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Efficacy of Siddha Formulation *Sathakuppai Choornam* Against CYP-17α-Hydroxylase Using In-Silico Model for Poly Cystic Ovarian Syndrome

Kavitha K[1], Bhagyalakshmi B[2], Ramya V[2], Muthukumar N J [3]

1.PG Scholar, Department of Varma Maruthuvam, National Institute of Siddha, Chennai 47, Tamil Nadu, India.

2.PG Scholar, Department of Pura Maruthuvam, National Institute of Siddha, Chennai 47, Tamil Nadu, India.

3.Head of the department, Department of Varma Maruthuvam, National Institute of Siddha, Chennai-47, Tamil Nadu, India.

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KEYWORDS

CYP- 17α-hydroxylase, Siddha Medicine, *Sathakuppai choornam*, PCOS

ABSTRACT:

Introduction: The polycystic ovarian syndrome *(Sinaipai neerkatti)* is a heterogeneous disorder characterized by hyper androgenism and chronic anovulation. Most women with PCOS have number of small cysts, or fluid-filled sacs, on their ovaries. They also experience menstrual cycle abnormalities, increased androgen (sex hormone) levels, excess hair growth, acne, and obesity. *Siddha* system plays an important role in treating and managing PCOS and its risks.

Objective: To find the efficacy of the *Siddha* formulation *Sathakuppai choornam* against CYP-17α-hydroxylase using the In-Silico method that may act as a potential therapeutic agent for management of PCOD.

Methods: The molecular docking study was used for docking against CYP- 17α-hydroxylase

Results: The phytochemical such as Oleic acid and Quercetin reveals maximum of 2-3 interactions with the core active amino acid residues present on the target enzyme CYP- 17α-hydroxylase.

Conclusion: The bio-active compounds like Oleic acid and Quercetin present in the herbal ingredients possess significant binding against the target enzyme CYP-17α-hydroxylase by interacting with active amino acids. Hence these phyto-components which inhibit the target enzyme CYP-17α-hydroxylase may act as a potential therapeutic agent for the management of PCOD.

1. Introduction

The polycystic ovarian syndrome *(Sinaipai neerkatti)* is a heterogeneous disorder characterized by hyper androgenism and chronic anovulation which is also called as Stein-Leventhal syndrome. Most women with PCOS have number of small cysts, or fluid-filled sacs, on their ovaries. The cysts are not harmful, but they cause hormonal imbalance. They also experience menstrual cycle abnormalities, increased androgen (sex hormone) levels, excess hair growth, acne, and obesity (1). Anxiety and depression are caused by PCOS. The metabolic consequences indicate impaired glucose tolerance, type 2 diabetes, obesity and high mortality of cardiovascular diseases. The various pathogenic mechanisms of PCOS include abnormal gonadotropin-releasing hormone (GnRH) regulation which leads to increased luteinizing hormone (LH) and decreased FSH; decreased response of ovarian follicles to FSH; increased anti-Mullerian hormone (AMH); follicular arrest and increased secretion of testosterone, estradiol and dehydro epiandrosterone (DHEA) (2).The

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global prevalence of PCOS is estimated between 4% and 20% [3]The World Health Organization (WHO) data suggests that approximately 116 million women (3.4%) are affected by PCOS globally (3).

Cytochrome P450 17alpha-hydroxylase/17, 20-lyase (CYP17) is a microsomal enzyme induce two distinct activities, 17alpha-hydroxylase and 17, 20-lyase, vital for the anabolism of adrenal and gonadal steroids. CYP17 is a potent oxidant, it is present in liver and non-steroid genic tissues, and it has distinct function in steroid metabolism (4).The variable enzyme cytochrome (CYP17A1), plays a critical role in the production of androgens, inducing two key reactions on pregnenolone (PREG) and progesterone (PROG), the first being a 17- hydroxylation to generate 17-OH PREG and 17-OH PROG (5).

Siddha system of medicine is one among the indigenous medical system of India. The ailments at the time of childhood, adolescent period, pregnancy and postmenopausal stages are treatable and managed well with Siddha system of Medicine. The common gynaecological disorders are Leucorrhoea, infertility, menorrhea, Pelvic Inflammatory Disease (PID),amenorrhea, Poly Cystic Ovarian Syndrome (PCOS),, etc.,(6)

The *Sathakuppai choornam* contains *Sathakuppai* (Anethum graveolens), *Karunjeeragam* (Nigella sativa),*Maramanjal* (Coscinium fenestratum) *and Vellam* (Saccharum officinarum). After the intake of this *choornam* drink *Sombu kudineer* (Pimpinella anisum) (7). The bioactive compound present in Anethum graveolens is Carvone, Limonene, Myristicin and Anethole (8); in Nigella sativa is Oleic acid and Phellandrene (9); in Coscinium fenestratumis Quercetin (10) and in Pimpinella anisum is Anisaldehyde (11).Thus, the *Sathakuppai choornam* mentioned in *Siddha* literature were selected for evaluating their ability to inhibit the enzyme CYP- 17α-hydroxylase.

2. Objectives

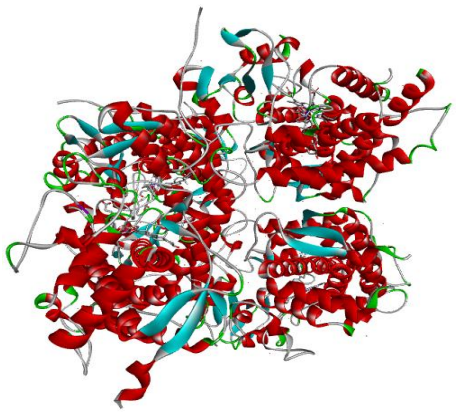
Phyto-components which suppress the target enzyme CYP- 17α-hydroxylase may act as a potential curative agent for the management of PCOD

3. Methods

Crystalline structure of the target enzyme CYP-17α-hydroxylase with PDB – 3RUK was retrieved from protein data bank and protein clean-up process was done and essential missing hydrogen atom were being added. Variant orientation of the lead molecules with regard to the target protein was evaluated by Auto dock program and the best dock pose was preferred based on the interaction study analysis.

3.1 Protein Preparation

Three-dimensional protein structure of the target protein CYP- 17α-hydroxylase with PDB – 3RUKwere retrieved from the online repository of Protein Data Bank and subjected to protein clean prior to docking simulation.



PDB	Name of the Target
3RUK	CYP- 17α-hydroxylase

Figure 1: 3D- Structure of CYP- 17α-hydroxylase (PDB) - 3RUK

3,2 Ligand Preparation

Phytochemical subjected to the investigation were retrieved from the herbs listed in the table based on the literature survey and 3D structure of the same were built using Chem Draw prof online

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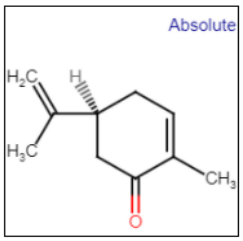
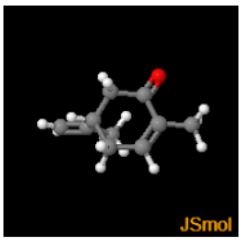
tool version 12.0. Ligands prepared through geometry optimization method (MMFF94).

Figure 2: 2D and 3D Structure of Phytocomponents

Ligand in 2D

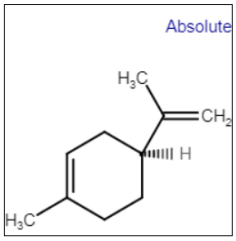


Ligand in 3D

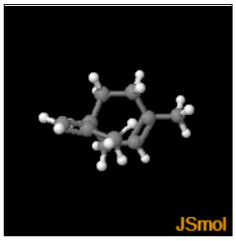


2.1 Carvone

Ligand in 2D

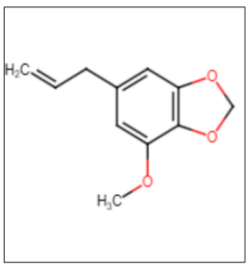


Ligand in 3D

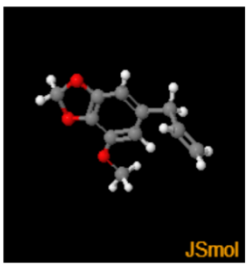


2.2 Limonene

Ligand in 2D

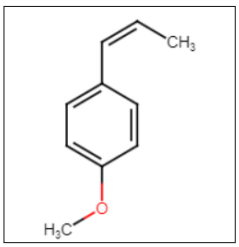


Ligand in 3D

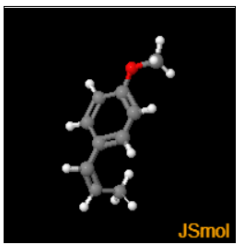


2.3 Myristicin

Ligand in 2D

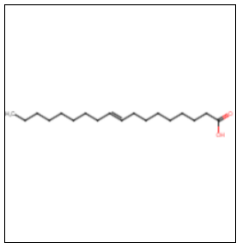


Ligand in 3D

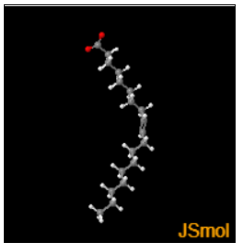


2.4 Anethole

Ligand in 2D

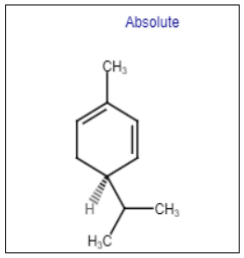


Ligand in 3D

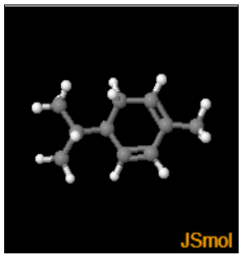


2.5 Oleic acid

Ligand in 2D

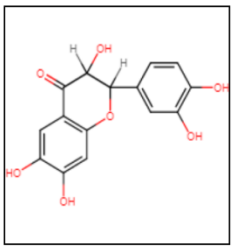


Ligand in 3D

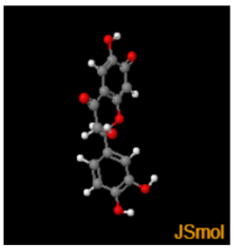


2.6 Phellandrene

Ligand in 2D

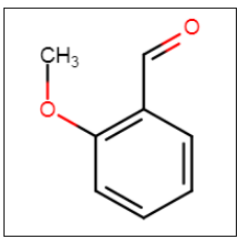


Ligand in 3D

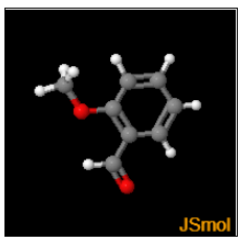


2.7 Quercetin

Ligand in 2D



Ligand in 3D



2.8 Anisaldehyde

3.3 Docking Methodology

Docking calculations were carried out for retrieved phyto-components against target enzyme CYP- 17α-hydroxylase. Essential hydrogen

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JCHR (2024) 14(2), 744-749 | ISSN:2251-6727

atoms, Kollman united atom type charges, and solvation parameters were adjunct with the aid of Auto Dock tools (*Morris, Goodsell et al., 1998*). Affinity (grid) maps of ×× Å grid points and 0.375 Å spacing were generated using the Autogrid program (*Morris, Goodsell et al., 1998*). AutoDock parameter set and distance-dependent dielectric functions were inclined in the assessment of the van der Waals and the electrostatic terms, respectively. Docking simulations were executed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method (*Solis and Wets, 1981*). Initial position, torsions of the ligand molecules and orientation were assigned at random. During docking torsions were released. Each docking experiment was concluded from 2 different runs that were set to terminable after a maximum of 250000 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 useful.

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
Carvone	150.221 g/mol	$C_{10}H_{14}O$	0	1	1
Limonene	136.23 g/mol	$C_{10}H_{16}$	0	0	1
Myristicin	192.21 g/mol	$C_{11}H_{12}O_3$	0	3	3
Anethole	148.20 g/mol	$C_{10}H_{12}O$	0	1	2
Oleic acid	282.5 g/mol	$C_{18}H_{34}O_2$	1	2	15
Phellandrene	136.23 g/mol	$C_{10}H_{16}$	0	0	1
Quercetin	302.23 g/mol	$C_{15}H_{10}O_7$	5	7	1
Anisaldehyde	136.148 g/mol	$C_8H_8O_2$	0	1	2

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

Compound	Est. Free Energy of Binding	Est. Inhibition Constant, Ki	Electrostatic Energy	Total Intermolec. Energy	Interact. Surface
Carvone	-6.18 kcal/mol	29.75 uM	-0.03 kcal/mol	-6.47 kcal/mol	361.948
Limonene	-6.05 kcal/mol	36.64 uM	-0.01 kcal/mol	-6.35 kcal/mol	357.491
Myristicin	-5.31 kcal/mol	128.62 uM	-0.10 kcal/mol	-6.13 kcal/mol	415.081
Anethole	-5.25 kcal/mol	142.54 uM	-0.02 kcal/mol	-5.85 kcal/mol	378.455
Oleic acid	-3.91 kcal/mol	1.36 mM	-0.05 kcal/mol	-4.21 kcal/mol	303.332
Phellandrene	-6.10 kcal/mol	33.51 uM	-0.12 kcal/mol	-6.40 kcal/mol	364.418
Quercetin	-7.50 kcal/mol	3.20 uM	-0.07 kcal/mol	-6.66 kcal/mol	491.771
Anisaldehyde	-4.67 kcal/mol	376.45 uM	-0.03 kcal/mol	-5.27 kcal/mol	332.341

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

Compounds	Interactions	Amino acid Residues											
Carvone	0	302ALA	306THR	366VAL	367ALA	371ILE	483VAL						
Limonene	0	302ALA	306THR	366VAL	367ALA	371ILE	483VAL						
Myristicin	0	113A	302A	305G	306T	366V	367A	483V					

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JCHR (2024) 14(2), 744-749 | ISSN:2251-6727

		LA	LA	LU	HR	AL	LA	AL					
Anethole	0	113ALA	302ALA	306THR	366VAL	367ALA	371ILE	483VAL					
Oleic acid	2	201TYR	202ASN	205ILE	239ARG	297GLY	298ASP						
Phellandrene	0	113ALA	302ALA	306THR	366VAL	367ALA	371ILE						
Quercetin	3	105ALA	113ALA	114PHE	202ASN	209LEU	239ARG	298ASP	302ALA	367ALA	371ILE	482VAL	483VAL
Anisaldehyde	0	113ALA	306THR	366VAL	367ALA	371ILE							

4. Results and Discussion

The CYP17A1 gene belongs to cytochrome P450 which is involved in the formation (synthesis) of steroid hormones. Cytochrome P450 17A1 (steroid 17α-monooxygenase, 17α-hydroxylase, 17-alpha-hydroxylase, 17,20-lyase, 17,20-desmolase) is an enzyme of the hydroxylase type that in humans is encrypted by the *CYP17A1* gene on chromosome 10CYP17A1 is a 57.4 kDa protein that belongs to the cytochrome P450 family(12,13). The protein coded by its cDNA is composed of 508 amino acid residues. As an enzyme, CYP17A1 possesses an active site that associates with a heme prosthetic group to catalyse biosynthetic reactions (14). Expression of CYP17A1 has been found in all of the traditional steroid genic tissues except the placenta, including the zona reticularis and zona fasciculata of the adrenal cortex, the Leydig cells of the testes, the thecal cells of the ovaries, and, more recently, in luteinized granulosa cells in ovarian follicles (15).The 17α-hydroxylase activity of CYP17A1 is required for the generation of glucocorticoids such as cortisol, but both the hydroxylase and 17,20-lyase activities of CYP17A1 are required for the production of androgenic and oestrogenic sex steroids by influencing 17α-hydroxy pregnenolone to dehydro epiandrosterone (DHEA) (15). Mutations in this gene are associated with isolated steroid -17α-hydroxylase deficiency, 17α-hydroxylase or 17, 20-lyase deficiency, pseudo hermaphroditism, and adrenal hyperplasia.

Total of 8 bioactive lead compounds were retrieved from the herbal ingredient in the *Siddha* formulation. On comparing binding affinities of the compounds it was found that quercetin showed highest binding affinity of -7.50 kcal/mol. Carvone showed the second highest binding affinity with the binding free energy of -6.18 kcal/mol. Phellandrene had -6.10 kcal/mol, Limonene had -6.05 kcal/mol, Myristicin had -5.31 kcal/mol, Anethole had -5.25 kcal/mol, Anisaldehyde had -4.67 kcal/mol, Oleic acid had -3.91 kcal/mol. The phytochemical such as Oleic acid and Quercetin reveals maximum of 2-3 interactions with the core active amino acid residues present on the target enzyme CYP- 17α-hydroxylase.

5. Conclusion

Based on the results of the computational analysis it was concluded that the bio-active compound's like Oleic acid and Quercetin present in the herbal ingredients possess significant binding against the target enzyme CYP-17α-hydroxylase by engaging 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.

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