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Identification of phytoconstituents for combating Polycystic ovarian syndrome through *in silico* techniques

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Polycystic ovarian syndrome is one of the leading causes for infertility in women. One in Five women of the population is affected by PCOS. The synthetic drugs currently used are targeted to provide an artificial support for the hormonal imbalance in the body which leads to various adverse effects. Natural herbs serve as a best remedy for many of the diseases as they cure the root cause and target the disease specifically. Selection of herbs is a crucial part in the formulation. *In silico* studies play an important role in analyzing the activity of the compound with the selected target. The herbs which had reported biological activity on uterus were selected and their vital chemical constituents were docked with the identified target of PDB ID 3RUK and 1E3K, respectively. The values obtained shows the potential effect of chemical constituent with the suitable target. Among the list of herbs selected, Sesamin from *Sesamum indicum* and lanosterol from *Ficus religiosa* had good binding affinity with both the selected proteins and had better drug likeliness properties. Hence, further studies on these compounds for targeting PCOS is expected to give potent activity and produce promising results.

Keywords: *Ficus religiosa*, *In silico* studies, Lanosterol, Polycystic ovarian syndrome, Sesamin, *Sesamum indicum*

Polycystic ovarian syndrome (PCOS) is one of the prevailing disorders in the current scenario. One among Five women of the Indian population and One among Ten women of world population are affected with this disorder. The main underlying cause for PCOS is not known. But some of the expected major causes are stress, Insulin resistance, excess androgen and heredity (Fig. 1). The syndrome is mainly caused due to the excessive hormonal imbalance in the body. The people affected with PCOS tend to have elevated levels of androgen hormones which is specified as the male sex hormones in the body[1]. These hormones are responsible for producing conditions like hirsutism which is the excessive growth of hair in the regions of body, thinning of hair, acanthosis nigricans which is denoted as the blackening of the neck and the armpit regions[2]. The major complications of PCOS include cardiovascular diseases, development of Type II diabetes mellitus and endometrial cancer[3]. Based on the Rotterdam criteria, PCOS is explained as a condition caused in the patients with the presence of two or three of the listed criterions like, oligo anovulation, polycystic ovaries with greater than or equal to 12 follicles and a measurement of 2-9 mm diameter and an ovarian volume greater than 10 mL[4]. The drugs which are currently used to supress the symptoms of PCOS include Metformin, clomiphene, letrozole and spironolactone. But these compounds tend to produce adverse reactions when taken for long duration[5].

Natural herbs play an important role in combating the menstrual problems. They have the potential effect to stabilise the hormones, regulate the menstruation and bypass the adverse effects produced by synthetic drugs[6].A list of the herbs was selected for the study based on their activity on the uterus and its hormones (Table 1)

Saracaasoka, commonly called as Ashoka belonging to the family caesalpinioideae act as a potent agent in treatment of various menstrual ailments like irregular bleeding, pain or cramps during the menstruation and improper vaginal discharge[7].

Cinnamomum zeylanicum, commonly called as cinnamom of the family lauraceae is a commonly used spices in kitchen. The flavonoids and polyphenols present in the compound rises the antioxidant properties and they relatively reduce the oxidative stress[8]. *Tephrosia purpurea*, commonly known as Sarapunkha belonging to the family Fabaceae, has a great wound healing property and the network pharmacological

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studies on this plant proves in regualting the Polycystic ovarian syndrome[9]. *Moringa oleifera*, commonly called as moringa of the family moringaceae has been proved to have potent effects in reducing the insulin resistance in the PCOS induced rats[10]. *Trigonella foenum-graecum* and *Sesamum indicum* belonging to the families, fabaceae and Pedaliaceae are reported to have potential effects on uterus and they treat the menstrual disroders by reducing the insulin resistance[11]. *Azadirachta indica* commonly known as neem of the family Meliaceae reduces the body weight in an effective manner and they help in regulating the hormones in the body[12,13]. *Ficus religiosa*, belonging to the family Moraceae is reported to have various biological activities like anti-inflammatory, anti-diabetic, anti-tumor, antioxidant, immunomodulatory, wound healing and other properties[14]. *Zingiber officinale*, commonly known as Ginger belonging to the family zingiberaceae is reported to have potential effects in treating the hirsutism and reduces the levels of androgen in the body[15].

Some of the other herbs like *Saccharum officinarum, Coscinium fenestratum, Plumbago rossea, Inula racemosa, Cinnamomum tamala, Mesua ferrea, Nelumbo nucifera, Myristica fragrans, Pterocarpus marsupium, Hedychium spicatum and Rubia cordifolia* have also been reported to have potential effects on the uterus[16].

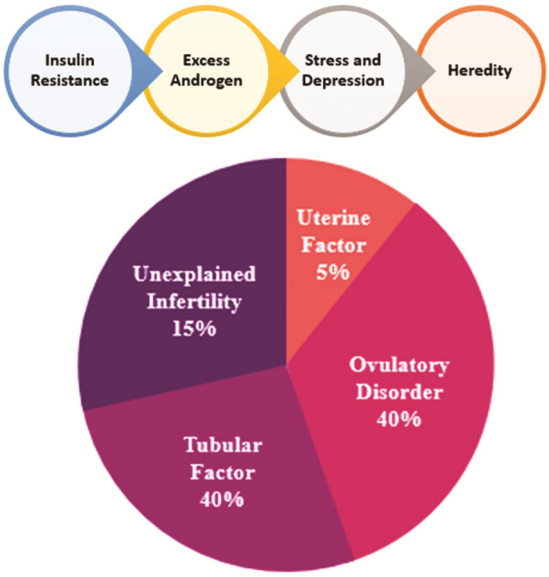


Fig. 1 — Causes of Polycystic ovarian syndrome and infertility in women

Selection of chemical constituents

Based on the chemical composition and the structural features of the compounds, one chemical constituent from each herb was being selected and taken into the study.

Materials and Methods

The main method for determination of the binding affinity and interactions of the ligands with the protein is through Molecular docking techniques. The docking technique commonly used is rigid- flexible docking where the target is placed rigid and the ligand is run with the flexible conformations[17].

Preparation of chemical structures

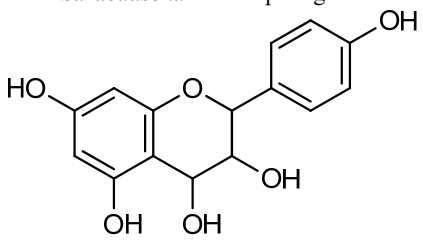
The compounds selected from each and every plant were downloaded from PubChem server and sketched in the ChemDraw Ultra 12.0 software (Table 2).

Table 1 — List of selected plants and chemical constituents

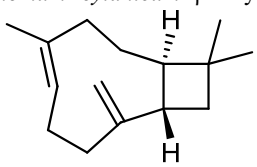
S. No	Medicinal plant	Family name	Parts used	Selected Chemical constituent
1	*Saracaasoka*	Fabaceae	Seed, bark, flowers	Leucopelargonidin
2	*Cinnamomum zeylanicum*	Lauraceae	Stem bark	β-caryophyllene
3	*Tephrosia purpurea*	Fabaceae	Seed	Rotenone
4	*Moringa oliefera*	Moringaceae	Root, stem, leaves, fruits	Moringyne
5	*Trigonella foenum-graceum*	Fabaceae	Seeds, leaves	Trigoneoside IIA
6	*Sesamum indicum*	Pedaliaceae	Seeds	Sesamin
7	*Saccharum officinarum*	Poaceae	Root, Stem	Swertisin
8	*Cosciniumfenestratum*	Menispermaceae	Root, stem	Jatrorrhizine
9	*Azadirachta indica*	Meliaceae	Fruit, bark, leaves, flower	Nimbolide
10	*Zingiber officinale*	Zingiberaceae	Rhizome	α-Zingiberene
11	*Plumbago rossea*	Plumbaginaceae	Leaves, root	Pumbagin
12	*Inula racemosa*	Asteraceae	Rhizomes, root	Alantolactone
13	*Cinnamomum tamala*	Lauraceae	Seeds	Eugenol
14	*Mesua ferrea*	Calophyllaceae	Flowers	Messuaferol
15	*Nelumbo nucifera*	Nelumbonaceae	Flowers, rhizome	Gallic acid
16	*Myristica fragrans*	Myristicaceae	Leaves, fruit	Myristicin
17	*Pterocarpus marsupium*	Fabaceae	Bark, leaves	Marsupin
18	*Ficus religiosa*	Moraceae	Bark, root, leaves	Lanosterol
19	*Hedychium spicatum*	Zingiberaceae	Root	Curzerene
20	*Rubia cordifolia*	Rubiaceae	Root	Rubiaidin

Table 2 — Structures of the selected chemical constituents

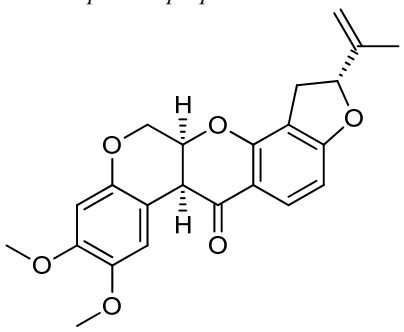
1. *Saracaasoka* - Leucopelargonidin



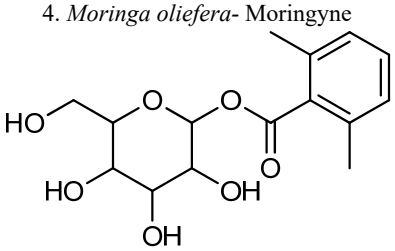
2. *Cinnamomum zeylanicum*- β-caryophyllene



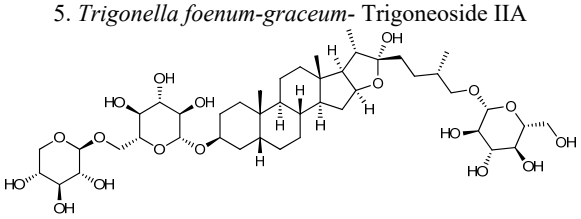
3. *Tephrosia purpurea*- Rotenone



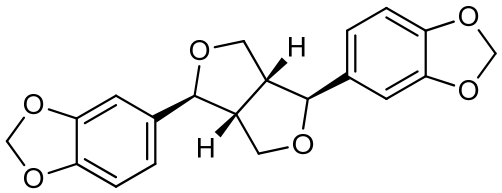
4. *Moringa oliefera*- Moringyne



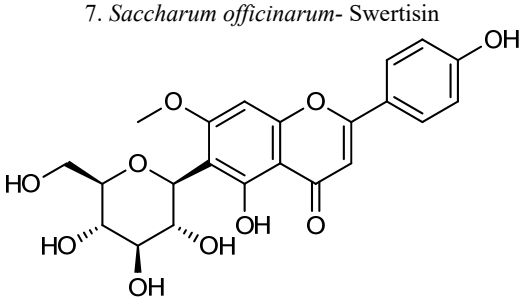
5. *Trigonella foenum-graceum*- Trigoneoside IIA



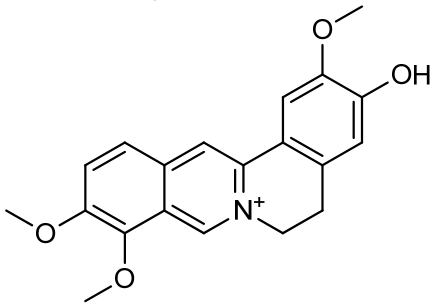
6. *Sesamum indicum*- Sesamin



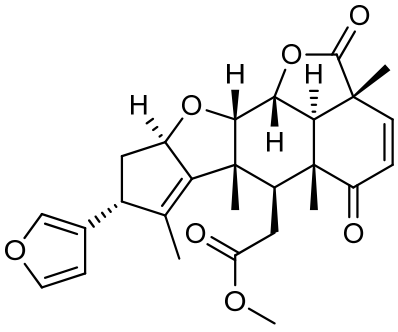
7. *Saccharum officinarum*- Swertisin



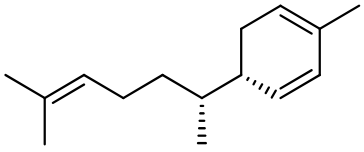
8. *Cosciniumfenestratum*- Jatrorrhizine



9. *Azadirachta indica*- Nimbolide



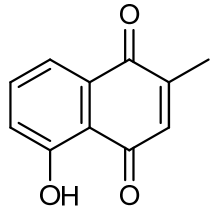
10. *Zingiber officinale*- α-Zingiberene



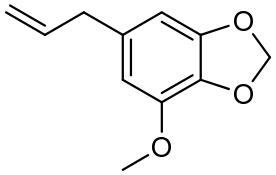
(*Contd*.)

Table 2 — Structures of the selected chemical constituents (*contd*.)

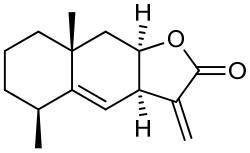
11. *Plumbago rossea*- Pumbagin



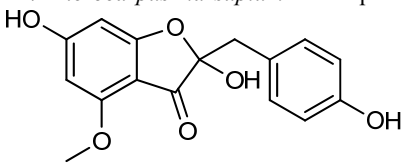
16. *Myristica fragrans*- Myristicin



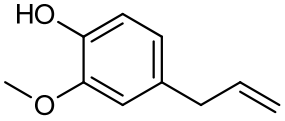
12. *Inula racemosa*- Alantolactone



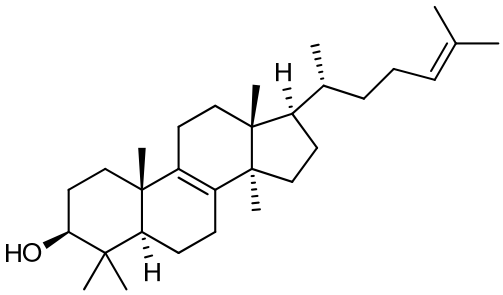
17. *Pterocarpus marsupium*- Marsupin



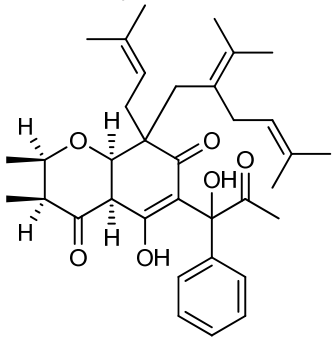
13. *Cinnamomum tamala*- Eugenol



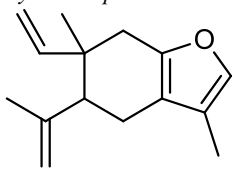
18. *Ficus religiosa*- Lanosterol



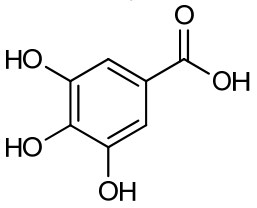
14. *Mesua ferrea*- Messuaferol



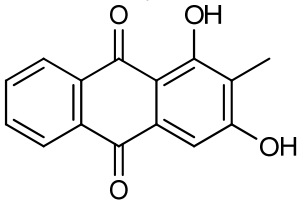
19. *Hedychium spicatum*- Curzerene



15. *Nelumbo nucifera*- Gallic acid



20. *Rubia cordifolia*- Rubiaidin



Physiochemical properties and toxicity prediction

All the selected chemical constituents were subjected to the determination of Physiochemical properties like absorption, distribution, metabolism and elimination by SWISS ADME online property prediction. The drug likeliness property mainly involves in the determination of the violations present in the compound like Lipinski, Egan, veber and ghose[18]. The toxicity parameters were determined using the OSIRIS toxicity predictor, an online tool.

The toxicity profiling for four different conditions like Mutagenicity, Tumorigenicity, Reproductive effect, eye and skin irritation levels were calculated using this tool.

Molecular docking studies

Human Cytochrome P450 CYP17A1 (3RUK) and Human progesterone receptor (1E3K) were obtained from Protein Data Bank (PDB), and they were used for the Docking studies. The water molecules and co-crystallized ligands were removed and the protein preparation was carried out by Molegro molecular viewer 2.5.

Preparation of docking structures

The lowest energy conformations of the sketched diagrams were obtained using Avogadro 1.2.0 software. MGL Tools 1.5.7 was used for the preparation of ligands and receptors for docking. All the allowed torsions in the ligands were set as flexible. Human Cytochrome P450 CYP17A1 (3RUK) and Human progesterone receptor (1E3K) were obtained, and all the heteroatoms, water molecules, and bound ligands in PDB crystal structures were removed. Grid generation was carried out after adding polar hydrogen, merging non-polar hydrogens, and adding Kollman charges. Grid with suitable dimensions was fixed (60*60*60 Å) with a grid spacing of 0.53 Å, and the grid file was obtained. Then the receptor was set as rigid with no flexible bonds to carry out the docking studies.

Docking method

Molecular docking studies were performed for the optimized compounds with the suitably prepared protein by an automated docking tool, AutoDock 1.5.7 which works by Lamarckian Genetic Algorithm[19]. The precise interaction of bioactive agents or candidate molecules with their targets is essential in drug development. AutoDock combines two methods to achieve these goals rapid grid-based energy evaluation and efficient search of torsional freedom. The final binding energy values were obtained, and the binding interaction was analyzed with suitable tools[20,21].

Visualisation tool

The Biovia Discovery studio (2017-release) was used to analyse the docking results by interpreting the ligand-protein binding sites, and the interaction plots were obtained[22,23].

Results and Discussion

Drug likeliness and ADME calculations

The physiochemical properties like absorption, distribution, metabolism and elimination were determined by the online tool named SWISS ADME Property calculator. The results and data obtained for the selected 20 chemical constituents explains the better drug likeliness properties of certain compounds and also gives the data of compounds which violate some rules. The values obtained from the tool is reported in the (Table 3). From the table it is clear that the values of the compounds Lanosterol, Sesamin,

Table 3 — Drug likeliness and ADME properties for the selected chemical compounds

Phytoconstituents	MW	iLogp	HBD	HBA	RB	MR
Leucopelargonidin	290.27	1.04	5	6	1	73.47
β-caryophyllene	204.35	3.29	0	0	0	68.78
Rotenone	394.42	3.78	0	6	3	106.15
Moringyne	311.35	2.00	3	6	4	78.36
Trigoneoside IIA	891.05	2.88	11	18	12	216.60
Sesamin	354.35	3.46	0	6	2	90.00
Swertisin	446.40	2.07	6	10	4	111.08
Jatrorrhizine	338.38	0.15	1	4	3	97.33
Nimbolide	466.52	3.51	0	7	4	120.99
α-Zingiberene	204.35	3.65	0	0	4	70.68
Pumbagin	188.18	1.79	1	3	0	51.07
Alantolactone	232.32	2.77	0	2	0	67.95
Eugenol	164.20	2.37	1	2	3	49.06
Messuaferol	598.51	3.80	5	12	3	160.28
Gallic acid	170.12	0.21	4	5	1	39.47
Myristicin	192.21	2.67	0	3	3	53.10
Marsupin;	302.28	1.20	3	6	3	77.24
Lanosterol	426.72	5.09	1	1	4	137.04

MW- Molecular weight, iLogp- Partition coefficient, HBD- Hydrogen bond Donor, HBA- Hydrogen bond acceptor, RB- Rotatable bonds, MR- Molar refractivity

Alantolactone and nimbolide stay within the limits of Lipinski rule of five hence they are determined to follow good drug likeliness properties.

Toxicity prediction

The values obtained from the OSIRIS toxicity predictor, gives a set of conditions which explains the extent of four different toxicity parameters. From the data obtained, it is visible that only few of the selected compounds show moderate to high toxicity values while the other compounds remain safe from toxicity. The compounds like Sesamin and lanosterol had no toxicity reported for all the four parameters. While, the compounds like α-Zingiberene, Nimbolide, alantolactone, eugenol and rubiadin were reported to produce high eye and skin irritation levels (Table 4).

Docking analysis

The docking studies was performed by Autodock 1.5.7 docking tool and the binding energy and interactions obtained were noted. The binding energy values of the list of 20 compounds are given in the (Table 5). From the list of the docking score obtained for selected 20 chemical constituents, 4 compounds obtained high docking score and good binding interaction (Fig. 2 and 3). Sesamin from *Sesamum indicum* obtained a docking score of –9.23 KJ/mol and –9.14 KJ/mol for the proteins 3RUK and 1E3K,

Table 4 — Toxicity parameters for the selected Chemical Constituents

Phytoconstituents	Mutagenic	Tumerigenic	Eye and Skin Irrritation	Reproductive Effect
Leucopelargonidin	No	No	No	No
β-caryophyllene	No	No	No	No
Rotenone	No	No	No	High
Moringyne	Mod	Mod	No	Mod
Trigoneoside IIA	No	No	No	No
Sesamin	No	No	No	No
Swertisin	No	No	No	No
Jatrorrhizine	No	No	No	No
Nimbolide	No	No	High	No
α-Zingiberene	High	No	High	High
Pumbagin	High	No	No	High
Alantolactone	No	No	High	No
Eugenol	High	High	High	No
Messuaferol	High	No	No	No
Gallic acid	High	No	No	High
Myristicin	High	High	No	No
Marsupin	No	No	No	No
Lanosterol	No	No	No	No

Table 5 — Binding energy of the selected Chemical Constituents with Proteins 3RUK and 1E3K

Chemical Constituent	Docking Score with 3RUK (KJ/Mol)	Docking Score with 1E3K (KJ/Mol)
Lanosterol	–9.37	–9.63
Sesamin	–.23	–9.14
Nimbolide	–8.37	–8.05
Alantolactone	–7.88	–8.61
Leucopelargonidin	–6.36	–7.92
β-caryophyllene	–6.6	–8.22
Rotenone	–8.04	–7.51
Moringyne	–5.98	–6.38
Trigoneoside IIA	–2.97	–4.72
Swertisin	–5.21	–5.30
Jatrorrhizine	–7.17	–7.92
α-Zingiberene	–7.16	–7.58
Pumbagin	–6.43	–6.59
Eugenol	–5.42	–5.51
Messuaferol	–5.98	–6.02
Gallic acid	–4.51	–4.15
Myristicin	–5.57	–5.40
Marsupin	–6.99	–7.48
Curzerene	–7.22	–7.21
Rubiaidin	–6.94	–7.47

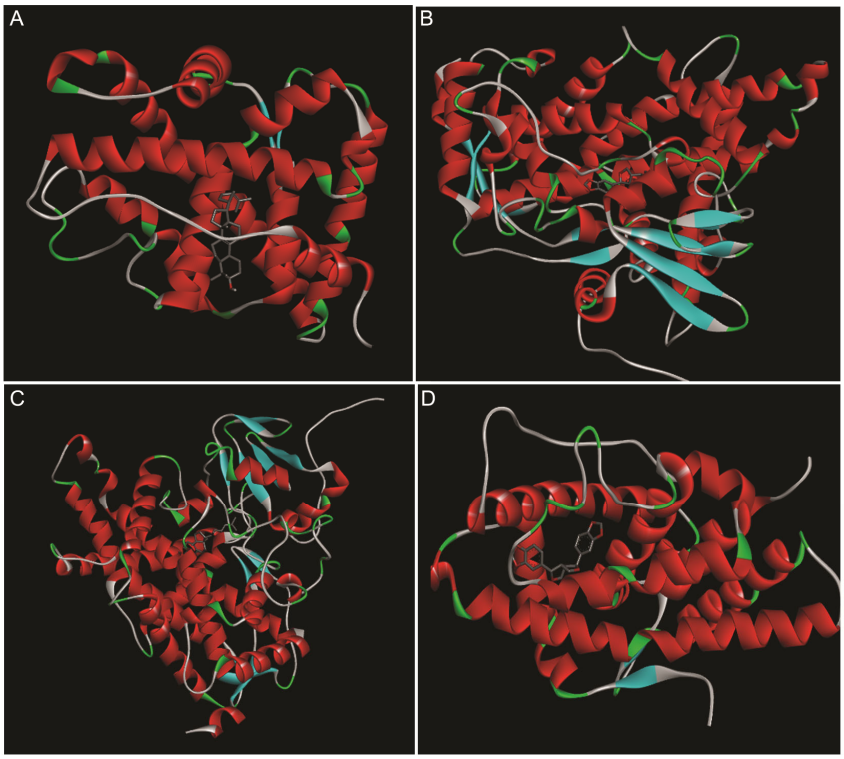


Fig. 2 — (A) Ligand protein complex of lanosterol with 3RUK protein; (B) Ligand protein complex of sesamin with 3RUK protein; (C) Ligand protein complex of lanosterol with 1E3K protein; and (D) ligand protein complex of sesamin with 1E3K protein

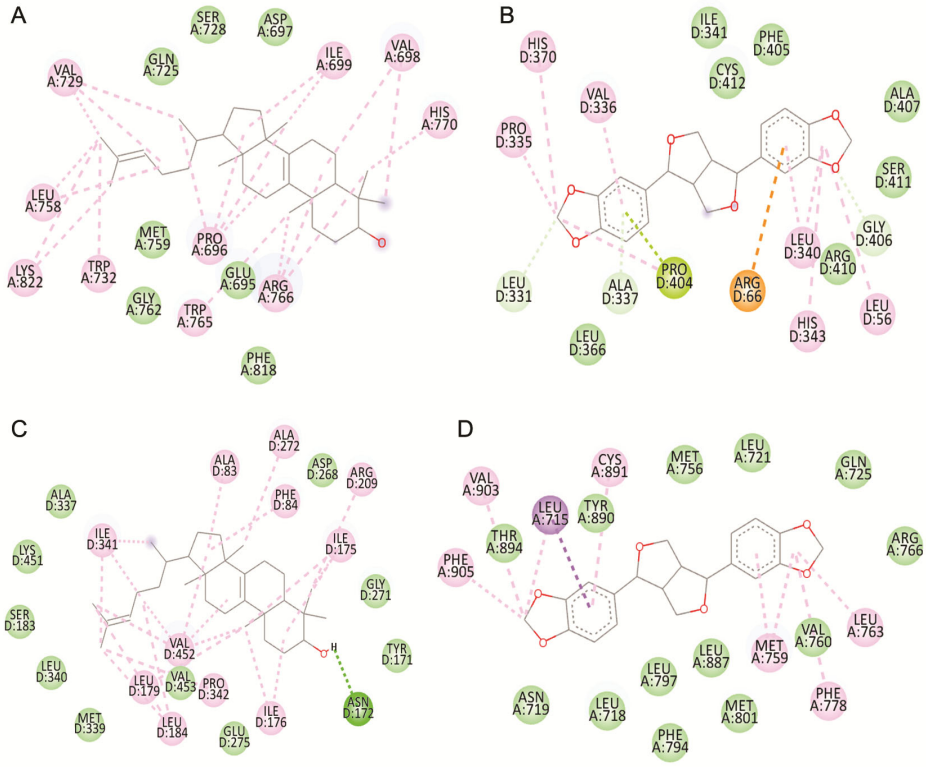


Fig. 3 — (A) Binding interaction of lanosterol with 3RUK protein; (B) Binding interaction of sesamin with 3RUK protein; (C) Binding interaction of lanosterol with 1E3K protein; and (D) Binding interaction of sesamin with 1E3K protein

respectively. While, Lanosterol from *Ficus religiosa* obtained −9.37 KJ/ mol and −9.63 KJ/mol, Nimbolide from *Azadirachta indica* with a values of −8.37 KJ/ mol and −8.05 KJ/mol and alantolactone from *Inula racemosa* with the values of −7.88 KJ/mol and −8.61 KJ/mol respectively (Table 5).

Conclusion

Treatment for Polycystic ovarian syndrome remains as a great need and demand in the present situation. Hence, works based on the natural products helps in paving a wave for the development of the new formulation techniques and a remedy for syndrome. In the current study, the *In silico* approaches were used for determining the critical constituent present in the particular herb that acts on the selected receptor and produce promising results. From the list of the 20 herbs selected for the study, the constituents like lanosterol from *Ficus religiosa* showed a good docking energy of −9.37 KJ/mol and −9.63 KJ/mol and Sesamin from *Sesamum indicum* showed docking energy of −9.23 KJ/mol and −9.14 KJ/mol for the targets with PDB ID 3RUK and 1E3K. They had better binding affinity with the amino acids LEU 340, ILE 699, TYR 890, PHE 705 and LEU 715. The same compounds had better drug likeliness properties and safe toxicity profile which denotes that these herbs are acting as potential agents in the particular receptor for the treatment of polycystic ovarian syndrome. Hence, further *in silico* studies like Molecular dynamic simulations and drug development techniques for these two is expected to produce promising results.

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Conflict of interest

All authors declare no conflict of interest.

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