Ameliorative Role of *In-Silico* Study in Phytoconstituents of Cordyceps Used for PCOS

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Abstract

***Background*:** Polycystic ovarian syndrome (PCOS) is a heterogeneous endocrine disease that impacts about one in 15 girls worldwide. It is a major sickness characterized through multiplied ranges of male hormones (androgens), acne and hirsutism.

***Objective*:** *In silico* study of different compounds from Cordyceps into active site of the target protein. (3RUK).

***Methods*:** Computer-aided drug plan principles should enhance the discovery of putative Cordyceps-derived medication inside much less time and low budget. The integration of computer-aided drug design techniques with experimental validation has contributed to the profitable discovery of novel drugs.

***Results*:** Results indicated that all the ligands have a strong binding affinity for the Human Cytochrome P450 CYP17A1 receptor as indicated by their docking score values that were found to be comparable with the docking score of the molecules1,6-di-o-glloyl-d-glucose (-12.172) Isoquercitrin acid (-11.366) etc.

***Conclusion*:** It has been concluded that computer-aided drug design techniques could influence the multiple target-focused drug design, *In silico* studies were performed on the different compounds from Cordyceps into active site of the target protein. (3RUK). Compound 1,6-di-o-glloyl-d-glucose was found to have highest affinity towards the Human Cytochrome P450 CYP17A1receptor(docking score = -12.172). Other different compounds from Cordyceps have also good dock scores.

***Keywords**:* PCOS, In-silico, Cytochrome P450 CYP17A1, Protein, Ligand.

1. Introduction:

Cordyceps sensulato are economically, medicinally and ecologically necessary entomo pathogenic fungi. Most of the species life-style are endo-parasitic, frequently relying on other insects and some arthropods, and few of them depend on different fungi to fulfill their parasitic necessities [1]. Cordyceps species originate in a variety of areas of Asia, North America and Europe [2]. Cordyceps products, cultured both naturally or artificially, are very well-known natural remedy and as properly as healthy meals for this universe. Cordyceps species incorporates an extensive range of nutritionally widespread components including crucial amino acids, proteins and polypeptides, essential oils, fatty acids, sterols, inorganic metals, polysaccharides, pyridines, phenols, almost all types of few aliphatic and aromatic ketones, aldehydes, etc[3]. Cordyceps and their extracts have fantastic medical effects including motion on cardiovascular, immunological systems, hepatic, renal, respiratory, nervous, sexual, anti-cancer, anti-oxidants, anti-inflammatory and anti-microbial things to do [1] . Cordyceps are additionally one of the possible sources of natural cosmetics. Cordyceps research on a variety of skin problems and its metabolites have been performed for a number of skin issues [4].

Polycystic ovary syndrome (PCOS) is a notably normal endocrine ailment and a main cause of infertility, affecting at least 10% of reproductive-age girls [5]. PCOS is a common reproductive ailment in female that is described by two out of three criteria[5]: menstrual irregularity(oligo-ovulation or anovulation), [6] hyper androgenism (clinical or biochemical) and [7] polycystic ovarian morphology [6]. One in every 5–6 female is facing serious complications regarding infertility and irregularity in their menstrual cycles. This endocrine disorder affects women underneath 18–44 age [7]. PCOS is associated with a huge expand in threat elements such as cardiovascular disease, type 2 diabetes, and infertility. Data estimates that 38–88% of women with PCOS are obese or overweight throughout the world, with an extended charge in the United States to reflect the greater weight problems quotes in the non-PCOS populace [8].

1.1.Etiology of PCOS:

The genetic and environmental component is accountable for the etiology of this condition. Unhealthy lifestyle, weight-reduction plan or any infectious mediators extend the danger of PCOS. Due to insulin resistance and its multiplied level, the ovaries feature disturbs that rises androgen level which leads to anovulation [9]. Apart from the environmental factors, there are genetic elements that are accountable for the etiology of PCOS. Its cause includes candidate genes, SNP's. According to databases PCOS etiology entails 241 gene variants [10]. Polymorphism or any change in nucleotide may results into defects in the transcriptional activity of a gene that results into PCOS. Mostly genes that encode for the androgen receptor, Luteinizing Hormone receptors, Follicular Stimulating Hormone receptors, Leptin receptors are accountable for PCOS [7]. Gene defect may alter the biochemical pathway and results into days functioning of an ovary. Polymorphism such as StAR polymorphs, FSHR polymorphism, FTO polymorphism, VDR polymorphism, IR and IRS polymorphism, GnRH polymorphism are determined to be involved in PCOS. PCOS development and severity increases with the rise in insulin level as properly as an androgen. Hyperinsulinemia impacts ovarian theca cells and increase androgen level. This situation reduces the hepatic biosynthesis of SHBG andIGFBP-1. Elevated androgen level, on the different hand, stimulates visceral adipose tissue

(VAT) that generates free fatty acids (FFA's)which contributes in insulin resistance [11]. Specific gene andits loci for PCOS as follows:

• Gene CYP 11A1 incorporates coding and promoter areas for the translation of protein, P450 SCC.

• Promoter vicinity of CYP 17 encodes a unique androgen regulating protein, P450 17α

• Gene which encoding the enzyme, leptin performs a fundamental position in reproductive characteristic and obesity.

The steroidogenic enzyme, CYP 17 features as hydroxylation and lyase. It is present in the zonareticularis and zona fasciculate of the respective gonad tissues and adrenal cortex. In the first step of enzymatic activity, hydroxylation of pre gnenolone and progesterone at the C17 function takes place ensuing in the formation of 17-hydroxypregnenolone and 17-hydroxyprogesterone.During the second step of enzymatic action, C17-C20 bond of 17-hydroxypregnenolone and 17 hydroxy progesterone are cleaved to generate dehydro epiandrosterone and androstene dione respectively. [12] Increase in CYP 17 exercise in adrenal and ovarian sites induces hyperandrogenism in PCOS. In PCOS patients, gene that encodes CYP 17 has been overexpressed and androgen has been converted greater correctly to testosterone than ordinary theca cells [13].

1.2.Pathophysiology:

Disrupted secretion of the pulsatile gonadotropin - releasing hormone (GnRH) from hypothalamus is a issue accountable for PCOS [14]. GnRH induces the pituitary gland to secrete follicle stimulating hormone (FSH) and luteinizing hormone (LH). These two hormones are essential for the two wonderful phases of menstrual cycle. In PCOS, as these hormones are scanty, the egg is both no longer formed, or can't be liberated from the follicle. So, the cycle is disrupted and amenorrhea occurs, which can be of two types, the main or secondary amenorrhea. While predominant amenorrhea is the incapability to attain menarche due to chromosomal or anatomic issues, secondary amenorrhea, additionally called hypothalamic amenorrhea, is characterized by way of the absence of menstrual cycles for three or greater consecutive months [15]. High stage of prolactin, a peptide hormone, blocks the GnRH [16]. As the human physique is a complicated machine and the metabolites are functionally-interlinked, disturbance in one can have an effect on the others as well. Upset in the stage of a quantity of hormones (prolactin, anti-Müllerian hormone (AMH), cortisol, androgen), neurotransmitters (dopamine), peptides, lipid, protein, and glucose are related with PCOS manifestation. Hyperprolactinemia motives hypogonadotropic hypogonadism, characterized with amenorrhea, galactorrhea (abnormal milk manufacturing from the breasts), and osteoporosis [17].

1.3.Symptoms:

Anovulation or oligovulation is a frequent symptom of PCOS. Some of the cysts produce androgens, which end result in the civilization or the expression of male-like characters in the females. So, PCOS leads to the appearance of a gamut of masculine signs or 'hyperandrogenism'. Visible symptoms of hyperandrogenism consist of weight gain, belly and subcutaneous fat, hirsutism (facial and physique hair), male-pattern alopecia (hair loss), clitoromegaly (enlargement of the clitoris), deep voice, seborrhea (oily skin), pimples etc [18].

Apart from these morphological features, alteration in metabolic profile occurs. Insulin resistance is a main symptom of PCOS. It causes hyper insulinemia, and can lead to diabetes mellitus. High insulin degree is responsible for the deposition of fats round the stomach or central adiposity. In a majority of ladies with PCOS, the physique mass index (BMI) is 30 or higher. Other than that, hypertension, cardiovascular issues, dyslipidemia, etc. are co-morbidities of PCOS [19, 20]. A healthy blood stress for women is one hundred twenty over eighty or less. PCOS sufferers are at a high danger for the improvement of early-onset cardiovascular disease. The PCOS sufferers regularly show sugar cravings, frequent urination, delayed healing, fatigue, blurred vision, tingling sensation, mood swing, anxiety, and melancholy episodes. It is understandable, as these conditions are tied to diabetes as well. The sufferers frequently experience pelvic pain, fever, nausea, vomiting, urinary conditions, constipation etc. Pressing of the large cysts towards the bladder or rectum is accountable for the anomalous urinary and bowl movement. Sleep apnea (sleep changes in which breathing persistently stops and starts) is any other symptom of PCOS, arising due to altered intercourse steroid stage [21]. PCOS can put woman at the threat for uterine cancer, as the prevailing excessive estradiol degree and the lack of progesterone due to ovarian malfunction will increase the danger of endometrial hyperplasia [22]. Mucus-deficient endocervix, and smooth vagina is a characteristic of PCOS, which can be determined at some stage in a pelvic examination. Due to the hormonal imbalance in PCOS, pores and skin develops light brown or black patches, a circumstance acknowledged as ‘acanthosisnigricans’. Skin of neck, armpits, thighs, and breasts are extra susceptible to this skin pigmentation. Also, pores and skin tags show up in these regions. In fact, the dark pigmentation is a cutaneous marker for insulin resistance [23]. The metabolic syndrome resultant of PCOS is vast. In fact, the pathologies are bilateral, as metabolic syndrome, and the consistent inflammations, can lead to PCOS. Based on evidences, the hyperlink between non-alcoholic fatty liver ailment (NAFLD), a persistent liver disorder characterized by hepatic injury from fatty liver infiltration main to end-stage liver disease, and PCOS has been traced, which has indicated a novel hepato ovarian axis [24].

Ovarian hyper stimulation syndrome (OHSS) is a circumstance of fluid collection in the stomach and chest (ascites and pleural effusion), resulting due to issues in ovulation induction. This shift of fluids into the third area i.e. stomach and pleural cavity is due to vascular hyper permeability. OHSS is graded primarily based on the symptoms. It can be mild, main to weight gain, stomach pain, nausea and vomiting, bloated stomach due to ovarian distension (from 5 to 12 cm), low urinary sodium excretion, oliguria etc. But sometimes, the situation is severe, manifesting in problem with breathing; ionic imbalance; deep vein thrombosis; hypovolemia, rupture of a cyst in an ovary main to serious bleeding; ovarian torsion; being pregnant loss from miscarriage, or termination due to the fact of complications; pulmonary embolism, kidney failure etc. Ovarian torsion is a scientific emergency, and it can reduce off blood to the ovaries, inflicting extreme ache and bleeding. In serious cases, death can happen due to hypovolemia, hypercoagulation, respiratory, and circulatory cave in [25, 26]. It potential the sodium and potassium pump performance is affected. The hormone HCG (human chorionic gonadotrophin) motives the ovary to bear giant luteinization, causing the release of extra estrogens, progesterone, and nearby cytokines. Vascular endothelial increase component (VEGF) is a substance that increases the vascular permeability. VEGF induces HCG to increase capillary permeability in OHSS. PCOS makes the woman inclined to OHSS [27]. Apart from

these effects, PCOS can have an effect on the psychiatric aspects of a patient's life. Anxiety, depression, binge consuming disorder, and bipolar disease have been found as PCOS co-morbidities [19, 28]. In postmenopausal females, with PCOS, cerebral white matter develops lesions. The neural pathology is possibly to be due to neural damages [25].

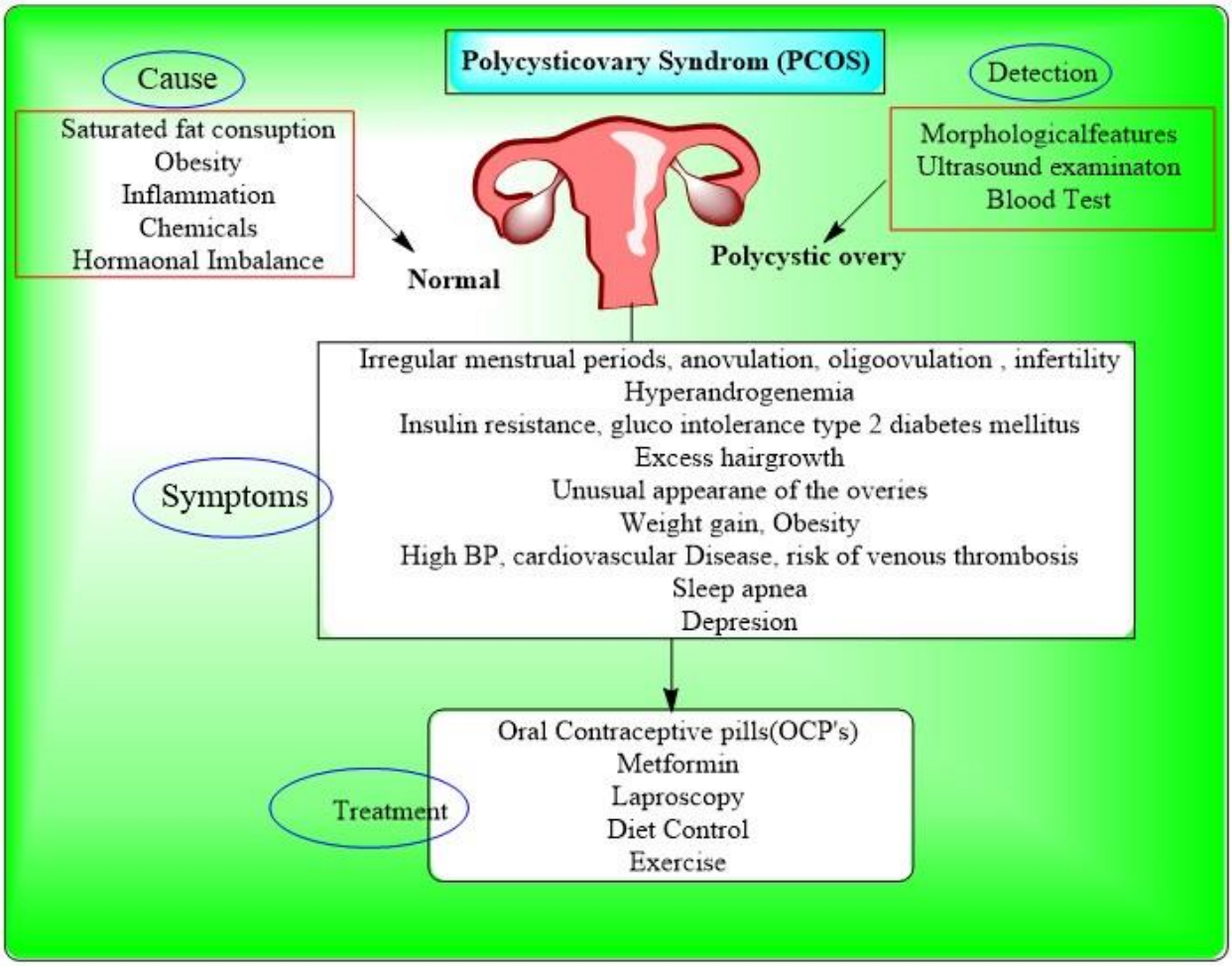


Figure 1 Causes, diagnosis, symptoms and allopathy treatment of PCOS

2. Material and Method

2.1. Mac Operating System platform installed on Apple I Mac was used to perform docking studies on Maestro 12.0 (Schrodinger 2020). The study was done to identify the possible binding modes of the selected ligands to the active site of the protein receptor.

2.2. Selection and Preparation of Ligands

Ligand preparation was done by using the application Ligrep wizard of Maestro 12.0 (Schrodinger 2020). In this step, ligand structure was converted into a 3D form, from 2D, hydrogen atoms were added, discrepancies between bond lengths and angle were resolved, low energy structure and ring conformation followed by minimization and OPLS 2015 force field were conducted for the preparation of data. Consecutively, the rest of the factors such as ionization state were not altered, and specified chirality was retained.

2.3.Preparation of the Protein Molecules

The PDB for the X-ray crystallographic structure of Human Cytochrome P450 CYP17A1 (PDB ID: 3-RUK) prepared by Maestro 12.0 protein preparation wizard was obtained from the Protein Data Bank (RSCB). Protein preparation was done in the protein preparation wizard of Schrodinger maestro software. Following steps were done to prepare protein:

- Assignment of bond orders.
- Additions of hydrogen atoms.
- Deletion of the bonds to metal.
- Setting of the formal charge on the metal
- Deletion of the neighboring atoms that were at a distance more than 5Å.
- The addition of any missing disulphide bonds was done.
- Optimization of hydrogen bonds was done at pH 7.0.
- Overlapping issues in the hydrogen bond network were solved by reorienting hydroxyl group, water molecules, and amino acids.
- In the tab "Review and modify", only the required part of the protein under study is kept by making a selection and the rest was deleted.
- Then, possible protonation states of the co-crystallized ligand were generated by clicking on generate states option. The structure of the ligand was reviewed to solve the problems, if any.
- Finally, with the help of restrained minimization, refinement of the structure was done.

2.4.Receptor Grid Generation

Ligand binded within the X-ray crystal structure of a protein was utilized by Glide molecular docking for the identification of active site receptor grid. Thus, the ligands were assisted by grid based molecular docking to bind in more than one possible conformations. 0.25 Å, scaling factor and 1.0 Å, partial charge cutoff of Van Der Waals radius and other parameters were also applied.

2.5.Glide Molecular Docking

After the preparation of the ligands, protein and the grid on the active site of the target protein, molecular docking was carried out. Glide molecular docking used computational simulation method for the evaluation of binding poses. Glide systematic method is a newer approach for the quick, precise molecular docking, and its output G- score (which is an empirical scoring function), is the combination of various factors. The binding energy which includes Ligand-protein interaction energies was calculated in kcal/mol. Determination of H-bond, lipophilic interaction, π-π stacking interactions, internal energy, and RMSD (Root Mean Square Deviation) and desolvation energy was also done. XP visualizer was used for the analysis of the specific ligand-protein interactions. All of the selective ligands with the X-ray crystal structure were docked including reference compound using Glide

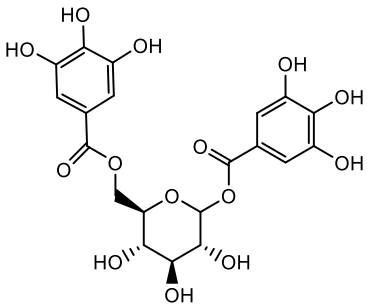
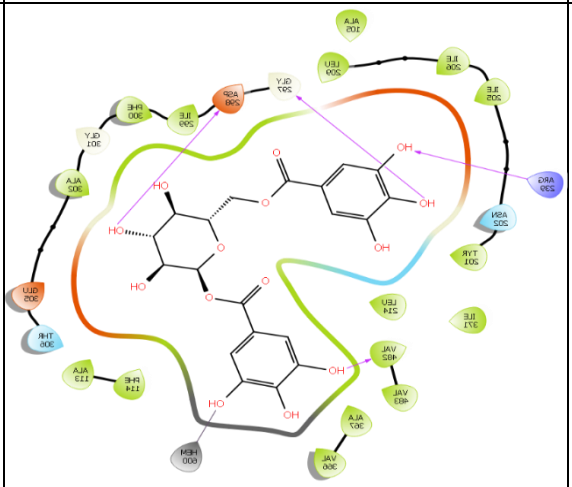
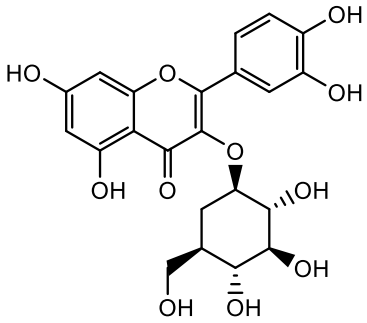
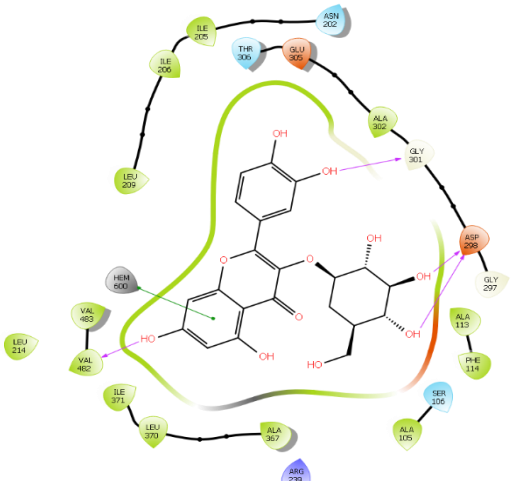
3. Results and Discussion

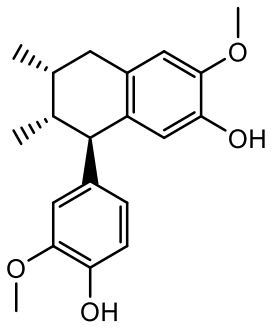
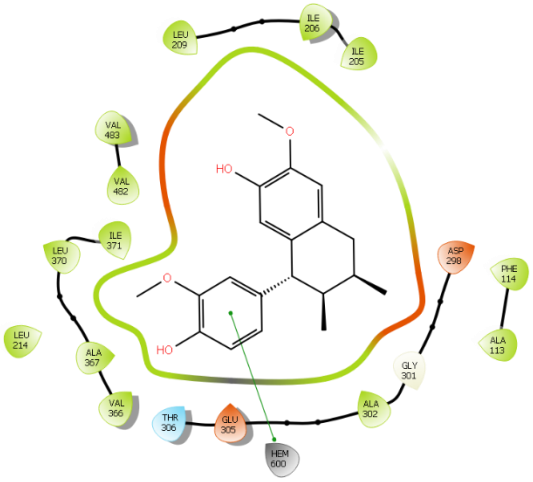
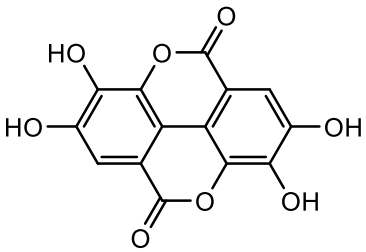
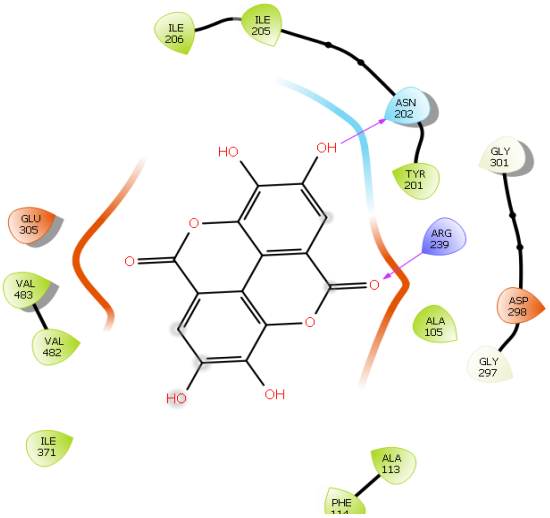
3.1. Evaluation of the Docking Study

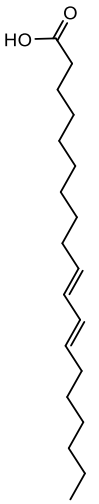
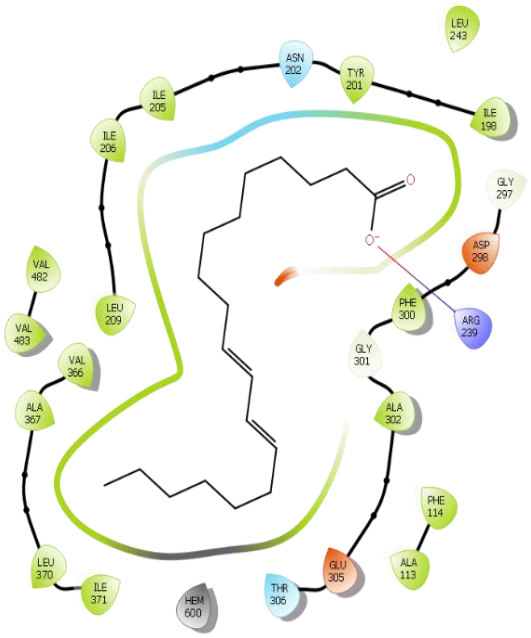
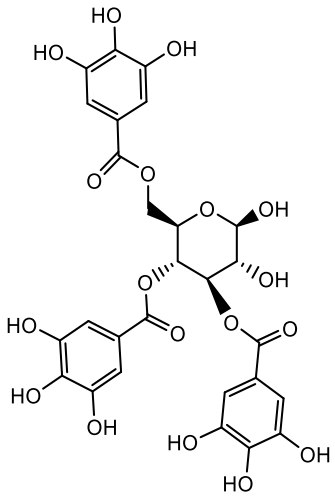
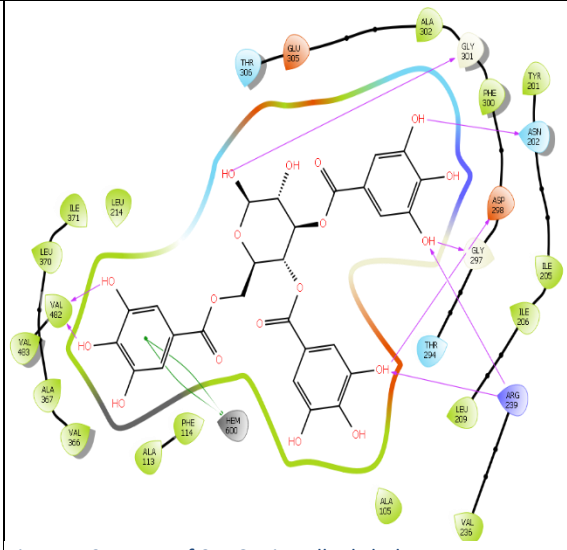
The docking score was obtained and the ligand-protein interaction pose was formed the basis of evaluation of the results of docking study. The compounds with highest dock score in

magnitude and having good interaction profile were most active compounds against the target receptor protein.

Table 1 2-D docking pose of the compounds and their docking score

Ligand	Docking Pose (2D)	Docking score (Kcal/mole)
 1,6-di-o-glloyl-d-glucose	 *Figure 2* 2D Pose of 1,6-di-o-glloyl-d-glucose	-12.172
 Isoquercitrin acid	 *Figure 3* 2D pose of Isoquercitrin acid	-11.366

 Isoguaicin	 *Figure 4* 2D Pose of Isoguaicin	-8.04
 Ellagic Acid	 *Figure 5* 2D Pose of Ellagic Acid	-7.938

 Linoleic acid	 *Figure 6* 2D pose of Linoleic acid	-5.463
 3,4,6-tri-o-glloyl-d-glucose	 *Figure 7*-2D Pose of 3,4,6-tri-o-glloyl-d-glucose	-5.079

Results indicated that all the ligands have a strong binding affinity for the Human Cytochrome P450 CYP17A1 receptor as indicated by their docking score values that were found to be comparable with the docking score of the molecules 1,6-di-o-glloyl-d-glucose (-12.172) Isoquercitrin acid (-11.366), Isoguaicin (-8.04), Ellagic Acid (-7.938), Linoleic acid (-5.463) & 3,4,6-tri-o-glloyl-d-glucose (-5.079). Binding interactions (2D) of all molecules are described in Table 1. Binding interactions of the compounds possessing good docking score are discussed below.

a. Docking Studies of Standard Inhibitor 1,6-di-o-glloyl-d-glucose

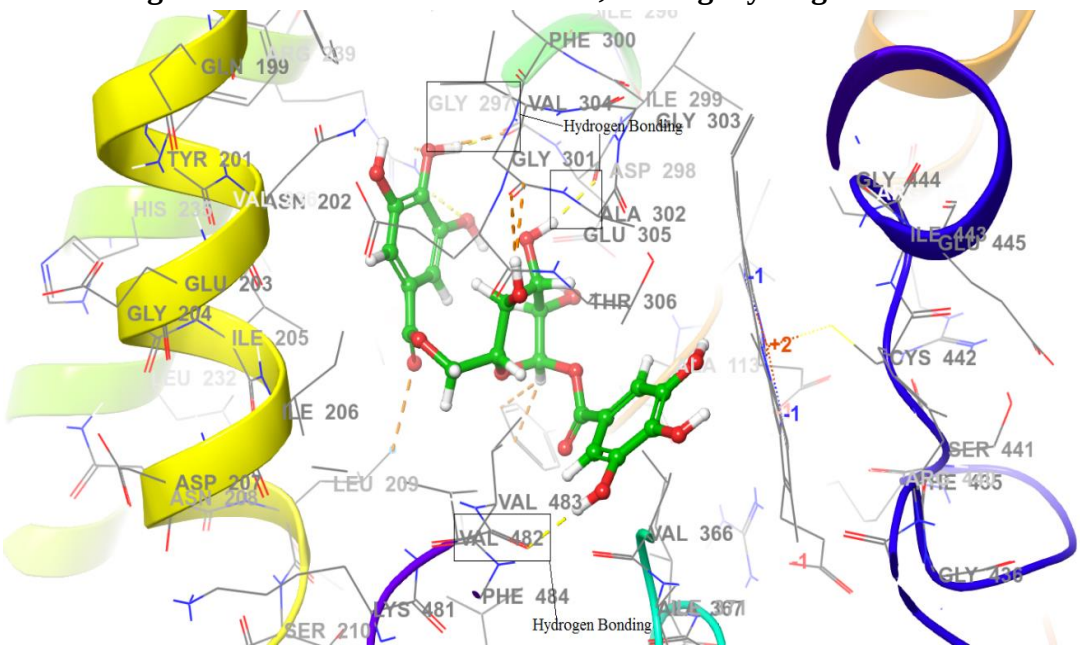


Figure 8 3D interaction pattern of 1,6-di-o-glloyl-d-glucose with Human Cytochrome P450 CYP17A1 protein (Code:3RUK)

Docking of 1,6-di-o-glloyl-d-glucose revealed that the inhibitor having various interactions with different amino acids of selected protein.

These interactions include:

1. Hydrogen Bonding with ARG-239, GLY-297, ASP-298 & VAL-482
2. Metal co-ordination with HEM-600

b. Docking Studies of Standard Inhibitor Isoquercitrin acid

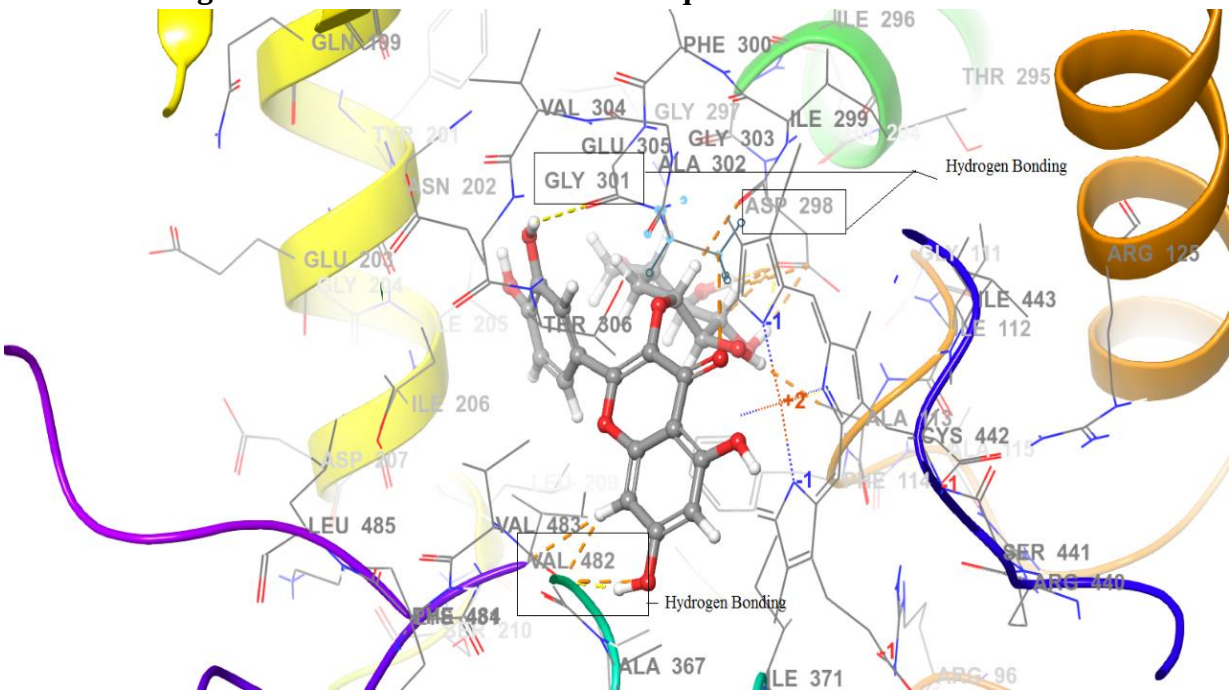


Figure 9 3D interaction pattern of Isoquercitrin acid with Human Cytochrome P450 CYP17A1 protein (Code: 3RUK)

Docking of Isoquercitrin acid revealed that the inhibitor having various interactions with different amino acids of selected protein.

These interactions include:

1. Hydrogen Bonding with GLY-301, VAL-482, ASP-298.
2. Pi-Pi stacking with HEM-600

c. Docking Studies of Standard Inhibitor Isoguaicin

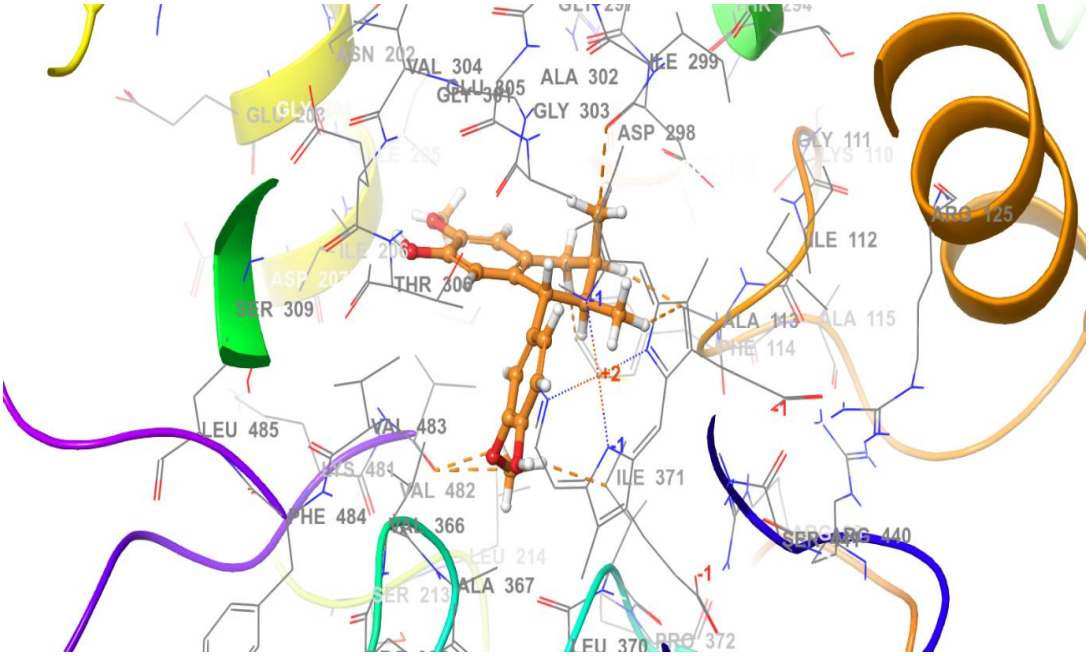


Figure 10 3D interaction pattern of Isoguaicin acid with Human Cytochrome P450 CYP17A1 protein (Code:3RUK)

Docking of Isoguaicin acid revealed that the inhibitor having various interactions with different amino acids of selected protein.

These interactions include:

1. Pi-Pi stacking with HEM-600

d. Docking Studies of Standard Inhibitor Ellagic Acid

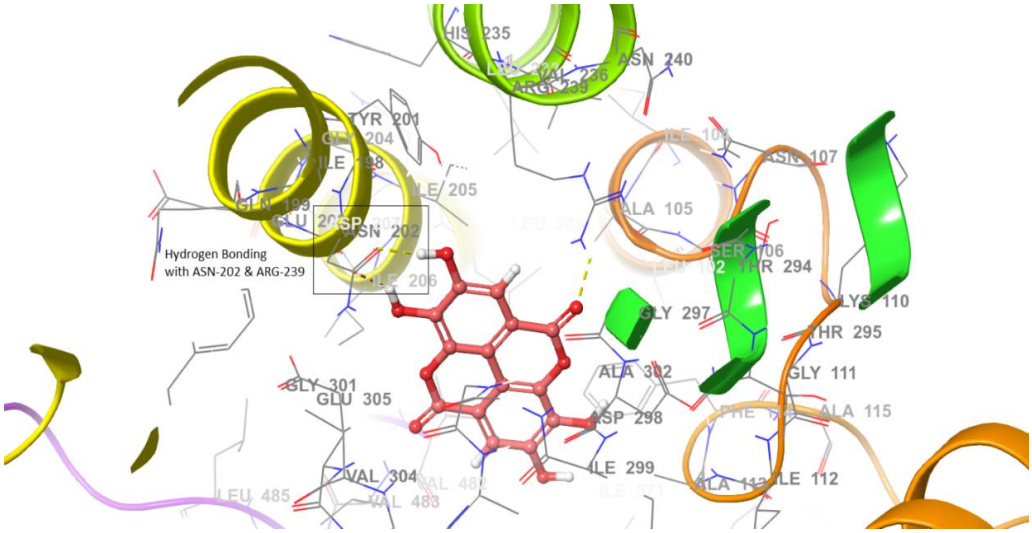


Figure 11 3D interaction pattern of Ellagic acid with Human Cytochrome P450 CYP17A1 protein (Code: 3RUK)

Docking of Ellagic acid revealed that the inhibitor having various interactions with different amino acids of selected protein.

These interactions include:

1. Hydrogen Bonding with ASN-202, ARG-239

e. Docking Studies of Standard Inhibitor Linoleic acid

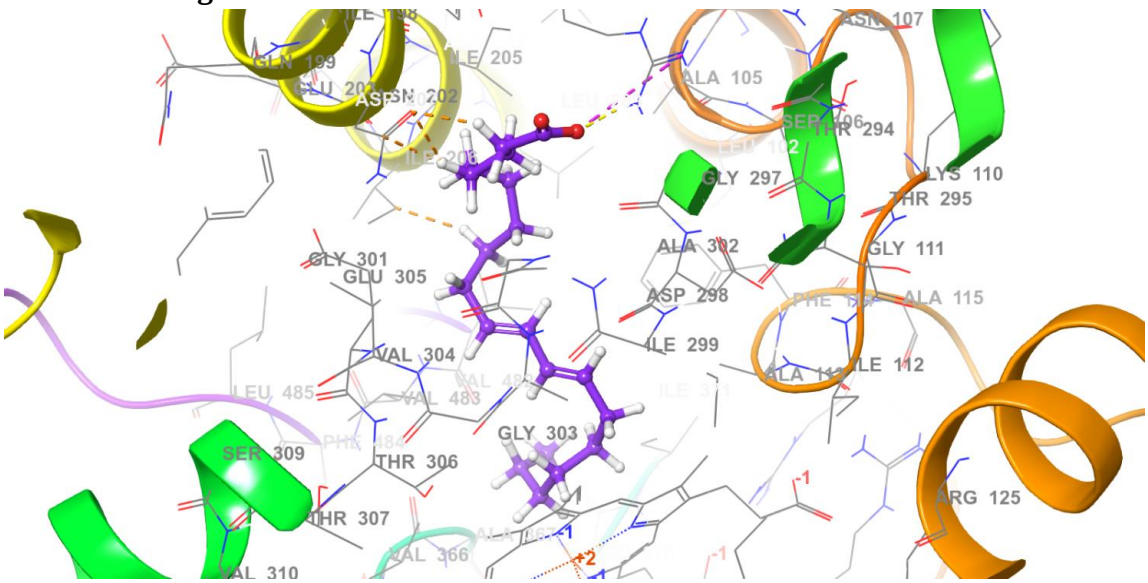


Figure 12 3D interaction pattern of Linoleic acid with Human Cytochrome P450 CYP17A1 protein (Code: 3RUK)

Docking of Linoleic acid revealed that the inhibitor having various interactions with different amino acids of selected protein.

These interactions include:

1. Salt bridge with ARG-239
2.

f. Docking Studies of Standard Inhibitor3,4,6-tri-o-glloyl-d-glucose

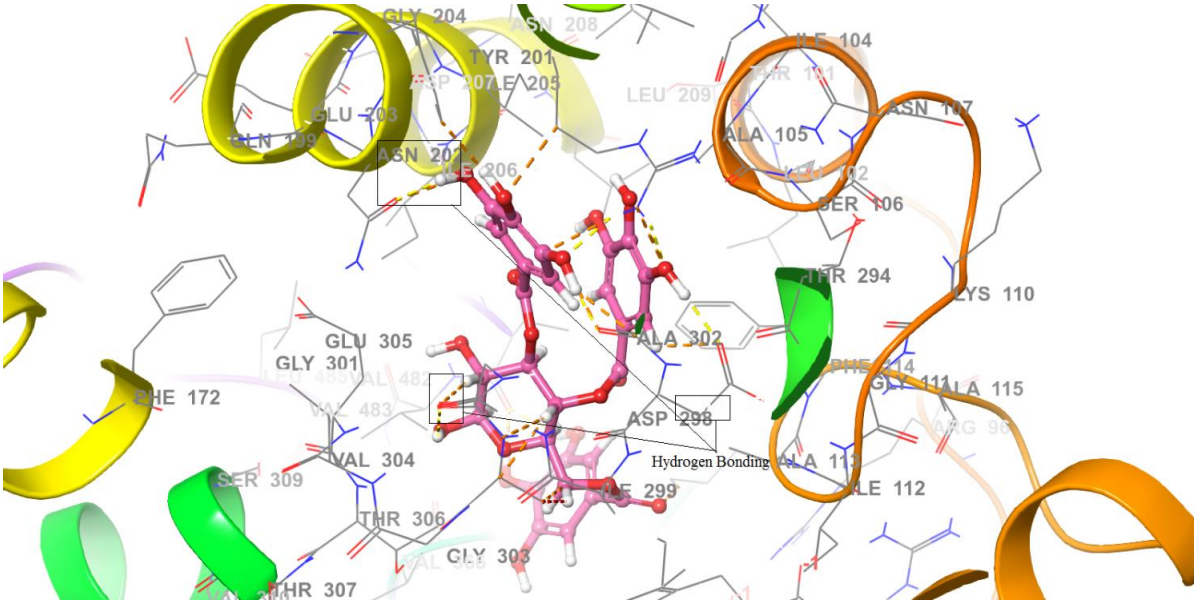


Figure 13 3D interaction pattern of 3,4,6-tri-o-glloyl-d-glucosewith Human Cytochrome P450 CYP17A1 protein (Code: 3RUK)

Docking of 3,4,6-tri-o-glloyl-d-glucoserevealed that the inhibitor having various interaction with different amino acids of selected protein.

These interactions include:

1. Hydrogen Bonding with VAL-482, GLY-297, ASP-298, ARG-239, ASN-202, GLY-301
2. Pi-Pi stacking with HEM-600

Conclusion:

Currently, there is huge interest in the scientific community, Physician and drug industry to exploit Poly cystic syndrome. Polycystic ovary syndrome is one of the most common hormonal disorder among women of reproductive age. “PCOS is a challenging experience for women; many remain undiagnosed and experience delays in diagnosis”. Polycystic ovary syndrome is common diagnosis in women presenting with infertility. PCOS is highly variable ranging from 2.2% to 26% globally. So far, as no effective single drug is identified for PCOS treatment, the treatment of PCOS is a challenging problem worldwide at present. Due to its complex pathogenesis and unrecognized etiology, there is no effective preventive measure available for PCOS until now. In spite of the tremendous progress made in the development of drug delivery system, there is an urgent need to find out alternate therapeutic regimen to control PCOS syndrome.

Conflict of Interest: There are no conflicts of interest.

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