Indian Journal of Biochemistry & Biophysics
Vol. 60, February 2023, pp. 108-121
DOI: 10.56042/ijbb.v60i2.70684

Network pharmacology and molecular docking study of the active ingredients in Saptasaram kashayam for the treatment of Polycystic ovary syndrome

T Santh Rani[1]*, P Premitha Rajya Lakshmi[1]* & Ch Manga Devi[1]

[1]Institute of Pharmaceutical Technology, Sri Padmavati Mahila Visvavidyalayam, Tirupati-517 502, Andhra Pradesh, India

Received 25 December 2022; *revised 07 January 2023*

Polycystic Ovary Syndrome (PCOS) is one of the most prevalent endocrine disorder in women of reproductive age characterized by hyperandrogenism (HA). Current treatment options for PCOS are either with adverse effects or ineffective. Saptasaram kashayam (SK), an ayurvedic formulation is often been a safe traditional alternative medicine to improve the PCOS symptoms as well as its pathological development. However, its principle phytoconstituents or underlying mechanisms have not been investigated. In order to achieve this, the current study systematically utilized computational tools, network pharmacology approaches and molecular docking studies. All identified phytoconstituents of SK were screened by QikProp ADME prediction and 47 were selected based on oral bioavailability and drug likeliness scores. Their 3D structures were submitted to three online target fishing webservers PharmMapper, ChemMapper and Swiss Target Prediction which produced 1084 biological targets for SK comprehensively. 350 known PCOS therapeutic targets were retreived as common targets from three different interrogative disease centric bioinformatic platforms DisGeNET, OMIM and GeneCards. Intersection of 1084 biological targets of SK and 350 PCOS therapeutic targets produced, 88 potential therapeutic targets of SK against PCOS. STRING PPI and Compound-Target-Pathway networks were constructed and analysed using Cytoscape software. GO & KEGG pathway enrichment analysis was performed using DAVID database. 15 PCOS therapeutic target proteins were short listed from network analysis report- PIK3CA, PDPK1, AKT1, PIK3R1, STAT3, MAPK1, MAPK3, EGFR, AR, ESR1, ESR2, SHGB, NOS3, F2 & CREBBP. Targets that were likely to be inhibited/modulated by SK for treatment of PCOS were docked against the screened phytoconstituents and their respective standard inhibitors using GLIDE-SP of Schrodinger suite, Maestro version- 13.0. Results showed that Quercetin, Catechin, Boeravinone J, Genistein, Protocatechuic Acid, Gentisic Acid, Xanthoarnol, Luteolin, Boeravinone F, Tyrosine, Kaempferol, Dalbergioidin, etc exhibited good binding affinities when compared to standard drugs and might be responsible for synergistic/additive protective effect of SK against PCOS. Meanwhile PI3K-Akt signaling pathway, Prolactin signaling pathway, AGE-RAG diabetic complications, HIF-1 signaling pathway and Estrogen signaling pathway were found to be involving the hub genes of interest and in this way, they might be intervened during treatment of PCOS by SK. Present study succeeded in identifying the drug like principle phytoconstituents, probable PCOS therapeutic targets and the underlying molecular mechanism of SK apart from providing reliable evidence for therapeutic potential of SK against PCOS. However further validation by *in vitro* and *in vivo* investigations is necessary.

Keywords: Molecular docking, Network pharmacology, Poly herbal formulation, Polycystic ovary syndrome, Saptasaram kashayam, Traditional medicine

Polycystic Ovary Syndrome (PCOS) is one of the most prevalent endocrine disorder in women of reproductive age characterized by hyperandrogenism. It accounts for 70-80% of infertility cases worldwide[1-3]. As per 2021 about 22.5% of women or every one in five women suffers from PCOS in India. The exact etiology of PCOS remains unknown yet.Many theories were postulated explaining the pathogenesis of PCOS which includes, increased Gonadotropin releasing hormone (GnRH) pulse frequency resulting in increased frequency and pulsatile secretion of Luteinising hormone (LH) that hyper stimulate ovaries to produce more testosterone[4].Another theory is hyperinsulinemia causing decreased secretion of sex hormone-binding globulin (SHBG) from the liver thus resulting in increased levels of free circulating testosterone in blood[5]. Besides this, hyperinsulinemia also increase GnRH pulse frequency and LH surge which predominently increases Follicle Stimulating Hormone

*Correspondence:
E-mail: drsanthrani@gmail.com (TSR);
premithaplummy78@gmail.com (PPRK)

Abbreviations: ADME, Absorption Distribution Metabolism and Excretion; AGEs, Advanced glycation end products; AKT1, RAC-alpha Serine/threonine protein kinase; AR, Androgen Receptor; Binding DB, Binding Database; BP, Biological Process; cAMP, Cyclic adenosine monophosphate; CC, Cellular Composition; CREB, cAMP-response element binding protein; CREBBP, CREB-Binding Protein; DAVID, Database for Annotation, Visualization and Integrated Discovery

Suppl. Data available on respective page of NOPR

(FSH) surge resulting in decreased follicular maturation, increased ovarian androgen production, and decreased SHBG. Also, alteration of adipocytokines leads to insulin resistance[6]. From this it is evident why till to date there is no single unified treatment for PCOS and the current treatment options are either with adverse effects or ineffective. They include life style changes (diet & exercise)[7], oral contraceptive pills[8], ovulation induction agents, antiandrogens and insulin sensitizers.

Ayurvedic formulations usenatural ingredients to treat diseases by restoring balance,with no side effects[9]. Recent studies reported that Ayurvedic formulations from various ayurvedic texts can be a better choice for the definitive cure of PCOS. Saptasaram Kashayam (SK), a poly herbal formulation is often been a safe traditional alternative ayurvedic medicine to improve the PCOS symptoms as well as its pathological development. It is made of seven plants, hence the name Saptasaram and it consists of *Boerhaaviadiffusa* (Punarnava), *Aegle marmelos* (Bilva), *Macrotyloma uniflorum* (Horse gram), *Ricinus communis* (castor), Barleriaprionitis (Sahachara), *Zingiber officinale* (Sunthi) and *Premna mucronate* (Agnimantha). This formulation is also used to treat amenorrhoea, dysmenorrhoea, pelvic inflammatory disease, dyspepsia, abdominal and pelvic pain. It was traditionally known to improve the ovarian functions and regulates menstrual cycle.However, the phytoconstituents in these herbs that alleviate PCOS or the underlying mechanism, have not been investigated.

Network pharmacology is based on systems biology,integrating pharmacology and bioinformatics to investigate the overall correlation between phytoconstituents and diseases.It helps in discovering drug targets, and also guide the development of new drugs[10]. In recent years, network pharmacology has been widely used to explore the pharmacological mechanisms of traditional medicine. Similarly, molecular docking emerged as an inevitable method for preliminary evaluation of binding affinity and prediction of intermolecular interactions of novel compounds with receptors. *In silico* studies provides a cost and time effective solution to *in vitro* and *in vivo* assays following 3R's principle- Reducing, Refining and Replacing animals for preliminary studies. Current study utilized computational tools, network pharmacology approaches and molecular docking studies to systematically unravel 1) active drug like phytoconstituents of SK, 2) probable PCOS therapeutic targets of SK and 3) the underlying pathophysiological mechanisms/pathways likely to be intervened by SK.

Materials and Methods

Identification and Screening of phytoconstituents

The phytoconstituents of all nine herbs present in SK were identified using NCBI PubMed database (http://pubmed.ncbi.nlm.nih.gov/) and their 3D-structures were obtained from PubChem database (http://pubchem.ncbi.nlm.nih.gov/). Oral Bioavailability (OB) and Druglikeliness (DL) are very important parameters and are significant indicators used for screening of phytoconstituents in research. Here QikPropmodule of Schrodinger suite was used to predict ADME parameters and Molsoft (www.molsoft.com) to predict an overall drug-likeness score.

Identificaton of probable biological targets of the phytoconstituents of SK

3D structures of screened phytoconstituents were submitted to three online target fishing web-servers. 1) PharmMapper(http://liab-ecust.cn)- it is designed to identify potential target candidates for the query molecules using pharmacophore mapping approach[11]. Filter criteria used in present study were 'Human protein targets'with normalized fit score > 0.6[12] 2) ChemMapper (http://www.lilab-ecust.cn/chemmapper /index.html)-a chemical database searching*via* molecular 3D similarity calculation strategies. Here 'SHAFTS 3D similarity' was chosen, setting similarity threshold at 1.2 and for every phytoconstituent three bioactivity databases-ChEMBL, Drug Bank and Binding DB were searched and all the results were pooled removing duplicates. 3) Swiss Target Prediction (http://www.swisstarget prediction.ch/)- itidentifies targets of tiny bioactive compounds in humans and other vertebrates based on a combination of 2D and 3D similarity. In our study, the targets of human species were separated and their respective gene symbols were retrieved for further use.

A comprehensive list of targets of all phytoconstituents was obtained when targets from PharmMapper, ChemMapper & SwissTargetPrediction were combined and duplicates removed.

Identification of known therapeutic targets of PCOS

One of the important steps in early stages of drug discovery is target identification and characterization. Here are three different interrogative disease-centric bioinformatic platforms- DisGeNET (https://www. disgenet.org/), GeneCards (https:// www.genecards. org) and OMIM (https://www.omim.org/) databases

were searched with the key word "Polycystic Ovary Syndrome". The overlapping targets from three sources were collected by drawing a Venn-diagram (https://bioinformatics. psb.ugent.be/webtools/Venn/).

Identification of potential therapeutic targets of SK againstPCOS

Another Venn diagram was drawn between two sets: 1) Comprehensive list of targets of phytoconstituents from PharmMapper, ChemMapper & SwissTargetPrediction and 2) Known therapeutic targets of PCOS obtained as the intersection targets of DisGeNET, GeneCards& OMIM. Finally, the intersection list of target proteins would be the *"potential therapeutic targets of SK against PCOS"* and were considered for further investigations.

STRING Protein-Protein Interaction

STRING (Search Tool for the Retrieval of Interacting Genes) is a database of known and predicted protein-protein interactions. The obtained *"potential therapeutic targets of SK against PCOS"* were imported into STRING (https://string-db.org/)[13] for generating the Protein-Protein Interaction network with a high confidence level (0.90) and the species defined as 'Human'. The PPI network was uploaded to cytoscape 3.9.0 for analysis of topological features. Top 10 potential therapeutic targets with highest degree were chosen for further consideration.

GO and KEGG pathway enrichment analysis

The DAVID (Database for Annotation, Visualization and Integrated Discovery) database is a tool for gene annotation, enrichment, and pathway analysis[14]. The "*potential therapeutic targets of SK against PCOS*" were inserted into DAVID Bioinformatics Resources 6.8 database (https:// david.ncifcrf.gov/tools.jsp) for GO and KEGG pathway enrichment analysis. The top 10 GO terms in each of 3 GO aspects- Biological Process (BP), Cellular Composition (CC) and Molecular Function (MF) with least P-value were selected. Similarly, the list of genes of interest was imported into DAVID for KEGG pathway enrichment analysis and top 20 enriched pathways with least P-value (or) highest $-\log_{10}$ (P value) were selected for the construction of Compound-Target-Pathway network.

Construction of Compound-Target-Pathway Network

'Formulation-Botanical- Bioactive-Target-Pathway' network (referred as 'Compound-Target-Pathway' network) was constructed using Cytoscape 3.9.1to better understand the complex relationship between compounds, disease targets and molecular pathways. The top 10 potential therapeutic targets ranked by degree, betweenness centrality and closeness centrality were obtained using CytoHubba plugin.

Molecular docking

Molecular Docking is a powerful computational method for identifying potential interactions between small molecules and pharmacological targets. In the present study, to assess the binding affinities of specific phytochemicals with specific target proteins GLIDE-SP module of Schrodinger suite- Maestro 13.0 was used. From among the top potential therapeutic targets of SK against PCOS obtained from STRING PPI network and Compound-Target-Pathway network analysis, the hub targets that were likely to be inhibited by SK in the treatment of PCOS were selected and docked against their respective known standard drugs and all screened phytoconstituents of SK.PDB IDs of all target proteinswere selected from Protein Data Bank (PDB) (http://www.rcsb.org/pdb*)*. The proteins were prepared using "protein preparation wizard". Both standard drugs and the phytoconstituents were prepared by using "LigPrep" module and receptor grids were generated with default setting of "Receptor grid generation" panel. All docking studies were performed using "Ligand docking" panel.

Interpretation of docking results

Docking score, docking pose, number of interactions, bond lengths and type of interactions were considered for interpreting docking results.

Results

Identification and Screening of phytoconstituents

A total of 299 phytoconstituents were reported in the nine herbs of SK and (Table 1) shows the number

Table 1 — Number of phytoconstituents present in each herb of Saptasaram kashaya (SK)

Sl. No	Herbs name	Number of Phytoconstituents	Reference
1	*Boerhaaviadiffusa*	23	15
2	*Aegle marmelos*	73	16
3	*Horse gram*	30	17
4	*Ricinus communis*	39	18
5	*Barleriaprionitis*	13	19
6	*Zingiber officinale*	74	20
7	*Premna mucronate*	17	21
8	*Long pepper* (additive used)	16	22
9	*Asafoetida* (additive used)	14	23

of phytoconstituents in each herb. 47 phytoconstituents were selected based on screening ADME properties (%OB>25), Lipinski's rule of five (zero violations) and druglikeliness score (DL-0 to 2) (Suppl. Table 1).

Identificaton of probable biological targets of the phytoconstituents of SK

For all the 47 phytoconstituents selected, PharmMapper produced 3858 targets, ChemMapper produced 1257 targets and SwissTargetPrediction produced 1791 targets. 1084 targets were remained after de-duplication.

Identification of known therapeutic targets of PCOS

DisGeNET produced 989 therapeutic targets, OMIM produced 4969 therapeutic targets and GeneCards produced 3068 therapeutic targets by setting limit as score ≥ 3.66. A venn diagram was drawn, which produced 350 intersecting therapeutic targets (Fig. 1).

Identification of potential therapeutic targets of SK againstPCOS

Another venn diagram Figure 2 was drawn between 1084 biological targets of SK and 350 therapeutic targets of PCOS. Finally, the obtained intersection list of 88 target proteins would be the *"potential therapeutic targets of SK against PCOS"*.

STRING Protein-Protein Interaction

PPI network was constructed by importing the above 88 targets to STRING web portal (Fig. 3). It consists of 87 nodes and 206 edges. Nodes represent proteins and the edges represent predicted functional associations. The top 10 potential therapeutic targets with highest degree were obtained from network analysis (Table 2) and were selected for further study.

GO and KEGG pathway enrichment analysis

The 88 potential targets were imported into DAVID database. GO annotation and enrichment of the genes encoded was conducted and the results were filtered off using the criteria P-value < 0.01.Dual axis bar and line graphs were plotted for the three GO aspects (BP, CC & MF) of potential target genes depicting GO term name on X-axis, percentage of input gene on Y-axis (bar graph) and -$\log_{10}$ (P value) on Secondary axis (line graph) (Fig. 4 A-C). Similarly, KEGG pathway enrichment analysis was done. The results are filtered off using the criteria P-value < 0.01, and top 20 enriched KEGG pathways (Table 3) were collected. Bubble chart was plotted for enriched pathways of genes of interest depicting KEGG pathway name on Y-axis and rich factor (ratio of number of input genes annotated in a

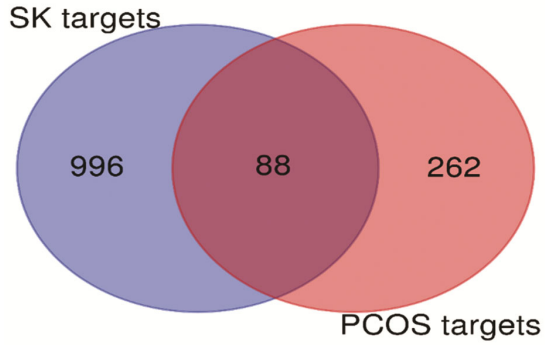

Fig. 1 — Venn diagram for PCOS therapeutic targets from the three web tools

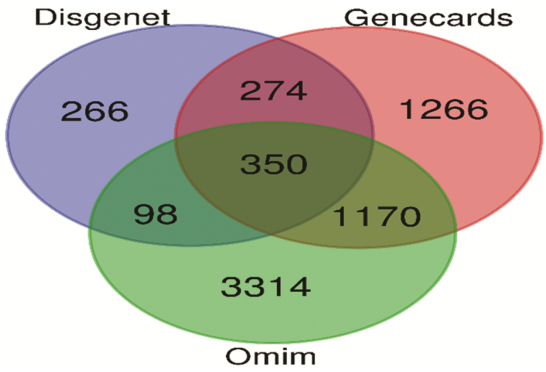

Fig. 2 — Venn diagram for probabletargets of SK and the therapeutic targets of PCOS

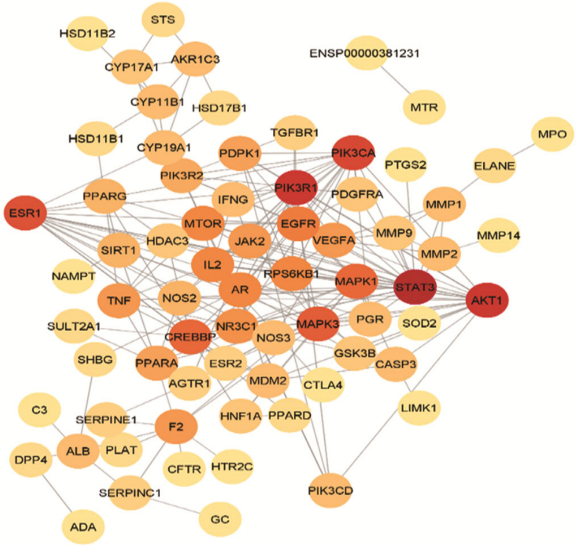

Fig. 3 — STRING Protein-Protein Interaction network. Change in node colour from yellow to brown indicates increase in degree value

Table 2 — STRING PPI Network analysis report showing top 10 hub protein targets (Degree ≥ Average value)

Sl. No	Gene	Protein name	Degree	Betweenness Centrality	Closeness Centrality
1	STAT3	Signal transducer and activator of transcription-3	23	0.22	0.48
2	AKT1	RAC-alpha Serine/threonine protein kinase	19	0.10	0.46
3	PIK3R1	Phosphotidylinositol-3-kinase regulatory subunit alpha	19	0.05	0.43
4	PIK3CA	Phosphatidylinositol 4,5-bisphosphate-3-kinase catalytic subunit alpha isoform	17	0.03	0.43
5	ESR1	Estrogen receptor	16	0.21	0.47
6	MAPK3	Mitogen activated protein kinase 3	15	0.13	0.47
7	CREBBP	CREB-Binding Protein	14	0.08	0.44
8	MAPK1	Mitogen activated protein kinase 1	14	0.11	0.47
9	EGFR	Epidermal Growth Factor Receptor	12	0.02	0.43
10	AR	Androgen Receptor	11	0.06	0.4

Table 3 — Top 20 KEGG Enriched signaling pathways

Sl. No	Pathway name	Number of genes of interest involved	Genes of interest involved
1	Pathway in cancer	33	GSK3B, PIK3CD, PIK3R2, PIK3R1, PTGS2, EGFR, CASP3, AKT1, MAPK1, JAK2, MAPK3, PDGFRA, CREBBP, NOS2, MMP1, MMP2, STAT3, F2, MMP9, ESR1, TGFBR1, IL2, MTOR, ESR2, VEGFA, AR, IFNG, PIK3CA, RPS6KB1, MDM2, AGTR1, PPARG, PPARD
2	Chemical receptor activation	22	EPHX1, STAT3, PIK3CD, PIK3R2, PIK3R1, ESR1, EGFR, MTOR, ESR2, VEGFA, AR, PIK3CA, RPS6KB1, ADRB3, AKT1, MAPK1, PGR, JAK2, PPARA, MAPK3
3	Proteoglycan in cancer	19	PDPK1, MMP2, STAT3, PIK3CD, PIK3R2, PIK3R1, ESR1, TNF, MMP9, EGFR, MTOR, VEGFA, PIK3CA, RPS6KB1, CASP3, MDM2, AKT1, MAPK1, MAPK3
4	Lipid and atherosclerosis	18	GSK3B, NOS3, PDPK1, MMP1, STAT3, PIK3CD, PIK3R2, PIK3R1, SOD2, TNF, MMP9, PIK3CA, CASP3, AKT1, MAPK1, PPARG, JAK2, MAPK3
5	Human cytomegalovirus infection	18	PDGFRA, GSK3B, STAT3, PIK3CD, PIK3R2, PIK3R1, PTGS2, TNF, EGFR, MTOR, VEGFA, PIK3CA, RPS6KB1, CASP3, MDM2, AKT1, MAPK1, MAPK3
6	PI3K-Ak signaling pathway	18	PDGFRA, GSK3B, NOS3, PDPK1, PIK3CD, PIK3R2, PIK3R1, EGFR, IL2, MTOR, VEGFA, PIK3CA, RPS6KB1, MDM2, AKT1, MAPK1, JAK2, MAPK3
7	Prolactin signaling pathway	17	GSK3B, LHCGR, STAT3, PIK3CD, PIK3R2, PIK3R1, ESR1, ESR2, GCK, CYP17A1, PIK3CA, TH, AKT1, MAPK1, JAK2, CGA, MAPK3
8	Prostate cancer	17	PDGFRA, GSK3B, CREBBP, PDPK1, PIK3CD, PLAT, PIK3R2, PIK3R1, MMP9, EGFR, MTOR, AR, PIK3CA, MDM2, AKT1, MAPK1, MAPK3
9	AGE-RAG diabetic complications	17	NOS3, MMP2, STAT3, SERPINE1, PIK3CD, PIK3R2, PIK3R1, TNF, TGFBR1, VEGFA, PIK3CA, CASP3, AGTR1, AKT1, MAPK1, JAK2, MAPK3
10	HIF-1 signaling pathway	17	CREBBP, NOS2, NOS3, STAT3, SERPINE1, PIK3CD, PIK3R2, PIK3R1, EGFR, MTOR, VEGFA, IFNG, PIK3CA, RPS6KB1, AKT1, MAPK1, MAPK3
11	MicroRNA in cancer	17	PDGFRA, CREBBP, STAT3, PIK3CD, PIK3R2, PIK3R1, PTGS2, SIRT1, MMP9, EGFR, MTOR, VEGFA, PIK3CA, CASP3, MDM2, MAPK1, MAPK3
12	Human papilloma virus infection	17	GSK3B, CREBBP, PIK3CD, PIK3R2, PIK3R1, PTGS2, TNF, EGFR, MTOR, VEGFA, PIK3CA, RPS6KB1, CASP3, MDM2, AKT1, MAPK1, MAPK3
13	Kaposi herpes virus infection	16	GSK3B, CREBBP, STAT3, PIK3CD, PIK3R2, PIK3R1, PTGS2, MTOR, VEGFA, C3, PIK3CA, CASP3, AKT1, MAPK1, JAK2, MAPK3
14	EGFR tyrosine kinase inhibitor resistance	15	PDGFRA, GSK3B, STAT3, PIK3CD, PIK3R2, PIK3R1, EGFR, MTOR, VEGFA, PIK3CA, RPS6KB1, AKT1, MAPK1, JAK2, MAPK3
15	Endocrine resistance	15	MMP2, PIK3CD, PIK3R2, PIK3R1, ESR1, MMP9, EGFR, MTOR, ESR2, PIK3CA, RPS6KB1, MDM2, AKT1, MAPK1, MAPK3
16	Relaxin signaling pathway	15	NOS2, NOS3, MMP1, MMP2, PIK3CD, PIK3R2, PIK3R1, MMP9, EGFR, TGFBR1, VEGFA, PIK3CA, AKT1, MAPK1, MAPK3

(Contd.)

Table 3 — Top 20 KEGG Enriched signaling pathways *(Contd.)*

Sl. No	Pathway name	Number of genes of interest involved	Genes of interest involved
17	Foxo signalling pathway	15	CREBBP, PDPK1, STAT3, PIK3CD, PIK3R2, PIK3R1, SOD2, SIRT1, EGFR, TGFBR1, PIK3CA, MDM2, AKT1, MAPK1, MAPK3
18	Chagas disease	14	NOS2, SERPINE1, PIK3CD, PIK3R2, PIK3R1, TNF, TGFBR1, IL2, C3, IFNG, PIK3CA, AKT1, MAPK1, MAPK3
19	Thyroid hormone signaling pathway	14	GSK3B, CREBBP, HDAC3, PDPK1, PIK3CD, PIK3R2, PIK3R1, ESR1, MTOR, PIK3CA, MDM2, AKT1, MAPK1, MAPK3
20	Estrogen signaling pathway	14	NOS3, MMP2, PIK3CD, PIK3R2, PIK3R1, ESR1, MMP9, EGFR, ESR2, PIK3CA, AKT1, MAPK1, PGR, MAPK3

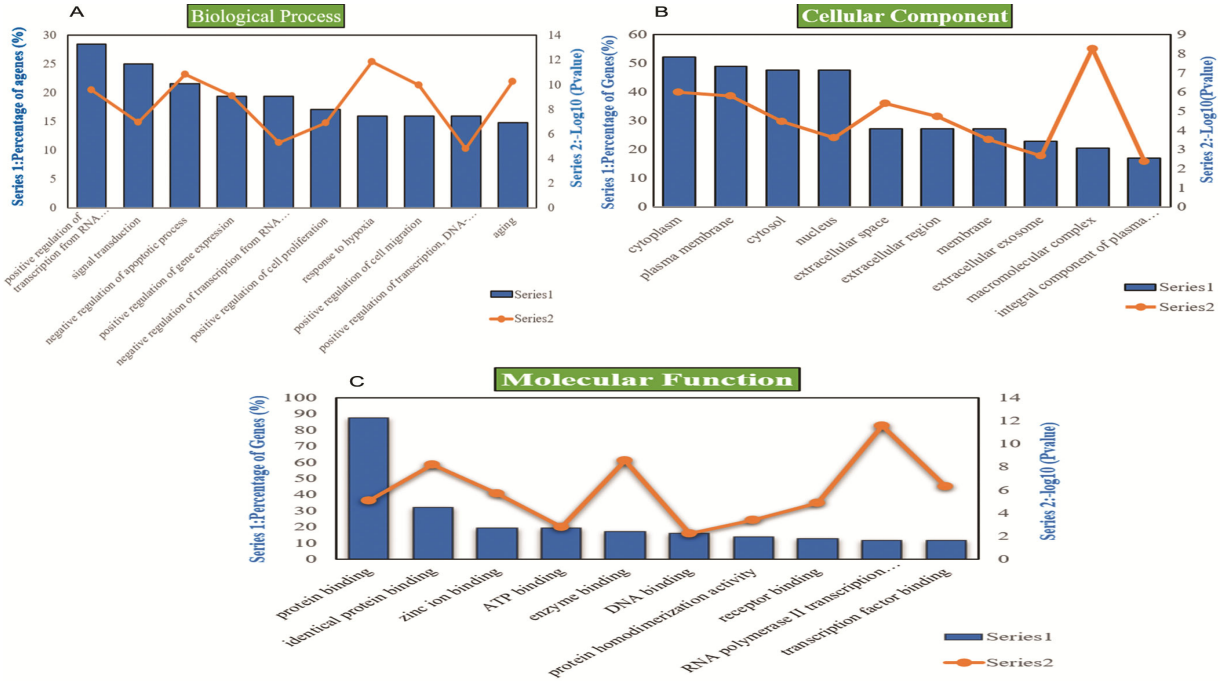

Fig. 4 — The GO functional enrichment analysis: (A) Enriched BP functions of potential target genes; (B) Enriched CC functions of potential target genes; and (C) Enriched MF functions of potential target genes

pathway to total number of genes annotated in that pathway) on X-axis. The size and color of the bubble represents count of input gene annotated in the pathway and -log10 P-value respectively (Fig. 5).

Construction of Compound-Target-Pathway Network

Network was constructed using Cytoscape 3.9.1 software (Fig. 6). The nodes describe formulation, botanical, phytoconstituents, potential targets and pathway, and the edges indicate the relationship between them. Network topological parameters were analyzed and top 10 hub targets (Table 4) were considered for further evaluation.

Molecular docking

From among the top hub target proteins obtained from STRING PPI network (Table 2) and Cytoscape Compound-Target-Pathway network (Table 5), the

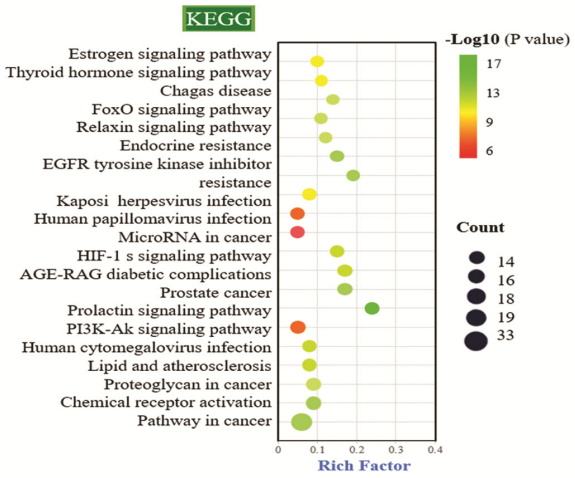

Fig. 5 — KEGG pathway enrichment analysis. KEGG pathway name on Y-axis and Rich factor on X-axis. Color from green to red reflects the adjusted -$\log_{10}$ (P value) from larger to small, and size of the bubble indicate the count of related genes involved

13 protein targets that are to be inhibited for alleviation of PCOS were selected for molecular docking studies (Table 6) and were docked with their respective standard inhibitors and with the 47 phytoconstituents of SK using GLIDE-SP. Their docking scores, number of interactions and interacting amino acid residues were tabulated in (Tables Suppl. 2-14). The 3D docking poses and 2D Ligand-Protein interaction images of each target against its respective standard and phytoconstituents with best binding affinity were depicted in (Fig. 7 and Suppl. 1), respectively.

Interpretation of docking results

Phytoconstituents of SK that show better affinity towards targets when compared to known standard drugs were listed in (Table 7) which also shows the number of

Table 4 — Phytoconstituents and number of associated target nodes in Compound-Target-Pathway network

Sl. No	Phytoconstituents	Number of target nodes
1	Quercetin	18
2	Catechin	16
3	Boeravinone J	18
4	Gentisic acid	11
5	Protocatecuic acid	16
6	Xanthoarnol	8
7	Luteolin	21
8	Kaempferol	16
9	Tyrosine	10
10	Boeravinone F	22
11	Genistein	22
12	Dalbergioidin	17

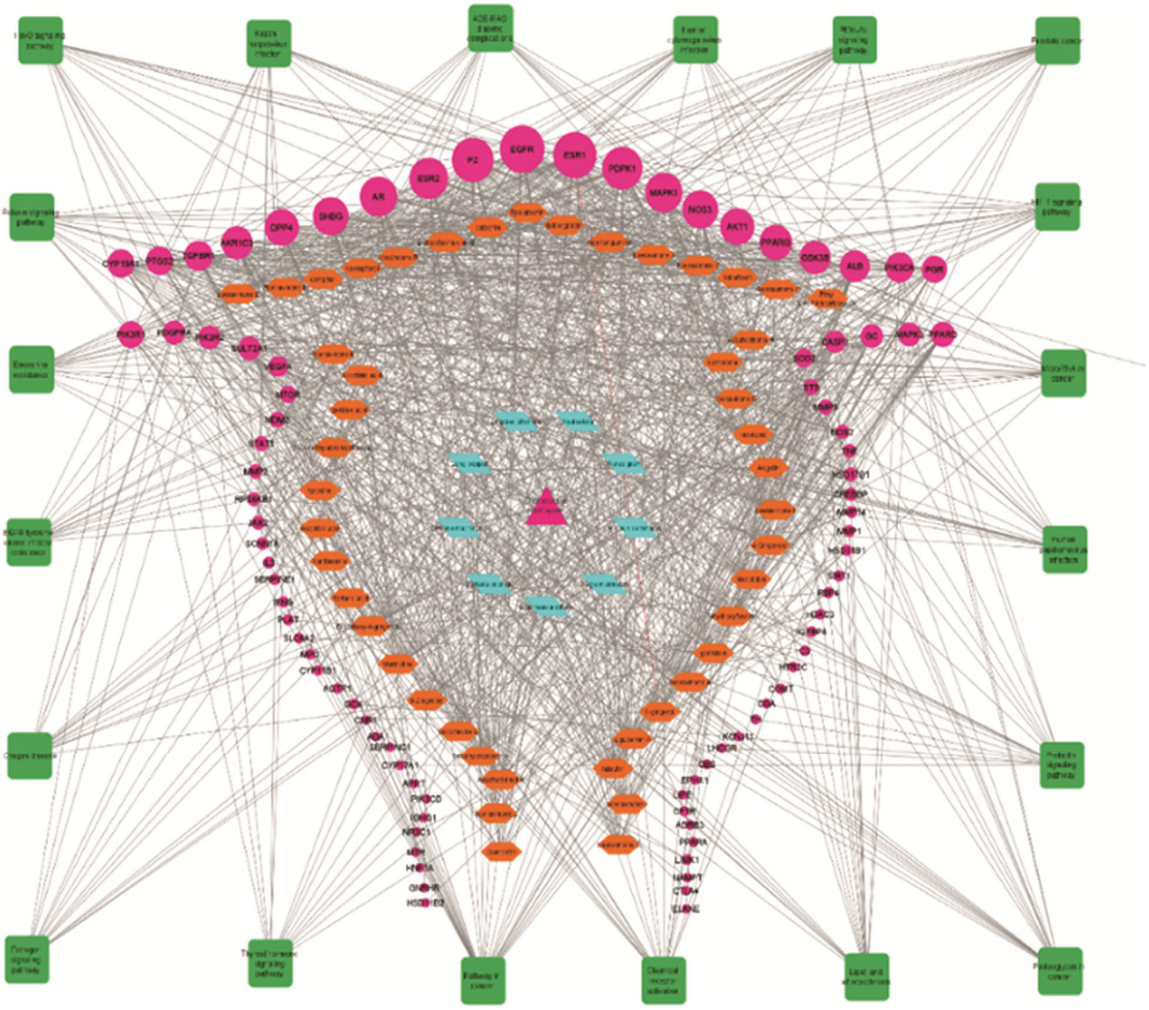

Fig. 6 — Formulation-Herb-Phytoconstituent-Target-Pathway network. Pink colour triangle at center represent formulation (SK), Sky blue colour parallelograms represent herbs, orange colour hexagonsrepresentsphytoconstituents, pink colour ellipse represents potential targets, rounded rectangles represent KEGG pathways. Increase in size of the pink colour ellipse node represent increase in the degree of respective target

Table 5 — Compound-Target-Pathway Network analysis report showing top 10 hub protein targets (Degree ≥ Average value)

Sl. No	Gene Symbol	Gene name	Degree	Betweenness Centrality	Closeness Centrality
1	EGFR	Epidermal Growth Factor Receptor	54	0.07	0.52
2	ESR1	Estrogen receptor	47	0.05	0.50
3	F2	Prothrombin	43	0.04	0.5
4	PDPK1	3-Phosphotidylinositol dependent protein kinase-1	42	0.04	0.48
5	ESR2	Estrogen receptor beta	40	0.04	0.49
6	MAPK1	Mitogen activated protein kinase 1	39	0.04	0.46
7	AR	Androgen Receptor	39	0.03	0.47
8	NOS3	Nitric Oxide Synthase, Endothelial	36	0.04	0.46
9	SHBG	Sex hormone binging globulin	36	0.01	0.44
10	AKT1	RAC-alpha Serine/threonine protein kinase	32	0.03	0.43

Table 6 — PDB ID's andstandard drugs of hub protein targets for docking studies

Sl. No	Hub gene	Protein name	PDBID	Standard drug
1	STAT3	Signal transducer and activator of transcription-3	6SM8	Napabucasin
2	AKT1	RAC-alpha Serine/threonine protein kinase	4GV1	Afuresertib
3	PIK3R1	Phosphotidylinositol-3-kinase regulatory subunit alpha	4L2Y	Isoproterenol
4	PIK3CA	Phosphatidylinositol 4,5-bisphosphate-3-kinase catalytic subunit alpha isoform	5DXT	Alpelisib
5	MAPK3	Mitogen activated protein kinase 3	4QTB	Ulixertinib
6	CREBBP	CREB-Binding Protein	4TQN	Inobrobid
7	MAPK1	Mitogen activated protein kinase 1	4ZZN	Ravoxertinib
8	EGFR	Epidermal Growth Factor Receptor	3W32	Erlotinib
9	ESR1	Estrogen receptor	1YLM	Fulvestrant
10	F2	Prothrombin	3DA9	Dabigatran
11	PDPK1	3-Phosphotidylinositol dependent protein kinase-1	3NAX	GSK2334470
12	ESR2	Estrogen receptor beta	1QKM	Estradiol
13	AR	Androgen Receptor	2AMA	Flutamide

Table 7 — List of phytoconstituents of SK that show better interaction with hub target proteins compared to standards

Sl. No	Potential multitargeting phytoconstituent	Number of targets	Names of the targets *(Showing better docking compared to standard)*
1	Quercetin	8	EGFR, MAPK3, PIK3R1, ESR2, STAT3, MAPK1, PIK3CA, F2
2	Catechin	8	ESR2, EGFR, MAPK3, PIK3CA, PIK3R1, STAT3, PDPK1, MAPK1
3	Boeravinone J	8	AR, AKT1, NOS3, PIK3R1, STAT3, ESR2, PDPK1, MAPK1
4	Genistein	7	ESR1, ESR2, NOS3, SHBG, PIK3R1, PDPK1, MAPK3
5	Protocatechuic acid	7	MAPK1, ESR1, ESR2, CREBBP, PIK3R1, STAT3, PIK3CA
6	Gentisic acid	7	STAT3, MAPK3, CREBBP, PDPK1, PIK3R1, PIK3CA, EGFR
7	Xanthoarnol	6	AKT1, ESR1, ESR2, AR, PDPK1, PIK3CA
8	Luteolin	6	PIK3CA, MAPK1, PIK3R1, PDPK1, ESR2, F2
9	Boeravinone F	6	SHBG, STAT3, ESR2, PIK3R1, CREBBP, MAPK3
10	Tyrosine	6	MAPK1, AKT1, MAPK3, CREBBP, PDPK1, PIK3CA
11	Kaempferol	6	STAT3, PDPK1, MAPK1, F2, EGFR, ESR2
12	Dalbergioidin	5	ESR2, EGFR, AKT1, SHBG, PIK3R1
13	Coccineone B	4	AR, AKT1, PIK3CA, SHBG,
14	Boeravinone B	4	AR, NOS3, EGFR, AKT1
15	Epicatechin	4	EGFR, SHBG, ESR2, AR
16	Diacetoxy-4-gingerdiol	4	PDPK1, NOS3, EGFR, ESR2
17	Kievitone	4	MAPK1, STAT3, PDPK1, PIK3CA
18	Riboflavin	4	PIK3R1, NOS3, PIK3CA, PDPK1
19	Boeravinone O	3	PIK3CA, STAT3, PDPK1
20	Boeravinone D	3	ESR1, SHBG, STAT3

(*Contd.*)

Table 7 — List of phytoconstituents of SK that show better interaction with hub target proteins compared to standards (*Contd.*)

Sl. No	Potential multitargeting phytoconstituent	Number of targets	Names of the targets *(Showing better docking compared to standard)*
21	Boeravinone E	3	NOS3, SHBG, AKT1
22	Boeravinone I	2	MAPK3, STAT3,
23	Tetrahydropiperine	2	NOS3, PIK3CA
24	Ligupersin A	2	MAPK3, NOS3
25	Boeravinone G	2	NOS3, AR
26	Boeravinone M	2	STAT3, PIK3CA,
27	Nicotinic acid	2	F2, PIK3CA
28	Aegelin	1	AKT1
29	8-Zingerine	1	EGFR
30	2-O Methylarabinoisoflavone	1	NOS3
31	Ethyl brevifolincarboxylate	1	PDPK1
32	4-Gingerdiol	1	ESR2
33	Marmeline	1	PIK3CA

Table 8 — List of hub target proteins andthe phytoconstituents that show good affinity with them compared to known standard drugs

Sl. No	Hub target protein	Number of phytoconstituents	Name of the Phytoconstituents *(Showing better binding affinity compared to standard)*
1	Phosphatidylinositol 4,5-bisphosphate-3-kinase catalytic subunit alpha isoform (PIK3CA)	16	Luteolin, Catechin, Coccineone B, Boeravinone O, Boeravinone M, Kaempferol, Quercetin, Riboflavin, Xanthoarnol, Tetrahydropiperine, Kievitone, Protocatechuic acid, Gentisic acid, Marmeline, Tyrosine, Nicotinic acid
2	3-Phosphotidylinositol dependent protein kinase-1 (PDPK1)	14	Kievitone, Luteolin, Catechin, Genistein, Tyrosine, Quercetin, Gentisic acid, Riboflavin, Boeravinone K, Ethyl brevifolincarboxylate, Kaempferol, Diacetoxy-4-gingerdiol, Xanthoarnol, Boeravinone J
3	Estrogen receptor beta (ESR2)	13	Genistein, Dalbergioidin, Catechin, Quercetin, Luteolin, Epicatechin, Xanthoarnol, 4-Gingerdiol, Boeravinone F, Boeravinone J, Protocatechuic acid, Kaempferol, Diacetoxy-4-gingerdiol
4	Signal transducer and activator of transcription-3 (STAT3)	13	Boeravinone F, Boeravinone J, Kievitone, Gentisic acid, Quercetin, Kaempferol, Boeravinone O, Boeravinone D, Boeravinone M, Boeravinone I, Catechin, Protocatechuic acid, 2-O Methylarabinoisoflavone
5	RAC-alpha Serine/threonine protein kinase (AKT1)	11	Aegelin, Dalbergioidin, Boeravinone J, Coccineone B, Xanthoarnol, Tyrosine, Boeravinone B, Boeravinone E
6	Phosphotidylinositol-3-kinase regulatory subunit alpha (PIK3R1)	10	Riboflavin, Luteolin, Catechin, Genistein, Boeravinone J, Dalbergioidin, Quercetin, Boeravinone F, Protocatechuic acid, Gentisic acid
7	Mitogen activated protein kinase 1 (MAPK1)	9	Luteolin, Tyrosine, Protocatechuic acid, Kievitone, Kaempferol, Quercetin, Boeravinone M, Boeravinone J, Catechin
8	Mitogen activated protein kinase 3 (MAPK3)	8	Catechin, Quercetin, Boeravinone I, Ligupersin A, Gentisic acid, Tyrosine, Genistein, Boeravinone F
9	Epidermal Growth Factor Receptor (EGFR)	7	Dalbergioidin, Catechin, Epicatechin, Quercetin, Kaempferol, Gentisic acid, Diacetoxy-4-gingerdiol
10	Androgen Receptor (AR)	6	Coccineone B, Boeravinone J, Boeravinone B, Epicatechin, Xanthoarnol, Boeravinone G
11	Estrogen receptor (ESR1)	4	Boeravinone D, Genistein, Protocatechuic acid, Xanthoarnol
12	Prothrombin (F2)	4	Quercetin, Kaempferol, Nicotinic acid, Luteolin
13	CREB-Binding Protein (CREBBP)	4	Gentisic acid, Boeravinone F, Protocatechuic acid, Tyrosine

potential targets for each constituent. This helps in the identification multitargeting active phytoconstituents of SK against PCOS. Similarly, another table was prepared postulating targets and the number of phytoconstituents that show good affinity against each when compared to standard drugs (Table 8). This helps in recognizing the PCOS proteins that may be targeted by multiple phytoconstituents.

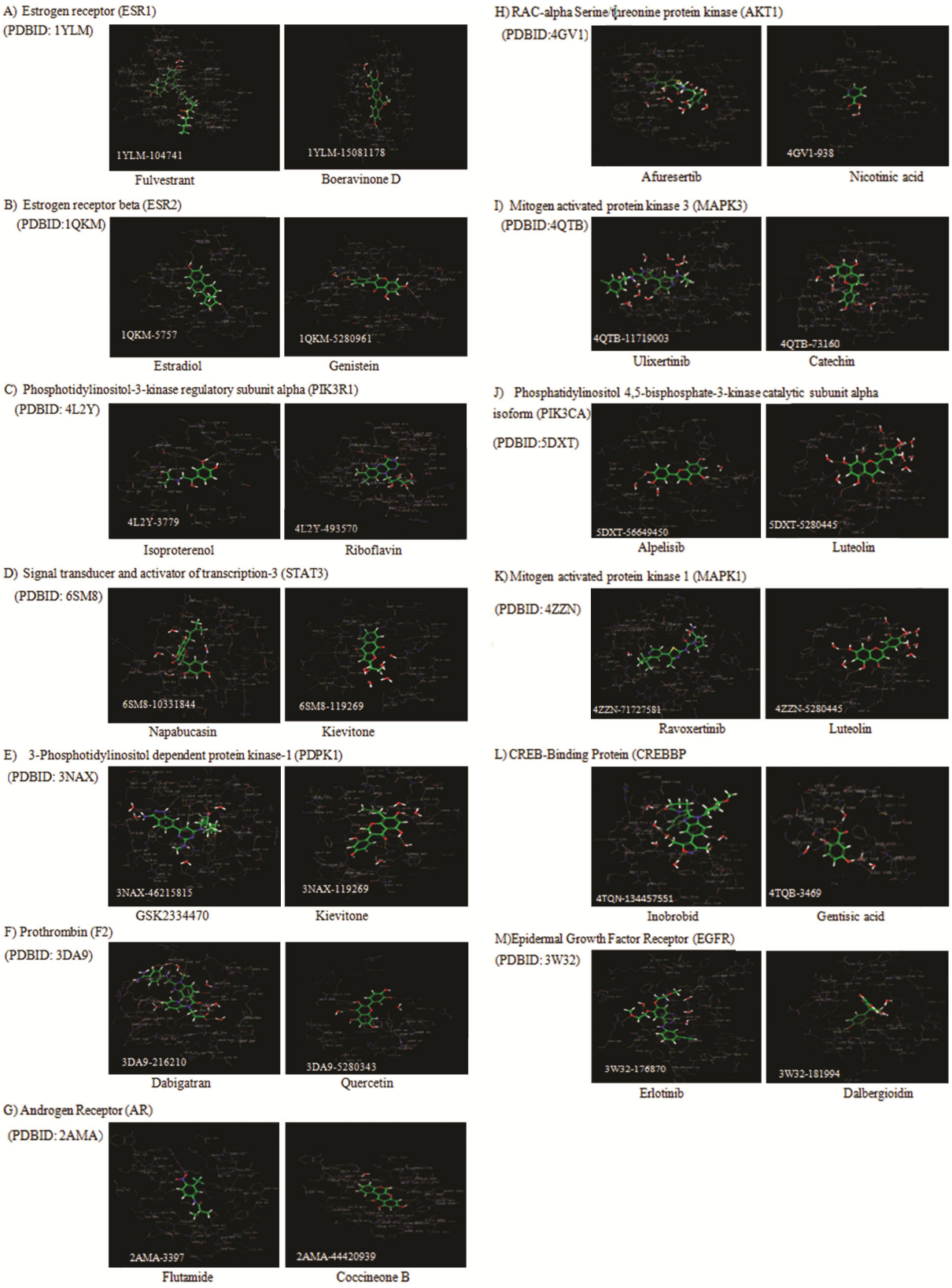

Fig. 7 — 3D interactions of standard and top scoring phytoconstituent against targets

Discussion

PCOS is a complex hormonal disorder affecting women of child bearing age. Currently there is no drug that is safe and effectively used clinically against PCOS. Owing to the complexicity of the disease a multidrug-mutitarget-multipath approach is preferable for the management of PCOS and here comes the role traditional medicine which use poly herbal formulations. SK is an ayurvedic formulation of seven herbs and traditionally being used for PCOS. But there are no scientific studies predicting the principle constituents and underlying mechanisms of its protective role. Thus present study was designed to investigate the components, targets and pathways that might be responsiblein a systematic way using network pharmacology approach. This facilitated the identification of 15 PCOS related hub target proteins of SK and for further validationthe protein targets were docked with their respective standard inhibitors and 47 phytoconstituents of SK using Glide-SP of Schrodinger suite. Phytoconstituents and their probable targets that showed good interactions and dock score when compared with standard drugs were short listed (Table 7). Among them are- Quercetin, Catechin, Boeravinone J, Genistein, Protocatechuic Acid, Gentisic Acid, Xanthoarnol, Luteolin, Boeravinone F, Tyrosine, Kaempferol, Dalbergioidin *etc*. Interestingly, it was evident from the Compound-Target-Pathway network that the above phytoconsituents were the core nodes with highest degrees (Table 4) reinforcing docking results. Quercetin showed good binding affinity against 8 out of 13 hub PCOS targets of SK compared with known standards (Table 7). Similarly, the multitargeting potential of several other phytoconstituents of SK was clear from (Table 7) and thus they might be the key components of SK. Quercetin, Catechin, Genistein, Protocatechuic Acid, Luteolin &Tyrosine were earlier reported to show anti PCOS effect in preclinical models. Quercetin showed an intrinsic potential of correcting hormonal disturbances, subsequent metabolic disorders occurred in PCOS and also exhibited a potential capacity to sensitize insulin receptors attributed to its antioxidant, anti-inflammatory property[24,25] and antitumour activity[26] Catechin was proven to have anti-inflammatory and antimatrix degradation effect in uterus[27,28]. Genistein may effectively mitigate PCOS related symptoms by decreasing insulin resistance and anthropometric indices and improving ovarian morphology, regulating reproductive hormones, reducing oxidative stress, inflammation[29] and shows estrogenic activity[30]. Protocatechuic acidimproved sex hormones by significantly upregulating FSH & estradiol and downregulating LH & testosterone levels owing to its antiandrogenic, antioxidant and antihyperglycemic activities[31]. Luteolin was reported to normalise estrous cycle, improve ovarian morphology, inhibit insulin resistance by prompting PI3K/Akt signaling pathway and restoring antioxidant Nrf2 pathway[32]. Tyrosine was found to regulate menstrual cycle and ovulation thus alleviating PCOS[33]. Boeravinone J, Gentisic acid, Xanthoarnol, Boeravinone F, Kaempferol and Dalbergiodin are not yet scientifically evaluated for anti PCOS effect elsewhere rising the scope for future research.

Further, set of 88 target genes of interest were analyzed using STRING PPI network, GO & KEGG pathway enrichment analysis and Compound-Target-Pathway network construction and analysis sequentially in order to illustrate the role of PCOS related targets of SK in gene function and signaling pathways. From the PPI network analysis STAT3, AKT1, PIK3R1, ESR1, MAPK3, CREBBP, MAPK1, EGFR &AR (Fig. 3 & Table 2) were the core proteins and similarly, from the Compound-Target-Pathway network analysis EGFR, ESR1, F2, PDPK1, ESR2, MAPK1, AR, NOS3, SHBG & AKT1 (Fig. 6 & Table 5) were obtained as the potential targets with highest nodal degree indicating that they might be modulated by SK in PCOS treatment. Among them PIK3CA, PDPK1, AKT1, PIK3R1, STAT3, MAPK1, MAPK3, EGFR, AR, ESR1, ESR2, F2 & CREBBP are the target receptors that are to be inhibited/modulated by SK to dampen PCOS pathological consequences. Docking studies showed that all these targets exhibit good docking scores with mutiple phytoconstituents of SK compared to standard drugs (Table 8, Fig. 7, Tables S2-14, and Suppl. Fig. 1).

PIK3CA, PDPK1, PIK3R1 & AKT1 are involved in PI3K-Akt signaling pathway, that regulates cell function by stimulating glucose metabolism, preventing the activation of apoptotic cascade, and promoting cell survival[34]. Over expression of PIK3-AKT signaling pathway causes insulin resistance and increase in VEGF & EGF levels leading to PCOS. Metformin, Spiranolactone and Letrozole used to treat PCOS act by inhibiting PI3K-Akt pathway and by lowering the VEGFA levels[35]. STAT3 activation is triggered by a number of cytokines and growth factors, including IL-6

signaling. STAT3 levels rise in PCOS, causing intrauterine inflammation.[27] MAPK1 & MAPK3 are members of the MAPK pathway whose inhibition impedes granulocyte proliferation. EGF inhibits estrogen synthesis and blocks antral follicle growth in granulosa cells[36]. It leads to follicular arrest in PCOS individuals. Pioglitazone used in PCOS is a specific antagonist of EGF that inhibit EGFR tyrosine kinase and also decreases insulin resistance. Hyperstimulation of AR alters the functions of ovaries and leads to anovulation in PCOS[37]. Antiandrogen drugs like Spironolactone, Flutamide and Finasteride are used to treat PCOS related symptoms. Upon phosphorylation CREB recruits its coactivators CREBBP and p300 to increase the transcription of CREB related genes that are involved in multiple complications associated with PCOS, including hyperglycemia, insulin resistance and compensatory hyperinsulinemia, adipose tissue inflammation, excess LH function, and androgen production[38]. Regulation of estrogen levels is crucial in ameliorating PCOS disease. Altered estrogen levels causes ovarian dysfunction leading to cysts formation. Clinically used drugs like Clomifene citrate & Flutamide mainly act by modulating Estrogen receptor (ESR2) and Fluvestrant act as an Estrogen receptor antagonist. ESR1&2 showed good binding affinities with many phytoconstituents of SK when compared with Estradiol and standard inhibitor Fluvestrant. NOS3 & SHBG play a protective role and are reported to be downregulated in PCOS disease[5,39]. Thus, our current workflow could fulfil the objective to identify potential therapeutic targets of SK against PCOS.

DAVID GO enrichment analysis performed on the 88 target genes of interest showed that SK might influence certain biological processes like apoptosis, proliferation, signal transduction, regulation of transcription, cell migration etc which may be related to the pathogenesis or amelioration of PCOS (Fig. 4). The KEGG enrichment pathways for the set of genes of interest include the following pathways that are well connected of PCOS disease complications- PI3K-Akt signaling pathway, Prolactin signaling pathway, AGE-RAG diabetic complications, HIF-1 signaling pathway and Estrogen signaling pathway, (Table 3). As discussed above PI3K-Akt pathway activation is detrimental in PCOS and is targeted by currently used anti PCOS drugs.[35] Prolactin (PRL) plays a vital role in numerous biological functions including reproduction, lactation, growth and development, endocrinology and metabolism, *etc*. PRL was reported to stimulate the expression of ESR1 and ESR2 in rat corpus luteum via JAK2/STAT5 pathway. It activates many signaling cascades like JAK2/STAT, PI3K/Akt and MAPK[40]. Altered deposition of AGEs (Advanced glycation end products) represent a common feature in PCOS and their interaction with specific receptor for AGEs (RAGE) activates many intracellular signaling pathways involving NADPH oxidase, protein kinase C, MAPKs and inflammatory NF-κB pathway there by increasing the production of ROS, expression of pro-inflammatory cytokines such as IL-1, IL-6, TNF-alpha[41] and a variety of atherosclerosis-related genes, including VCAM-1, tissue factor, VEGF, and RAGE. This leads to oxidative stress, inflammation, hyperandrogenism, insulin resistance, and ovulatory dysfunction[42,43]. In addition, JAK-STAT-mediated and PI3K-Akt-dependent pathways are induced *via* RAGE, which in turn participate in cell proliferation and apoptosis respectively. HIF-1 signaling pathway is crucial for the regulation of oxygen homeostatis. Hypoxic conditions prevailing in intrafollicular microenvironment during follicular growth may trigger expression of ovarian HIF-1α in granulosa cells[44-46]. Which upon stabilization interacts with its co activators CREBBP/p300 and eventually may result in increased transcription of CREB related PCOS genes as just discussed above[38]. Despite its name, HIF-1 is induced not only in response to reduced oxygen availability but also by other stimulants, such as nitric oxide, or various growth factors. Perturbation of Estrogen signaling affect cellular activities like ovulation, ovarian steroidogenesis, cell cycle, cell proliferation, migration and invasion. However its role in PCOS is still debatable and remains elusive. There are two types of oestrogen signalling pathways: nuclear-initiated steroid signalling and membrane-initiated steroid signalling. In the former, estrogen binds to ESR1 or ESR2 and translocates to the nucleus to bind to DNA at ERE elements and activates the expression of ERE-dependent genes. Later pathway, involves membrane bound G-protein coupled E2 receptors (GPER) which upon binding with estrogen rapidly activates various signaling pathways (*i.e*. Ca^{2+}, cAMP, protein kinase cascades) and ultimately influence downstream transcription factors[47]. By this we arrived at a better understanding of the complex interrelating pathophysiological pathways of PCOS and uncovered the likely mechanisms underlying

therapeutic potential of SK in counteracting PCOS. The result of present study provides an evidence for the protective effect of SK against PCOS in a systematic and scientific perspective.

Conclusion

Current research utilized the combination of bioinformatics, network pharmacology and molecular docking tools for understanding the multicomponent-mutitarget-multipathway characteristics of treatment of PCOS with ayurvedic polyherbal formulation SK. Quercetin, Catechin, Genistein, Protocatechuic Acid, Luteolin, and Tyrosine were recognized as principle phytoconstituents earlier reported in animal models for anti PCOS effect and might be responsible for a synergistic/ additive protective effect of SK against PCOS. Boeravinone J, Gentisic acid, Xanthoarnol, Boeravinone F, Kaempferol and Dalbergiodin also show good docking scores with hub targets compared to standard drugs but were not scientifically proven. Simultaneously, a total number of 88 target proteins were obtained common for SK and PCOS. And from network analysis PIK3CA, PDPK1, AKT1, PIK3R1, STAT3, MAPK1, MAPK3, EGFR, AR, CREBBP, ESR1, ESR2, SHBG, NOS3 & F2 were predicted as hub PCOS related targets of SK and docking studies showed that they were likely to be inhibited by multiple phytoconstituents of SK with good binding affinities when compared with their known standard drugs. Moreover, PI3K-Ak signaling pathway, Prolactin signaling pathway, AGE-RAG diabetic complications,HIF-1 signaling pathway and Estrogen signaling pathway were found to be involving the hub genes of interest and in this way, they might be intervened during treatment of PCOS by SK. However, further *in vitro* and *in vivo* research is necessary to scientifically prove the therapeutic role and complex mechanisms of SKin the treatment of PCOS.

Acknowledgement

We are thankful to DST-CURIE-AI test facility, Sri Padmavati Mahila Visvavidyalayam for providing all the facilities required for my project.

Conflicts of interest

All authors declare no conflicts of interest.

References

1 Brakta S, Lizneva D, Mykhalchenko K, Imam A, Walker W, Diamond MP & Azziz R, Perspectives on Polycystic Ovary Syndrome: Is Polycystic Ovary Syndrome Research Underfunded? *J Clin Endocrinol Metab,* 12(2017) 4421.

2 Melo AS, Ferriani RA & Navarro PA, Treatmentof infertility in women with Polycystic Ovary Syndrome: approach to clinical practice. *Clinics,* 11 (2015) 765.

3 Zafar U, Memon Z, Moin K, Agha S, Hassan JA & Zehra D, Prevalence of PCOS with associated symptoms and complications at tertiary care hospital of Karachi. *J Adv Med Res*, 4 (2019) 1.

4 McPhee SJ, Papadakis MA & Rabow MW, Current medical diagnosis & treatment. New York: *McGraw-Hill Medical*, (2010).

5 Wallace IR, McKinley MC, Bell PM, Hunter SJ, Sex hormone binding globulin and insulin resistance. *Clin Endocrinol*, 3 (2013) 321.

6 Sanchez-Garrido MA & Tena-Sempere M, Metabolic dysfunction in polycystic ovary syndrome: pathogenic role of androgen excess and potential therapeutic strategies. *Mol Metab,* 35 (2020) 100937.

7 Panidis D, Tziomalos K, Papadakis E, Vosnakis C, Chatzis P & Katsikis I, Lifestyle intervention and anti-obesity therapies in the polycystic ovary syndrome: impact on metabolism and fertility. *Endocrine,* 44 (2013) 583.

8 Yildiz BO, Approach to the Patient: Contraception in Women with Polycystic Ovary Syndrome. *J Clin Endocrinol Metab,* 100 (2015) 794.

9 Ramya G, Dwarampudi LP, Vijayanandhan V, Shanmugam R, Dharshini SP & Raj KR, Polycystic ovarian syndrome (PCOS): Approach to traditional systems, natural and bio-chemical compounds for its management. *Indian J Biochem Biophys*, 59 (2022) 521.

10 Zhou Z, Chen B, Chen S, Lin M, Chen Y, Jin S, Chen W & Zhang Y, Affiliations expand, Applications of network pharmacology in traditional Chinese medicine research. *Evid Based Complement Alternat Med*, (2020) 9.

11 Ammar O, *In silico* pharmacodynamics, toxicity profile and biological activities of the Saharanmedicinal plant *Limoniastrum feei. Braz J Pharm Sci*, (2017) 53.

12 Zhen-Dong L, Liu Y, Li L, Xue B, Wang W & Xiu-Feng Y, Potential Target Screening and Molecular Docking of Newly Isolated Compound from Inonotus Obliquus. *Res Sq*, (2021) doi.org/10.21203/rs.3.rs-250772/v1.

13 Szklarczky & Morris JH, The STRING Database: quality-controlled protein-protein association networks made broadly accessible. *Nucleic Acids Res*, 42 (2017) 362.

14 Dennis Jr, Brad T Sherman, Douglas A Hosack, Jun Yang, Wei Gao, Lane HC & Lempicki RA, DAVID: Database for annotation, visualization, and integrated discovery. *Genome Biol*, 4 (2003) P3.

15 Mishra S, Aeri V, Gaur PK & Jachak SM, Phytochemical, therapeutic, and ethnopharmacological overview for a traditionally important herb: *Boerhavia diffusa* Linn. *Biomed Res Int*, 2014 (2014) 808302.

16 Murthy H N, Bhat M A & Dalawai D, Bioactive Compounds of Bael (*Aegle marmelos* (L.) Correa). In: Murthy, H., Bapat, V. (eds) Bioactive Compounds in Underutilized Fruits and Nuts. *Reference Series in Phytochemistry*. *Springer Cham,* (2019) 1.

17 Ingle KP, Al-Khayri JM, Chakraborty P, Narkhede GW & Suprasanna P, Bioactive Compounds of Horse Gram (*Macrotyloma uniflorum* Lam. [Verdc.]). *Reference Series in Phytochemistry*, (2021).

18 Ribeiro, PR, de Castro RD & Fernandez, LG, Chemical constituents of the oilseed crop *Ricinus communis* and their

pharmacological activities: A review. *Ind Crops Prod,* 91 (2016) 358.

19 Gangaram S, Naidoo Y, Dewir YH & El-Hendawy S, Phytochemicals and biological activities of Barleria (Acanthaceae). *Plants*, 11 (2022) 82.

20 Liu Y, Liu J & Zhang Y, Research Progress on Chemical Constituents of *Zingiber officinale* Roscoe. *Biomed Res Int,* (2019) 5370823.

21 Rambhau SS, Ravindra DP, Prabhakharrao PP & Chandrakant SV, Phytoconstituents and pharmacological activity of Agnimanta - A Review. *J Ayurvedic Herb Med,* 5 (2014) 1615.

22 Gajurel PR, Kashung S, Nopi S, Panmei R & Singh B, Can the Ayurvedic pippali plant (*Piper longum L.)* be a good option for livelihood and socio-economic development for Indian farmers? *Curr Sci*, 120 (2021) 25.

23 Amalraj A & Gopi S, Biological activities and medicinal properties of Asafoetida: A review. *J Tradit Complement Med*, 7 (2016) 347.

24 Wang Z, Zhai D, Zhang D, Bai L, Yao R, Yu J, Cheng W & Yu C, Quercetin decreases insulin resistance in a polycystic ovary syndrome rat model by improving inflammatory microenvironment. *Reprod Sci*, 24 (2017) 682.

25 Jahan S, Abid A, Khalid S, Afsar T, Shaheen G, Almajwal A & Razak S, Therapeutic potentials of Quercetin in management of polycystic ovarian syndrome using Letrozole induced rat model: a histological and a biochemical study. *J Ovarian Res,* 11 (2018) 26.2.

26 Malsawmawngliana, Zohmachhuana A, Vebeiryureilai M, Thangjam NM, Lalrinzuali K, Kumar NS & Kumar A, Antioxidant efficacy and cytotoxicity of ethanolic extract of *Clerodendrum infortunatum* against different cell lines. *Indian J Biochem Biophys,* 58 (2021) 572.

27 Hong G, Wu H, Ma ST & Su Z, Catechins from Oolong tea improves uterine defects by inhibiting STAT# signaling in Polycystic Ovary Syndrome mice. *Chin Med*, 15 (2020) 125.

28 Shimada K, KawarabayashiT, Tanaka A, Fukuda D, Nakamura Y, Yoshiyama M & Yoshikawa J, Oolong tea increases plasma adiponectin levels and low-density lipoprotein particle size in patients with coronary artery disease. *Diabetes Res*. *Clin Pract,* 65 (2004) 227.

29 Ramin Nasimi DA, Arezoo Maini J, Arash K & Sanaz P, Potential roles of genistein in polycystic ovary syndrome: A comprehensive systematic review. *Eur J Pharmacol*, 933 (2022) 175275.

30 Nadal-Serrano M, Pons DG, Sastre-Serra J, Blanquer-Rosselló MDM, Roca P & Oliver J, Genistein modulates oxidative stress in breast cancer cell lines according to ERα/ERβ ratio: Effects on mitochondrial functionality, sirtuins, uncoupling protein 2 and antioxidant enzymes. *Int J Biochem Cell Biol*, 45 (2013) 2045.

31 Rupavathi CH, Bakshi V, Jagruthi J, Nelavelli LB & Bhavani R, Protective and therapeutic effect of protocatechuic acid in assessment of letrozole–induced polycystic ovary syndrome in rats. *Asian Pac J Reprod*, 9 (2020) 230.

32 Huang Y & Zhang X, Luteolin alleviates polycystic ovary syndrome in rats by resolving insulin resistance and oxidative stress. *Am J Physiol Endocrinol Metab*, 320 (2021) E1085.

33 Cooper RL & Linnoila M, Effects of centrally and systemically administered L-tyrosine and L-leucine on ovarian function in the old rat. *Gerontology*, 26 (1980) 270.

34 Villavicencio A, Goyeneche A, Telleria C, Bacallao K, Gabler F, Fuentes A & Vega M, Involvement of Akt, Ras and cellcycle regulators in the potential development of endometrial hyperplasia in women with polycystic ovarian syndrome. *Gynecol Oncol*, 115 (2009)102.

35 Wu Y, Pengfen L, Zhang D & Sun Y, Metformin and pioglitazone combination therapy ameliorate polycystic ovary syndrome through AMPK/PI3K/JNK pathway. *Exp Ther Med,* 15 (2018) 2120.

36 Giovanni Artini P, Monteleone P, Toldin MRP, Matteucci C, Ruggiero M, Cela V & Genazzani AR, Growth factors and folliculogenesis in polycystic ovary patients. *Expert Rev Endocrinol Metab*, 2 (2007) 215.

37 Xue-Ying G, Liu Y, Lv Y, Huang T, Lu G, Hong-Bin L & Shi-Gang Z, Role of Androgen Receptor for Reconsidering the "True" Polycystic Ovarian Morphology in PCOS. *Sci Rep*, 10 (2020) 8993.

38 Chu-Chun H, Nei-Jou C, Chen-Wei L, Chia-Eng W, Mei-Chi H, Hung-Chih K & Hong-Nerng H, Hyperactive CREB Signaling pathway involved in the Pathogenisis of Polycystic Ovary Syndrome revealed by patient-specific induced pluripotent stem cell modelling. *Fertil Steril*, 112 (2019).

39 Krishna MB, Josepha A, Thomasa PL, Dsilvaa B, Pillai SM & Laloraya M, Impaired Arginine Metabolism Coupled to a Defective Redox Conduit Contributes to Low Plasma Nitric Oxide in Polycystic Ovary Syndrome. *Cell Physiol Biochem,* 43 (2017) 1880.

40 Telleria CM, Zhong L, Deb S, Srivastava RK, Park KS, Sugino N, Park-Sarge OK & Gibori G, Differential expression of the estrogen receptors a and b in the rat corpus luteum of pregnancy: regulation by prolactin and placental lactogens. *Endocrinology,* 139 (1998) 2432.

41 Aktaş HŞ, Ozel Y, Ahmad S, Pençe HH, Sayir N, Sapmaz T, Özçelik F & Elçioğlu HK, Influence of walnuts on hepatic ischemic-reperfusion injury in streptozocin induced diabetic rats. *Indian J Biochem Biophys,* 58 (2021) 13.

42 Erusalimsky JD, The use of the soluble receptor for advanced glycation-end products (sRAGE) as a potential biomarker of disease risk and adverse outcomes. *Redox Biol*, 42(2021) 101958.

43 Tatone C, Emidio GD, Placidi M, Rossi G, Ruggieri S, Taccaliti C, D'Alfonso A, Fernanda Amicarelli F & Guido M, AGEs-related dysfunctions on PCOS: evidence from animal and clinical research. *J Endocrinol*, 251 (2021) R1.

44 Fischer B, Kunzel W, Kleinstein J & Gips H, Oxygen tension in follicular fluid falls with follicle maturation. *Eur J Obstet Gynecol Reprod Biol,* 43 (1992) 39.

45 Nishimura R & Okuda K, Hypoxia is important for establishing vascularization during corpus luteum formation in cattle. *J Reprod Dev*, 56 (2010)110.

46 Wang Z, Zhang Z, Wu Y, Chen L, Luo Q, Zhang J, Chen J, Luo Z, Huang X & Cheng Y, Effects of echinomycin on endothelin-2 expression and ovulation in immature rats primed with gonadotropins. *Exp Mol Med*,44 (2012) 615.

47 Xu XL, Deng SL, Lian ZX & Yu K, Estrogen receptors in Polycystic ovary syndrome. *Cells,* 10 (2021) 459.