International Journal of Advanced Research and Development
www.multistudiesjournal.com
ISSN: 2455-4030
Received: 31-01-2025, Accepted: 02-03-2025, Published: 18-03-2025
Volume 10, Issue 1, 2025, Page No. 46-55

Menstruation problem due to polycystic ovarian syndrome: Himalayan medicinal plant, *Zanthoxylum armatum*, DC, (Timur), may block CYP-17, 5α-reductase and human androgen receptors

Vineeta Sharma[1], Suraj Joshi[1], Suman Bhandari[1], Shankar Mondal[2*]
[1] Department of Zoology, Immunology laboratory, LSM Government Postgraduate College, Pithoragarh, Uttarakhand, India
[2] Department of Zoology, PNG Government Postgraduate College, Ramnagar, Uttarakhand, India

Abstract
Polycystic ovarian syndrome (PCOS) is primarily an endocrinological disorder responsible for anovulatory related infertility. PCOS is characterized by symptoms like hyperandrogenism, irregular menses and chronic metabolic syndromes. PCOS manifests due to overexpression of genes like CYP-17 [Cytochrome P-450 superfamily gene] along with 5α-reductase and human androgen receptors. Therapeutic drugs like metformin, spironolactone and cyproterone acetate are used to treat PCOS butit shows side effects. The Himalayan medicinal plant *Zanthoxylum armatum*, DC, [ZA]is traditionally used in *Ayurveda* for many illnesses like asthma, stomach-ache and menstrual disorders. In this study, a comparative screening was done for least binding energy (ΔG) of phytochemicals for it potential to inhibit the target receptor (CYP-17, 5α-reductase and human androgen receptor) with the reference drug. PyRx and Biovia Discovery studio visualizer 2021softwares were used for virtual screening and analysis. The potential oftoxicity ZA phytochemicals was also screened using Swiss ADME software. Sixteen molecules of ZA have shown binding affinity with CYP-17, 5α-reductase and human androgen receptors. Phytochemical lupeol has shown the least binding energy [ΔG] -10.8kcal/mol with CYP-17, while hesperidin showed ΔG -12.2kcal/mol with 5α-reductase and Asarinin exhibitedΔG -9.8kcal/mol with human androgen receptors. The drugs metformin, spironolactone and cyproterone acetate have shown ΔG ranging from -5.0 to -11.2kcal/mol. Toxicity study showed that 12phytochemicals followed Lipinski's rule of five. In summary, ZA phytochemicals have exhibited significant least binding energy as compared to current drugs. Thus, these phytochemicals may be used as potential lead drug molecules for target-specific *in-vitro* studies.

Keywords: Ayurved, hesperidin, asarinin, lupeol, infertility

Introduction

Polycystic ovarian syndrome (PCOS) is one of the most common endocrine disorders in young women of reproductive ages. It was first reported in 1935 by Stein and Leventhal**,** so this disease is also known as Stein-Leventhal syndrome [1]. It is responsible for 70% anovulatory related infertility [2]. The PCOS ranges from 4 to 21% in reproductive age women in worldwide depending on the diagnostic criteria. There are mainly three sets of diagnostic criteria mainly – National Institute Health (NIH), Androgen excess polycystic ovarian syndrome (AE-PCOS) society and European Society of Human Reproduction and Embryology/American Society for Reproductive Medicine (ESHRE/ASRM) Rotterdam criteria. According to NIH (1990) criteria, prevalence of PCOS ranges from 5% to 10%, AE-PCOS society (2006) criteria ranges from 10% to 15% and ESHRE/ASRM Rotterdam (2009) criteria reported 6% to 21%. The estimated prevalence of PCOS in India ranged from 3.7 to 22.5% depending on the diagnostic criteria and population studies [3, 4]. Small cysts [2 to 9 mm diameter] may be developed on single or both ovaries in PCOS patients. The women suffering from PCOS present with a constellation of symptoms associated with menstrual dysfunction and androgen excess, which significantly impacts their quality of life [5]. The actual pathogenesis or cause of PCOS is unclear [6]; however, PCOS is characterized by both hormonal and metabolic disturbances [7]. PCOS is mainly linked with hyperandrogenaemia, hyperinsulinemia, hirsutism (excessive hair growth on face), irregular menses, oligo-ovulation, central obesity and insulin resistance is an important element in the development of PCOS [8]. Thus, steroid hormone synthesis pathways have an interplay of excess androgen production which leads to characteristics symptoms and related disorders.

Role of Cytochrome P-450 super family [CYP] genes, CYP11A1, CYP17A1 and CYP19, in the steroidogenesis

Ovary is the chief organ, which maintains female reproductive physiology. The ovaries are involved in synthesis of steroid hormone of female reproductive system and production of ova. Steroid hormones are synthesized in a cascade of events from precursor cholesterol molecule, the process is called steroidogenesis**.** There are mainly 3 genes responsible for the synthesis of steroid hormone, namely CYP11A1, CYP17A1 and CYP19 gene.

CYP11A1Gene

Cytochrome P450 family 11 subfamily A member 1, CYP 11A1, is located on chromosome 15q24.1. CYP 11A1 encodes forP-450 cholesterol side-chain cleavage [SCC] enzyme which is essential for the synthesis of pregnenolone from cholesterol in three sequential steps [9].

CYP17A1Gene

Cytochrome P450 family 17 subfamily A member 1, CYP17A1 gene, located on chromosome 10q24.3, encodes a microsomal enzyme, essential for the biosynthesis of adrenal and gonadal steroid hormones. It has both 17α-hydroxylase and 17, 20-lyase activities. This enzyme is

mainly expressed in zona fasciculata and zona reticularis of the adrenal cortex and gonadal tissues. With the help of 3βHSD [Hydroxysteroid Dehydrogenase], pregnenolone converts into progesterone. During the enzymatic activity, CYP17α-hydroxylase first acts upon the pregnenolone and progesterone at the C17 position and converts into 17α-OH pregnenolone and 17α-OH progesterone, respectively. CYP17, 20 lyase activity cleaves C17-C20 bond of 17α-OH pregnenolone and 17α-OH progesterone to form dehydroepiandrosterone [DHEA] and 4- androstenedione. DHEA converts into 4-androstenedione with the help of 3β-HSD enzyme. 17β-HSD causes DHEA and 4-androstenedione to convert into 5-androstenediol and testosterone. 3β-HSD causes 5-androstenediol converts into testosterone. All these processes take place in the theca cell. Luteinizing hormone [LH] is responsible to stimulate CYP 17 enzyme in theca cells to produce androgen and progesterone. Cytochrome P450 side-chain cleavage enzyme (CYP11A1), steroidogenic acute regulatory protein (StAR), 3β-HSD (3β-hydroxysteroid dehydrogenase), 17α-hydroxylase/C17-20 lyase Cytochrome P450 (CYP17A1), and 17β-HSD are responsible for the androgen and progesterone synthesis [10].

CYP19Gene

CYP 19 gene is located on chromosome 15q21.2, it encodes a P450 aromatase enzyme, essential for estradiol (potent estrogen) hormone. In normal condition the level of FSH is higher than LH. FSH mainly stimulates the P450 aromatase, resultant 4-androstenedione convert into estrone, now estrone converts into 17β-estradiol by the action of 17β-HSD. Similarly, testosterone is also converted into 17β-estradiol and dihydrotestosterone [DHT], a potent androgen with the help of P450 aromatase and 5α-reductase. These are normal processes when the ovary works in its normal way. In abnormal conditions, the level of FSH is lower than LH, now all the normal processes will not be able to do their work properly, due to which the level of estradiol will decrease. This may cause hyperandrogenaemia (excessive amount of androgen) in the ovary. Hyperandrogenism is mainly seen in PCOS due to dysregulation of the hormonal level. Hyperinsulinemia (type-2 diabetes) is one of the major reasons of PCOS, this is because high level of insulin (hyperinsulinemia) also behaves synergistically like LH hormone and activates the CYP17 enzyme, resultant more androgen hormone synthesized. Hyperinsulinemia is also associated with the lowering the SHBG (sex hormone binding globin) and IGFBP (Insulin growth factor binding protein) protein, primarily synthesized in the liver, SHBG and IGFBP binds with free androgen and high level of insulin with higher affinity and leads to lowering the free circulating steroid hormone and insulin hormone. SHBG gene located on chromosome 17p13.1. Hyperandrogenaemia is responsible for alopecia (loss of hairs), acne, hirsutism (unwanted hair growth on a woman's face), metabolic disorder, anovulation and irregular menses [11].

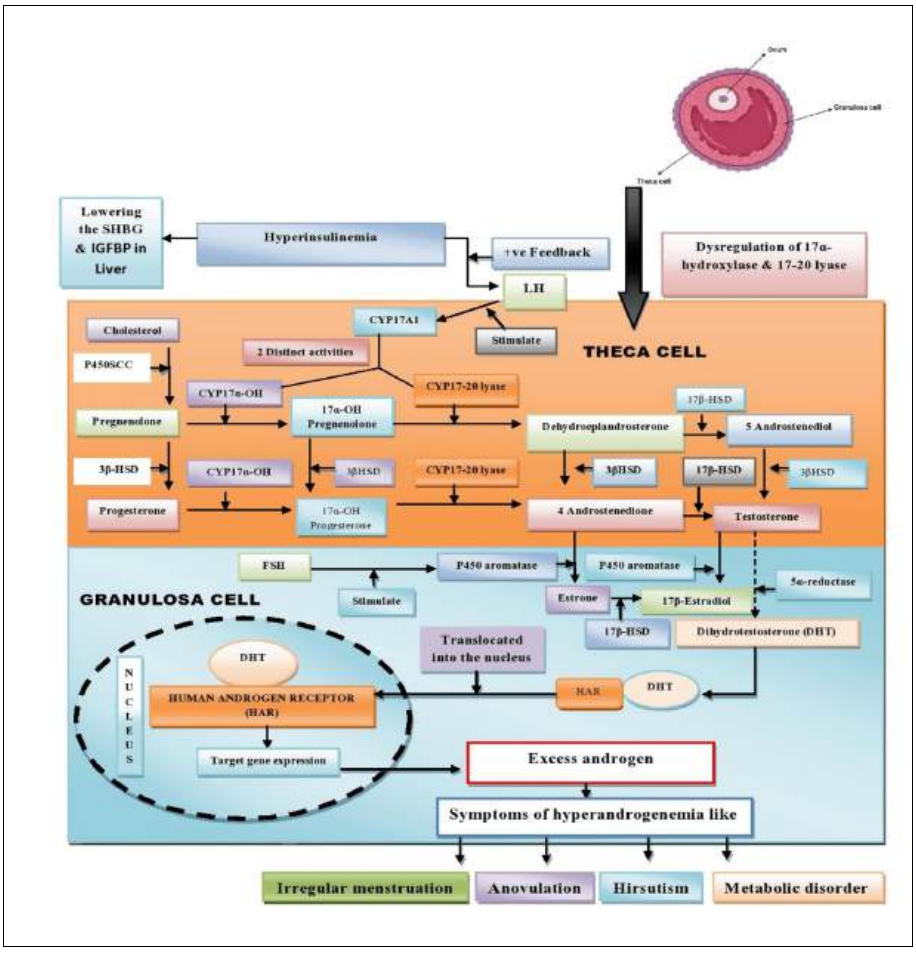


Fig 1: A proposed model of steroidogenesis in PCOS [Polycystic Ovarian Syndrome] which leads to symptoms and metabolic disorders. Dotted lines indicate the high level of hormones (FSH<LH) and solid lines indicate the resultant outcomes in the process. [SHBG- Sex Hormone Binding Globulin, IGFBP- Insulin-like Growth Factor Binding Protein, CYP- Cytochrome P-450, LH- Luteinizing Hormone, FSH- Follicular Stimulating Hormone, HSD- Hydroxysteroid Dehydrogenase, P450SSC-P450 cholesterol side-chain cleavage]

Some of the drugs currently used to treat PCOS and their side effects

The drugs available for PCOS are mainly used to manage the symptoms like anovulation, androgenic symptoms and aromatase inhibitors. Some of the choice of drugs to manage infertility and other symptoms are-

1. Metformin
2. Spironolactone
3. Cyproterone acetate

Metformin: Metformin is an anti-hyperglycaemic drug commonly used in the patients of PCOS. Metformin inhibits the androgen production by blocking 3β-HSD and CYP17-lyase [14]. Gastrointestinal symptoms (vomiting, diarrhoea, abdominal pain) are the most common side effects of metformin. It have the risk of lowering blood glucose and affects the absorption of glucose, vitamin B12 and bile salts in the intestine [15].

Spironolactone: It is mainly inhibiting the androgen production by blocking 5α-reductase and human androgen receptors and increasing the levels of sex hormone binding globulin (SHBG) by decreasing the free testosterone [16]. Gynaecomastia, headache, fatigue, vomiting, diarrhoea, facial swelling, abdominal cramps and decreased the libido is the most common side effects of spironolactone [17].

Cyproterone acetate (CPA): CPA is mainly inhibiting the conversion of DHEA to 4-androstenedione by 3β-HSD and it is also block the human androgen receptor, thus, decreases the level of androgen production. CPA also reduces the menstrual irregularities when combined with an oestrogen. Menstrual irregularities, breast tenderness, headache, nausea and liver toxicity are the most common side effects of cyproterone acetate. [18]. A model of current drug action at different receptors level is proposed in the following figure 2.

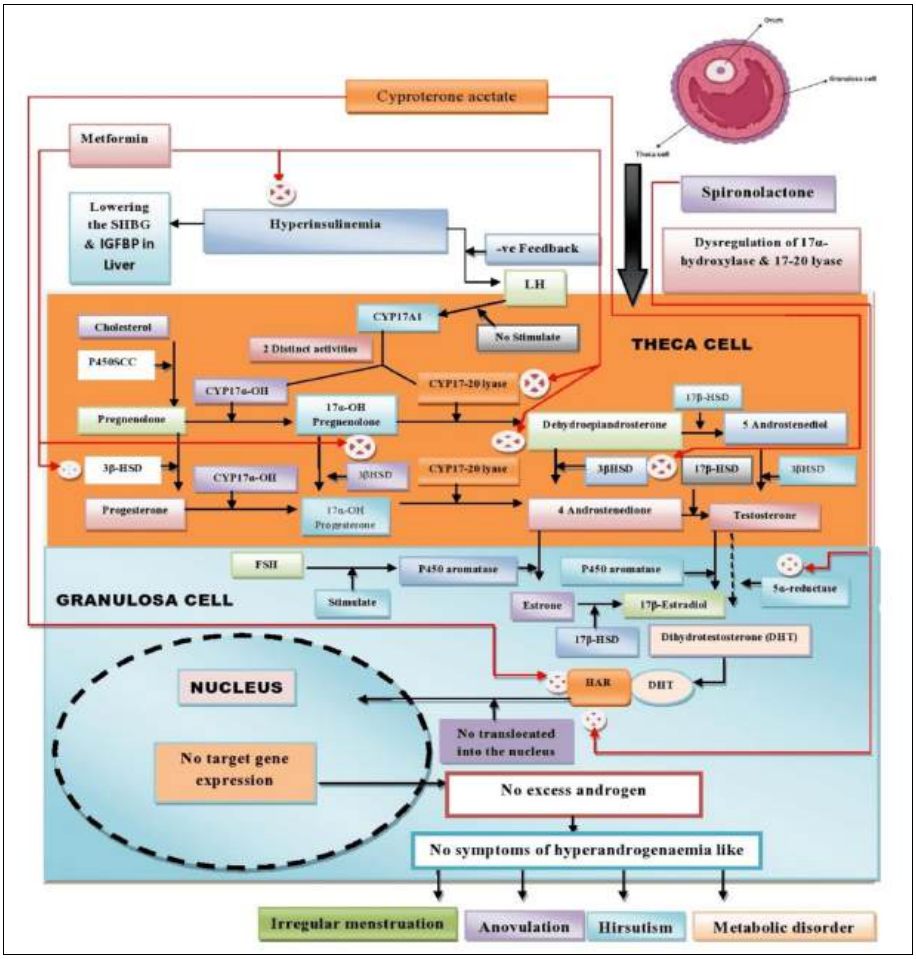


Fig 2: A proposed model showing the plausible mechanism of action of metformin, spironolectone and cyproterone acetace in steroidogenesis. Red lines and crosses indicate inhibition. Dotted lines indicate the abnormal level of hormones and solid lines indicates the resultant outcomes in the process. [SHBG- Sex Hormone Binding Globulin, IGFBP- Insulin-like Growth Factor Binding Protein, CYP- Cytochrome P-450, LH- Luteinizing Hormone, FSH- Follicular Stimulating Hormone, HSD- Hydroxysteroid Dehydrogenase, P450SSC- P450 cholesterol side-chain cleavage]

Androgen Receptor [AR] Gene

AR gene provides instructions for synthesis a protein called an androgen receptor. Androgen hormone is present in both sexes, male and female, responsible for maintenance of muscles and normal male secondary sexual characteristics and reproductive system. Dihydrotestosterone (DHT) is the most potent androgen hormone than testosterone, produced in the cytoplasm of granulosa cell of the ovary, then binding of DHT to human androgen receptor (HAR) in the cell cytoplasm and translocation of the hormone-receptor complex into the nucleus where it binds with androgen receptor complex DNA with strong affinity and controls the expression of specific genes [12, 13]

Thus, these gene and their products have an interplay to maintenance of steroidogenesis. A model of hormonal dysregulation of these genes and manifestation of PCOS is shown in the following figure 1.

***Zanthoxylum armatum*, DC, [ZA] and its medicinal properties**

Since the current medicines that used to treat PCOS are having its undesired side effects. It is imperative to explore the other possibilities using plant-based drug molecules. It is a fact that about 30% of modern drugs are derived from plant-based molecules [19]. India is a biodiversity rich country with its own indigenous medicine system, *Ayurveda*, the science of life. *Ayurveda* is continuously being practiced from 2500 BC to till date. It primarily describes plant-based products, especially Himalayan herbs, to treat various ailments. *Zanthoxylum armatum*, DC, [ZA] which is known as *Timur* in vernacular languages is one the plants that traditionally being used. Figure 3 is showing the whole tree of ZA. It is an aromatic medicinal plantof the Rutaceae family. The plant parts like leaves, bark, seeds and fruits are used in indigenous medicine preparation against various diseases like asthma, bronchitis, indigestion, diarrhoea, rheumatism, toothache [20] locally it is also used for menstruation related problems**.**Various phytochemical constituents like hesperidin, asarinin,isovitexin, tambuletin, chelerythrine, lupeol, vitexin, xanthyletin, kaempferol, kobusin, β-sitosterol, fargesin, eudesmin, epieudesmin, catechin and planinin have been reported from different parts of the plant. The main components of the leaves are hesperidin, isovitexin, vitexin, kobusin, eudesmin, epieudesmin and catechin. Bark containasarinin, chelerythrine, lupeol, xanthyletin, kaempferol, β-sitosterol, fargesin and planinin. It is also reported that seeds contain Tambuletin and β-sitosterol [21, 22].

Fig 3: Showing the *Zanthoxylum armatum*, DC, (Timur) plant [Upper panel shows the plant and lower panel showing the spines on the stems]

Phytochemical compounds of ZA like- hesperidin, asarinin, lupeoletc.... have been reported to have potential antiviral, antibacterial, larvicidal, antifungal and anti-plasmodial activity.

Since modern drug of choice like metformin, spironolactone and cyproterone acetate have its own side effects, a new drug based on herbal origin should be investigated for its potential to find a lead molecule which may be used in PCOS. The current study was undertaken to study the molecular docking and *in-silico* toxicity study to target 3 major receptors of steroidogenesis which are overexpressed in PCOS. The phytochemicals present in ZA have been screened for its potential to block steroidogenesis receptors and its binding efficacies were also compared with current drugs as a reference molecule

3. Materials and Methods

A. Retrieval and preparation of protein structure

The three-dimensional (3D) structures of CYP17 (PDB ID: 3RUK) 5α-reductase (PDB ID: 7BW1) and human androgen receptor (PDB ID: 2AM9) were retrieved from the protein data bank (PDB) (https://www.rcsb.org). All existing co-crystallized ligands, water molecules, ions and cofactors were removed from the protein receptors (CYP-17, 5α-reductase and human androgen) using open source molecular visualization software PyMOL [23].

B. Ligand preparation

The 3D structure of each phytochemical compounds of ZA, were retrieved from the PubChem (https://pubchem.ncbi.nlm.nih.gov) in SDF format and converted to PDB format using Open Babel software [24]. Auto Dock tools were used to convert protein and ligand into proper readable-file format (Pdbqt) [25]. Standard drug molecules metformin, spironolactone and cyproterone acetate were retrieved from PubChem and used as a reference drug in the current study. These drugs are known inhibitors of CYP-17, 5α-reductase and human androgen receptors.

C. Active site prediction

This was executed using CAST$_P$ 3.0 (http://sts.bioe.uic.edu/) and PLIP (https://plip-tool.biotec.tu-dresden.de/plip-web/plip/index) web server to find the active sites/binding pockets in the selected target proteins where the drugs are most likely to bind with stable free energy.

D. Virtual screening and molecular docking

In the present investigations, PyRx virtual screening tool (http://sourceforge.net/projects/pyrx) were used. It is a virtual screening opensource software for computational drug discovery that can be used to screening the compounds against a potential drug target [25]. PyRx uses both Autodock vina and autodock 4.2 and contribute higher docking accuracy. CYP17, 5α-reductase and human androgen receptor were docked with the lead molecule using PyRx. The grid centre between CYP-17 receptor and phytochemical compounds of ZA and reference drug was set X= 2.012, Y= 18.474 and Z= 45.844. The grid centre between 5α-reductase and phytochemical compounds of ZA and reference drug was set X= -32.310, Y=14.101 and Z=31.023 and for human androgen receptor were set as X= 25.118, Y= 9.514 and Z= 8.216 by using Autodock vina. where 8 maximum exhaustiveness was calculated for each lead molecules of ZA. These are 56 phytochemical compounds were found in Pubchem, all 56 molecules were screened for its potential.

Result and Discussion

PCOS is primarily an endocrinological disorder affecting multiple aspect of women's overall health, responsible for anovulatory related infertility. Therapeutic drugs like, metformin, spironolactone and cyproterone acetate are used to treat the PCOS but it shows side effects. Therefore, Himalayan medicinal plant ZA, phytochemicals were used to treat the fertility and reproductive health of the affected women. In this study, Molecular docking studies were carried out between receptor proteins (CYP-17, 5α-reductase and human androgen receptors) and its inhibitors (*Zanthoxylum armatum*, DC compounds). Before testing the phytochemical compounds of ZA and its inhibitors, a commercial drug (metformin, spironolactone and cyproterone acetate) were docked against docking proteins for comparative study. In the present study, 56 phytochemical compounds of ZA were taken for docking studies, out of which 16 compounds of ZA has shown the good binding energy. After molecular docking, these docking protein and ligands display the result with a particular docking score. Simply, least binding energy is regarded as the best mode of binding as it is stable for the ligand. i.e., lower binding energy means higher docking scores. Figure 4 is showing the docking score of phytochemical compounds of ZA and its inhibitors (CYP-17, 5α-reductase and human androgen receptor). After successfully docking of these compounds into target receptor, the final visualization of the docked structure was performed using the Biovia Discovery studio visualizer 2021. The experimental ligands of ZA were taken in this study, whose binding energy is better than the reference molecule. So, these potential lead compounds could be effective and balance the level of ovarian hormones in PCOS women.

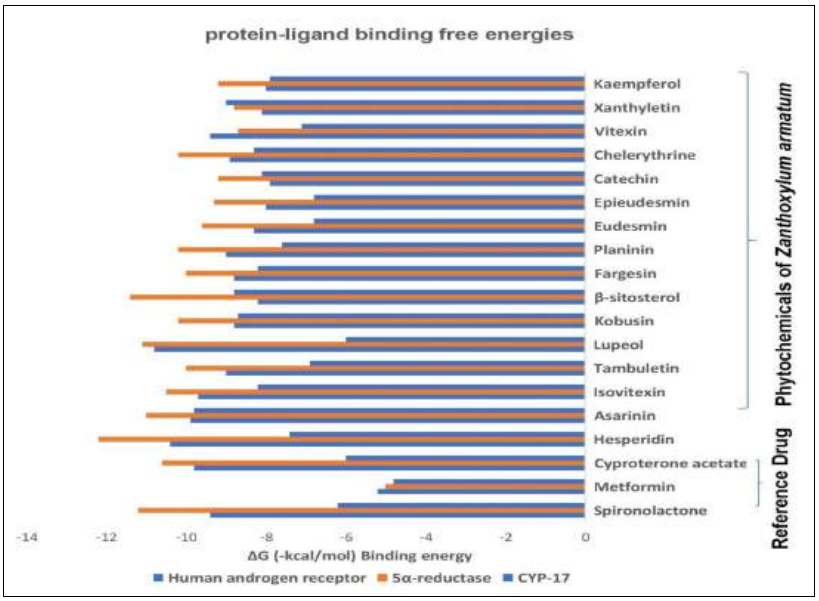


Fig 4: Graph showing the binding energy value ΔG (-kcal/ mol) of CYP-17, 5α-reductase and human androgen receptor with several inhibitor compound candidate

Interaction between phytochemical compounds of *Zanthoxylum armatum*, DC, with CYP-17 receptor

Before the screening, we used the reference drugs for molecular docking. The target protein (CYP-17) was docked with phytochemical compounds of ZA and reference drugs (metformin, spironolactone and cyproterone acetate). The binding energy of metformin with the CYP-17 protein was -5.2kcal/mol, -9.4kcal/mol for spironolactone and -9.8kcal/mol for cyproterone acetate. Now virtual screening resulted in the top phytochemicals from the target showing significantly lower binding energy. The binding energy of 16phytochemical compounds (hesperidin, asarinin, isovitexin, tambuletin, chelerythrine, lupeol, vitexin, xanthyletin, kaempferol, kobusin, β-sitosterol, fargesin, eudesmin, epieudesmin, catechin and planinin) were showing lower binding energy than metformin. 4phytochemical compounds (hesperidin, asarinin, isovitexin and lupeol) were showing lower binding energy than spironolactone and 3 phytochemical compounds (hesperidin, asarinin and lupeol) were showing lower binding energy than cyproterone acetate. The more negative value of the docking score represents better docking affinity as compared to a more positive value and the best docking result of the ligand with the CYP-17 receptors were -10.8kcal/mol for lupeol, -10.4kcal/mol for hesperidin, -9.9kcal/mol for asarinin, -9.7kcal/mol for isovitexin, -9.4kcal/mol for vitexin, -9kcal/mol for planinin and tambuletin, -8.9kcal/mol for chelerythrine, -8.8kcal/mol for

kobusin and fargesin, -8.3kcal/mol for eudesmin, -8.2kcal/mol for β-sitosterol, -8.1kcal/mol for xanthyletin, -8kcal/mol for kaempferol and epieudesmin, -7.9kcal/mol for catechin. These natural ligands could be good inhibitors of the CYP-17 receptor. Figure 5 is showing the 2D and 3D interaction of cyproterone acetate (reference drug) and lupeol with the CYP-17 receptor. Hesperidin, asarinin, isovitexin, tambuletin, chelerythrine, lupeol, vitexin, kaempferol, kobusin, β-sitosterol, fargesin eudesmin, epieudesmin, catechin, xanthyletin and planinin shows better binding affinities with CYP-17 receptor as compared to reference drugs.

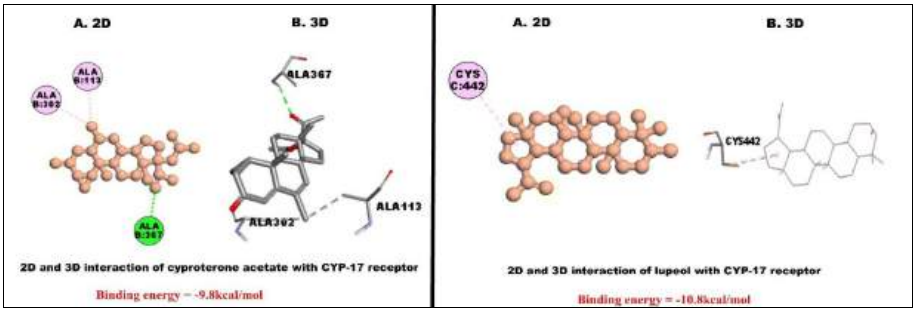


Fig 5: A proposed model showing the 2D and 3D interaction of reference molecules (cyproterone acetate) and lupeol with CYP-17 receptor

Interaction between phytochemical compounds of *Zanthoxylum armatum*, DC, with 5α-reductase receptor

The target protein (5α-reductase) was docked with phytochemical compounds of ZA and reference drugs (metformin, spironolactone and cyproterone acetate). The binding energy of metformin with 5α-reductase was -5kcal/mol, -11.2kcal/mol for spironolactone and -10.6kcal/mol for cyproterone acetate (CPA). Now virtual screening resulted in the top phytochemicals from the target showing significantly lower binding energy. The binding energy of 16phytochemical compounds (hesperidin, asarinin, isovitexin, tambuletin, chelerythrine, lupeol, vitexin, xanthyletin, kaempferol, kobusin, β-sitosterol, fargesin, eudesmin, epieudesmin, catechin and planinin) were showing lower binding energy than metformin. 2 phytochemical compounds (hesperidin and β-sitosterol) were showing lower binding energy than spironolactone and 4 phytochemical compounds (hesperidin, asarinin, β-sitosterol and lupeol) were showing lower binding energy than cyproterone acetate. The best docking result of ligand with 5α-reductase were -12.2kcal/mol for hesperidin, -11.4kcal/mol for β-sitosterol, -11.1kcal/mol for lupeol, -11kcal/mol for asarinin, -10.5kcal/mol for isovitexin, -10.2kcal/mol for kobusin, planinin and chelerythrine, -10kcal/mol for fargesin and tambuletin, -9.6kcal/mol for eudesmin, - 9.3kcal/mol for epieudesmin, -9.2kcal/mol for catechin and kaempferol, -8.8kcal/mol for xanthyletin, -8.7kcal/mol for vitexin. These natural ligands could be good inhibitors of 5α-reductase. **Figure 6** is showing the 2D and 3D interaction of spironolactone (reference drug) and hesperidin with the 5α-reductase receptor. Hesperidin, asarinin, isovitexin, tambuletin, chelerythrine, lupeol, vitexin, kaempferol, kobusin, β-sitosterol, fargesin, eudesmin, epieudesmin, catechin, xanthyletin and planinin shows better binding affinities with the target protein as compare to reference drugs and hence act as potent inhibitors of 5α-reductase enzyme

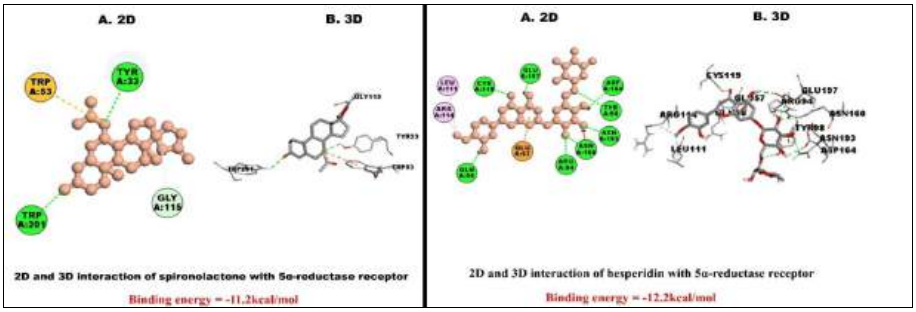


Fig 6: A proposed model showing the 2D and 3D interaction of reference molecules (spironolactone) and hesperidin with 5α-reductase receptor

Interaction between phytochemical compounds of *Zanthoxylum armatum*, DC, and human androgen receptor

The target protein (human androgen receptor) was docked with phytochemical compounds of ZA and reference drugs (metformin, spironolactone and cyproterone acetate). The binding energies with human androgen receptor were -4.8kcal/mol with metformin, -6.2kcal/mol with spironolactone and -6kcal/mol with cyproterone acetate (CPA). Now virtual screening resulted in the top phytochemicals from target showing significantly lower binding energy. The binding energy of 16phytochemical compounds (hesperidin, asarinin, isovitexin, tambuletin, chelerythrine, lupeol, vitexin, xanthyletin, kaempferol, kobusin, β-sitosterol, fargesin, eudesmin, epieudesmin, catechin and planinin) were showing lower binding energy than metformin. 15phytochemical compounds (hesperidin, asarinin, isovitexin, tambuletin, chelerythrine, vitexin, xanthyletin, kaempferol, kobusin, β-sitosterol, fargesin, eudesmin, epieudesmin, catechin and planinin) were showing lower binding energy than spironolactone and 16phytochemical compounds (hesperidin, asarinin, isovitexin, eudesmin, epieudesmin, tambuletin, chelerythrine, lupeol, vitexin, xanthyletin, kaempferol,

kobusin, β-sitosterol, fargesin, Catechin and Planinin) were showing lower binding energy than cyproterone acetate. The best docking result of ligand with human androgen receptorwere -9.8kcal/mol for asarinin, -9kcal/mol for xanthyletin, -8.8kcal/mol for β-sitosterol, -8.7kcal/mol for kobusin, -8.3kcal/mol for chelerythrine, -8.2kcal/mol for isovitexin and fargesin, -8.1kcal/mol for catechin, -7.9kcal/mol for kaempferol, -7.6kcal/mol for planinin, -7.4kcal/mol for hesperidin, -7.1kcal/mol for vitexin,-6.9kcal/mol for tambuletin, -6.8kcal/mol for eudesmin and epieudesmin, -6kcal/mol forlupeol. These natural ligands could be a good inhibitor of human androgen receptors. Figure 7 is showing the 2D and 3D interaction of spironolactone (reference drug) and asarinin with the Human androgen receptor. Hesperidin, asarinin, isovitexin, tambuletin, chelerythrine, lupeol, vitexin, xanthyletin, kaempferol, kobusin, β-sitosterol, fargesin, eudesmin, epieudesmin, catechin and planinin shows better binding affinities with the target protein as compare to reference drugs and hence act as potent inhibitors of human Androgen receptor. Figure 8 A proposed model showing the inhibitory action of top 3 phytochemical compounds (asarinin, hesperidin and lupeol) of ZA with overexpressed receptor involved in steroidogenesis.

Drug-likeness Profile

In-silico or computational modelling is one of the important human based tools for preclinical evaluation. The need of *in*- silico testing was because it reduces the animal testing phase, reduce the time for pre-clinical testing and reduce the overall cost for drug development. Swiss ADME (Absorption, Distribution, Metabolism, Excretion and Toxicity is the free online web server, which is used for the determining the drug- likeness properties of the selected ligand molecules. The most important and famous drug like filter is the Lipinski's rule-of-five (Ro5) that helps distinguishing between drug-like and non-drug molecules. Drug-likeness properties of the selected ligand molecules depends on the four simple physiochemical parameter ranges: (1) Molecular weight (MW) should be less than 500g/mol, (2) Lipophilicity (MlogP) should be less than 4.15, (3) Less than 5 hydrogen bond donors, (4) Less than 10 hydrogen bond acceptors. Each threshold is a multiple of 5, so the rule was called Lipinski's rule of five. If a compound does follow two or more violations of this rule, then the probability of a compound being an oral drug is reduced. Poor ADME is one of the major reason for drug failure in clinical experiments. [26, 27, 28]. The important properties based on Lipinski Rules of Five (RO5) are shown in the following (Table 1).

Table 1: Lipinski's rule of five for drug- likeness prediction of control and selected hits using SwissADME

Lipinski's Rule of five (Ro5)							
S. No.	**Name**	**Molecular weight (g/mol)**	**Lipophilicity (MLogP)**	**H-bond donors**	**H-bond acceptors**	**No. Of rule violations**	**Drug-Likeness Lipinski's**
		Less than 500 dalton	Less than 4.5	Less than 5	Less than 10	Less than 2 violations	Rule Follows
1.	Hesperidin	610.56	-3.04	8	15	3	No
2.	Asarinin	354.35	1.98	0	6	0	Yes
3.	Isovitexin	432.38	-2.02	7	10	2	No
4.	Tambuletin	508.43	-2.64	7	13	3	No
5.	Chelerythrine	348.37	2.53	0	4	0	Yes
6.	Lupeol	426.72	6.92	1	1	1	Yes
7.	Vitexin	432.38	-2.02	7	10	2	No
8.	Xanthyletin	228.24	2.37	0	3	0	Yes
9.	Kaempferol	286.24	-0.03	4	6	0	Yes
10.	Kobusin	370.40	1.79	0	6	0	Yes
11.	β-sitosterol	414.71	6.73	1	1	1	Yes
12.	Fargesin	370.40	1.79	0	6	0	Yes
13.	Eudesmin	386.44	1.61	0	6	0	Yes
14.	Epieudesmin	386.44	1.61	0	6	0	Yes
15.	Catechin	290.27	0.24	5	6	0	Yes
16.	Planinin	370.40	1.79	0	6	0	Yes
17.	Metformin	129.16	-0.56	3	2	0	Yes
18.	Spironolactone	416.57	3.58	0	4	0	Yes
19.	Cyproterone acetate	416.94	3.71	0	4	0	Yes

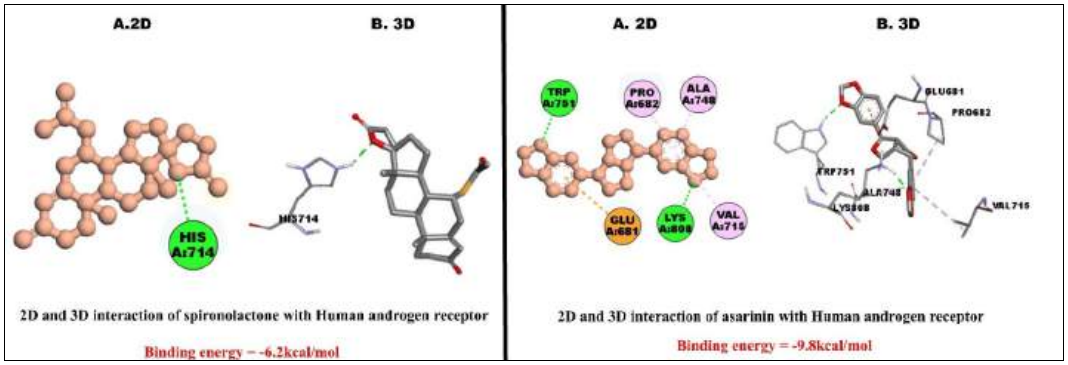


Fig 7: A proposed model showing the 2D and 3D interaction of reference molecules (spironolactone) and asarinin with human androgen receptor

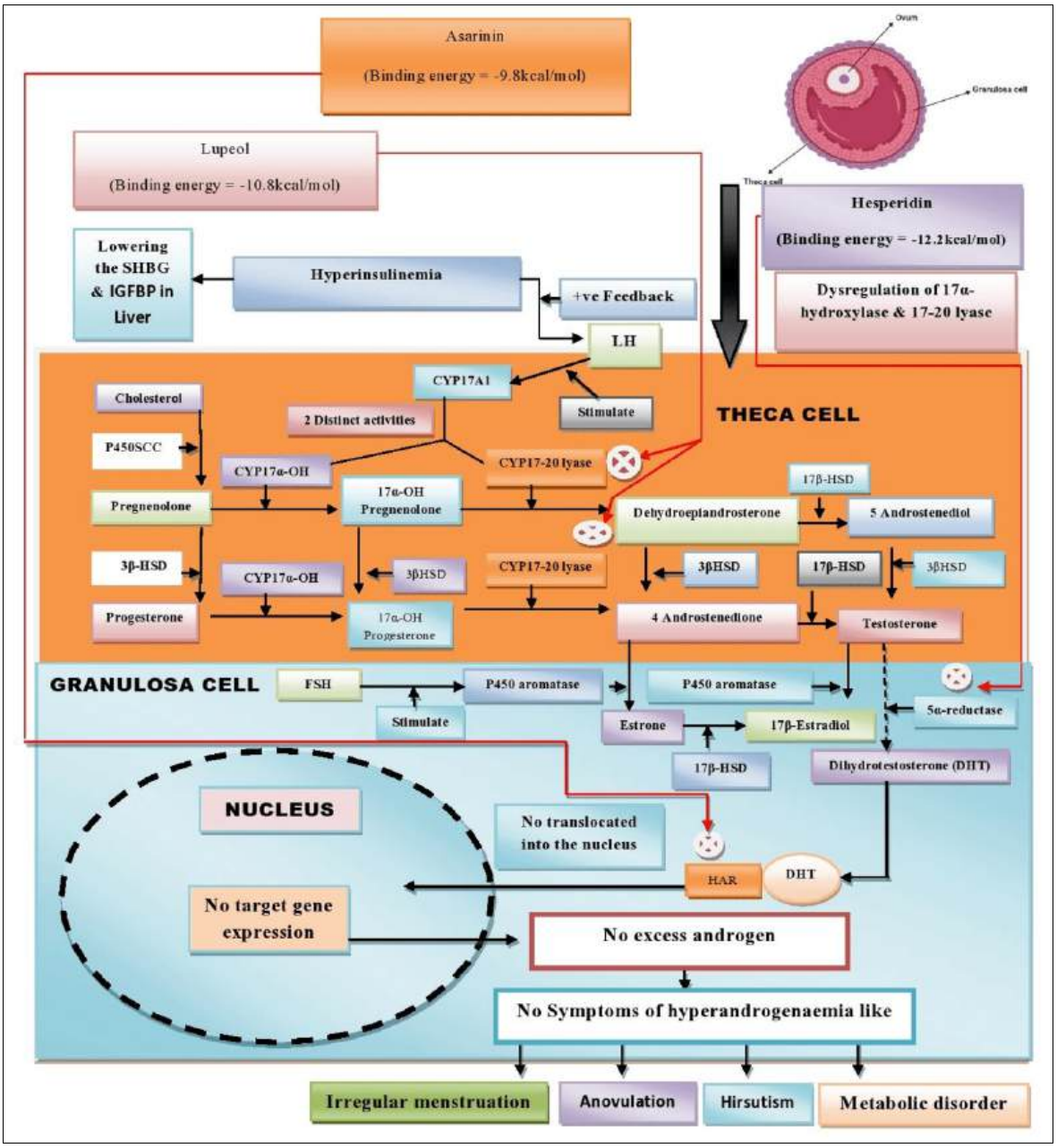
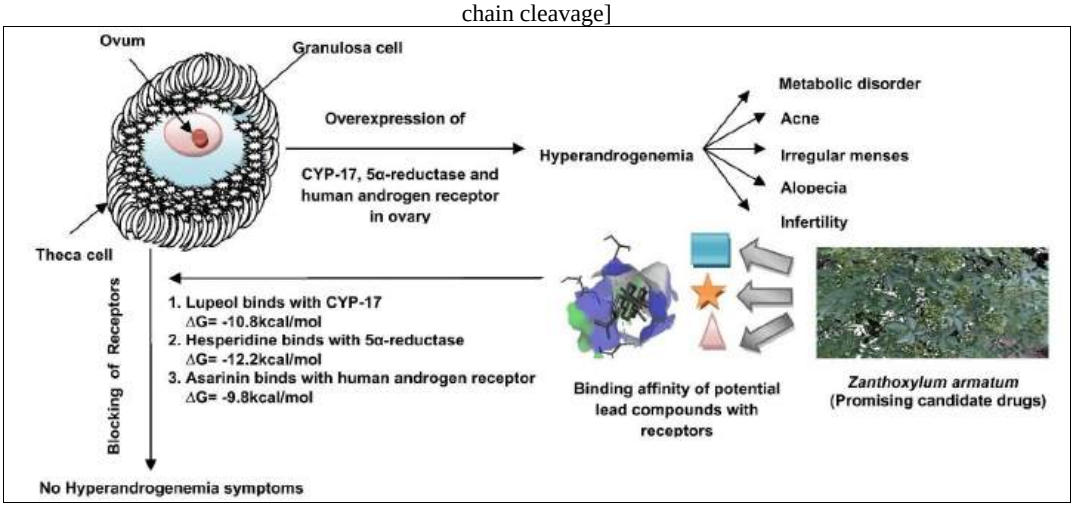


Fig 8: A proposed model showing the inhibitory action of top 3 phytochemical compounds (asarinin, hesperidin and lupeol) of *Zanthoxylum armatum*with overexpressed receptor involved in steroidogenesis. Asarinin showed the lowest binding energy [ΔG] -9.8kcal/mol with human androgen receptor, Hesperidin showed ΔG -12.2kcal/mol with 5α-reductase and Lupeol showed ΔG -10.8kcal/mol with CYP-17 receptor. Red lines and crosses indicate a blockage. Dotted lines indicate the high level (FSH<LH) of hormones and solid lines indicate the normal level of hormones. [SHBG- Sex Hormone Binding Globulin, IGFBP- Insulin-like Growth Factor Binding Protein, CYP- Cytochrome P-450, LH- Luteinizing Hormone, FSH- Follicular Stimulating Hormone, HSD- Hydroxysteroid Dehydrogenase, P450SSC-P450 cholesterol side-chain cleavage]



In-silico prediction of toxicity

The selected control and hit compounds were screened for toxicity analysis using admetSAR online server (URL: http://lmmd.ecust.edu.cn/admetsar2/). Toxicity prediction showed that the compounds are non-carcinogens and all of them could cross blood brain barrier (except -hesperidin, isovitexin, tambuletin, vitexin and catechin) and be absorbed by human intestine **(Table 2),** which allows them to achieve high concentration at the target site of the selected protein.

Table 2: Toxicity prediction of control and selected compounds using admetsar

S. No.	Name of Phytochemicals	Human Intestinal Absorption	Blood -Brain Barrier	Carcinogens
1.	Hesperidin	HIA+	BBB-	Non-carcinogen
2.	Asarinin	HIA+	BBB+	Non-carcinogen
3.	Isovitexin	HIA+	BBB-	Non-carcinogen
4.	Tambuletin	HIA+	BBB-	Non-carcinogen
5.	Chelerythrine	HIA+	BBB+	Non-carcinogen
6.	Lupeol	HIA+	BBB+	Non-carcinogen
7.	Vitexin	HIA+	BBB-	Non-carcinogen
8.	Xanthyletin	HIA+	BBB+	Non-carcinogen
9.	Kaempferol	HIA+	BBB+	Non-carcinogen
10.	Kobusin	HIA+	BBB+	Non-carcinogen
11.	β-sitosterol	HIA+	BBB+	Non-carcinogen
12.	Fargesin	HIA+	BBB+	Non-carcinogen
13.	Eudesmin	HIA+	BBB+	Non-carcinogen
14.	Epieudesmin	HIA+	BBB+	Non-carcinogen
15.	Catechin	HIA+	BBB-	Non-carcinogen
16.	Planinin	HIA+	BBB+	Non-carcinogen
17.	Metformin (Reference)	HIA+	BBB+	Non-carcinogen
18.	Spironolactone (Reference)	HIA+	BBB+	Non-carcinogen
19.	Cyproterone acetate (Reference)	HIA+	BBB+	Non-carcinogen

Conclusion

Phytochemical compounds of ZA have shown a higher -ΔG as compared to reference drugs used. Thus, an *in-vitro* study should be undertaken further and phytochemical compounds of *Timur* have a good pose with the CYP17, 5α-reductase and human androgen protein and can be used to treat infertility in PCOS patients. These results are only preliminary screening of phytochemical compounds of ZA to facilitate subsequent tests for *in-vitro* study and human clinical trials (*in-vivo*).

Author Contributions

Suraj Joshi performed the simulations and prepared manuscript. Vinita Sharma and Suman Bhandari conducted literature search and prepared manuscript. Shankar Mondal conceptualized the project and supervised final preparation of manuscript.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgement

The authors would like to thank the Director of Higher Education, Uttarakhand and the Principal LSM Government Postgraduate College, Pithoragarh, Uttarakhand for providing necessary infrastructure to for the study.

Abbreviations

ΔG Binding Energy
ADME Adsorption, Distribution, Metabolism, Excretion
AE Androgen Excess
AR Androgen Receptor Gene
ASRM American Society for Reproductive Medicine
βHSD Beta-Hydroxysteroid Dehydrogenase
$CAST_P$ Computed Atlas of Surface Topography of proteins
CPA Cyproterone Acetate
CYP-17 Cytochrome P-450 superfamily
DC Augustin Pyramus de Candolle
DHEA Dehydroepiandrosterone
DHT Dihydrotestosterone
ESHRE European Society of Human Reproductive and Embryology
FSH Follicular Stimulating Hormone
HSD Hydroxysteroid Dehydrogenase
IGFBP Insulin-like Growth Factor Binding Protein
LH Luteinizing Hormone
Log Logarithm
NIH National Institute of Health
PCOS Polycystic Ovarian Syndrome
PLIP Protein-Ligand Interaction Profiler
SCC Side-Chain Cleavage
SHBG Sex Hormone Binding Globin
StAR Steroidogenic Acute Regulatory Protein
ZA *Zanthoxylum armatum*, DC

References

1. Mohamed-Hussein ZA, Harun S. Construction of a polycystic ovarian syndrome (PCOS) pathway based on the interactions of PCOS-related proteins retrieved from bibliomic data. Theor Biol Med Model,2009:6(1):1-7. doi: 10.1186/1742-4682-6-18.
2. Roy KK, Baruah J, Moda N, Kumar S. Evaluation of unilateral versus bilateral ovarian drilling in clomiphene citrate resistant cases of polycystic ovarian syndrome. Arch Gynecol Obstet,2009:280(4):573-578. doi: 10.1007/s00404-009-0961-z.
3. Lizneva D, Suturina L, Walker W, Brakta S, Gavrilova-Jordan L, Azziz R. Criteria, prevalence, and phenotypes of polycystic ovary syndrome. Fertil Steril,2016:106(1):6-15. doi: 10.1016/j.fertnstert.2016.05.003.
4. Ganie MA, Vasudevan V, Wani IA, Baba MS. Epidemiology, pathogenesis, genetics & management of polycystic ovary syndrome in India,2019:10:517-520. doi: 10.4103/ijmr.IJMR.

5. El Hayek S, Bitar L, Hamdar LH, Mirza FG, Daoud G. Poly Cystic Ovarian Syndrome: An updated overview. Front Physiol,2016:7:1-15. doi: 10.3389/fphys.2016.00124.
6. Avery JC, Braunack-Mayer AJ. The information needs of women diagnosed with Polycystic Ovarian Syndrome - Implications for treatment and health outcomes. BMC Womens Health,2007:7:1-10. doi: 10.1186/1472-6874-7-9.
7. De Leo V, Musacchio MC, Cappelli V, Massaro MG, Morgante G, Petraglia F. Genetic, hormonal and metabolic aspects of PCOS: An update. Reprod Biol Endocrinol,2016:14(1):1-17. doi: 10.1186/s12958-016-0173-x.
8. Iatrakis G, *et al*. Polycystic ovarian syndrome, insulin resistance and thickness of the endometrium. Eur J Obstet Gynecol Reprod Biol,2006:127(2):218-221. doi: 10.1016/j.ejogrb.2006.01.023.
9. Pusalkar M, Meherji P, Gokral J, Chinnaraj S, Maitra A. CYP11A1 and CYP17 promoter polymorphisms associate with hyperandrogenemia in polycystic ovary syndrome. Fertil Steril,2009:92(2):653-659. doi: 10.1016/j.fertnstert.2008.07.016.
10. Prapas N, Karkanaki A, Prapas I, Kalogiannidis I, Katsikis I, Panidis D. Genetics of polycystic ovary syndrome. Hippokratia,2009:13(4):216-223. doi: 10.1016/s1472-6483(11)60572-5.
11. Dadachanji R, Shaikh N, Mukherjee S. Genetic Variants Associated with Hyperandrogenemia in PCOS Pathophysiology. Genet Res Int,2018:2018:7624932. doi: 10.1155/2018/7624932.
12. Franks S, Hardy K. Androgen Action in the Ovary,2018:8:9:1-7. doi: 10.3389/fendo.2018.00452.
13. Wang L, Hsu CL, Chang C. Androgen receptor corepressors: An overview. Prostate,2005:63(2):117-130. doi: 10.1002/pros.20170.
14. Hirsch A, *et al*. Metformin inhibits human androgen production by regulating steroidogenic enzymes HSD3B2 and CYP17A1 and complex I activity of the respiratory chain. Endocrinology,2012:153(9):4354-4366. doi: 10.1210/en.2012-1145.
15. Bouchoucha M, Uzzan B, Cohen R. Metformin and digestive disorders. Diabetes Metab,2011:37(2):90-96. doi: 10.1016/j.diabet.2010.11.002.
16. Jing G, Anjali M. Anti-Androgen Therapy in Female Adult Acne. J Dermatology Res Ther,2016:2(2):20-22. doi: 10.23937/2469-5750/1510023.
17. Yemisci A, Gorgulu A, Piskin S. Effects and side-effects of spironolactone therapy in women with acne. J Eur Acad Dermatology Venereol,2005:19(2):163-166. doi: 10.1111/j.1468-3083.2005.01072.x.
18. Bettoli V, Zauli S, Virgili A. Is hormonal treatment still an option in acne today? Br J Dermatol,2015:172(S1):37-46. doi: 10.1111/bjd.13681.
19. Mondal SB. Pharma Review, 2009.
20. Brijwal L, Pandey A. An overview on phytomedicinal approaches of Zanthoxylum armatum DC.: An important magical medicinal plant. J Med Plants Res,2013:7(8):366-370. doi: 10.5897/JMPR12.743.
21. Bhattacharjee A, D B K, Das B, Dkhar D, Kachari D. Zanthoxylum Armatum: A Systemic Review of its Ethno-Medicinal Properties, Phytochemistry, Pharmacology and Toxicology. Adv Pharmacol Clin Trials,2019:4(3):1-11. doi: 10.23880/apct-16000162.
22. Paul A, Kumar A, Singh G, Choudhary A. Medicinal, pharmaceutical and pharmacological properties of Zanthoxylum armatum: A Review. J Pharmacogn Phytochem,2018:7(4):892-900. Available from: https://www.researchgate.net/publication/374152067.
23. Schiffrin B, Radford SE, Brockwell DJ, Calabrese AN. PyXlinkViewer: A flexible tool for visualization of protein chemical crosslinking data within the PyMOL molecular graphics system. Protein Sci,2020:29(8):1851-1857. doi: 10.1002/pro.3902.
24. Kim S, *et al*. PubChem substance and compound databases. Nucleic Acids Res,2016:44(D1):D1202-D1213. doi: 10.1093/nar/gkv951.
25. Jacob RB, Andersen T, McDougal OM. Accessible high-throughput virtual screening molecular docking software for students and educators. PLoS Comput Biol,2012:8(5):e1002499. doi: 10.1371/journal.pcbi.1002499.
26. Ursu O, Rayan A, Goldblum A, Oprea TI. Understanding drug-likeness. Wiley Interdiscip Rev Comput Mol Sci,2011:1(5):760-781. doi: 10.1002/wcms.52.
27. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev,2012:64(SUPPL):4-17. doi: 10.1016/j.addr.2012.09.019.
28. Silva J, Nunes da Rocha M, Machado Marinho E, Machado Marinho M, Silva Marinho E, Silva dos Santos H. Evaluation of the ADME, toxicological analysis and molecular docking studies of the anacardic acid derivatives with potential antibacterial effects against staphylococcus aureus. J Appl Pharm Res,2021:10(5):84-94. doi: 10.15406/japlr.2021.10.00384.