Published online in http://ijam. co. in

ISSN No: 0976-5921

International Journal of Ayurvedic Medicine, Vol 14 (2), 2023; 511-516

An Insilico Computational Screening of Siddha Formulation *Kalingathy Thailam* against Cyp- 17α-Hydroxylase in PCOS

Research Article

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Abstract

Background: PCOS is characterized by ovulatory failure, hirsutism, obesity, glucose intolerance, resistance to insulin, dyslipidemia and infertility. Siddha system of medicine has a worthwhile therapeutic effect in treating PCOS. The bioactive compounds of *Kalingathy Thailam* mentioned in Siddha literature was selected for evaluating their ability to inhibit CYP- 17α-hydroxylase. Objective: The study is aimed to perform the In Silico computational screening of the formulation *Kalingathy Thailam* against the target enzyme CYP-17 α -hydroxylase in PCOS. Methods: Autodock program was used against the target enzyme CYP-17 α -hydroxylase for the phytocomponents such as Sinapic acid, Cucurbitacin B of *Citrullus colocynthis* (L.) Schrader, Aromadendrene, Linolenic acid of *Melia dubia* Linn., Onionin A, Protocatechuic acid of *Allium cepa* Linn., Ascorbic acid, Limonene of *Citrus limon* (L.)Burm. f., Ricinine of *Ricinus communis* Linn. Results: A total of 9 components were screened, out of these Protocatechuic acid, Ascorbic acid, Linolenic acid and Cucurbitacin B reveals maximum of 2 to 3 interactions accounting for 75-100% binding efficacy with the core active amino acid residues present on the target enzyme CYP-17α-hydroxylase. Conclusion: Based on the results of the computational analysis it was concluded that the bio-active compounds present in the herbal ingredients possess significant binding against the target enzyme CYP-17α-hydroxylase by interacting with active amino acids. Hence these phytocomponents which inhibit the target enzyme may act as a potential therapeutic agent for management of PCOD.

Key Words: Docking study, PCOS, Siddha, Herbal formulation, *Kalingathy thailam,* CYP-17α -hydroxylase.

Introduction

Polycystic Ovarian Syndrome (PCOS) or Stein-Leventhal syndrome, a heterogenous disorder, clinically characterized by ovulatory failure, hirsutism, glucose intolerance, resistance to insulin, dyslipidemia and infertility. The ovaries are enlarged, multicystic and show hyperplastic theca cells around the cysts. The various pathophysiological changes in PCOS are: a) Increased Luteinizing hormone (LH) frequency due to increased sensitivity of pituitary to GnRH stimulation. b) LH stimulated increased androgen production by the ovary. c) Chronic anovulation leads to steady state increased estrogen production. d)Reduced sex hormone binding globulin (SHBG) levels. e) Follicular growth is continually stimulated but not to the point of full maturation or ovulation (1).

CYP17 is described as a causative gene in the etiology of PCOS. Polymorphism C > T in the CYP17 is responsible for PCOS progression which was proved in a study, conducted on the Chilean population (2). The human gene which encodes cytochrome P450c17 (CYP17) belongs to the family cytochrome P450. This gene contains 8 exons, localized on chromosome 10 (10q24. 3) (3) . This gene encodes 17-α hydroxylase enzyme that is mostly expressed in the theca cells. The 17- hydroxylase enzyme regulates the conversion of pregnenolon to 17-hydroxyl pregnenolon and progesterone to hydroxyl progesterone for limiting androgen expression. Some studies showed that rs743572 polymorphism of the CYP17A1 gene is associated with hyper expression of androgen in PCOS patients in Greek population(4-6).

Therefore, CYP- 17α-hydroxylase was chosen as the target for this study. Binding of phytocomponents with the core amino acids (Ala105, Arg239 and Asn202) of the target by forming hydrogen bond will hinder the function of the enzyme CYP- 17α-hydroxylase with PDB – 3RUK. These amino acid residues are functionally responsible for binding of substrate and inhibitors. Thereby phytocomponents which inhibit the target enzyme CYP- 17α-hydroxylase may act as a potential therapeutic agent for management of PCOD. *Kalingathy Thailam* is the *Siddha* herbal formulations that has been using for centuries. *Kalingathy Thailam* contains *Citrullus colocynthis* (L.) Schrader, *Melia dubia* Linn., *Allium cepa* Linn., *Citrus limon* (L.) Burm. f., *Ricinus communis* Linn. is used for

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treating PCOS(7). Thus, the bioactive compounds of *Kalingathy Thailam* mentioned in *Siddha* literature was selected for evaluating their ability to inhibit CYP- 17α-hydroxylase.

Objectives

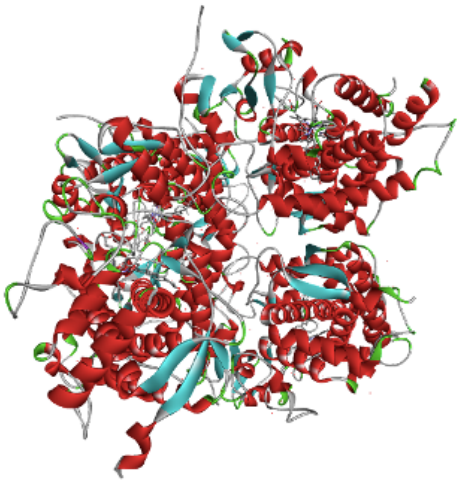
The objectives of the study is to find the efficacy of the lead molecules to bind with these core bio active amino acid residues Ala105, Arg239 and Asn202 which mediates the enzymatic action of the CYP- 17α-hydroxylase that has higher level of significance in the management of PCOS.

Materials and Methods

Target protein preparation

The crystalline structure of the target enzyme CYP- 17α-hydroxylase with PDB – 3RUK(Fig. 1) was retrieved from protein data bank and protein clean-up process was done and essential missing hydrogen atom were added. Different orientation of the lead molecules with respect to the target protein was evaluated by Autodock program and the best dock pose was selected based on the interaction study analysis.

Fig. 1: 3D- Structure of CYP- 17α-hydroxylase (PDB) - 3RUK -Receptor structure



Ligand Preparation

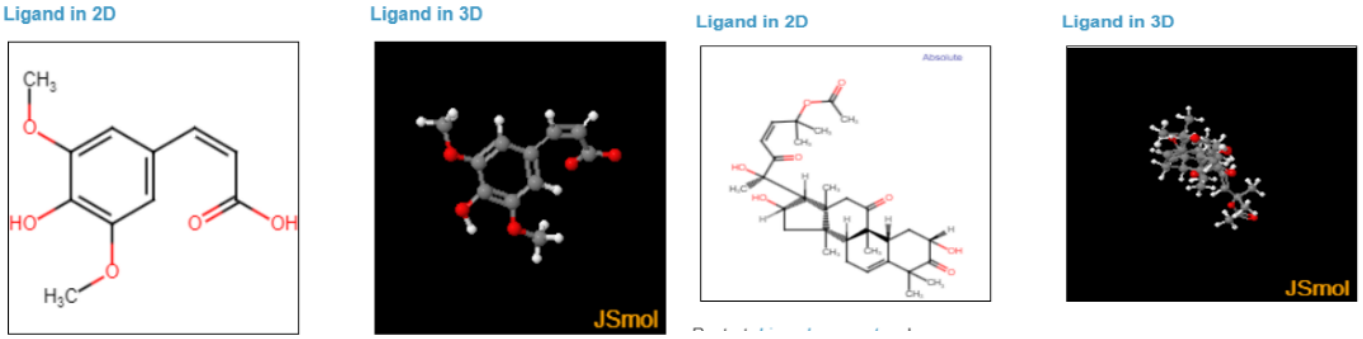
This herbal formulation *Kalingathy Thailam* contains *Citrullus colocynthis* (L.) Schrader, *Melia dubia* Linn., *Allium cepa* Linn., *Citrus limon* (L.)Burm. f., *Ricinus communis* Linn. They have the phytocomponents like Sinapic acid, Cucurbitacin B from *Citrullus colocynthis* (L.) Schrader, Aromadendrene, Linolenic acid from *Melia dubia* Linn., Onionin A, Protocatechuic acid from *Allium cepa* Linn., Ascorbic acid, Limonene from *Citrus limon* (L.)Burm. f., Ricinine from *Ricinus communis* Linn. They were retrieved from systematic literature review and IMPPAT database.

Methodology

Docking calculations were carried out for retrieved phytocomponents Sinapic acid, Cucurbitacin B of *Citrullus colocynthis*(8), Aromadendrene, Linolenic acid of *Melia dubia* (9), Onionin A, Protocatechuic acid of *Allium cepa*(10, 11) , Ascorbic acid, Limonene of *Citrus limon* (12), Ricinine of *Ricinus communis* (13) against target enzyme CYP-17α-hydroxylase. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools. By using the Autogrid program, Affinity (grid) maps of ×× Å grid points and 0. 375 Å spacing were generated. AutoDock parameter set- was used in the calculation of the van der Waals force and distance-dependent dielectric functions was used in the calculation of electrostatic terms. By using the Solis & Wets local search method and Lamarckian genetic algorithm (LGA), Docking simulations were performed. Initial position, torsions and orientation of the ligand molecules were set arbitrarily. All rotatable torsions were released during docking. Each docking experiment was obtained from 2 different runs which were set to conclude after a maximum of 250000 energy evaluations. The size of the population was set to 150. Translational step of 0. 2 Å, quaternion and torsion steps of 5 were applied during the search. (14-17).

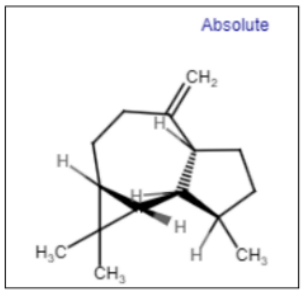
Fig. 2: 2D & 3D Structure of Selected Ligands and 2D & 3D Structure of Phytocomponents

Sinapic acid **Cucurbitacin B**

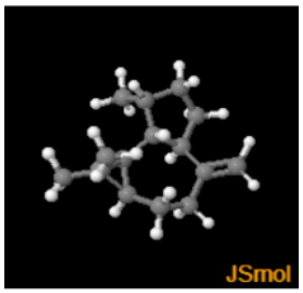


Aromadendrene

Ligand in 2D

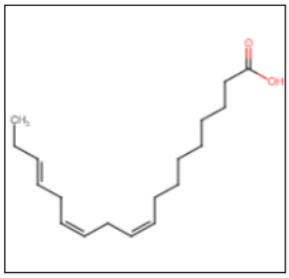


Ligand in 3D

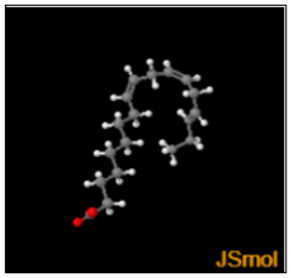


Linolenic acid

Ligand in 2D

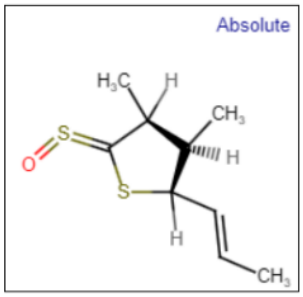


Ligand in 3D

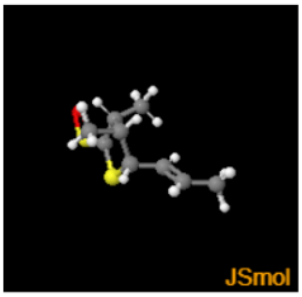


Onionin A

Ligand in 2D

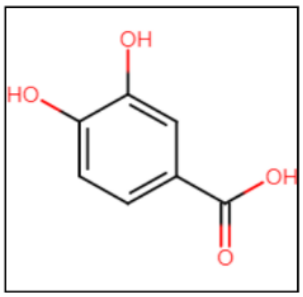


Ligand in 3D

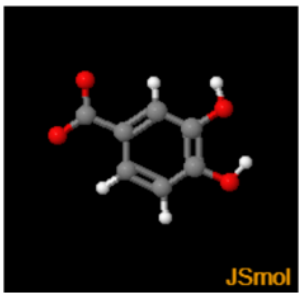


Protocatechuic acid

Ligand in 2D

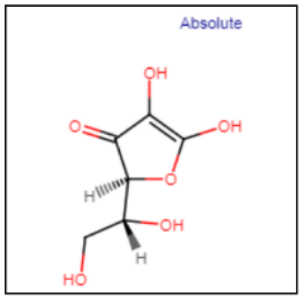


Ligand in 3D

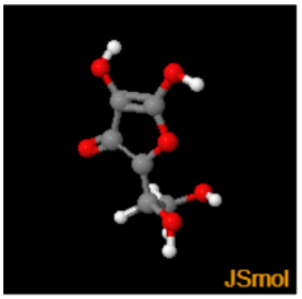


Ascorbic acid

Ligand in 2D

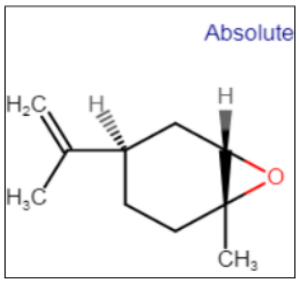


Ligand in 3D

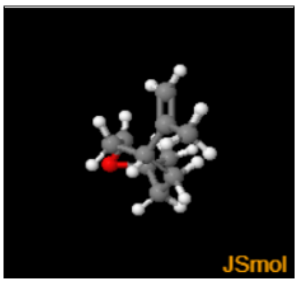


Limonene

Ligand in 2D

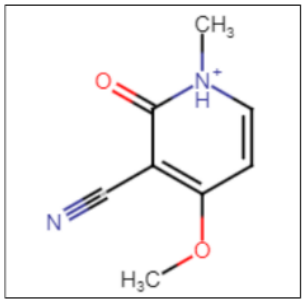


Ligand in 3D



Ricinine

Ligand in 2D



Ligand in 3D

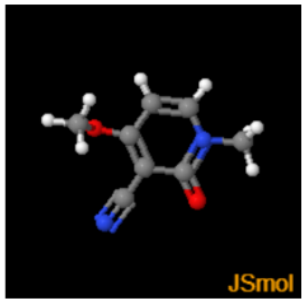


Table 1: Ligand Properties of the Compounds Selected for Docking Analysis

Compound	Molar weight g/mol	Molecular Formula	H Bond Donor	H Bond Acceptor	Rotatable bonds
Sinapic acid	224. 21 g/mol	C11H12O5	2	5	4
Cucurbitacin B	558. 7 g/mol	C32H46O8	3	8	6
Aromadendrene	204. 35 g/mol	C15H24	0	0	0
Linolenic acid	278. 4 g/mol	C18H30O2	1	2	13
Onionin A	218. 3 g/mol	C9H14O2S2	2	3	4
Protocatechuic acid	153. 112 g/mol	$C_7H_6O_4$	3	1	1
Ascorbic acid	176. 12 g/mol	$C_6H_8O_6$	4	6	2
Limonene	136. 23 g/mol	C10H16	0	0	1
Ricinine	164. 16 g/mol	$C_8H_8N_2O_2$	0	3	1

Table 2: Summary of the molecular docking studies of compounds against CYP- 17α-hydroxylase (PDB) - 3RUK

Compound	Estimated Free Energy of Binding	Estimated Inhibition Constant, Ki	Electrostatic Energy	Total Intermolecular Energy	Interaction Surface
Sinapic acid	-5. 45 kcal/mol	101. 21 uM	-0. 12 kcal/mol	-6. 26 kcal/mol	442. 621
Cucurbitacin B	-7. 21 kcal/mol	5. 22 uM	-0. 26 kcal/mol	-9. 63 kcal/mol	815. 966
Aromadendrene	-7. 89 kcal/mol	1. 66 uM	-0. 01 kcal/mol	-8. 18 kcal/mol	441. 854
Linolenic acid	-6. 58 kcal/mol	15. 09 uM	-0. 40 kcal/mol	-9. 06 kcal/mol	542. 907
Onionin A	-7. 14 kcal/mol	5. 85 uM	-0. 02 kcal/mol	-7. 64 kcal/mol	396. 768
Protocatechuic acid	-4. 58 kcal/mol	441. 44 uM	-0. 45 kcal/mol	-4. 35 kcal/mol	318. 436
Ascorbic acid	-5. 20 kcal/mol	154. 57 uM	-0. 56 kcal/mol	-4. 87 kcal/mol	335. 287
Limonene	-6. 03 kcal/mol	38. 14 uM	-0. 01 kcal/mol	-6. 33 kcal/mol	358. 589
Ricinine	-4. 61 kcal/mol	415. 23 uM	-0. 07 kcal/mol	-5. 21 kcal/mol	364. 488

Table 3: Amino acid Residue Interaction of Lead against CYP- 17α-hydroxylase (PDB) - 3RUK

Compounds	Interactions	Amino acid Residues										
Sinapic acid	0	206 ILE	302 ALA	306 THR	367 ALA	371 ILE	482 VAL	483 VAL				
Cucurbitacin B	3	105 ALA	113 ALA	114 PHE	201 TYR	202 ASN	205 ILE	206 ILE	209 ILE	236 VAL	239 ARG	298 ASP
Aromadendrene	0	114 PHE	302 ALA	306 THR	366 VAL	367 ALA	371 ILE	482 VAL	483 VAL			
Linolenic acid	3	105 ALA	201TYR	202 ASN	205 ILE	206 ILE	239 ARG	298 ASP	300 PHE	302 ALA	305 GLU	
Onionin A	0	113 ALA	302 ALA	305 GLU	306 THR	366 VAL	367 ALA	371 ILE	483 VAL			
Protocatechuic acid	3	105 ALA	114 PHE	201 TYR	202 ASN	205 ILE	239 ARG	298 ASP				
Ascorbic acid	2	201 TYR	202 ASN	205 ILE	239 ARG	298 ASP						
Limonene	0	302 ALA	306 THR	366 VAL	367 ALA	371 ILE	483 VAL					
Ricinine	0	113 ALA	114 PHE	302 ALA	306 THR	366 VAL	367 ALA	371 ILE	482 VAL			

Results and Discussion

Poly Cystic Ovarian Syndrome (PCOS) is characterized by menstural irregularities, hyperandrogenism and long term metabolic disturbances(18). Women with PCOS have higher rates of endometrial cancer, cardiovascular disease, dyslipidemia and type-2 diabetes mellitus(19). CYP-17α-hydroxylase inhibitors play an important role in the management of poly cystic ovarian syndrome. Due to overexpression of CYP17, hyperandrogenism occurs and leads to PCOS(20).

According to the systematic literature review, the most bioactive lead compounds were retrieved from each ingredient of the herbal formulation *Kalingathy Thailam*. Total of 9 bioactive lead compounds Sinapic acid, Cucurbitacin B of *Citrullus colocynthis* (L.) Schrader, Aromadendrene, Linolenic acid of *Melia dubia* Linn., Onionin A, Protocatechuic acid of *Allium cepa* Linn., Ascorbic acid, Limonene of *Citrus limon* (L.)Burm. f., Ricinine of *Ricinus communis* Linn. were retrieved from the systematic literature review. From reported data of the herbs, it was found that Aromadendrene showed the highest binding

affinity of -7. 89 kcal/mol. Then, Cucurbitacin B showed the second highest binding affinity of -7. 21 kcal/mol to the amino acids Ala105, Arg239 and Asn202, followed by Onionin A, Linolenic acid, Limonene, Sinapic acid, Ascorbic acid, Ricinine, Protocatechuic acid with binding energies of -7. 14 kcal/mol, -6. 58 kcal/mol, -6. 03 kcal/mol, -5. 45 kcal/mol, -5. 20 kcal/mol, -4. 61 kcal/mol, -4. 58 kcal/mol respectively (Table 2).

Cucurbitacin B, Linolenic acid, Protocatechuic acid shared three active site amino acid in common and Ascorbic acid shared two active site amino acid (Table 3). Though the compound Aromadendrene has the highest binding energy among all the compounds, while considering the interactions, it did not show any interactions with the amino-acid residues. Similarly, Onionin A, Limonene, Sinapic acid, Ricinine did not show any interactions with the amino-acid residues. As a whole, phytochemicals such as Cucurbitacin B of *Citrullus colocynthis*, Linolenic acid of *Melia dubia*, Protocatechuic acid of *Allium cepa* and Ascorbic acid of *Citrus limon* revealed maximum of 2 to 3 interactions accounting for 75-100% binding efficacy with the core active amino acid residues present on the target enzyme CYP- 17α-hydroxylase.

Conclusion

Based on the results of the computational analysis it was concluded that the bio-active compounds Cucurbitacin B, Linolenic acid, Protocatechuic acid and Ascorbic acid present in the herbal ingredients possess significant binding against the target enzyme CYP-17α-hydroxylase by interacting with active amino acids. Hence these phytocomponents which inhibit the target enzyme CYP-17α-hydroxylase may act as a potential therapeutic agent for management of PCOD. Based on further clinical trials and experiments, the formulation *Kalingathy Thailam* could be proved to be effective in treatment of PCOS.

Competing interst

There is no conflict of interest among the authors**.**

Fnding

No funding was received for the study.

Acknowledgement

I would like to thank all the faculties of Department of Maruthuvam**.**

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