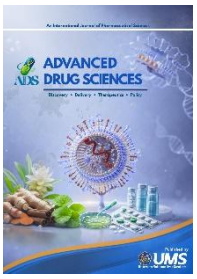
Advanced Drug Sciences

Volume 1 *https://doi.org/to be assigned* *Issue 1*

Journal Homepage: https://journals2.ums.ac.id/ads/index



RESEARCH ARTICLE

Elucidating the Multi-Target Mechanisms of *Camellia sinensis* in Human Fertility Disorders Through Network Pharmacology and Molecular Docking

Md. Mainuddin Hossain[1*] and Jannatul Fardous[1]

[1] Department of Biotechnology and Genetic Engineering, Mawlana Bhashani Science and Technology University, Santosh, Tangail -1902, Bangladesh.

***Corresponding Author:** Email: mainuddinbge@gmail.com; Tel: +880 1568931181; Fax: +880-921-51900.*

Citation: Hossain, M. M. & Fardous, J. (2026). Elucidating the Multi-Target Mechanisms of Camellia sinensis in Human Fertility Disorders Through Network Pharmacology and Molecular Docking. *Adv. Drug Sci., 1*(2026), e000003. DOI: To Be Assigned

ABSTRACT

Background: Human fertility disorders (HFDs) are multifactorial reproductive conditions influenced by genetic, hormonal, inflammatory, and environmental factors. Although *Camellia sinensis* has been widely recognized for its medicinal properties, its molecular mechanisms in fertility regulation remain insufficiently understood. **Objective:** This study aimed to investigate the therapeutic potential and underlying molecular mechanisms of *C. sinensis* against HFDs using an integrated approach combining network pharmacology and molecular docking. **Method:** A total of 123 phytochemicals were retrieved from the IMPPAT database and screened using SwissADME and pkCSM for pharmacokinetic and toxicity evaluation. Targets were predicted using SwissTargetPrediction. Protein–protein interactions network analysis was performed to determine hub proteins, followed by Gene Ontology and KEGG pathway enrichment analyses. Molecular docking was conducted to evaluate the binding affinities of selected compounds with target proteins. **Results:** 6 compounds—Typhasterol, Teasterone, Brassinolide, Castasterone, A1-Barrigenol, and Theasapogenol B—exhibited favorable pharmacokinetic characteristics and non-toxic profiles. 29 overlapping targets were identified between compound and HFDs-related proteins. PPIs analysis revealed IGF1R, EGFR, PIK3CA, mTOR, and ERBB2 as key regulatory targets. GO and KEGG analyses highlighted significant enrichment of the PI3K/Akt, VEGF, HIF-1, EGFR, and JAK–STAT signaling pathways, which are involved in reproductive regulation, gametogenesis, implantation, and endometrial receptivity. Molecular docking demonstrated strong binding affinities of the compounds toward the hub proteins, with docking scores below −7.0 kcal/mol. **Conclusion:** *C. sinensis* may exert therapeutic effects against HFDs through multi-target and multi-pathway mechanisms. These findings provide mechanistic insights into its fertility-regulating potential and support further experimental validation to confirm its therapeutic applicability.

ARTICLE INFORMATION

Keywords:
Camellia sinensis
Human Fertility Disorders
Network pharmacology
Molecular docking
Signaling pathways

Received	22 May 2026
Revision	02 Jun 2026
Accepted	08 Jun 2026
Published	29 Jun 2026

1. INTRODUCTION

Human fertility disorders (HFDs) encompass a broad spectrum of conditions affecting the reproductive system, clinically manifested as impaired gametogenesis, hormonal imbalances, and reduced reproductive capacity [1]. The global prevalence of infertility is increasing, driven by lifestyle factors, environmental exposures, and advancing parental age, posing significant social and economic challenges worldwide [2]. The etiology of HFDs is multifactorial and complex, involving genetic, endocrine, immunological, and environmental components [3,4]. Disruptions in key reproductive signaling pathways and gamete quality are central to the pathophysiology of fertility disorders. For example, aberrations in hormonal regulation or oxidative stress can impair follicular development and sperm function, leading to compromised fertility [5]. Current therapeutic approaches, including hormone replacement, assisted reproductive technologies, and surgical interventions, primarily address symptoms and specific causes but often have limitations such as side effects and variable success rates [6]. In recent years, bioactive compounds derived from traditional medicinal plants have attracted considerable attention owing to their potential therapeutic efficacy, reduced adverse effects, and cost-effectiveness in the management of fertility-related disorders [7,8].

Camellia sinensis occupies an important position in traditional medicinal systems, including Ayurveda and Traditional Chinese Medicine, owing to its broad spectrum of therapeutic properties. Various formulations such as extracts, infusions, and powdered preparations of *C. sinensis* have been traditionally employed in the treatment of diverse health conditions, including infectious diseases, metabolic disorders, inflammatory conditions, and reproductive system-related ailments [9]. This plant exhibits a wide range of pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, and endocrine-modulatory effects, which have been documented in both traditional and modern scientific contexts with relevance to reproductive health [10]. Emerging evidence indicates that bioactive constituents of *C. sinensis* may exert therapeutic effects in human fertility disorders by modulating key reproductive processes, including enhancement of gamete quality, regulation of hormonal homeostasis, and attenuation of oxidative stress and inflammation within reproductive tissues [11]. Although increasing data support the potential role of *C. sinensis* in managing human fertility disorders, the specific molecular targets, signaling pathways, and underlying mechanisms remain insufficiently characterized. Accordingly, the present study aims to elucidate the mechanistic basis of *C. sinensis* in the treatment of HFDs and to identify key signaling pathways associated with reproductive function and fertility regulation.

Traditional "disease–target–drug" paradigms are often insufficient to comprehensively represent the complex, multifactorial interactions between therapeutic agents and disease pathways. In contrast, network pharmacology, an integrative and systems biology–based approach, offers a holistic framework for systematically elucidating drug mechanisms of action [12], identifying novel therapeutic targets [13] and improving understanding of how bioactive compounds influence cellular signaling networks. In parallel, molecular docking is a widely used computational strategy in modern drug discovery that enables the virtual screening and assessment of candidate compounds by predicting their binding interactions with target proteins [14].

In the present study, an integrated network pharmacology and molecular docking approach was applied to explore the potential therapeutic effects and underlying molecular mechanisms of *C. sinensis* in the management of HFDs. This combined computational framework is intended to provide novel mechanistic insights into HFDs pathology and to support the development of more effective and innovative therapeutic strategies.

2. MATERIALS AND METHODS

2.1. Compounds Retrieval and Filtration

Phytochemicals derived from the leaves of *Camellia sinensis* were retrieved from the Indian Medicinal Plants, Phytochemistry and Therapeutics 2.0 (IMPPAT 2.0) (https://cb.imsc.res.in/imppat/) [15] and PubChem (https://pubchem.ncbi.nlm.nih.gov/) [16] databases. IMPPAT is a manually curated resource compiled from traditional medicinal texts and extensive scientific literature, providing one of the most comprehensive repositories of Indian medicinal plant–derived phytochemicals, while PubChem is a large-scale chemical database maintained by the NIH that includes diverse small and complex molecules.

The pharmacokinetic and drug-likeness properties of the retrieved compounds were evaluated using SwissADME (http://www.swissadme.ch/) [17] and pKCSM (https://biosig.lab.uq.edu.au/pkcsm/prediction) [18],

focusing on ADMET (absorption, distribution, metabolism, excretion, and toxicity) profiles, bioavailability, and drug-likeness parameters. SMILES representations of the compounds were used as input for computational screening. These integrated analyses enabled the selection of compounds with favorable pharmacokinetic behavior and acceptable drug-likeness characteristics for further study.

2.2. Identification of Compound Targets and Fertility Disorders-Associated Targets

Putative compound targets were predicted using the SwissTargetPrediction server (http://www.swisstargetprediction.ch/) [19], a web-based tool designed to identify the most likely protein targets of small molecules based on the principle of chemical similarity through reverse screening approaches. For this analysis, the SMILES representations of the compounds, obtained from the PubChem database, were submitted to the SwissTargetPrediction platform via SwissADME to obtain potential target profiles.

Disease-associated targets related to human fertility disorders (HFDs) were retrieved from the GeneCards database (https://www.genecards.org/) [20], a comprehensive integrative knowledge base that consolidates gene-centric information from approximately 200 curated data sources, including genomic, transcriptomic, proteomic, genetic, clinical, and functional datasets [21]. HFDs-related genes were identified using the keyword "Human Fertility Disorders," with a relevance score threshold set at >30 to ensure higher-confidence associations.

Finally, overlapping targets between compound-associated proteins and HFDs-related genes were identified using Venny v2.1.0, enabling the determination of shared molecular targets potentially involved in the therapeutic effects of the studied compounds (https://bioinfogp.cnb.csic.es/tools/venny/) [22].

further study.

2.3. Protein-Protein Interaction Network Analysis

Protein–protein interaction (PPI) networks were generated using the STRING database (https://string-db.org/) [23], which integrates known and predicted interactions derived from experimental evidence, curated pathway databases, and computational predictions. This platform enables comprehensive assessment of functional protein associations and facilitates the prediction of interactions among common targets. For analysis, the "Multiple proteins" option was selected, and all shared target genes were submitted with the organism specified as Homo sapiens.

Subsequently, network visualization and hub gene identification were performed using Cytoscape v3.10.3 [24]. The CytoHubba plugin was applied to evaluate the topological properties of the constructed PPI network and to identify key hub genes. The top ten hub genes were prioritized based on multiple centrality algorithms, including maximal clique centrality (MCC), maximum neighborhood component (MNC), edge percolated component (EPC), degree, and closeness, ensuring high-confidence selection of biologically significant nodes within the network.

2.4. GO and KEGG Pathway Analysis

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses of the core targets were conducted using the DAVID database (https://davidbioinformatics.nih.gov/) [25] with a significance threshold set at $P < 0.05$. GO functional annotation encompassed three categories: biological process (BP), cellular component (CC), and molecular function (MF). For analysis, all core target genes were submitted to the DAVID interface, with "OFFICIAL_GENE_SYMBOL" selected as the identifier, "Homo sapiens" specified as the organism, and "Gene List" chosen as the list type prior to submission. In addition, the SRplot web server [26] was employed to visualize the enrichment results, facilitating clearer interpretation of functional and pathway associations.

2.5. Compound-target biological process-pathway network analysis

The compound–target–biological process–pathway network was constructed and analyzed using Cytoscape v3.10.3, with key regulatory nodes identified through topological parameters such as degree, closeness, MCC, and EPC. This systems-level analytical framework facilitates the integration of multi-dimensional biological data to characterize interactions among bioactive compounds, their molecular targets, and associated signaling pathways. Furthermore, it underscores the ability of the investigated compounds to modulate multiple targets and signaling cascades concurrently, thereby extending beyond the traditional "one drug–one target" model and supporting a multi-target therapeutic paradigm [27].

2.6. Molecular docking analysis

The 3D structures of the six selected compounds were retrieved from the PubChem database, while the crystal structures of the five target proteins—IGF1R (PDB ID: 3D94), EGFR (PDB ID: 1M17), PIK3CA (PDB ID: 7R9V), mTOR (PDB ID: 4JSV), and ERBB2 (PDB ID: 8U8X)—were obtained from the RCSB Protein Data Bank (https://www.rcsb.org/) [28]. Prior to docking, protein structures were prepared by removing co-crystallized ligands, water molecules, and heteroatoms using Discovery Studio 4.5 [29], followed by energy minimization using SWISS-PDBViewer [30] to optimize structural stability.

Molecular docking was then performed using PyRx Virtual Screening Tools and AutoDock Vina v1.2.0 [31] to evaluate the binding affinities between the selected compounds and target proteins. The binding pockets were defined using grid boxes with specified coordinates for each receptor, enabling systematic exploration of ligand conformations within the active sites. The designated grid box for IGF1R: Dimensions (Angstrom) of X: 52.1443 Y: 53.7617 Z: 52.4716; with a center of X: 32.5168 Y: 20.1715 Z: -3.886 Å, for EGFR: Dimensions (Angstrom) of X: 113.5075 Y: 75.7168 Z: 51.5337; with a center of X: 9.9716 Y: 7.0761 Z: 59.4064 Å, for PIK3CA: Dimensions (Angstrom) of X: 83.8916 Y: 87.9266 Z: 85.4042; with a center of X: 1.7056 Y: 5.6110 Z: 21.0043 Å, for mTOR: Dimensions (Angstrom) of X: 89.9849 Y: 104.5680 Z: 117.1629; with a center of X: 69.5604 Y: -12.490 Z: -49.8992 Å, and for ERBB2: Dimensions (Angstrom) of X: 54.3016 Y: 59.5439 Z: 59.6839; with a center of X: -13.955 Y: 13.4823 Z: 14.4906 Å, respectively. The most favorable binding poses were selected based on the lowest binding energy, indicating the highest predicted binding affinity and stability of the ligand–protein complexes.

To validate the docking protocol, the co-crystallized reference ligands, namely 3-[cis-3-(4-methylpiperazin-1-yl)cyclobutyl]-1-(2-phenylquinolin-7-yl)imidazo[1,5-a]pyrazin-8-amine (D94) for IGF1R, [6,7-bis(2-methoxyethoxy)quinazoline-4-yl]-(3-ethynylphenyl)amine (AQ4) for EGFR, N-[2-(4-{4-[2-amino-4-(difluoromethyl)pyrimidin-5-yl]-6-(morpholin-4-yl)-1,3,5-triazin-2-yl}piperazin-1-yl)-2-oxoethyl]-1-(prop-2-enoyl)piperidine-4-carboxamide (2Q7) for PIK3CA, adenosine-5′-diphosphate (ADP) for mTOR, and 1-{(1R,3r,5S)-3-[(3M)-4-methyl-3-{3-methyl-4-[(1-methyl-1H-benzimidazol-5-yl)oxy]phenyl}-1H-pyrazolo[3,4-d]pyrimidin-1-yl]-8-azabicyclo[3.2.1]octan-8-yl}propan-1-one (W9N) for ERBB2 were re-docked into their respective binding pockets using the same docking parameters employed for the screened compounds. The RMSD values between the re-docked and crystallographic ligand conformations were calculated using UCSF Chimera [32]. All RMSD values were below 2.0 Å, indicating that the docking protocol was reliable and suitable for predicting ligand–protein interactions.

3. RESULTS

3.1. Compounds Retrieval and Filtration

A total of 123 phytochemicals derived from *C. sinensis* leaves were retrieved from the IMPPAT 2.0 database by querying the plant name, and all available phytochemicals associated with *C. sinensis* were included for subsequent analyses, while the corresponding PubChem CID identifiers and SMILES representations for each compound were retrieved and downloaded from the PubChem database for downstream analysis.

To assess their pharmacological suitability, the compounds were subjected to virtual screening using SwissADME and pkCSM platforms, focusing on drug-likeness, bioavailability, and ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties. Based on these evaluations, six compounds—Typhasterol, Teasterone, Brassinolide, Castasterone, A1-Barrigenol, and Theasapogenol B—were shortlisted as promising candidates, demonstrating non-toxic profiles and no predicted skin sensitization. Furthermore, these compounds exhibited favorable pharmacokinetic behavior, including acceptable absorption, distribution, metabolism, and overall drug-likeness characteristics. Although catechins such as EGCG, epicatechin, epigallocatechin, and epicatechin gallate are major bioactive constituents of *C. sinensis*, they were not retained after SwissADME and pkCSM screening due to comparatively less favorable pharmacokinetic and drug-likeness properties. In contrast, Typhasterol, Teasterone, Brassinolide, Castasterone, A1-Barrigenol, and Theasapogenol B exhibited superior ADMET and drug-likeness profiles and were therefore selected for further analyses. Detailed screening outcomes are presented in **Supplementary Table S1**.

3.2. Compound Targets and HFDs-Associated Targets Retrieval

Target prediction using SwissTargetPrediction identified 100 putative protein targets for each of the six bioactive compounds, yielding a total of 600 predicted targets across all compounds. Detailed target information is provided in **Supplementary Table S2**.

For disease-associated target identification, the GeneCards database was employed to retrieve HFDs-related genes, resulting in 7,517 associated targets (**Supplementary Table S3**). Among these, 501 targets were further prioritized based on a relevance score greater than 30 (**Supplementary Table S4**), indicating higher confidence in their association with HFDs. Subsequently, the intersection between compound-associated targets and HFDs-related targets was analyzed using the venn diagram, leading to the identification of 29 shared targets (**Supplementary Table S5**). These common targets are visually represented in a venn diagram (**Figure 1A**), highlighting the potential molecular overlap between the bioactive compounds and HFDs-related biological processes.

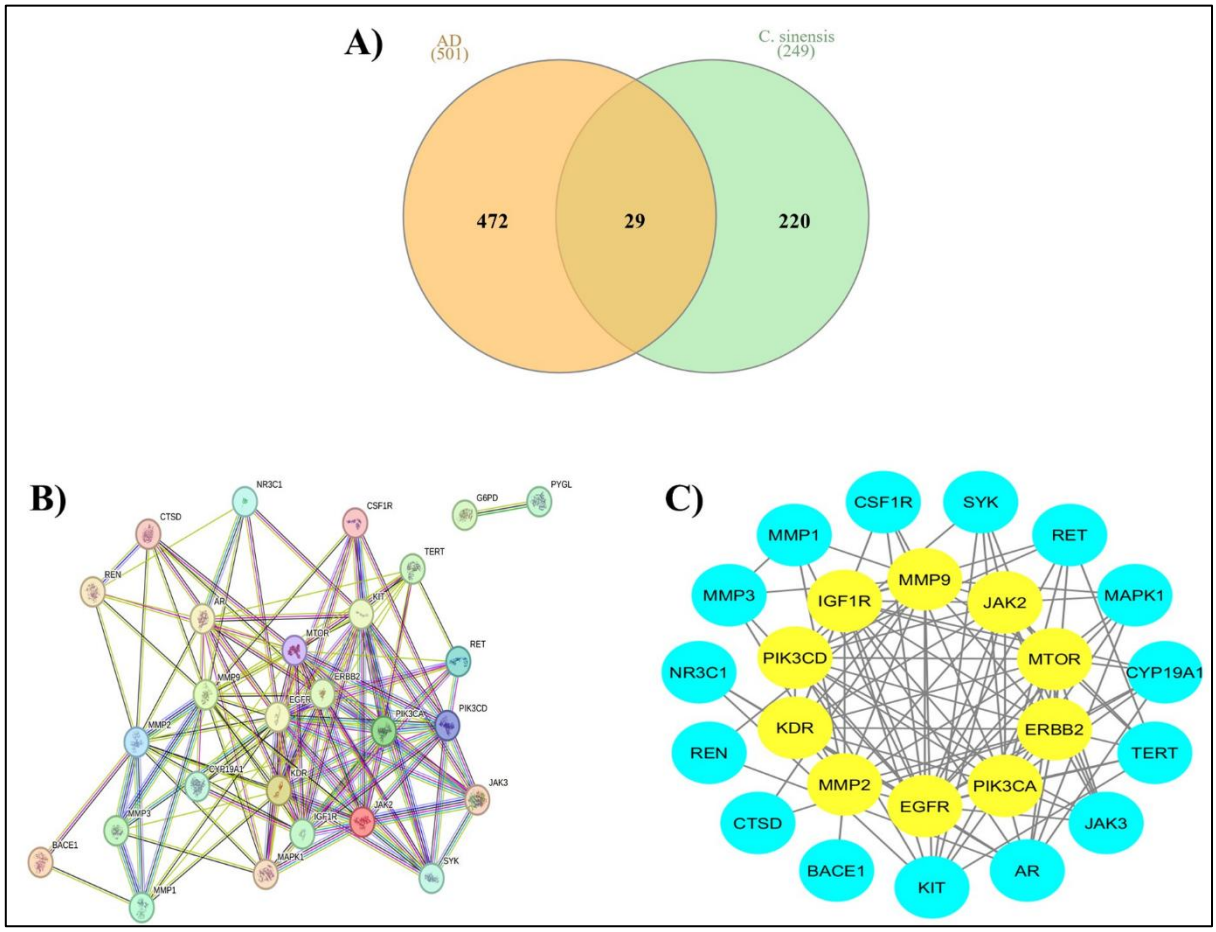


Figure 1. A) Venn diagram illustrating the shared targets between *C. sinensis* and HFDs; B) the PPI network of identified targets generated using the STRING database; and C) the refined PPI network of core targets constructed using Cytoscape. In the network visualization, yellow octagonal nodes represent the 10 core target genes, whereas cyan circular nodes denote the broader set of interacting target proteins, highlighting the hierarchical organization of the interaction network.

3.3. Analysis of PPI Network

The PPI network was constructed using the STRING database, revealing a highly interconnected network with 29 nodes and 144 edges. The network exhibited a high interaction density, with an average node degree of 9.93, a local clustering coefficient of 0.682, and a statistically significant PPI enrichment ($p < 1.0 \times 10^{-16}$) (**Figure 1B** and **Supplementary Table S6**). After applying a confidence score threshold (> 0.5), a refined network comprising 27 nodes and 107 edges was obtained (**Supplementary Table S7**).

Subsequent topological analysis using Cytoscape v3.10.3 facilitated the identification of 10 hub genes (HubGs) within the interaction network (**Figure 1C** and **Table 1**). These key targets, including EGFR, PIK3CA, ERBB2, mTOR, JAK2, MMP9, IGF1R, PIK3CD,

MMP, and KDR, were selected for further investigation based on their high centrality scores across multiple network topology measures, including degree, closeness, MCC, MNC, and EPC.

Table 1. Top 10 targets (HubGs) identified by PPI network analysis.

Degree	Score	Closeness	Score	MCC	Score	MNC	Score	EPC	Score
EGFR	19	EGFR	21.5	EGFR	11,894	EGFR	19	EGFR	14.702
PIK3CA	16	PIK3CA	19.833	mTOR	11,880	PIK3CA	16	ERBB2	14.31
ERBB2	15	ERBB2	19.5	ERBB2	11,786	ERBB2	15	mTOR	14.301
mTOR	15	mTOR	19.5	JAK2	11,184	mTOR	15	PIK3CA	14.232
JAK2	14	JAK2	19	IGF1R	11,064	JAK2	14	JAK2	13.979
MMP9	14	MMP9	19	KDR	10,092	IGF1R	13	IGF1R	13.886
IGF1R	13	IGF1R	18.5	PIK3CA	6,990	MMP9	13	MMP9	13.585
PIK3CD	11	PIK3CD	17.166	PIK3CD	6,048	PIK3CD	11	PIK3CD	13.224
MMP2	10	MMP2	17	MMP9	5,121	KDR	10	KDR	12.946
KDR	10	KDR	17	MMP2	5,065	MMP2	9	MMP2	12.16

Abbreviations: MCC = Maximal Clique Centrality; MNC = Maximum Neighborhood Component; EPC = Edge Percolated Component.

3.4. Analysis of GO and KEGG Pathway

GO functional enrichment analysis identified a total of 21 significantly enriched terms ($P < 0.05$), comprising 13 BP, 2 CC, and 6 MF categories (**Figure 2A–C**). The most prominently enriched BP terms included the vascular endothelial growth factor (VEGF) signaling pathway, the ephrin receptor signaling pathway, the positive regulation of phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) signaling, and the positive regulation of cell migration. For CC classification, the enriched terms were mainly associated with the plasma membrane and receptor complexes. MF analysis indicated significant involvement in protein binding, ATP binding, identical protein binding, protein tyrosine kinase activity, as well as histone kinase activities targeting H3Y41 and H2AXY142 modifications (**Supplementary Table S8**). Collectively, these results suggest that the 10 target genes identified are predominantly involved in key signaling cascades and molecular functions closely linked to reproductive physiology, including gametogenesis, hormonal regulation, embryo implantation, and overall fertility outcomes.

KEGG pathway enrichment analysis further identified 25 significantly associated pathways, including pathways in cancer, proteoglycans in cancer, PI3K-Akt signaling pathway, EGFR tyrosine kinase inhibitor resistance, endocrine resistance, prostate cancer, hypoxia-inducible factor 1 (HIF-1) signaling pathway, focal adhesion, JAK-STAT signaling pathway, and microRNAs in cancer, among others (**Figure 2D and Supplementary Table S9**). Notably, pathways such as PI3K-Akt, EGFR, HIF-1, JAK-STAT, microRNA regulation, and focal adhesion signaling have well-established roles in reproductive processes, highlighting their potential fertility-related implications.

3.5. Compound-target biological process-pathway network analysis

A compound–target–biological process–pathway interaction network was constructed using Cytoscape v3.10.3 to identify central molecular targets based on network topological properties. The network analysis revealed that the six bioactive compounds derived from *C. sinensis* interacted with multiple targets and signaling pathways, indicating their potential synergistic therapeutic effects (**Figure 3**).

To determine the most influential nodes within the network, several centrality parameters, including degree, closeness, MCC, and EPC, were applied. Based on these topological algorithms, five hub targets were identified: IGF1R, EGFR, PIK3CA, mTOR, and ERBB2 (**Table 2**). These hub genes may play biologically significant regulatory roles and could serve as potential therapeutic targets or key molecular intervention points.

3.6. Molecular docking analysis

Molecular docking analysis demonstrated favorable binding interactions between the selected phytochemicals and the five target proteins, including IGF1R, EGFR, PIK3CA, mTOR, and ERBB2 (**Table 3** and detailed in **Supplementary Table S10**). Among all compounds, Theasapogenol B exhibited the strongest bind-

ing affinities against PIK3CA and mTOR, with docking scores of −10.3 kcal/mol for both targets, indicating a highly stable ligand–receptor interaction. Teasterone also showed notable binding potential, particularly against IGF1R (−9.7 kcal/mol) and PIK3CA (−9.2 kcal/mol). A lower binding energy indicates a stronger binding affinity and reflects the formation of a more stable ligand–receptor complex [33].

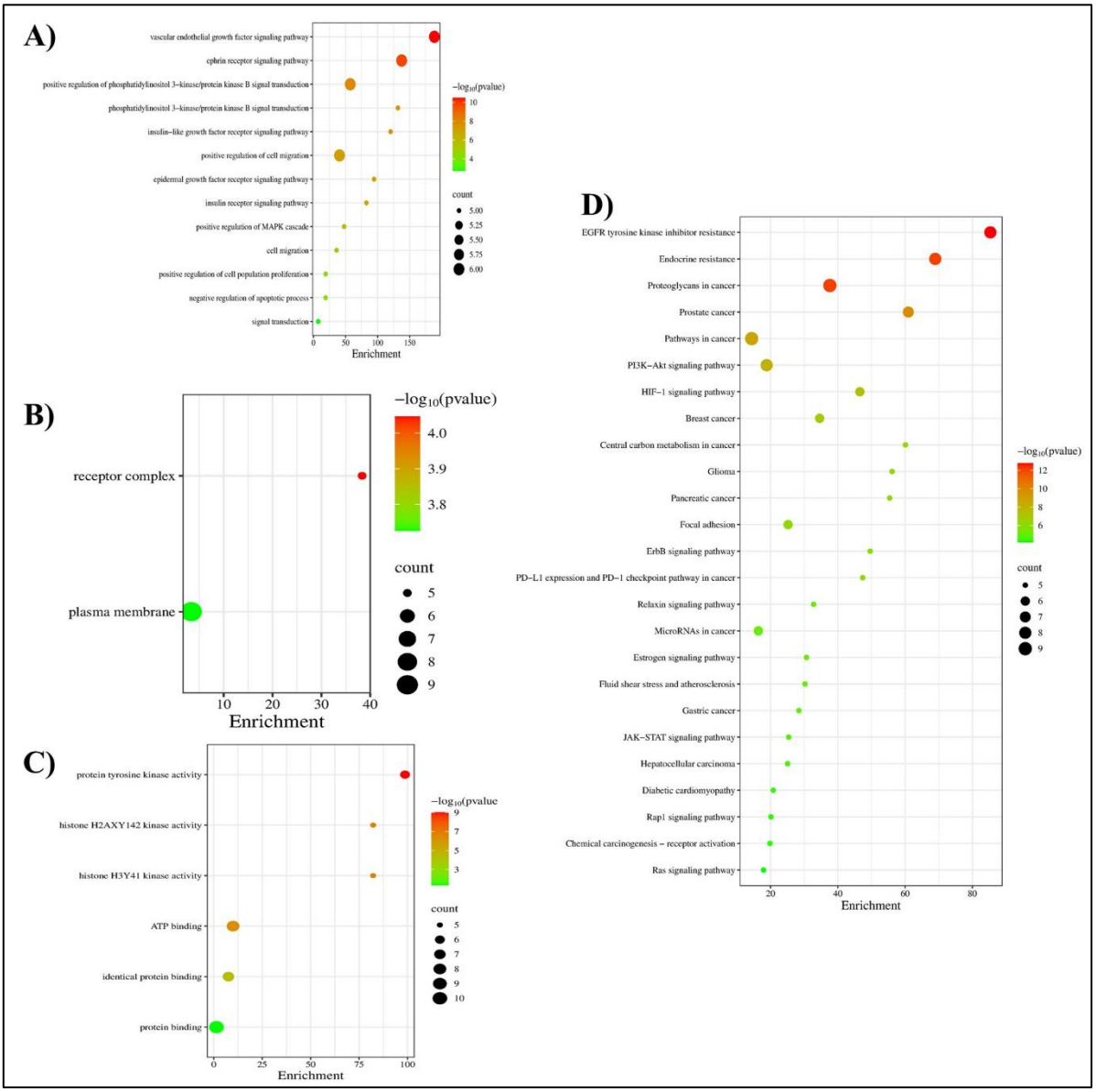


Figure 2. The ontological and pathway enrichment analysis of the 10 core targets is visualized using bubble plots. A) Biological Process, B) Cellular Component, C) Molecular Function, and D) KEGG pathway categories. In these representations, bubble size reflects the degree of gene enrichment: larger bubbles indicate more genes involved in a specific biological process or pathway, whereas smaller bubbles denote fewer associated genes. Additionally, the color gradient corresponds to the statistical significance of enrichment, represented by −log10(p-value), with distinct colors indicating varying levels of significance across the analyzed terms.

For IGF1R, Teasterone demonstrated the highest affinity, followed by Theasapogenol B (−9.3 kcal/mol) and A1-Barrigenol (−8.9 kcal/mol), whereas Castasterone showed the lowest affinity (−7.0 kcal/mol). In the case of EGFR, Teasterone again exhibited the strongest interaction with a binding affinity of −7.8 kcal/mol, followed closely by Theasapogenol B (−9.1 kcal/mol) and A1-Barrigenol (−8.8 kcal/mol). For PIK3CA, multiple compounds, including Teasterone, Typhasterol, and Castasterone, displayed identical docking scores of −9.2 kcal/mol, while Brassinolide showed comparatively lower affinity (−8.3 kcal/mol).

Table 2. Top 5 targets identified by compound-target biological process-pathway network analysis based on their Degree, Closeness, MCC, and EPC methods.

Degree	Score	Closeness	Score	MCC	Score	EPC	Score
IGF1R	38	EGFR	40.83333	EGFR	35	IGF1R	16.186
EGFR	35	PIK3CA	38.83333	PIK3CA	32	EGFR	15.909
PIK3CA	32	mTOR	38.16667	mTOR	31	PIK3CA	15.627
mTOR	31	IGF1R	33.91667	IGF1R	25	mTOR	15.595
ERBB2	24	ERBB2	33.5	ERBB2	24	ERBB2	14.072

Abbreviations: MCC = Maximal Clique Centrality; EPC = Edge Percolated Component.

Similarly, docking against mTOR revealed strong interactions for Theasapogenol B (−10.3 kcal/mol), whereas Teasterone and Typhasterol each demonstrated binding affinities of −8.6 kcal/mol. In the ERBB2 complex, Theasapogenol B again produced the best docking score (−9.3 kcal/mol), followed by A1-Barrigenol (−7.8 kcal/mol). Overall, the docking results suggest that all investigated phytochemicals exhibit substantial binding affinity for the selected targets, supporting their potential as multi-target therapeutic candidates.

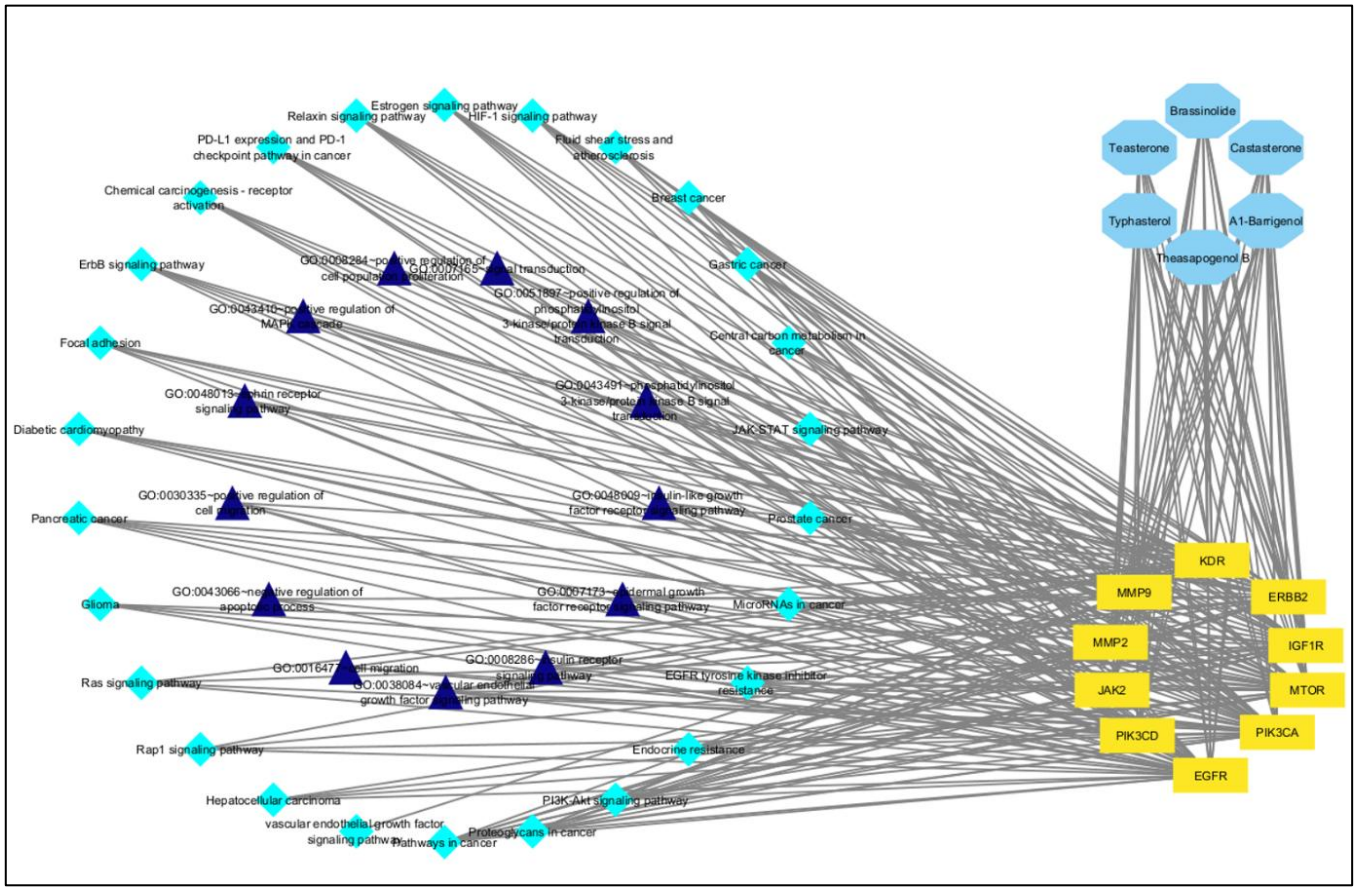


Figure 3. The predicted compound–target–biological process–pathway interaction network. Within the network, bioactive compounds are represented as sky-blue octagonal nodes, while the ten core target genes are illustrated as yellow rounded rectangles. Biological processes are depicted using purple triangular nodes, whereas signaling pathways are represented as cyan diamond-shaped nodes, providing a clear visualization of the multi-layered interactions among compounds, targets, biological functions, and pathways.

3.7. Docking Validation

To evaluate the reliability of the molecular docking protocol, a re-docking validation procedure was performed using the co-crystallized ligands associated with each target protein. The reference ligands included D94 for IGF1R, AQ4 for EGFR, 2Q7 for PIK3CA, ADP for mTOR, and W9N for ERBB2. Each ligand was re-

docked into its respective binding pocket using the same docking parameters employed for the screened phytochemicals. Subsequently, the RMSD values between the re-docked poses and the corresponding crystallographic conformations were calculated using UCSF Chimera. The RMSD values obtained were below the generally accepted threshold of 2.0 Å for all protein–ligand complexes (**Table 4**), confirming the docking protocol's ability to accurately reproduce experimentally observed binding orientations. These findings validate the reliability and reproducibility of the molecular docking workflow employed in the present study.

Furthermore, the selected phytochemicals demonstrated favorable binding interactions within the validated active sites of IGF1R, EGFR, PIK3CA, mTOR, and ERBB2, supporting the robustness of the docking results and strengthening confidence in the predicted ligand–target interactions.

4. DISCUSSION

In the present study, a network pharmacology approach was utilized to elucidate the potential therapeutic mechanisms of *C. sinensis* against HFDs. Six bioactive compounds, namely Typhasterol, Teasterone, Brassinolide, Castasterone, A1-Barrigenol, and Theasapogenol B, were identified as pharmacologically relevant constituents. Toxicological assessment revealed that all selected compounds were non-toxic, non-skin sensitizing, and unable to penetrate the BBB. Additionally, the compounds exhibited favorable pharmacokinetic and drug-likeness properties, including satisfactory bioavailability, absorption, distribution, and metabolic profiles. Target screening using publicly accessible databases identified 29 overlapping targets between the active compounds and HFDs-related genes, suggesting their possible involvement in mediating therapeutic effects. PPI network analysis, integrating the six active compounds with HFDs-associated targets, identified 10 hub genes, including EGFR, PIK3CA, ERBB2, mTOR, JAK2, MMP9, IGF1R, PIK3CD, MMP, and KDR, using five topological algorithms implemented in Cytoscape.

GO enrichment analysis demonstrated that the anti-HFDs activity of these compounds is primarily associated with cellular components such as the plasma membrane and receptor complexes. Molecular function analysis highlighted involvement in protein binding, ATP binding, identical protein binding, protein tyrosine kinase activity, and kinase activities associated with histone modifications, including phosphorylation of H3Y41 and H2AXY142.

Table 3. Binding affinities of the 6 compounds with the top 5 targets.

Receptor	Ligand	Binding Affinity (kcal/mol)
IGF1R	Teasterone	-9.7
	Theasapogenol B	-9.3
	A1-Barrigenol	-8.9
	Typhasterol	-8.5
	Brassinolide	-7.3
	Castasterone	-7.0
EGFR	Teasterone	-7.8
	Theasapogenol B	-9.1
	A1-Barrigenol	-8.8
	Typhasterol	-7.8
	Brassinolide	-7.4
	Castasterone	-7.5
PIK3CA	Teasterone	-9.2
	Theasapogenol B	-10.3
	A1-Barrigenol	-8.4
	Typhasterol	-9.2
	Brassinolide	-8.3
	Castasterone	-9.2
mTOR	Teasterone	-8.6
	Theasapogenol B	-10.3
	A1-Barrigenol	-8.5
	Typhasterol	-8.6
	Brassinolide	-8.3
	Castasterone	-8.3
ERBB2	Teasterone	-7.1
	Theasapogenol B	-9.3
	A1-Barrigenol	-7.8
	Typhasterol	-7.1
	Brassinolide	-7.5
	Castasterone	-7.4

Furthermore, biological process enrichment revealed significant enrichment for pathways related to VEGF signaling, ephrin receptor signaling, positive regulation of the PI3K/Akt signaling pathway, and pos-

itive regulation of cell migration, collectively suggesting the multi-target therapeutic potential of *C. sinensis* against HFDs.

Table 4. Docking validation through re-docking of co-crystallized ligands and corresponding RMSD values.

Proteins (Receptors)	Reference Ligand	RMSD (Å)
IGF1R	D94	1.24
EGFR	AQ4	1.51
PIK3CA	2Q7	0.98
mTOR	ADP	1.37
ERBB2	W9N	1.12

While KEGG enrichment often highlights broad, pleiotropic pathways (e.g., "pathways in cancer", PI3K–Akt, JAK–STAT, HIF-1), these pathways also have discrete, well-documented roles in reproductive biology; therefore, their presence in our enrichment results is not necessarily non-specific but rather points to mechanistic routes by which the identified phytochemicals could affect fertility. For example, PI3K–Akt signaling is a central regulator of ovarian folliculogenesis, oocyte growth, and testicular function, so modulation of PI3K–Akt by plant compounds could plausibly alter gametogenesis or steroidogenic support [34]. EGFR/ErbB signaling is required for endometrial receptivity and embryo implantation, linking EGFR-related hits to implantation biology rather than only to oncogenesis [35]. HIF-1 activity is a physiological regulator of early placental development and angiogenesis (with dysregulation linked to implantation/placentation disorders), so phytochemical effects on HIF-1 could impact implantation and early pregnancy [36]. JAK–STAT signaling contributes to spermatogenesis and somatic support cells in the gonad, indicating a direct route to male fertility phenotypes from compounds that alter this axis [37]. Finally, microRNAs and focal-adhesion signaling are intimately involved in oocyte competence, follicle–granulosa communication, and embryo development—processes that are fertility-specific despite appearing in cancer-related KEGG categories [38,39]. Compound–target–biological process–pathway network analysis revealed that the 6 active compounds interact with multiple potential targets and signaling pathways, indicating possible synergistic therapeutic effects against HFDs. Five key hub genes (IGF1R, EGFR, PIK3CA, mTOR, and ERBB2) were identified as biologically significant nodes, potentially serving as critical regulators and therapeutic targets in the context of HFDs.

The IGF1R gene and its associated signaling pathways are integral to the regulation of spermatogenesis and sperm function. Clinical and experimental studies have demonstrated that reduced circulating levels of IGF-1, the primary ligand activating IGF1R, correlate with impaired sperm quality, including abnormalities in sperm morphology and decreased motility [40]. The EGFR gene and its associated signaling pathways are essential during early pregnancy, playing a pivotal role in embryo implantation and placental development [35]. Studies have demonstrated that PIK3CA is involved in the development of uterine glands, a process critical for successful embryo implantation and pregnancy maintenance. Experimental evidence from murine models revealed that mutations in PIK3CA lead to abnormal hyperproliferation of endometrial epithelial cells, suggesting a significant role for PIK3CA in regulating uterine tissue homeostasis and reproductive health [41]. The mechanistic target of mTOR is a key regulator of primordial follicle activation and subsequent growth, and its targeted modulation offers potential applications in ovarian preservation strategies [42]. Furthermore, mTOR signaling is essential for establishing endometrial receptivity, a critical determinant of the uterine lining's capacity to support successful embryo implantation [43]. Although ERBB2, which encodes the HER2 protein, is not typically classified as a direct "fertility disorder" gene, it has been implicated in female infertility associated with endometriosis. Research indicates that loss of mitogen-inducible gene 6 (MIG-6) in the endometrium leads to progesterone resistance and promotes the development of endometriosis, ultimately resulting in infertility. The ERBB2 signaling pathway is involved in this pathological mechanism, and targeted inhibition of ERBB2 has been shown to mitigate certain adverse reproductive outcomes associated with MIG-6 loss [44]. Collectively, these hub genes and molecular pathways influence spermatogenesis, follicular development, endometrial receptivity, and implantation, underscoring their significance as potential therapeutic targets for human fertility disorders.

To further explore the interactions between the bioactive constituents of *C. sinensis* and the proteins encoded by the identified hub genes, molecular docking simulations were performed. The docking results demonstrated favorable binding affinities of all selected compounds toward the target proteins, suggesting their potential as promising therapeutic agents against HFDs.

Based on the integrated findings from network pharmacology, enrichment analysis, and molecular docking, we have a moderate to strong level of confidence that the identified active compounds of *C. sinensis* and their interactions with the prioritized targets (IGF1R, EGFR, PIK3CA, mTOR, and ERBB2) are biologically relevant to human fertility disorders. The consistency observed among the compound–target–biological process–pathway network analysis, the known reproductive functions of these targets, and the favorable docking interactions provides computational support for the proposed mechanisms.

However, we acknowledge that these findings are currently based on in silico predictions and therefore remain exploratory until confirmed experimentally. Further in vitro studies, such as receptor-binding assays, cellular signaling analyses, and reproductive cell-based functional assays, together with in vivo validation in appropriate fertility-related experimental models, will be essential for verifying the biological activity, therapeutic relevance, and mechanistic pathways of the six identified phytochemicals. Accordingly, the conclusion has been framed cautiously to emphasize that the present study provides a strong computational foundation and mechanistic hypothesis for future experimental validation, rather than definitive biological confirmation.

To further confirm their therapeutic potential, we recommend the following laboratory investigations: (i) in vitro studies using reproductive cell models such as granulosa cells, Sertoli cells, Leydig cells, or endometrial epithelial cells to evaluate cellular responses, proliferation, apoptosis, oxidative stress, and hormone-related signaling; (ii) molecular validation of target engagement and pathway modulation, including IGF1R/PI3K/AKT/mTOR and EGFR/ERBB2 signaling, using RT-PCR and western blot analyses; (iii) reproductive toxicity and dose-response assessments to determine safety and optimal concentrations; and (iv) in vivo studies using established infertility or reproductive dysfunction animal models to evaluate effects on spermatogenesis, follicular development, endometrial receptivity, implantation, and fertility outcomes.

5. CONCLUSION

The present study employed an integrated network pharmacology and molecular docking approach to elucidate the potential therapeutic mechanisms of *C. sinensis* against HFDs. Six bioactive compounds, including Typhasterol, Teasterone, Brassinolide, Castasterone, A1-Barrigenol, and Theasapogenol B, demonstrated favorable pharmacokinetic, drug-likeness, and safety profiles. Network analysis identified multiple HFDs-associated targets and key hub genes, particularly IGF1R, EGFR, PIK3CA, mTOR, and ERBB2, which are closely associated with spermatogenesis, follicular development, endometrial receptivity, implantation, and reproductive regulation. Functional enrichment analyses further indicated the involvement of VEGF, PI3K/Akt, ephrin receptor, and JAK–STAT signaling pathways in mediating the observed therapeutic effects. Molecular docking analysis confirmed favorable binding interactions between the active compounds and the identified hub proteins, supporting their multi-target therapeutic potential. Although the present findings are based solely on computational analyses and require further in vitro and in vivo validation, this study provides important mechanistic insights and a valuable theoretical basis for future investigations aimed at developing novel fertility-related therapeutic agents derived from *C. sinensis*.

Supplementary Materials

The supplementary materials supporting the findings of this study are available with the online version of this article as Supplementary Tables S1-S10.

Author(s) ORCID iDs

Md. Mainuddin Hossain: https://orcid.org/0009-0005-7076-3235
Jannatul Fardous: https://orcid.org/0009-0005-7022-3263

Authorship Contribution

Md. Mainuddin Hossain: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Supervision, Validation, Visualization, Writing–original draft, Writing–review & editing. Jannatul Fardous: Data curation, Formal analysis, Methodology, Writing–original draft.

Funding Information

This research received no external funding.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgments

None.

Artificial Intelligence (AI) Declaration

No artificial intelligence (AI) was used in the design, conduct, analysis, interpretation, or preparation of this manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

References

1. Zegers-Hochschild F, Adamson GD, Dyer S, Racowsky C, de Mouzon J, Sokol R, et al. The International Glossary on Infertility and Fertility Care, Fertil Steril. 2017;108(3):393–406. doi:10.1016/j.fertnstert.2017.06.005
2. Mascarenhas MN, Flaxman SR, Boerma T, Vanderpoel S, Stevens GA. National, regional, and global trends in infertility prevalence since 1990: a systematic analysis of 277 health surveys. PLoS Med. 2012;9(12):e1001356. doi:10.1371/journal.pmed.1001356
3. Kumar N, Singh AK. Trends of male factor infertility, an important cause of infertility: A review of literature. J Hum Reprod Sci. 2015;8(4):191–6. doi:10.4103/0974-1208.170370
4. Agarwal A, Baskaran S, Parekh N, Cho C-L, Henkel R, Vij S, et al. Male infertility. Lancet (London, England). 2021;397(10271):319–33. doi:10.1016/S0140-6736(20)32667-2
5. Agarwal A, Gupta S, Sharma R. Oxidative stress and its implications in female infertility - a clinician's perspective. Reprod Biomed Online. 2005;11(5):641–50. doi:10.1016/s1472-6483(10)61174-1
6. Wyns C, Bergh C, Calhaz-Jorge C, De Geyter C, Kupka MS, Motrenko T, et al. ART in Europe, 2016: results generated from European registries by ESHRE. Hum Reprod open. 2020;2020(3):hoaa032. doi:10.1093/hropen/hoaa032
7. Rafieian-Kopaei M. Medicinal plants for renal injury prevention. J Ren Inj Prev. 2013;2(2):63–5. doi:10.12861/jrip.2013.21
8. El-Saadony MT, Saad AM, Mohammed DM, Korma SA, Alshahrani MY, Ahmed AE, et al. Medicinal plants: bioactive compounds, biological activities, combating multidrug-resistant microorganisms, and human health benefits - a comprehensive review. Front Immunol. 2025;16:1491777. doi:10.3389/fimmu.2025.1491777
9. Cabrera C, Artacho R, Giménez R. Beneficial effects of green tea--a review. J Am Coll Nutr. 2006;25(2):79–99. doi:10.1080/07315724.2006.10719518
10. Sánchez M, González-Burgos E, Iglesias I, Lozano R, Gómez-Serranillos MP. The Pharmacological Activity of Camellia sinensis (L.) Kuntze on Metabolic and Endocrine Disorders: A Systematic Review. Biomolecules. 2020;10(4):603. doi:10.3390/biom10040603
11. Granja A, Frias I, Neves AR, Pinheiro M, Reis S. Therapeutic Potential of Epigallocatechin Gallate Nanodelivery Systems. Biomed Res Int. 2017;2017:5813793. doi:10.1155/2017/5813793
12. Hossain MM, Evamoni FZ, Morshed MM. Integrative network pharmacology, molecular docking and network-based drug repurposing reveal therapeutic targets and repurposable drugs for obesity-associated type 2 diabetes mellitus. Silico Res Biomed. 2025;1:100117. doi:10.1016/j.insi.2025.100117
13. Zhang M, Su S, Bhatnagar RK, Hassett DJ, Lu LJ. Prediction and analysis of the protein interactome in Pseudomonas aeruginosa to enable network-based drug target selection. PLoS One. 2012;7(7):e41202. doi:10.1371/journal.pone.0041202
14. Hossain MM, Apu MJH, Aziz MFBA, Tanjil MTR, Das LC, Kar A, et al. Exploring Dolichos lablab compounds as potential inhibitors for fusion (F) protein of human metapneumovirus (HMPV): A systematic computational approach. PLoS One. 2025;20(9):e0332170. doi:10.1371/journal.pone.0332170
15. Vivek-Ananth RP, Mohanraj K, Sahoo AK, Samal A. IMPPAT 2.0: an enhanced and expanded phytochemical atlas of Indian medicinal plants. ACS omega. 2023;8(9):8827–45. doi:10.1021/acsomega.3c00156
16. Kim S, Chen J, Cheng T, Gindulyte A, He J, He S, et al. PubChem 2023 update. Nucleic Acids Res. 2023;51(D1):D1373–80. doi:10.1093/nar/gkac956
17. Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep. 2017;7:42717. doi:10.1038/srep42717
18. Pires DE V, Blundell TL, Ascher DB. pkCSM: Predicting Small-Molecule Pharmacokinetic and Toxicity Properties Using Graph-Based Signatures. J Med Chem. 2015;58(9):4066–72. doi:10.1021/acs.jmedchem.5b00104
19. Daina A, Michielin O, Zoete V. SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules. Nucleic Acids Res. 2019;47(W1):W357–64. doi:10.1093/nar/gkz382
20. Stelzer G, Rosen N, Plaschkes I, Zimmerman S, Twik M, Fishilevich S, et al. The GeneCards Suite: From Gene Data Mining to Disease Genome Sequence Analyses. Curr Protoc Bioinforma. 2016;54:1.30.1-1.30.33. doi:10.1002/cpbi.5
21. Safran M, Rosen N, Twik M, BarShir R, Stein TI, Dahary D, et al. The GeneCards Suite BT - Practical Guide to Life Science Databases. In: Abugessaisa I, Kasukawa T, editors. Singapore: Springer Nature Singapore; 2021. p. 27–56. doi:10.1007/978-981-16-5812-9_2
22. Oliveros JC. VENNY. An interactive tool for comparing lists with Venn Diagrams. http//bioinfogp cnb csic es/tools/venny/index html. 2007;
23. Szklarczyk D, Kirsch R, Koutrouli M, Nastou K, Mehryary F, Hachilif R, et al. The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. Nucleic Acids Res. 2023;51(D1):D638–46. doi:10.1093/nar/gkac1000
24. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003;13(11):2498–504. doi:10.1101/gr.1239303
25. Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc. 2009;4(1):44–57. doi:10.1038/nprot.2008.211
26. Tang D, Chen M, Huang X, Zhang G, Zeng L, Zhang G, et al. SRplot: A free online platform for data visualization and graphing. PLoS One. 2023;18(11):e0294236. doi:10.1371/journal.pone.0294236
27. Sakle NS, More SA, Mokale SN. A network pharmacology-based approach to explore potential targets of Caesalpinia

pulcherima: an updated prototype in drug discovery. Sci Rep. 2020;10(1):17217. doi:10.1038/s41598-020-74251-1

28. Rose PW, Prlić A, Altunkaya A, Bi C, Bradley AR, Christie CH, et al. The RCSB protein data bank: integrative view of protein, gene and 3D structural information. Nucleic Acids Res. 2017;45(D1):D271–81. doi:10.1093/nar/gkw1000

29. Oyesakin YM, George DE, Fadare RY, Idris AY, Fadare OA. Molecular Docking and In-Silico ADME Prediction of Substituted (E)-4-Styryl-7, 8-dihydroquinazolin-5 (6H)-ones and 5-((E)-Styryl) pyrimidine [4, 5-d] pyrimidine-2, 4 (1H, 3H)-diones as Potential SERT Inhibitors and Antidepressants. Am J Pharmacol Sci. 2018;6(1):25–32. doi:10.12691/ajps-6-1-5

30. Guex N, Peitsch MC. SWISS-MODEL and the Swiss-Pdb Viewer: an environment for comparative protein modeling. Electrophoresis. 1997;18(15):2714–23.

31. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. 2010;31(2):455–61. doi:10.1002/jcc.21334

32. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, et al. UCSF Chimera--a visualization system for exploratory research and analysis. J Comput Chem. 2004;25(13):1605–12. doi:10.1002/jcc.20084

33. Ferreira LG, Dos Santos RN, Oliva G, Andricopulo AD. Molecular docking and structure-based drug design strategies. Molecules. 2015;20(7):13384–421. doi:10.3390/molecules200713384

34. Deng C-Y, Lv M, Luo B-H, Zhao S-Z, Mo Z-C, Xie Y-J. The Role of the PI3K/AKT/mTOR Signalling Pathway in Male Reproduction. Curr Mol Med. 2021;21(7):539–48. doi:10.2174/1566524020666201203164910

35. Large MJ, Wetendorf M, Lanz RB, Hartig SM, Creighton CJ, Mancini MA, et al. The epidermal growth factor receptor critically regulates endometrial function during early pregnancy. PLoS Genet. 2014;10(6):e1004451. doi:10.1371/journal.pgen.1004451

36. Rath G, Aggarwal R, Jawanjal P, Tripathi R, Batra A. HIF-1 Alpha and Placental Growth Factor in Pregnancies Complicated With Preeclampsia: A Qualitative and Quantitative Analysis. J Clin Lab Anal. 2016;30(1):75–83. doi:10.1002/jcla.21819

37. Alnajem A, Al-Maghrebi M. The Regulatory Effects of JAK2/STAT3 on Spermatogenesis and the Redox Keap1/Nrf2 Axis in an Animal Model of Testicular Ischemia Reperfusion Injury. Cells. 2023;12(18). doi:10.3390/cells12182292

38. Chico-Sordo L, García-Velasco JA. MicroRNAs as Biomarkers and Therapeutic Targets in Female Infertility. Int J Mol Sci. 2024;25(23). doi:10.3390/ijms252312979

39. Orozco-Galindo BV, Sánchez-Ramírez B, González-Trevizo CL, Castro-Valenzuela B, Varela-Rodríguez L, Burrola-Barraza ME. Folliculogenesis: A Cellular Crosstalk Mechanism. Curr Issues Mol Biol. 2025;47(2). doi:10.3390/cimb47020113

40. Cannarella R, Condorelli RA, La Vignera S, Bellucci C, Luca G, Calafiore R, et al. IGF2 and IGF1R mRNAs Are Detectable in Human Spermatozoa. World J Mens Health. 2020;38(4):545–51. doi:10.5534/wjmh.190070

41. Chang HJ, Shin HS, Kim TH, Yoo J-Y, Teasley HE, Zhao JJ, et al. Pik3ca is required for mouse uterine gland development and pregnancy. PLoS One. 2018;13(1):e0191433. doi:10.1371/journal.pone.0191433

42. Guo Z, Yu Q. Role of mTOR Signaling in Female Reproduction. Front Endocrinol (Lausanne). 2019;10:692. doi:10.3389/fendo.2019.00692

43. Makker A, Goel MM, Nigam D, Makker I, Pandey A. mTOR signaling and endometrial receptivity in infertile women with intramural uterine leiomyomas. Middle East Fertil Soc J. 2023;28(1):13. doi:10.1186/s43043-023-00138-6

44. Yoo J-Y, Kim TH, Shin J-H, Marquardt RM, Müller U, Fazleabas AT, et al. Loss of MIG-6 results in endometrial progesterone resistance via ERBB2. Nat Commun. 2022;13(1):1101. doi:10.1038/s41467-022-28608-x