INSILICO STUDY OF PHYTOCHEMICAL COMPONENT OF *ANGELICA SINENSIS* WITH GENES OF POLYCYSTIC OVARIAN SYNDROME

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Abstract

To investigate the efficiency of phytochemical components from *Angelica sinensis* against CYP 19A1 and CYP17A1 utilising computational molecular docking techniques. Polycystic ovarian syndrome is distinguished by hyperinsulinemia, menstrual abnormalities, long-term metabolic problems, and hyperandrogenism. An enzyme, CYP 19A1 and CYP17A1, stimulates the synthesis of androgens in the adrenal and ovarian glands. Hyperandrogenism is mostly caused by an increase in the activity of this enzyme. Excess androgen production in ovarian theca cells can be prevented by decreasing enzymatic activity.

Using CB docking, a phytochemical study of A sinensis was performed using CYP 19A1 and CYP17A1. These findings were compared to the docked scores of the fertility drugs letrozole and clomiphene citrate. The findings demonstrated that the chemicals found in A sinensis can help treat PCOS. This paper sheds light on the experimental approaches used to develop a novel medication for infertility.

Key words: Polycystic ovarian syndrome, Molecular docking, CYP 19A1, CYP17A1, *Angelica sinensis*

1. Introduction

PCOS primarily affects 5–10% of women in reproductive age. Polycystic ovarian syndrome (PCOS) is an endocrine disorder, which is characterized by hyperinsulinemia, hyperandrogenism, menstrual irregularities, and long-term metabolic disturbances in female [1]. Obesity, diabetes, and metabolic syndrome are typically present in women with PCOS, and these conditions increase the risk of infertility since ovulatory dysfunction is linked to poor reproductive outcomes. It is widely accepted that PCOS is a hereditary condition. Hyperandrogenism is the most typical sign of PCOS [2].

A multitude of anomalies contribute to the multifactorial condition known as PCOS. It is linked to all genes and mutations that directly or indirectly impact the ovaries [3].

Specific gene and its role in PCOS as follow [4]:

On chromosome 15q21.2, there is the CYP19 gene, which produces the aromatase p450 enzyme necessary for the production of estrogen. Both obese and lean PCOS women have been observed to have lower aromatase activity.

R. Somashekhar et al. (eds.), *Proceedings of the International Conference on Advances in Nano-Neuro-Bio-Quantum (ICAN 2023)*,
Advances in Intelligent Systems Research 69,
https://doi.org/10.2991/978-94-6463-294-1_17

An enzyme (P450c17), which is encoded by CYP17, catalyzes the conversion of progesterone and pregnenolone into 17- hydroxyprogesterone and 17- hydroxypregnenolone, respectively.

As a result of the overexpression of the CYP17 gene in PCOS patients, androgen is converted to testosterone more quickly than it is in healthy theca cells.

An enzyme that is necessary for an intermediate phase in the conversion of cholesterol to progesterone is encoded by the CYP11a gene. This phase of the conversion of cholesterol is rate-limiting.

An enzyme CYP21 catalyzes the conversion of 17-hydroxyprogesterone to 11-deoxycortisol during the production of steroid hormones. Ineffective anabolism of steroidogenesis results from a less efficient enzyme as a result it contributes to PCOS.

Today, a wide variety of medications are available to treat PCOS's symptoms and underlying causes. Congenital heart disease and congenital malformation are the primary adverse effects of the commercial synthetic medication clomiphene citrate and letrozole, which are used to treat infertility.

In order to treat infertility diseases, naturally extracted chemicals from medicinal plants have been chosen and developed as an effective therapeutic agent. Finding plant-based medications for all disease-related issues must focus on being easily accessible, frequently available, and having less adverse effects.

The herb *Angelica sinensis*, also referred to as dong quai or female ginseng, is a member of the Apiaceae family. In East Asia, it grows in the chilly, high highlands. One of the oldest and most revered plants in the Chinese pharmacopeia is dong quai. Major essential oil compounds like ligustilide, butylidenphthalide, and butylphthalide are found to be present in *A. sinensis* [5].

Molecular docking studies are frequently used in the contemporary drug design process to pinpoint protein-ligand interactions. To treat female reproductive problems, in silico docking analysis must be used to take advantage of naturally occurring active molecules with strong therapeutic potential and little side effects.

Objective:

The objective of the study, using molecular docking experiments, focuses on determining the phytoconstituents found in Angelica sinensis' binding efficiency against CYP 17 and CYP19A1. The results were also compared to the docking scores of the recognized fertility-inducing drugs letroze and clomiphene citrate against CYP 17 and CYP19A1. For the development of new PCOS medications, this study offers a good foundation.

2. Materials and Methodology:

2.1Materials:

PUBCHEM: PubChem is a database of help to identify structure of the chemical compounds and their biological functions.https://pubchem.ncbi.nlm.nih.gov

PDB: The Protein Data Bank (PDB) is a database that contains three-dimensional structural data for biological entities including proteins and nucleic acids. https://www.rcsb.org/

Molinspiration: Molinspiration cheminformatics is a software application used to process molecules, including SMILES and SDfile conversion, molecule normalization, tautomer synthesis, molecule fragmentation, and molecular property calculation. https://www.molinspiration.com/

SwissADME: To aid in drug discovery, compute physicochemical descriptors as well as forecast ADME parameters, pharmacokinetic features, druglike nature, and medicinal chemistry friendliness of one or more small compounds. http://www.swissadme.ch/index.php

Drug bank: Drug databases are sites where information about drugs, drug targets, and more, is used by researchers and health professionals globally. https://go.drugbank.com/

SWISS–MODEL: automated protein structure homology-modeling server. https://swissmodel.expasy.org

CB-Dock: CB-Dock is a tool for protein-ligand docking that automatically finds binding sites, determines center and size, and adjusts the docking box size based on the query ligand. It employs either Open Babel or Auto Dock Tools. http://clab.labshare.cn/cb-dock/php/index.php

2.2 Methodology - Molecular docking analyses:

2.2.1 Ligand preparation:

To identify ligands for molecular docking studies, the PubChem web site was used to study the structure of *A. sinensis* phytochemical components [5]. Phytochemical components were analyzed using the Lipinski rule of five and then chosen as ligand molecules using Molinspiration. Components of letrozole and clomiphene were used as a positive control (**Table 1)**.

SwissADME datasets were used to predict ADME features, pharmacokinetic properties, and drug similarity, assisting in drug discovery.

S. NO	Compound name	Smile	Molecular weight g/mol	Hydrogen bond acceptor	Hydrogen bond donor	M log P
1	Z-ligustilide	CCCC=C1C2=C(C=CCC2)C(=O)O1	190.24	2	0	2.75
2	Z-butylideneph	CCCC=C1C2=CC=CC=C2C(=O)O1	188.22	2	0	2.94

	thalide					
3	3-butylphthalide	CCCCC1C2=CC=CC=C2C(=O)O1	190.24	2	0	2.81
4	senkyunolide F	CCC(C=C1C2=C(C=CCC2)C(=O)O1)O	206.24	3	1	1.91
5	6,7-dihydroxyligustilide	CCCC=C1C2=C(C(C(CC2)O)O)C(=O)O1	224.25	4	2	1.22
6	bergapten	COC1=C2C=CC(=O)OC2=CC3=C1C=CO3	216.19	4	0	2.16
7	6-methoxy-7-hydroxycoumarin (scopoletin)	COC1=C(C=C2C(=C1)C=CC(=O)O2)O	192.17	4	1	1.52
8	umbelliferone	C1=CC(=CC2=C1C=CC(=O)O2)O	162.14	3	1	1.51
9	xanthotoxin	COC1=C2C(=CC3=C1OC=C3)C=CC(=O)O2	216.19	4	0	2.16
10	6-methoxycoumarin	COC1=CC2=C(C=C1)OC(=O)C=C2	176.17	3	0	1.80
11	coniferyl ferulate	COC1=C(C=CC(=C1)C=CCOC(=O)C=CC2=CC(=C(C=C2)O)OC)O	56.37	6	2	3.25

Table 1 - Lipinski rule of 5 of Phytochemical compound of *A. sinensis*

2.2.2 Target Preparation:

The raw X-ray crystal structures of CYP19A1 (3EQM) and CYP17A1 (3RUK) were obtained from the PDB database, but the raw structure could not be used directly for molecular docking experiments. Hence, the original PDB structure was modified using SWISS - MODEL to find the Q MEAN score and generate a pdb file.

2.2.3 Docking:

The CB-Dock (Cavity-detection guided Blind Docking) server was used to simulate the docking of the target proteins CYP19A1 (3EQM), CYP17A1 (3RUK) with the ligand phytochemicals and Letrozole, Clomiphene components. Using a unique curvature-based cavity identification approach, this server predicts the binding sites of a given protein and estimates the centers and sizes. The server collaborates with AutoDock Vina and has been rigorously optimized to achieve a success rate of more than 70% in the models built. Using protein files in.pdb format and ligand files in pdb format, five possible coupling cavities were discovered throughout the tests. The option with the lowest binding energy was chosen based on the lowest Vina value discovered. The ligands and the proteins were then visualized with the space fill and secondary structure respectively.

3. Results:

The size of the active site was established by creating the receptor grid. The most likely orientation of the ligands in the binding pocket is predicted, and the strength of the interaction in that orientation is assessed using a scoring system. Docking analysis was performed using CB dock software between ligands and the target proteins CYP19A1, CYP17A1, and the docked pictures are shown in Fig1 and Fig2. The docking of ligand and protein was graded according to the relevant scoring function, with the larger negative value on the scoring function indicating better docking.

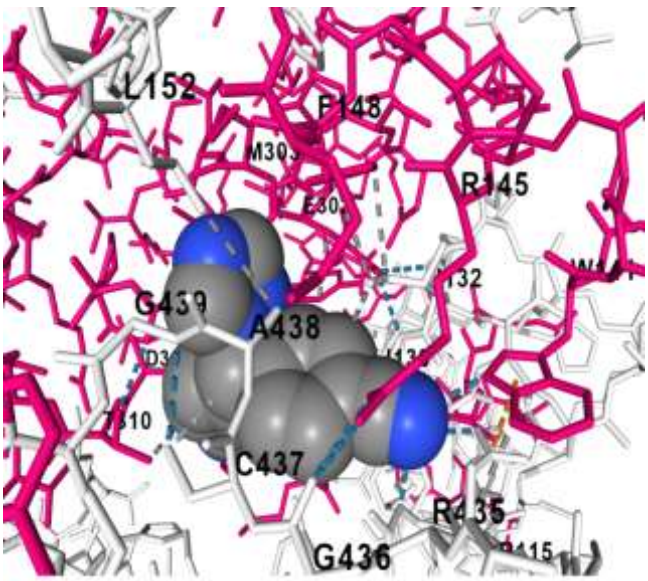

Fig1 a. Docking image of letrozole with CYP19A1

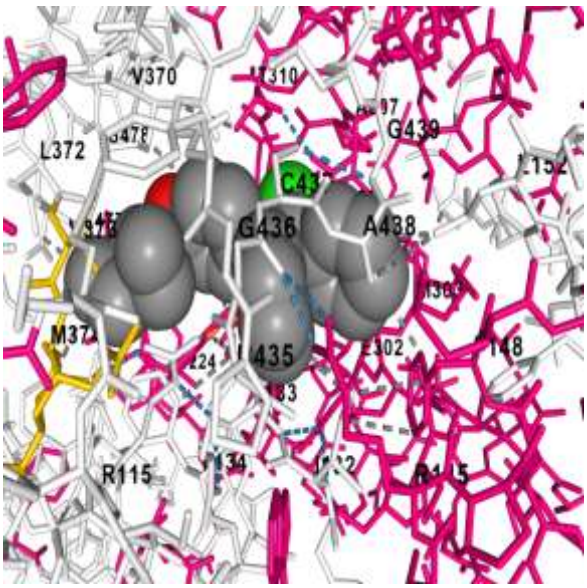

Fig1 b. Docking image of Clomiphene with CYP19A1

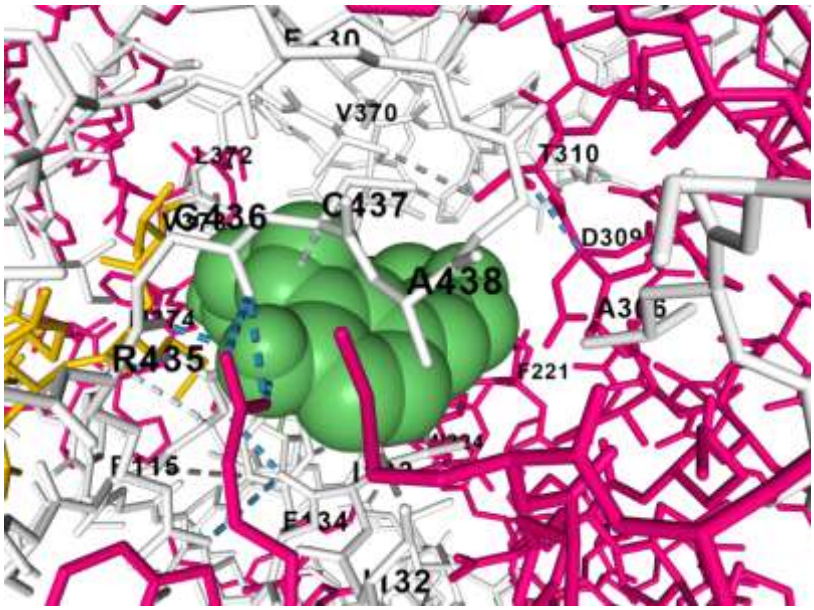

Fig1c. Docking image of coniferyl ferulate with CYP19A1

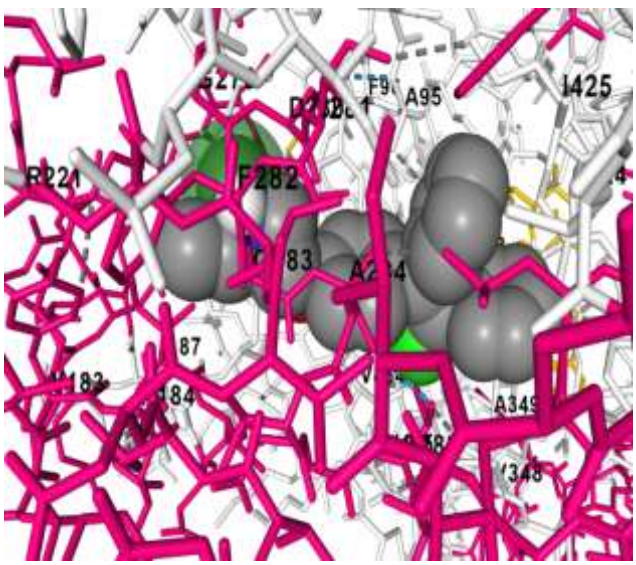

Fig2 a. Docking image of letrozole with CYP17A1

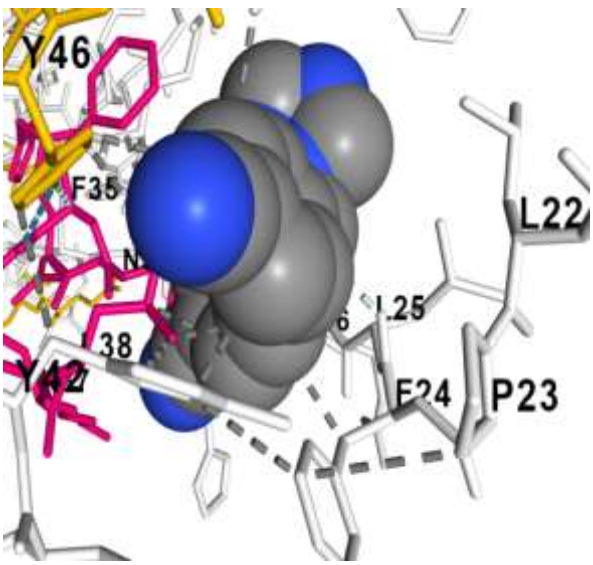

Fig2 b. Docking image of Clomiphene with CYP17A1

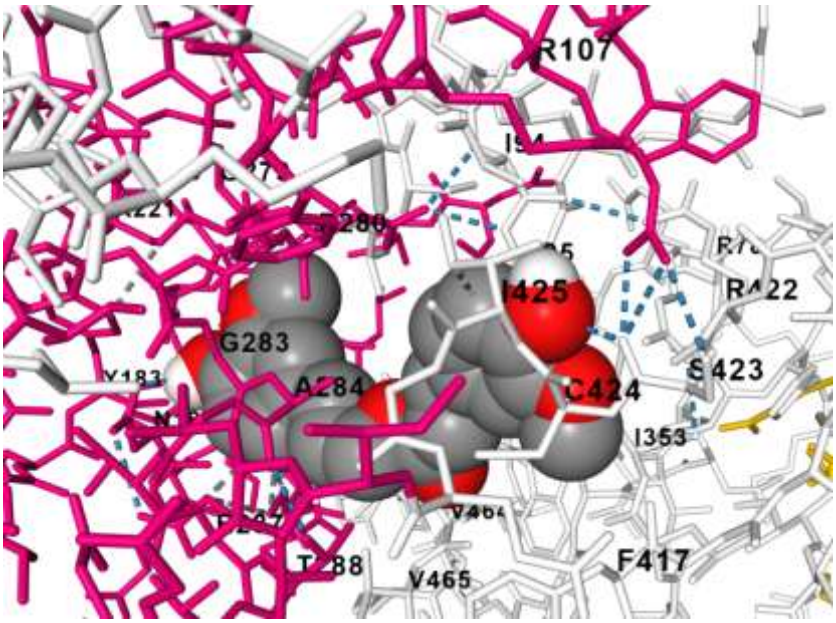

Fig2c. Docking image of coniferyl ferulate with CYP17A1

Molecular interaction and binding affinity of ligand compounds with target proteins, CYP19A1 , CYP17A1, were evaluated by docking. The docking results of the ligands were tabulated in CYP19A1 (Table 2) and CYP17A1 (Table 3).

The various interaction energies, such as van der Waals energy, intermolecular hydrogen bonding, and electrostatic energy, were calculated for each minimised complex. Vina-score for ligands ranging from 5.9 to 8.3 against CYP19A1.

Vina-score for the recognised fertility medication letrozole -8.3 clomiphene citrate -8.1 docked against CYP19A1. A. sinensis phytochemical compounds include coniferyl ferulate (-8.2), Z-butylidenephthalide, and Bergapten, xanthotoxin (-7.0), Z-ligustilide, senkyunolide F (-6.6), 3-butylphthalide (-6.6), 6-methoxyligustilideScopoletin (-7-hydroxycoumarin), umbelliferone (-6.1), and 6-methoxycoumarin (5.9).

S.NO	Ligand molecules	**Target protein - CYP19A1**	
		Cavity Size	Vina Score
1.	Letrozole	2054	-8.3
2.	Clomiphene	2054	-8.1
3.	Z-ligustilide	890	-6.8
4.	Z-butylidenephthalide	890	-7.0
5.	3-butylphthalide	890	-6.6
6.	senkyunolide F	2054	-6.8
7.	6,7-dihydroxyligustilide	537	-7.0
8.	bergapten	537	-7.0
9.	6-methoxy-7-hydroxycoumarin (scopoletin)	537	-6.1
10.	umbelliferone	537	-6.1
11.	xanthotoxin	2054	-7.0
12.	6-methoxycoumarin	2054	-5.9
13.	coniferyl ferulate	2054	-8.2

Table 2: Docking score of phytochemical component of *A. sinensis and infertility drugs with CYP19A1*

S.NO	Ligand molecules	Target protein - CYP17A1	
		Cavity Size	**Vina Score**
1.	Letrozole	909	-8.8
2.	Clomiphene	2118	-8.2
3.	Z-ligustilide	2118	-7.1
4.	Z-butylidenephthalide	2118	-7.2
5.	3-butylphthalide	2118	-6.8
6.	senkyunolide F	2118	-6.9
7.	6,7-dihydroxyligustilide	2118	-7.0
8.	bergapten	2118	-7.4
9.	6-methoxy-7-hydroxycoumarin (scopoletin)	2118	-6.6
10.	umbelliferone	2118	-6.0
11.	xanthotoxin	2118	-7.6
12.	6-methoxycoumarin	2118	-6.1
13.	coniferyl ferulate	2118	-8.2

Table 3: Docking score of phytochemical component of *A. sinensis and infertility drugs with CYP17A1*

Letrozole has a vina-score of -8.8 and clomiphene citrate has a vina-score of -8.2 when docked against CYP17. Phytochemical *A. sinensis* compounds such as coniferyl ferulate (-8.2) had the same binding score as clomiphene citrate. xanthotoxin (-7.6), bergapten (7.3), Z-butylidenephthalide (-7.2), Z-ligustilide (-7.1), and 6,7-dihydroxyligustilide (-7.0). These substances are less active than medicines. As a result, it had a higher vina score than the CYP19A1.

This indicated that the coniferyl ferulate (-8.2) compounds have the maximum binding capacity when docked against the protein's structure. This study concluded that the phytoconstituents found in A. sinensis (coniferyl ferulate could be potential compounds for the development of reproductive medicines.

4.**Discussion:**

One of the important genes in PCOS follicular arrest is CYP19A1, which produces cytochrome P450 aromatase, which converts androgens to 17 estradiol (E2) [6] .

Letrozole is a non-steroidal aromatase inhibitor of type II. It inhibits the active site of CYP19A1 and thus the electron transport chain. This competitive inhibition inhibits androgens from being converted to estrogen. This action causes a decrease in uterine weight and an increase in luteinizing hormone. Aromatase is responsible for the majority of estrogen production in postmenopausal women [7,8].

The promoters of the CYP17A1 gene were studied because this region regulates the enzymes that control the rate-limiting steps in androgen production [9].

Clomiphene citrate contains both estrogenic and anti-estrogenic effects. It appears to stimulate the secretion of gonadotropins, follicle-stimulating hormone (FSH), and luteinizing hormone (LH), which leads to ovarian follicle development and maturation, ovulation, and subsequent development and function of the corpus luteum, resulting in pregnancy [10,11].

CYP19A1 and CYP17A1 were play an important role in PCOS was showed in the Fig 3

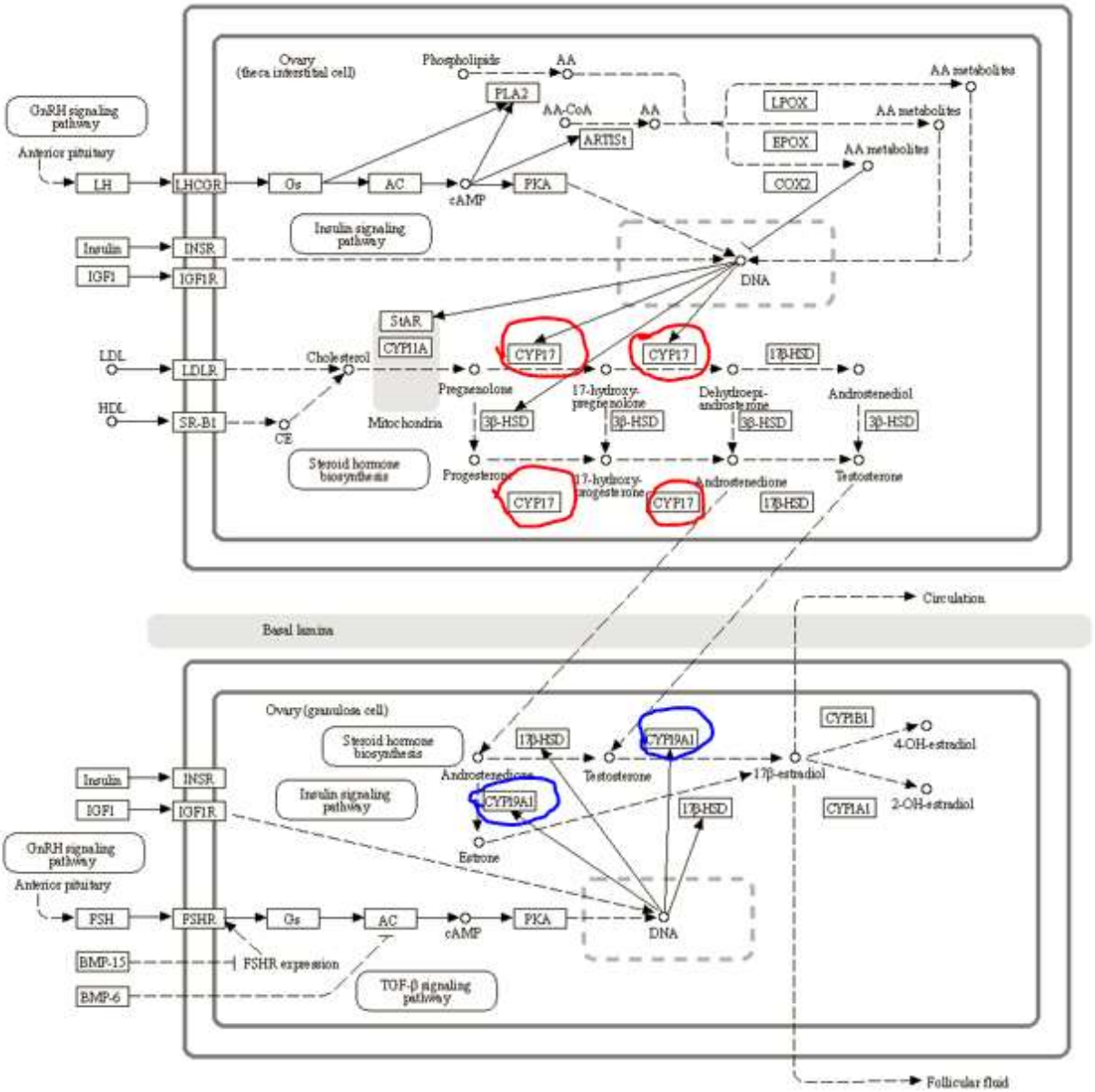

Fig 3. Ovarian steroidogenesis - KEGG pathway (https://www.genome.jp/pathway/hsa04913)

Gonadotropin release can be caused by either direct stimulation of the hypothalamic-pituitary axis or by estrogens losing their inhibitory influence on the axis by competing with endogenous estrogens from the uterus, pituitary, or hypothalamus. According to the findings of this investigation, A. sinensis photochemical such as coniferyl ferulate have the same impact as Clomiphene citrate.

5. Conclusion

Effective screening processes could be used during drug discovery to minimise costs and time. In this study, effective approaches of molecular docking with CB docking software, was utilised to analyse the binding ability of phytochemical compounds with CYP19A1 and CYP17A1. Coniferyl ferulate, a chemical from A. sinensis, has a high binding score.

This demonstrated the significance of compounds from various herbals as docking agents. As a result of this work, preliminary screening for possible fertility medicines derived from A. Sinensis has been created for PCOS. This paper sheds light on the experimental approaches used to develop a novel medication for infertility.

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