredibly bitter. This study shows that it is a dependable *Siddha* medicine for PCOS-like conditions. Another traditional *Siddha* medicine named *K. choornam* is documented in the *Siddha* book *Gunapadam Mooligai Vaguppu*[11], a monoherbal formulation containing *Phyllanthus niruri* with a bitter taste and deobstruent action.

Phytochemicals in *Kalarchi* and *Keezharnelli choornam* were seen to inhibit the CYP17 hydroxylase enzyme, which is crucial for steroid hormone synthesis. Through virtual screening of phytochemicals from the plants *C. bonduc, P. nigrum*, and *P. niruri*, this study discovered a potent inhibitor of the CYP17 hydroxylase enzyme. Over expression of CYP17 hydroxylase leads to increased androgen production, causing hyper androgenism in Polycystic Ovary Syndrome (PCOS). Inhibiting CYP17 hydroxylase activity may reduce ovarian androgen synthesis[19]. This study reveals that the phytochemicals from the *C. bonduc*, *P. nigrum*, and *P. niruri* are potent inhibitors of the CYP-17 hydroxylase enzyme. As a result, the *Siddha* medications *Kalarchi* and *Keezharnelli choornam* can potentially be used as brand-new treatments for PCOS-related infertility.

4. Conclusion

From a wider research perspective, in this *in silico* analysis, the lead molecules from the phytochemicals of the *Kalarchi* and *Keezharnelli choornam* were interpreted for their CYP-17-hydroxylase inhibitor activity. The computational analysis revealed that the active ingredients in these herbal ingredients, including rutin, piperine, campesterol, beta-sitosterol, linoleic acid, and piperic acid, interact with active amino acids on the active site of the target enzyme CYP-17-hydroxylase to produce a significant binding effect. This indicates that these compounds may have encouraged PCOS amelioration effects by inhibiting this enzyme. This may help in the scientific validation of these drugs and the broader acceptability of *Kalarchi* and *Keezharnelli choornam* as efficient herbal formulations to treat polycystic ovarian syndrome.

5. References

1. Adone A, Fulmali DG. Polycystic ovarian syndrome in adolescents. Cureus. 2023; 15(1):e34183. PMID: 36843701. PMCID: PMC9951123. https://doi.org/10.7759/cureus.34183
2. Singh S, Pal N, Shubham S, Sarma DK, Verma V, Marotta F, Kumar M. Polycystic ovary syndrome: Etiology, current management, and future therapeutics. J Clin Med. 2023; 12(4):1454. PMID: 36835989, PMCID: PMC9964744. https://doi.org/10.3390/jcm12041454. PMID: 36835989
3. Rosenfield RL, Ehrmann DA. The pathogenesis of Polycystic Ovary Syndrome (PCOS): The hypothesis of PCOS as functional ovarian hyperandrogenism revisited. Endocr Rev. 2016; 37(5):467-520. https://doi.org/10.1210/er.2015-1104. PMID: 27459230
4. Sanchez-Garrido MA, Tena-Sempere M. Metabolic dysfunction in polycystic ovary syndrome: Pathogenic role of androgen excess and potential therapeutic strategies. Mol Metab. 2020; 35:100937. https://doi.org/10.1016/j.molmet.2020.01.001
5. Liu X, Xu M, Qian M, Yang L. CYP17 T/C (rs74357) gene polymorphism contributes to polycystic ovary syndrome susceptibility: Evidence from a meta-analysis. Endocr Connect. 2021; 10(12):R305-R316. PMID: 34788226, PMCID: PMC8679930. https://doi.org/10.1530/EC-21-0327
6. Banerjee U, Dasgupta A, Khan A, Ghosh MK, Roy P, Rout JK, Roy P, Dhara S. A cross-sectional study to assess any possible linkage of C/T polymorphism in CYP17A1 gene with insulin resistance in non-obese women with polycystic ovarian syndrome. Indian J Med Res. 2016; 143(6):739-747. PMID: 27748298 PMCID: PMC5094113. https://doi.org/10.4103/0971-5916.191990
7. Jiao M, Liu X, Ren Y, Wang Y, Cheng L, Liang Y, Li Y, Zhang T, Wang W, Mei Z. Comparison of herbal medicines used for women's menstruation diseases in different areas of the world. Front Pharmacol. 2022; 12:751207. PMID: 35185533, PMCID: PMC8854496. https://doi.org/10.3389/fphar.2021.751207
8. Pillai EI. Pararasasekeram. Garpa roga nithanam. Vada Maakaana Sabai: Maakaana Sudesha Maruthuvathinaikkalam. 1928;1:1-2.
9. Shanmugavelan S. Noinaadal noimuthalnaadal thirattu. Department of Indian Medicine and Homeopathy. 1967;1:18.
10. Muthaliyar KNK, Uthamarayan KC. Siddha vaidya thirattu. Department of Indian Medicine and Homeopathy. 2009; 1.
11. Mudhaliar KNM. Gunapadam mooligai vaguppu. Department of Indian Medicine and Homeopathy. 2002:1(2).
12. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ. AutoDock4 and AutoDock tools4: Automated docking with selective receptor flexibility. J Comput Chem. 2009; 30(16):2785-2791. PMID: 19399780. PMCID: PMC2760638. https://doi.org/10.1002/jcc.21256
13. Sasidharan S, KP Srinivasakumar, Bhaumik A, Das SK, Nair JH. Administration of *Caesalpinia bonduc* seed extracts ameliorates testosterone-induced Benign Prostatic Hyperplasia (BPH) in male Wistar rats. Res Rep Urol. 2022; 14:225-239. https://doi.org/10.2147/rru.s365598
14. Salehi B, Zakaria ZA, Gyawali R, *et al*. Piper species: A comprehensive review on their phytochemistry, biological activities, and applications. Molecules. 2019; 24(7):1364. https://doi.org/10.3390/molecules24071364
15. Daina A, Michielin O, Zoete V. Swiss ADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep. 2017; 7(1):42717. https://doi.org/10.1038/srep42717

16. Singh MK, Ahmad S. Phytochemical profile of *Phyllanthus niruri* and evaluation of its potent bioactive compounds. Biosc Biotech Res Comm. 2020; 13(3):1-10. https://doi.org/10.21786/bbrc/13.3/82
17. Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, Olson AJ. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. J Comput Chem. 1998; 19(14):1639-1662. https://doi.org/10.1002/(sici)1096-987x(19981115)19:14<1639::aid-jcc10>3.0.co;2-b
18. Du X, Li Y, Xia YL, Ai SM, Liang J, Sang P, Ji XL, Liu SQ. Insights into protein-ligand interactions: Mechanisms, models, and methods. Int J Mol Sci. 2016; 17(2):144. https://doi.org/10.3390/ijms17020144
19. Chen Z, Liu L, Xi X, Burn M, Karakaya C, Kallen AN. Aberrant H19 expression disrupts ovarian Cyp17 and testosterone production and is associated with polycystic ovary syndrome in women. Reprod Sci. 2022; 29(4):1357-1367. https://doi.org/10.1007/s43032-021-00700-5