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Gymnema sylvestre as a potential therapeutic agent for PCOS: insights from mRNA differential gene expression and molecular docking analysis

Divya Vora[1], Hardi Kapadia[1], Susha Dinesh[2], Sameer Sharma[2] and Dinesh Sosalagere Manjegowda[1*]

Abstract

Background In spite of the increased prevalence of Polycystic Ovary Syndrome in bilateral ovaries (PCOS) in females globally (~ 20%), the rate of decisive treatment is limited due to late and invasive diagnostics and the unavailability of stand-alone drugs against phenotypic symptoms associated with PCOS. This study aims at unravelling molecular mechanisms allied with this disorder and identifying genes and pathways that are altered causing PCOS as a prerequisite need. Furthermore, this study intends at assessing the therapeutic potentials of *Gymnema sylvestre* phytocompounds mainly triterpene saponins against targeted proteins and helps in seeking exemplar drug candidates. The study is designed in 2 major parts—firstly identifying key proteins involved in pathophysiology by tracing back the deregulated genes in mRNA expression profiles of PCOS Patients obtained from GEO datasets database further compared and analysed by GEO2R Analyzer and significantly deregulated genes were subjected to PPI network, KEGG and GO analysis using STRING and ShinyGO tools. The second half of the study involved molecular docking its visualisation and in silico pharmacological analysis of imminent phytocompounds revealing plausible drug candidates.

Results In the study, most implied deregulated genes were MRP gene family, ICT1, NDUFA12, VDAC3, APOO, TOMM5, COX6C, COX7B, EDN1 and EDN3 genes whose functional enrichment suggests flawed metabolism of proteins and damaged mitochondrial translation. With high binding affinity, less toxicity at higher dose level Lupeol, Beta-Amyrin, Beta-Elemene, Stigmasterol, Gymnestrogenin and Dammarane proves to be efficient drug candidates.

Conclusion The findings of this study imply better understanding of the molecular mechanism of the disorder and encourage further clinical studies pertaining to medicinal natural phytocompounds of *Gymnema sylvestre*.

Keywords PCOS, *Gymnema sylvestre*, mRNA differential gene expression, MRP gene family, Pharmacological analysis, Lupeol

Background

In current times, the most burdening heterogenous endocrine disorder is Polycystic Ovarian syndrome in bilateral ovaries (PCOS) affecting 6–20% women of reproductive age globally [1]. Ovulatory dysfunction along with hyperandrogenism and chronic anovulation, which inhibits the development of follicles, and formation of cysts in both ovaries detected by ultrasound are the clinical features of PCOS [2] paired with, menstrual irregularities, insulin resistance obesity, infertility, hyperandrogenism (androgen excess causing alopecia, hirsutism, and acne). Occurrence of elevated plasma insulin levels to recompense insulin resistance is prevalent in 70–80% of obese women whereas 30–40% in lean women having PCOS. Recently revealed mechanism associated with hyperandrogenism revealed

*Correspondence:
Dinesh Sosalagere Manjegowda
dineshmgowda1@gmail.com
[1] Department of Human Genetics, School of Basic and Applied Sciences, Dayananda Sagar University, Bengaluru 560078, Karnataka, India
[2] Department of Bioinformatics, Bionome, Bengaluru 560043, Karnataka, India

synthesis of androgens in the theca cells of ovaries is directly triggered by insulin leading to production of sex hormone-binding globulin (SHBG) in the liver, thereby rising concentration of free testosterone in the circulation that worsens the syndromic effects caused to the body [3, 4]. Female health issues predominantly surround around infertility for instance Endometriosis and PCOS, and research on them has been insufficient which brings in concerns regarding delays in diagnosis and indecisive treatment options.

Keeping in mind the achievements on therapeutic effects made by omics-based polypharmacology in modulating molecular network of varied complex disorders highlights the upcoming importance given to traditional herbal medicines for developing novel drugs to treat complex multi-aetiology syndromes. One such indigenous perennial medicinal wood climber *Gymnema sylvestre* belonging to Asclepiadaceae family is an acclaimed antidiabetic herb by many ancient Indian medical practices such as Siddha, Ayurveda, Unani, and also has reliable reports in modern medicine. According to Sushruta Samhita, one of the oldest Sanskrit text (sixth century BCE) [5] holds immense value to medical domain due to numerous chapters describing surgical instruments and procedures on which modern science of surgery is build, *G. sylvestre* was given a name gur-mar, which means sugar killer and aids in curing *Madhumeha,* an ancient Sanskrit term, referring to hyperglycaemia and diabetes mellitus and other urinary ailments [6]. The literature so far suggests that *G. sylvestre* holds a treasure of benefits and acts as anthelmintic, liver restorative, anti-inflammatory, emetic, laxative, antipyretic, gastrointestinal, diuretics, thermogenic, cardiotonic, expectorant, stimulants and even is considered to be "uterus tonic". All these therapeutic effects attributes to secondary metabolites predominantly triterpene saponins, acidic glycosides, gymnemic acids, and anthraquinones [7]. In a preclinical study, the effect of ethanolic leaves extract of *G. sylvestre* on the menstrual irregularity was reported to achieve normal regularity in cycles [8]. Latest study designed to evaluate the therapeutic potential of *G. sylvestre* leaf extract against PCOS rat models, with 28 days of continuous ingestion recorded reduced blood glucose level, improved hormonal irregularity, reverted abnormal cysts and atretic follicles and presented evidences of mature follicles with corpus luteum that indicated regular oestrous cycle and prevented ovarian damage [9]. Considering all these recent studies *G. sylvestre* might be considered as an alternative remedy to treat reproductive and metabolic complications in PCOS women and further understanding of this effective therapeutic is determined in this in silico study.

Methods

Major pre-requisite for Insilco-pharmacology is to rule out what are the key target proteins involved in the aetiology of PCOS. The conventional goal of RNA-Seq and its data analysis is to find differentially expressed genes (DEGs) which are resulted from varied expression of genes under altered cellular state, and that transcriptomic data are registered in the form of datasets that can be retrieved and studied for investigating the potential molecular targets involved and can help with novel drug discovery or drug repurposing.

Screening and selection of mRNA datasets

Mining for mRNA datasets was done through the Gene Expression Omnibus (GEO) repository, [10] meant for storing curated expression profiles of many in vivo and clinical trial studies aiming varied disorders across the globe. Keywords "PCOS" and "mRNA" were searched as query which gave a result of 31 datasets out of which 27 belonged to humans. The selection criteria are as follows: (i) datasets should be obtained through high-throughput sequencing, (ii) datasets must be recorded recently from year 2020 onwards, (iii) must have minimum of 3 control and 3 patient samples involved and (iv) sources of sample should be from various tissue to have an overall representation of pathophysiology involved in PCOS, and 3 datasets with GSE ID, GSE155489, GSE156067, and GSE226146 were selected for further investigation, details mentioned in (Table 1).

GEO2R analysis and identification of differentially expressed genes (DEGs)

To identify DEGs across PCOS patients GEO2R analyser, [11] an interactional web tool was used that helped to visualize and assess mRNA data comparing healthy control v/s patients and gave top expressed deregulated genes table in an order by p values and a collection of graphic plots to ease the assessment of significantly deregulated genes.

Classification of common significantly deregulated genes

After manual sorting of all top expressed deregulated genes, they were compared between all 3 datasets by constructing a Venn diagram using tool [12] that helped to conclude with common significant genes that might be involved in pathophysiology of all different patients having different tissue and status of disorder.

Table 1 Description of mRNA datasets retrieved from GEO database

GSE ID	Sequencing platform	Source of sample	No. of samples	No. of control	No. of test (PCOS patients)	Description
GSE155489	HiSeq X Ten (*Homo sapiens*)	Oocytes and cumulus granulosa cells (GCs)	20	10	10	Transcriptome analysis to unravel molecular features of PCOS (2020) (PMID: 34552933)
GSE156067	Illumina HiSeq 4000 (*Homo sapiens*)	Newly-Formed Adipocytes derived from abdominal adipose stem cell (ASC)	18	9	9	Studied abnormal adipogenic gene transcription using RNA-sequencing to examine differential transcription patterns in PCOS vs controls and concluded predisposition to adipose dysfunction due to possible altered expression of genes in PCOS (2020) (PMID: 33228780)
GSE226146	Illumina HiSeq 2500	Endometrium tissue of IVF-treatment undergoing infertile females	6	3	3	Expression Profile Analysis of LncRNAs and mRNAs in endometrium of women with PCOS undergoing in vitro fertilization-embryo transfer (IVF-ET) (2023) (PMID: NA)

Establishing PPI network of key genes and functional enrichment studies to identify potentially involved pathways

All the common significantly expressed and top 20 deregulated genes among all 3 datasets were then considered for establishing protein–protein interaction (PPI) network using String database, [13] which evaluated the interactions and gave direct (physical) as well as indirect(functional) associations of proteins in disease causing pathways. The results from STRING also enabled the finding of major Kyoto encyclopaedia for genes and genomes (KEGG) pathways and gene ontology (GO) linked to PCOS but was not able to give functional enrichment related to GO molecular function. To overcome this and for aiding the visualization between all associated pathways tool ShinyGO 0.77, [14] was used which is better at network summarizing and clustering that gave insights about the disease associations based on genes involved [15].

Identification, retrieval, purification, and structural validation of target proteins

Analysing the PPI network, 4 important protein targets were identified namely Mitochondrial 39S ribosomal protein L21 (MRPL21), Cytochrome C Oxidase Subunit6C (COX6C), NADH:ubiquinone oxidoreductase subunit A12 (NDUFA12) and mitochondrial peptidyl-tRNA hydrolase (ICT1).The experimentally-determined 3D structures of these proteins by electron microscopy were downloaded from Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB), [16] having PDB ID 6ZSA, 5Z62, 5XTD and 3J7Y and resolution of 4.00A, 3.60A, 3.70 A and 3.40 A respectively in.pdb format. The purification of protein including removal of water molecules, heteroatoms, bounded extra ligands and other protein chains and addition of polar H bonds to stabilise protein chemistry were done using Biovia Discovery Studio [17]. Validating structures of purified proteins before performing molecular docking is essential as it gives valuable insights about protein folding and stability, topology and irregularities about protein structure which was assessed by generating Ramachandran plot using PDB sum generate tool, [18] wherein the Ramachandran favoured regions for all the proteins fell under accepted criteria (~ 90%) and with the help of ProSA tool, [19] the Z score of purified proteins were from − 0.76 to − 10.57 studying them the purified structures were considered further for docking against potential phytocompounds.

Retrieval of ligands

Based on ancient literature and recent animal model studies *G. sylvestre* is credited as effective therapeutics against PCOS especially the secondary metabolites obtained from the leaf extract. About 88 unique phytocompounds found in leaves of *G. sylvestre* avoiding repeated entries were retrieved from Indian Medicinal Plants, Phytochemistry and Therapeutics (IMPPAT) database, [20] which is exceptional collection of

phytochemicals and their therapeutic uses of the Indian medicinal plants.

Primary screening of phytocompounds

Out of 88 unique phytocompounds primary screening was done based on criteria such as non-permeable to blood brain barrier and accepted under LIPINSKI rule of drug-likeness by using analysis tool SwissADME, [21] Screening was necessary as the chemicals that violate LIPINSKI rule of 5 are not ideal drug candidates and can cause lethal effects to body. Results were downloaded from SwissADME tool, later manually curated by screening criteria, as a result of which 34 ligands were selected for further docking. The Canonical SMILES (Simplified Molecular Input Line Entry System), identifiers (PubChem CID) and 2D structures for all 34 ligands were downloaded, with the help of PubChem chemical database, in SDF (Structure Data File) format as it is accepted chemical structure format where the coordinates of bonds and atoms along with hybridisation is encoded in plain text which is further decoded by docking software. [22]

Molecular docking

For structure-based drug designing of phytocompounds against potential biomarkers of PCOS identified, the most crucial step was molecular docking performed through virtual screening and docking software PyRx (Version 0.8) being conventionally used and one of the open source Computer-Aided Drug Design software, employed to screen chemical molecules library against promising drug targets. PyRx serves better for structure-based drug design owing to its chemical spreadsheet-like functionality, [23] and with its AutoDock Vina plugin purified protein structures were labelled as macromolecules whereas 2D SDF files of 34 ligands were set to pdbqt format after minimization obtained by applied universal force field. A blind docking was performed against all 4 target proteins and the best docked complexes were studied in depth by means of visualisation.

Visualization

The key aspect of computational modelling is understanding mechanisms of interaction of phytocompounds to target protein fulfilled by visualisation and in this study was done by Biovia Discovery Studio, representing top ligands for each target protein [24].

Pharmacological analysis of top phytocompounds showing drug-likeness, including ADMET properties

Unwanted pharmacokinetics and toxicity of probable compound becomes inevitable reasons for drug development failure and hence absorption, distribution, metabolism, excretion, and toxicity (ADMET) along with medicinal chemistry of best docked phytocompounds were evaluated using ADMETlab 2.0, [25] and conclusive decisions on therapeutic benefits were suggested.

Results

GEO2R analysis and identification of differentially expressed genes (DEGs)

The obtained results were generated by comparing designated control samples with test samples. These results were reported in the form of significantly expressed genes, represented through a volcano plot illustrating the statistical significance (− 10log P) against the magnitude of change (log 2), as depicted in Fig. 1. The number of genes that were deregulated was manually sorted and documented in Table 2.

Classification of common significantly deregulated genes

Considering all the significantly expressed genes among 3 datasets the common genes were classified by Venn diagram as shown in Fig. 2 and the same along with top 20

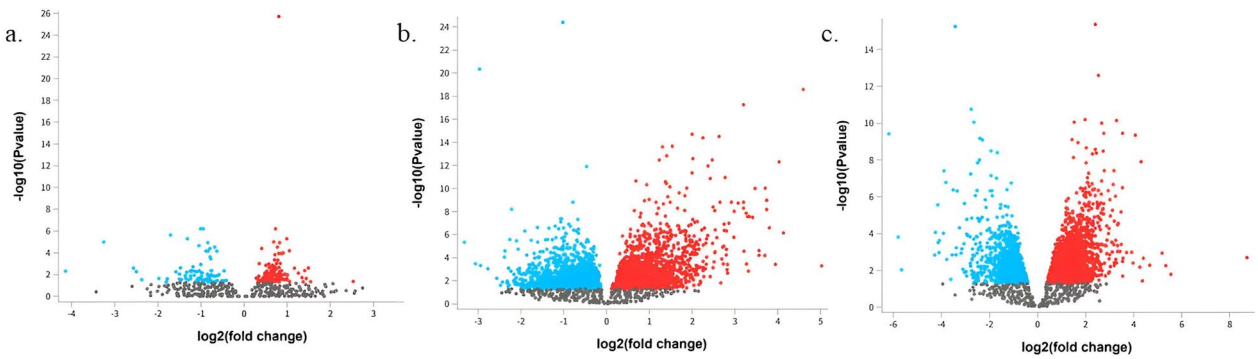

Fig. 1 Volcano plot analysis where adjusted p value cut-off is 0.05 (red = upregulated, blue = downregulated) and the datasets represented are **a** GSE155489 **b** GSE156067 **c** GSE226146

Table 2 GEOR2 Analysis results

Dataset GSE ID	Total No. of expressed genes	No. of significantly expressed genes	No. of upregulated genes	No. of downregulated genes
GSE155489	13738	225	13	212
GSE156067	15988	4046	660	3384
GSE226146	22439	5022	1584	3436

Significantly expressed genes—adjusted *p*-value(< 0.05); criteria to sort dysregulated genes upregulated (LogFC > 1); downregulated (LogFC < 1)

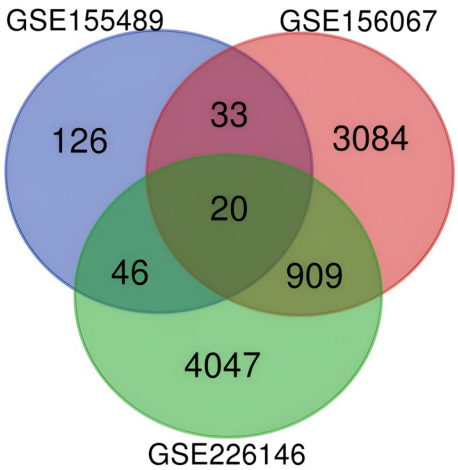

Fig. 2 Venn diagram resulted from 3 datasets gave 20 common genes between dataset

expressed deregulated genes of each dataset were taken into account for construction of PPI network.

Establishing PPI network of key genes and functional enrichment studies to identify potentially involved pathways

STRING database

At high confidence level, the interaction between nodes were clear and key players were identified as illustrated in Fig. 3, were MRPS28, MRPS33, MRPL20, MRPL21, 1CT1, NDUFA8, NDUFA12, VDAC3, APOO, TOMM5, COX6C, COX7B, EDN1 and EDN3 and helped to understand the molecular mechanisms in PCOS. The role of MRP family of genes was evidently seen in the network obtained which majorly codes for mitochondrial ribosomal proteins and here suggests that mitochondrial translation is deregulated. Interestingly the involvement of ICT1 with maximum interaction suggested errors in protein biosynthesis and inappropriate assembly of mitochondrial ribosome intracellular signaling and apoptosis owning to it being crucial termination factor in mitochondria [26]. On the other end, involvement of COX6C as major interactors implicates oxidative phosphorylation deviations and many recent researches have reported aberrant levels in diabetes, uterine leiomyoma, familial hypercholesteremia and other chronic disorders such as cancers and kidney failure [27]. Indications for association to mitochondrial and neurodegenerative disorders are supposed as NADH:ubiquinone oxidoreductase subunit A12 is part of respiratory chain complex, and dysfunction in them leads to distinctive range of disorders specially neuromuscular [28]. The detailed functional enrichment is mentioned in (Table 3) wherein KEGG pathway implies major neurochemical disorders which may be the reason for stress associated symptoms of PCOS.

SHINY GO

The major pathways ruled out in this study were oxidative phosphorylation, non-alcoholic fatty liver disease, diabetic cardiomyopathy which are in alignment to the literature about known pathophysiology of PCOS alongside various neurodegenerative disease and metabolic pathways as depicted from Fig. 4. Biological processes, cellular locations and molecular functions affected by PCOS are directed by investigating GO Network obtained where two pathways that share genes are connected by nodes. Interpretation of these networks illustrated in Fig. 5a–c was done as per standards stated, such as larger gene sets are represented by bigger nodes and darker the node more significantly gene enriched whereas overlapped genes are implied by thicker nodes [15]. The diseases associated with these genes, determined through their phenotypic correlations, were also identified in Fig. 5d. Notably, there is a stronger emphasis on glaucoma, metabolic acidosis, and hypogonadism.

Molecular docking

Macromolecules are the target proteins which are generally considered rigid while the ligands are flexible assumed by virtual screening software PyRx, where efficacy of phytocompounds were estimated by binding affinity scores, more negative the value better binding. The best docked phytocompounds out of 34 that were subjected to blind docking were documented with their binding affinity against target proteins as in (Table 4). Highest affinity was found to be of Lupeol for all the

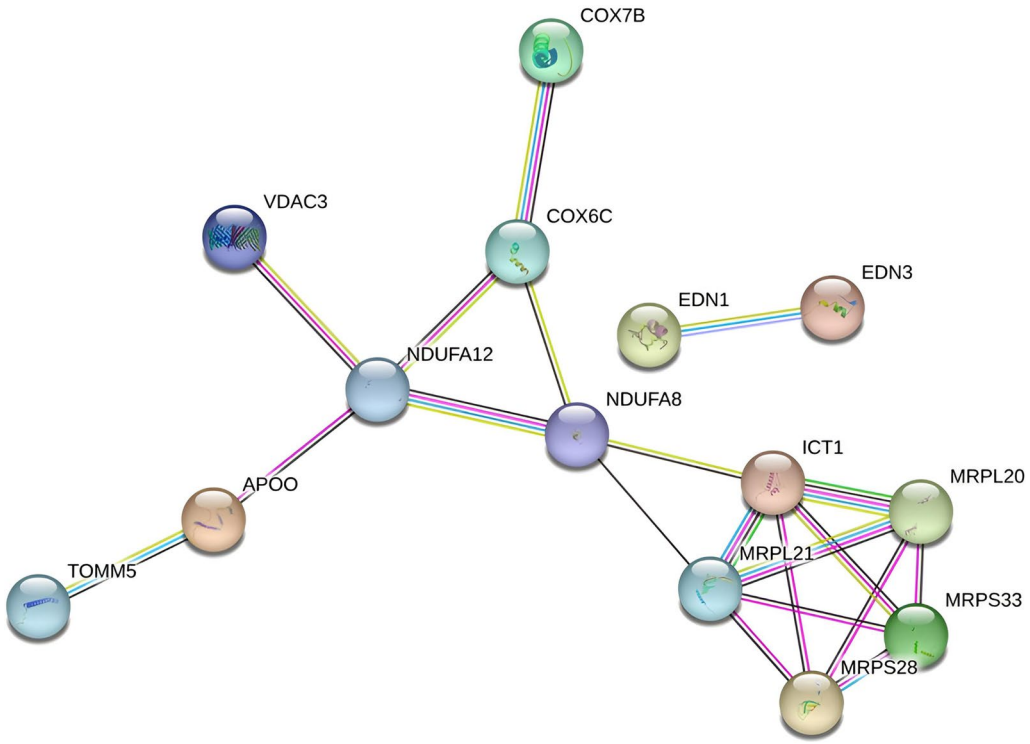

Fig. 3 PPI network constructed using STRING database at mean confidence level of 0.7 and gave total 65 nodes; 20 edges with an average node degree of 0.615 and PPI enrichment p-value of 0.000316

Table 3 Functional enrichment of network obtained from STRING database

	GO-term	Description	Count in network	Strength	False discovery rate
Biological process (Gene Ontology)	GO:0032543	Mitochondrial translation	6 of 114	1.2	0.0364
Cellular Component (Gene Ontology)	GO:0005739	Mitochondrion	16 of 1611	0.48	0.0102
	GO:0005743	Mitochondrial inner membrane	12 of 480	0.88	5.51e-05
	GO:0005761	Mitochondrial ribosome	5 of 87	1.24	0.0038
	GO:0031966	Mitochondrial membrane	14 of 722	0.77	5.62e-05
	GO:0098798	Mitochondrial protein complex	10 of 262	1.06	3.44e-05
	Pathway	**Description**	**Count in network**	**Strength**	**False discovery rate**
KEGG Pathways	hsa05010	Alzheimer disease	7 of 355	0.77	0.0152
	hsa05012	Parkinson disease	7 of 240	0.94	0.0058
	hsa05014	Amyotrophic lateral sclerosis	7 of 352	0.78	0.0152
	hsa05016	Huntington disease	7 of 298	0.85	0.0074
	hsa05020	Prion disease	7 of 265	0.9	0.0058

potential targets which makes it most suitable therapeutic agent, followed by Beta-Elemene, Stigmasterol and Gymnestrogenin which are proven to manage insulin resistance in patients of PCOS.

Visualization

The interactions of lupeol with amino acids of target proteins were in depth studied by visualisation, and the details can be assessed through Fig. 6, where lupeol

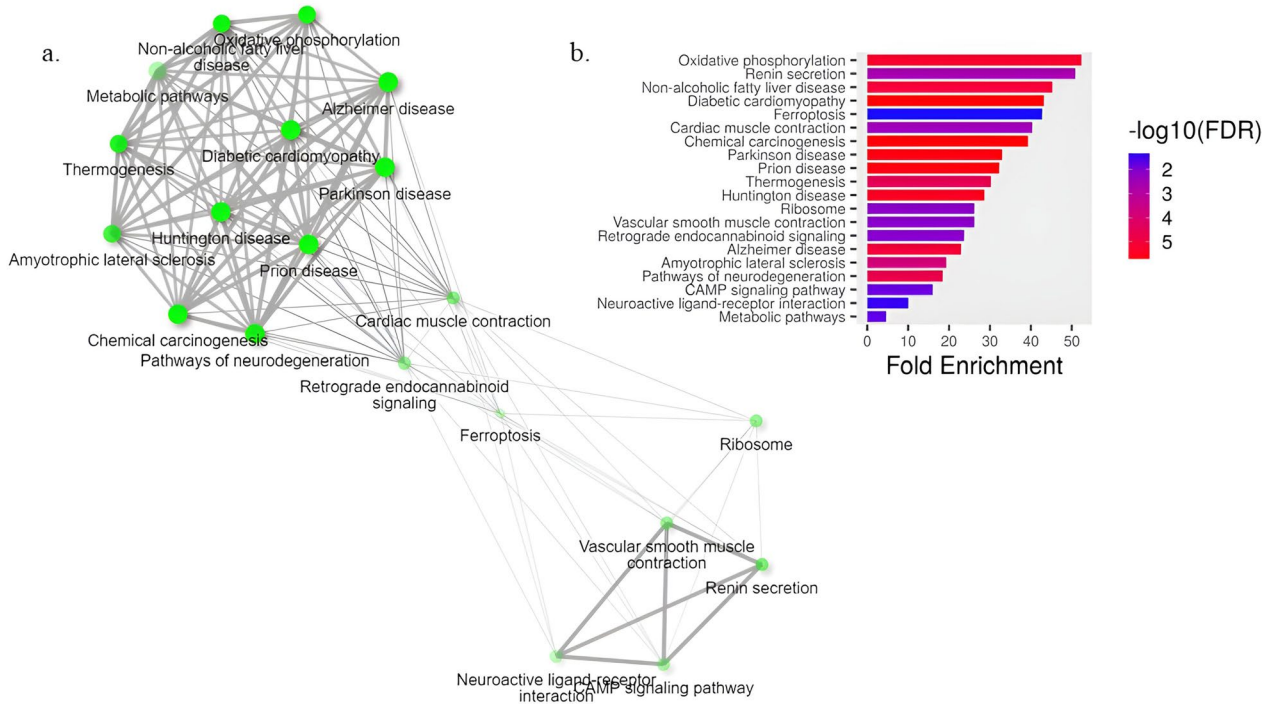

Fig. 4 KEGG pathway generated with the help of Shiny GO webserver where **a** indicates network of pathways suggested at edge cut-off 0.2, while **b** is a bar chart highlighting statistically significant pathways alongside its fold enrichment levels

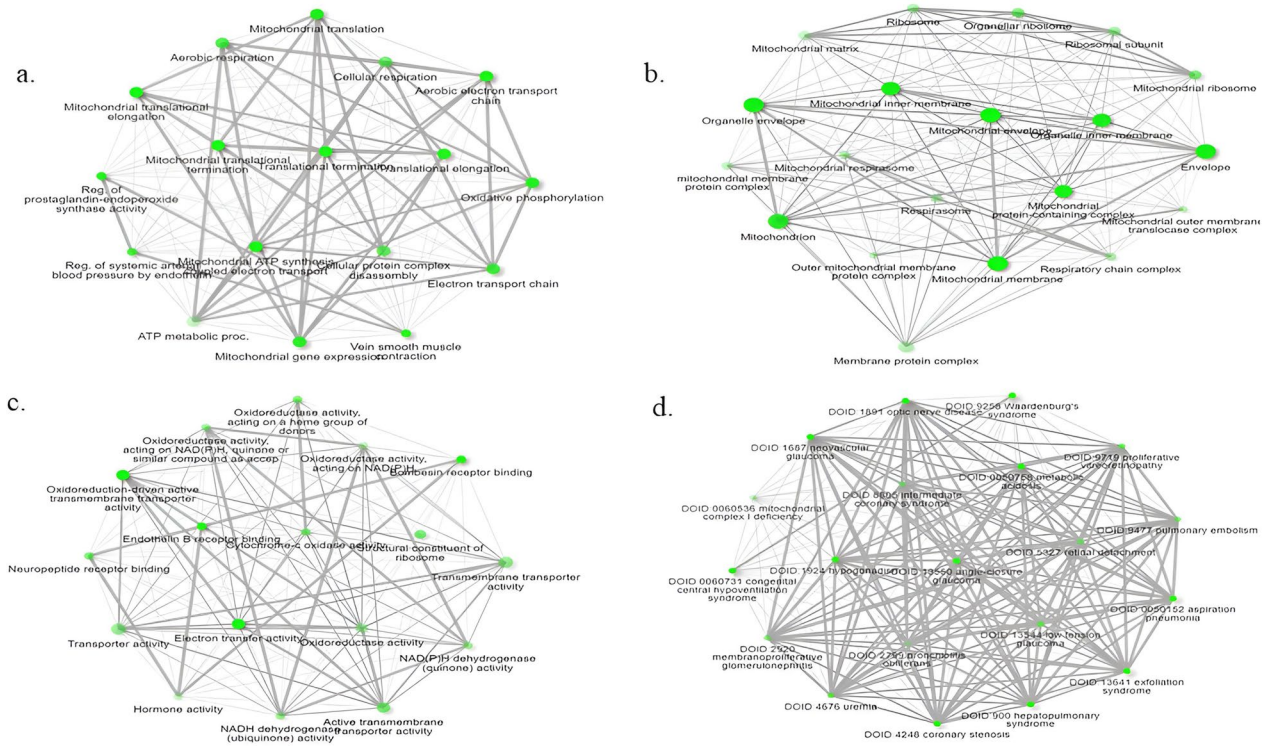

Fig. 5 Functional enrichment networks obtained from Shiny GO where **a** GO biological processes, **b** GO cellular component, **c** GO molecular functions and **d** Disease alliances are represented at edge cut-off 0.1

Table 4 List of ligands that give best binding score with the target proteins in terms of stronger binding affinity calculated via blind docking

Serial number	Ligand	PubChem CID	Binding affinity of target proteins			
			6ZSA	5Z62	5XTD	3J7Y
1	Lupeol	259,846	−10.3	−11.1	−14.6	−10.9
2	Beta-Amyrin	73,145	−7.4	−6.9	−8.8	−6.7
3	Beta-Elemene	6,918,391	−6.6	−7.4	−9.2	−6.5
4	Stigmasterol	5,280,794	−7.0	−6.5	−7.8	−6.2
5	Gymnestrogenin	15,560,302	−6.4	−6.6	−8.1	−6.1
6	Dammarane	9,548,714	−6.6	−6.2	−8.8	−5.7

6ZSA-Mitochondrial 39S ribosomal protein L21(MRPL21); 5Z62-Cytochrome C Oxidase Subunit6C(COX6C); 5XTD-NADH:ubiquinone oxidoreductase subunit A12(c); 3J7Y-mitochondrial peptidyl-tRNA hydrolase (ICT1)

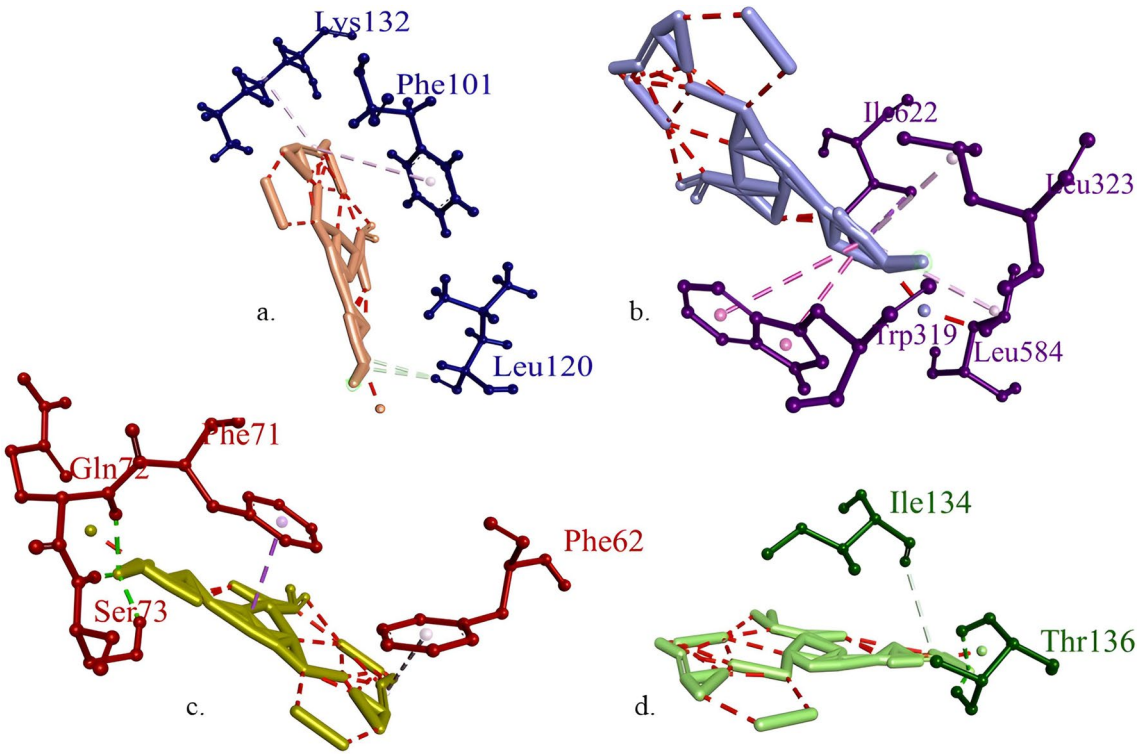

Fig. 6 Visualization of best phytocompound lupeol against target proteins: **a** Mitochondrial 39S ribosomal protein L21, **b** NADH:ubiquinone oxidoreductase subunit A12, **c** Cytochrome C Oxidase Subunit6C and **d** mitochondrial peptidyl-tRNA hydrolase

occupies space in binding pockets of protein targets and the interacting amino acids are also mentioned.

Pharmacological analysis of top phytocompounds showing drug-likeness, including ADMET properties

The bioactive phytocompounds derived from *G. sylvestre* were showing promising therapeutic role in the literatures till date and were also translated in this study. The focussed ADMET screening performed revealing binding absorption and distribution across systemic circulation is summarized in Table 5, where the findings related to plasma protein binding indicate that Beta-Elemene and Gymnestrogenin exhibit less binding to plasma proteins, suggesting better tissue penetration capability but faster excretion rates. On the other hand, Table 6 presents the medicinal chemistry data, clearance rates, and half-life values of the compounds. Lupeol displayed a longer clearance rate and half-life, making it a strong candidate for drug delivery systems. The industrial feasibility of drug

Table 5 Adsorption and distribution properties of best docked phytocompounds when considered to have drug-likeness

Ligand	PubChem CID	Pgp-sub	HIA	Caco-2	BBB	PPB	VDs
Lupeol	259846	0	0.008	−5.02	0.792	98.80%	1.731
beta-Amyrin	73145	0	0.03	−5.034	0.749	99.78%	1.82
beta-Elemene	6918391	0	0.003	−4.485	0.216	85.36%	2.128
Stigmasterol	5280794	0.001	0.005	−4.668	0.691	98.67%	2.408
Gymnestrogenin	15560302	0.006	0.268	−4.886	0.877	87.16%	1.077
Dammarane	9548714	0	0.008	−5.041	0.515	98.46%	2.491

Pgp-sub: the substrate of P-glycoprotein; HIA: Human intestinal absorption; Caco-2: Caco-2 Permeability; BBB: Blood–brain barrier; PPB: Plasma protein binding; VDs: Volume Distribution

Table 6 Medicinal chemistry of best docked phytocompounds having drug-likeness

Ligand	PubChem CID	QED	SA Score	PAINS	Lipinski	CL	T1/2
Lupeol	259846	0.421	4.663	0	Accepted	17.929	0.01
beta-Amyrin	73145	0.387	4.56	0	Accepted	15.308	0.009
beta-Elemene	6918391	0.582	4.188	0	Accepted	6.199	0.063
Stigmasterol	5280794	0.457	4.571	0	Accepted	15.958	0.014
Gymnestrogenin	15560302	0.376	5.237	0	Accepted	8.78	0.06
Dammarane	9548714	0.42	4.366	0	Accepted	10.902	0.007

QED: A measure of drug-likeness based on the concept of desirability; SA Score: Synthetic accessibility score; PAINS: Pan Assay Interference Compounds; Lipinski Rule of 5: Molecular weight less than 500 daltons, nHD < 5, nHA < 10, and lipophilicity < 4.15; CL: Clearance rate; T1/2: Half-life of the small molecules

Table 7 Toxicity analysis of promising phytocompounds

PubChem CID	hERG	DILI	Ames	FDAMMD	CG	IGC50	LC50	NR-AR	NR-ER	NR-PPAR-gamma
259846	0.034	0.018	0.015	0.845	0.009	5.481	6.898	0.002	0.298	0.009
73145	0.004	0.008	0.018	0.715	0.017	5.442	6.786	0.005	0.3	0.094
6918391	0.027	0.013	0.007	0.718	0.146	3.963	4.793	0.016	0.091	0.005
5280794	0.012	0.055	0.029	0.539	0.054	4.978	5.511	0	0.369	0.005
15560302	0.008	0.007	0.027	0.824	0.044	4.54	5.131	0.031	0.119	0.572
9548714	0.078	0.051	0.016	0.554	0.007	5.603	6.69	0.001	0.378	0.006

hERG: The human ether-a-go-go related gene; DILI: Drug-induced liver injury; AMES: The Ames test for mutagenicity; FDAMMD: maximum recommended daily dose; CG: Carcinogenicity; IGC50: Tetrahymena pyriformis 50 per cent growth inhibition concentration; LC50: 96-h fathead minnow LC_{50}; NR-AR: Androgen receptor; NR-AR: Oestrogen receptor; NR-PPAR-gamma: Peroxisome proliferator-activated receptor gamma

manufacturing and processing was evaluated using the Synthetic Accessibility Score (SA Score) in Table 6. The scores ranged from 4 to 5.3 for all six compounds, indicating moderate ease of synthesis with potential alterations to improve their solubility and other biochemical properties. To assess the drug-likeness and safety of the phytocompounds, toxicity studies were conducted, and the results are summarized in Table 7. The recommended daily doses for all six phytocompounds were found to be higher than 500 mg, inferring less threat of ingestive overdosing. Additionally, mutagenicity and drug-induced liver injury were estimated to be very low, supported by the high lethal dose of these compounds suggesting a low risk of toxic effects even at high doses.

Discussion

According to statistical report more prevalence of adult women suffering with menstrual abnormality, infertility and hirsutism registered in Turkey (33%), France (23%), Portugal (18%), Greece (9%), India (6%), etc. owns to varied alleles of genes across specific populations, [29] and their regulations involved in PCOS reflects more on androgen excess in spite of adrenal insufficiency. Based

on recent studies mutations within cytochrome P450 oxidoreductase (POR) gene, CYP9 and CYP11 are believed to be related to phenotypic spectrum of symptoms observed in PCOS like low sex steroid hormone levels, infertility, and amenorrhea [30]. Along with multiple genes the aetiology is still unclear whereas for one third of females with PCOS, from initial complaints to precise diagnosis can take as long as 10 years which comes with ultimate invasive procedures like laparoscopy, hence studies to understand molecular mechanism in depth becomes quintessential. The current study revealed involvement of oxidative stress, impaired mitochondrial translation and majorly impaired metabolism of proteins based on gene expression profiles of PCOS patients reflecting deregulation of MRP family genes, COX, NDUF and EDN genes. To summarise the deregulated pathways, an illustration was generated using Reactome pathway database, [31] which in sync with the literature evidences suggested the role of insulin-growth regulators Fig. 7, explains the predominant insulin resistance in PCOS patients.

Further this interpretation helps to understand why current therapeutic drugs target phenotypic symptoms, which is due to the fact that if insulin resistance, obesity and hyperandrogenism are brought under normal levels, PCOS can be effectively managed. On-going therapies for PCOS such as Letrozole and Clomiphene Citrate for menstrual irregularities while Oral Contraceptive Pills involving Metformin and Gonadotropins are segmented and are prescribed to cure symptoms but fails against multiple pathophysiology alterations involved. Moreover, they have limitations associated such as ovarian gonadotropin stimulation promises elevated ovarian function in PCOS, however possesses threat for multiple births and ovarian hyperstimulation syndrome (OHSS) [32]. In the same manner, Clomiphene citrate is an ovarian stimulant but elevates chances of multiple pregnancies and do not affect the other metabolic and psychosocial indicators of PCOS and hence limits its potential as stand-alone drug. Letrozole hinders the formation of oestrogen from androgen and is most commonly prescribed third-generation contraceptives composed of less oestrogen levels; alongside of Isotretinoin, Flutamide, and Finasteride to treat Hyperandrogenism but long-term cardio-metabolic effects still remain unanswered [33]. In spite of drugs available in most cases, still surgical interventions like Laparoscopic Ovarian Drilling (LOD) are being practiced which transiently alters ovarian function [34]. Hence, in

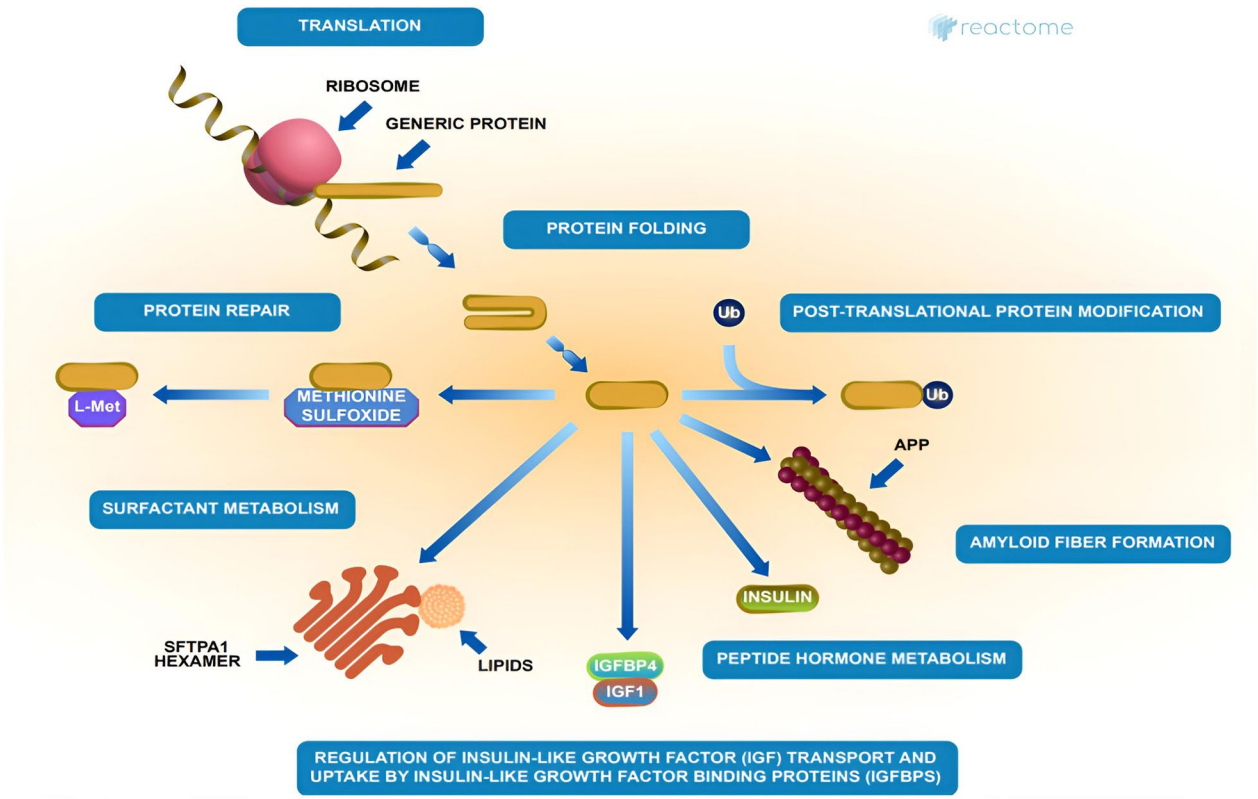

Fig. 7 Summarized pathways whose disbalanced regulations are co-related to PCOS patients focussing on metabolic errors of protein synthesis and impaired repair of IGF1 leading to insulin resistance

recent times, focus of scientific community is bended towards alternative medicines such as plant-based drug therapies for ultimate solution for PCOS.

As mentioned prior investigations suggest extract of *G. sylvestre* to be most efficient against hyperglycaemia which also has powerful antioxidant activities [35, 36] having both properties, it is peculiarly advisable to treat PCOS and inputs from this study also aligns. Considering the affinity of phytocompounds towards target proteins few phytocompounds were exceptionally good and researches in current time supports their therapeutic values. For example, Gymnemasaponins (aglycone saponins) exhibit dual mechanisms by elevating blood plasma levels of insulin and lowering free testosterone in blood circulation [6]. Beta-Amyrin and Beta-Elemene possess primarily Hepatoprotective properties along with antigastritic and antihomocystinuric effects. In terms of dosage, higher dose of Stigmasterol is relatively safe and helps to manage steoarthritic, hypercholesterolemic, cytotoxic and hypoglycaemic conditions by its antioxidant and anti-inflammatory effects [37]. This study highlights Lupeol and its potential to become most efficient drug compound against PCOS because of the fact that it is pharmacologically safe, with less threat of ingestive overdosing. Even evidences of treating malaria, rheumatism and urinary tract infections using Lupeol are reported owing to its anti-microbial properties [38]. In a novel in vivo study for reverting endometrial carcinoma by blocking potent target Matrix metalloproteinase-9 (MMP-9) involved in metastasis; using Lupeol as natural anti-cancerous and anti-inflammatory phytocompound with the least side effects the results supported therapeutic values pertaining to Lupeol [39]. Its efficacy in healing array of metabolic disorders was proved in a remarkable study where it acted as α-glucosidase inhibitor [40].

Further investigations with validating protein targets and their binding to natural phytocompounds with comparable efficacy in animal model studies (in vivo) are recommended which later can be translated to clinical trials after satisfactory observations to overcome the limitation of this study that is majorly sample size analysed. On the other hand, the availability of mRNA datasets was also very limited which brings in view that more RNA-Sequencing should be carried out with larger sample size and should be available as datasets so better conclusive studies are carried out in near future.

Conclusion

Investigating gene expression levels of PCOS patients revealed deregulation of MRPS28, MRPS33, MRPL20, MRPL21, 1CT1, NDUFA8, NDUFA12, VDAC3, APOO, TOMM5, COX6C, COX7B, EDN1 and EDN3 genes whose functional enrichment suggests erroneous metabolism of proteins and impaired mitochondrial translation to be causative of phenotypic spectrum of symptoms observed in PCOS. Therapeutic potential value obtained from *G. sylvestre* phytocompounds is high especially for Lupeol, Betaine and Stigmasterol against potential molecular targets (MRPL21, 1CT1, NDUFA12 and COX6C) and hence becomes eligible candidates for drug development.

Abbreviations

PCOS	Polycystic ovarian syndrome
G. sylvestre	*Gymnema sylvestre*
mRNA	Messenger ribonucleic acid
GEO	Gene expression omnibus
PPI	Protein–protein interaction
KEGG	Kyoto encyclopaedia for genes and genomes
GO	Gene ontology
STRING	Search tool for the retrieval of interacting genes/proteins
SHBG	Sex hormone-binding globulin
DEGs	Differentially expressed genes
GCs	Granulosa cells
ASC	Adipose stem cell
IVF	In vitro fertilization
LncRNAs	Long non-coding ribonucleic acids
IVF-ET	In vitro fertilization-embryo transfer
MRPL21	Mitochondrial 39S ribosomal protein L21
COX6C	Cytochrome C Oxidase subunit6C
NDUFA12	NADH: ubiquinone oxidoreductase subunit A12
ICT1	Mitochondrial peptidyl-tRNA hydrolase
RCSB PDB	Research collaboratory for structural bioinformatics protein data bank
PDB	Protein data bank
IMPPAT	Indian medicinal plants, phytochemistry and therapeutics
SMILES	Simplified molecular input line entry system
2D	Two-dimensional
SDF	Structure data file
ADMET	Absorption, distribution, metabolism, excretion, and toxicity
POR	P450 oxidoreductase
OHSS	Ovarian hyperstimulation syndrome
LOD	Laparoscopic ovarian drilling
MMP-9	Matrix metalloproteinase-9

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Author contributions

DSM (DSM) and DV conceptualized the study, DV contributed to literature survey, manuscript writing and data interpretation. (HK), (SS) and (SD) helped with editing along with proofreading. All authors have read and approved the final manuscript.

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Availability of data and materials

The datasets analysed during the current study are available in the GEO database repository NCBI with following Id's: GSE155489 https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE155489. GSE156067 https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE156067. GSE226146 https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE226146.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
The authors declare no conflict of interest.

Competing interests
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