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Uncovering Male Fertility–Modulating Potential of *Murraya koenigii* through Network Pharmacology and *In Silico* Targeting of Reproductive Proteins

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ABSTRACT

The *Murraya koenigii* (L.) Spreng., commonly known as curry leaf, is widely utilized in traditional medicine and valued for its richness in bioactive compounds, including carbazole alkaloids, flavonoids, tannins, and essential oils. While its antioxidant, anti-inflammatory, and metabolic regulatory activities are well established, its potential role in reproductive biology remains insufficiently studied. This study aimed to investigate the potential of *M. koenigii* phytochemicals to modulate male fertility by targeting key reproductive proteins and receptors. Network pharmacology and molecular docking analyses were employed to examine interactions with gonadotropin-releasing hormone receptor (GNRHR), follicle-stimulating hormone receptor (FSHR), cAMP-responsive element modulator (CREM), and protamine 2 (PRM2). The constructed interaction network revealed biologically relevant associations, particularly the CREM–PRM2 regulatory axis, which is crucial for spermatogenesis and sperm chromatin remodeling. Docking analyses suggested that selected phytoconstituents possess favorable binding affinities toward gonadotropin-related receptors, supporting their potential to influence the hypothalamic–pituitary–gonadal axis and downstream transcriptional events. These findings provide mechanistic insights into the traditional use of *M. koenigii* in reproductive health and highlight its promise as a natural source for developing botanical-based therapeutics in male infertility management.

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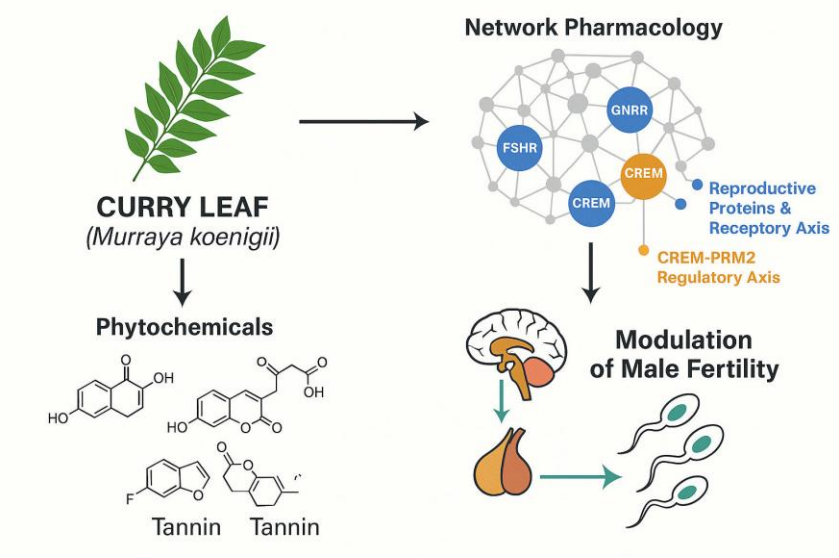
GRAPHICAL ABSTRACT



Introduction

Male infertility, a condition once shrouded in silence, is now recognized as a pressing global health challenge, affecting up to 15% of couples and contributing to approximately half of all infertility cases worldwide [1]. Far from a singular diagnosis, it encompasses a complex interplay of hormonal imbalances, impaired spermatogenesis, oxidative stress, environmental toxins, and genetic disruptions. With rising exposure to endocrine disruptors and lifestyle-related risk factors, modern living now poses unprecedented challenges to reproductive health, underscoring the urgent need for safe, effective, and holistic treatment approaches [2]. Among the molecular regulators essential to male reproductive function are the luteinizing hormone receptor (LHR), follicle-stimulating hormone receptor (FSHR), proprotein convertase subtilisin/kexin type 4 (PCSK4), cAMP response element modulator (CREM), and protamine 2 (PRM2) [3]. These targets control key aspects of hormonal signaling, spermatid gene expression, and sperm maturation [4]. Despite advances in assisted reproductive technologies, conventional therapies often fail to address the underlying pathophysiology and can pose considerable side effects, making natural alternatives an attractive research frontier. The use of plant-derived bioactive compounds has garnered increasing attention for their multi-target efficacy and reduced toxicity. *Murraya koenigii* (L.) Spreng., commonly known as curry or "*kari*" leaf, has long been revered in traditional medicine [5]. *M. koenigii* belongs to the family Rutaceae and is widely distributed throughout the Indian subcontinent. In Indonesia, this species is particularly abundant in Aceh Province, where it is commonly cultivated and used for both culinary and medicinal purposes. *M. koenigii* is widely used in traditional Ayurvedic and Southeast Asian medicine to treat ailments such as diabetes, gastrointestinal disorders, skin conditions, and inflammation [6]. Its leaves are rich in bioactive compounds, including carbazole alkaloids, flavonoids, terpenoids, phenolics, vitamin E, and essential oils [7]. Many of these constituents are known to exhibit antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, neuroprotective, and immunostimulatory properties, which are relevant to the pathophysiology of male infertility [8]. The diverse pharmacological properties of *M. koenigii* support its potential as a plant-derived

agent for improving reproductive function. This study aims to evaluate whether the diverse phytochemical constituents of *M. koenigii* (curry leaf) can be translated into biologically meaningful molecular interactions with key male fertility–related targets, including the LHR, FSHR, and protamine-associated proteins. By integrating phytochemical profiling with *in silico* molecular docking and network pharmacology analyses, this work seeks to determine whether bioactive compounds from *M. koenigii* possess the structural and physicochemical attributes necessary to modulate receptor function and the signaling pathways governing spermatogenesis, steroidogenesis, and sperm maturation. *In silico* molecular docking was employed in this study to evaluate the binding affinity, interaction patterns, and structural compatibility of *M. koenigii*–derived phytochemicals with key male fertility–related targets. This approach enables rapid, cost-effective screening of multiple compounds, provides chemical insight into ligand–receptor recognition, and helps prioritize bioactive candidates with the highest likelihood of modulating reproductive signaling pathways before experimental validation [9,10]. Elucidation of these interactions not only provides mechanistic support for the traditional use of *M. koenigii* in reproductive health, but also establishes a rational foundation for the development of plant-derived therapeutic candidates to address persistent unmet needs in the treatment of male infertility and subfertility.

Materials and Methods

Plant material and extraction processing

Fresh, disease-free *M. koenigii* (L.) Spreng leaves were collected from mature plants, air-dried at 40 °C to constant mass, and powdered to 40–60 mesh. Approximately 50 g powder was macerated (1:10, w/v) with 70% ethanol (3 × 24 h, room temperature) under light-protected conditions and filtered; pooled filtrates were rotary-evaporated (≤40 °C) to dryness and stored at −20 °C until analysis [11].

GC–MS conditions

The chemical constituents of the ethanolic extract were analyzed using a Gas Chromatography–Mass Spectrometry (GC–MS) system (Agilent 7890B GC coupled with 5977A MSD) equipped with an HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm film thickness). The injector temperature was set at 250 °C with a splitless injection of 1 µL of sample (1 mg/mL in methanol). Helium was used as the carrier gas at a constant flow rate of 1.0 mL min^{-1}. The oven temperature was programmed as follows: 60 °C (held 2 min), ramped to 280 °C at 10 °C min^{-1}, and held for 10 min. The MS was operated in electron ionization (EI, 70 eV) mode, with an ion source temperature of 230 °C and a mass scan range of m/z 40–550. Individual compounds were identified by comparing their mass spectra and retention indices with those of the NIST 17 library and published literature values for *M. koenigii* volatiles. Only peaks with a match probability ≥ 80% were considered. Relative peak areas (%) were used to estimate the proportional abundance of each compound. Non-redundant compounds detected by GC–MS were curated by cross-mapping SMILES against PubChem and exported as 3D PDBQT for docking. File conversions and hydrogen/valence checks were performed using Open Babel.

Male-fertility–related target collection

Male-reproduction targets (keywords: "*spermatogenesis*", "*steroidogenesis*", "*male fertility/infertility*", and "*sperm motility*") were compiled from GeneCards (human entries; relevance score filtered) and harmonized to UniProt IDs. This set defined the disease module for overlap analyses with compound-predicted targets. Compound-predicted targets were intersected with the male-fertility module. The

union set was submitted to STRING (organism: *Homo sapiens*; interaction sources: experiments, curated databases, text mining, co-expression; confidence ≥0.7; False Discovery Rate (FDR) stringency ON) to generate the protein–protein interaction (PPI) network and to perform Gene Ontology, including biological process, molecular function, and cellular component, as well as Kyoto Encyclopedia Genes and Genome pathway enrichment (Benjamini–Hochberg FDR) [12,13]. Networks and enrichment tables were exported from STRING and visualized in Cytoscape; degree and betweenness were computed to identify hubs [14].

Molecular docking analysis

Molecular docking was conducted to predict the binding affinity and interaction mode between the selected ligands and their respective receptor targets. The three-dimensional (3D) crystal structures of the target proteins were retrieved from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) [15]. Polar hydrogens were added, and Kollman partial charges were assigned to each receptor using AutoDockTools 1.5.7. Ligand structures were prepared by energy minimization and conversion into the PDBQT format, followed by the assignment of Gasteiger charges. Docking simulations were carried out using AutoDock Vina, with the exhaustiveness parameter set to 8 to balance computational efficiency and conformational sampling accuracy [16]. The grid box was centered on the experimentally defined binding pocket, extending by at least 10 Å in all spatial dimensions to allow sufficient ligand flexibility within the active site. For each ligand–receptor pair, multiple docking runs were performed to ensure reproducibility. The binding poses were ranked based on predicted binding affinity (ΔG, kcal/mol), and only the top-ranked conformations within a root-mean-square deviation (RMSD) ≤ 2.0 Å were retained as representative complexes. The consistency of binding orientation across independent runs was assessed to confirm convergence toward a stable docking solution.

Prediction of drug-likeness and pharmacokinetic properties

The drug-likeness and pharmacokinetic properties of the selected alkaloids from *M. koenigii* were evaluated using the SwissADME web server developed by the Swiss Institute of Bioinformatics [17]. The Simplified Molecular Input Line Entry System (SMILES) of each compound was obtained from the PubChem database and submitted to SwissADME for prediction. Key physicochemical descriptors, including molecular weight, number of hydrogen bond donors and acceptors, and octanol–water partition coefficient (Log $P_o/_t$), were calculated to assess compliance with Lipinski's Rule of Five. Additional pharmacokinetic parameters such as gastrointestinal (GI) absorption, blood–brain barrier (BBB) permeability, and bioavailability score were also predicted to evaluate oral bioavailability potential. Compounds satisfying all four of Lipinski's criteria (molecular weight < 500 Da, Log P ≤ 5, H-bond donors ≤ 5, and H-bond acceptors ≤ 10) were considered drug-like. Visualization of molecular properties, including the bioavailability radar and BOILED-Egg model, was used to qualitatively evaluate absorption and lipophilicity balance.

Results and Discussion

The GC-MS analysis of ethanol extract from *M. koenigii* revealed the presence of multiple bioactive compounds, several of which are known for their pharmacological significance. The most dominant peaks were observed at retention times of 48.00 and 48.39 minutes, corresponding to koenimbine ($C_{19}H_{19}NO_2$), with a cumulative area percentage of 26.24% (Table 1). Koenimbine and its derivatives are carbazole alkaloids reported to

exhibit potent antioxidant, antimicrobial, and cytoprotective effects, which may contribute to the therapeutic efficacy of *M. koenigii* in treating oxidative stress-related disorders such as male infertility [18].

Another major compound group appeared at 20.75% and 10.48% area, consisting of phytol ($C_{20}H_{40}O$) and hexadecanoic acid, methyl ester ($C_{17}H_{34}O_2$). Phytol is a diterpene alcohol known for its antioxidant, antimicrobial, and anti-inflammatory properties, which could help mitigate testicular inflammation and oxidative stress—two key factors in impaired male fertility [19]. The identification of hexadecanoic acid derivatives also supports the extract's potential as a bioactive agent, as alcohol-based terpenoids have been associated with hormonal modulation and reproductive health [20]. The presence of alkaloids and terpenoids with strong antioxidant and anti-inflammatory potential supports the hypothesis that *M. koenigii* leaf extract may contribute positively to male reproductive health. The diversity and dominance of these compounds in the ethanol extract suggest that *M. koenigii* has a promising phytochemical profile that warrants further investigation in fertility-related models.

The circular network diagram (Figure 1) illustrates the overall interaction topology among genes involved in reproductive regulation and steroidogenic pathways. Each diamond-shaped node represents a gene target, while the connecting circular nodes depict their interaction partners inferred from protein–protein interaction (PPI) and pathway co-association data.

Table 1. Identified bioactive compounds in ethanol extract of *M. koenigii* leaves based on GC-MS analysis

Peak	Retention time (min)	Identified compound	Area (%)	Compound class
1	23.77	Dodecanoic acid, methyl ester	1.30	Fatty acid ester
2	25.46	Caryophyllene oxide	2.56	Sesquiterpenoid
3	26.69	11,11-Dimethyl-4,8-dimethylenebicyclo[7.2.0]undecan-3-ol	0.63	Terpenoid
4	27.47	*trans*-α-Bisabolene epoxide	0.64	Terpenoid
5	28.35	Methyl tetradecanoate	1.11	Fatty acid ester
6	29.83	Tetradecanoic acid, ethyl ester	0.49	Fatty acid ester
7	30.76	Neophytadiene	2.89	Diterpenoid
8	30.91	2-Pentadecanone, 6,10,14-trimethyl-	2.02	Ketone
9	31.64	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.89	Terpenoid
10	32.34	Hexadecanal, 2-methyl-	0.48	Fatty aldehyde
11	32.56	Hexadecanoic acid, methyl ester	10.48	Fatty acid ester
12	33.87	Hexadecanoic acid, ethyl ester	4.58	Fatty acid ester
13	35.96	6-Octadecenoic acid, methyl ester (*Z*)-	2.93	Fatty acid ester
14	36.32	Phytol	20.75	Diterpenoid
15	36.48	Methyl stearate	1.56	Fatty acid ester
16	37.16	(*E*)-9-Octadecenoic acid ethyl ester	1.82	Fatty acid ester
17	37.62	Ethyl 13-methyl-tetradecanoate	0.90	Fatty acid ester
18	44.81	Girinimbine	6.28	Alkaloid
19	48.00	Koenimbin	26.24	Alkaloid

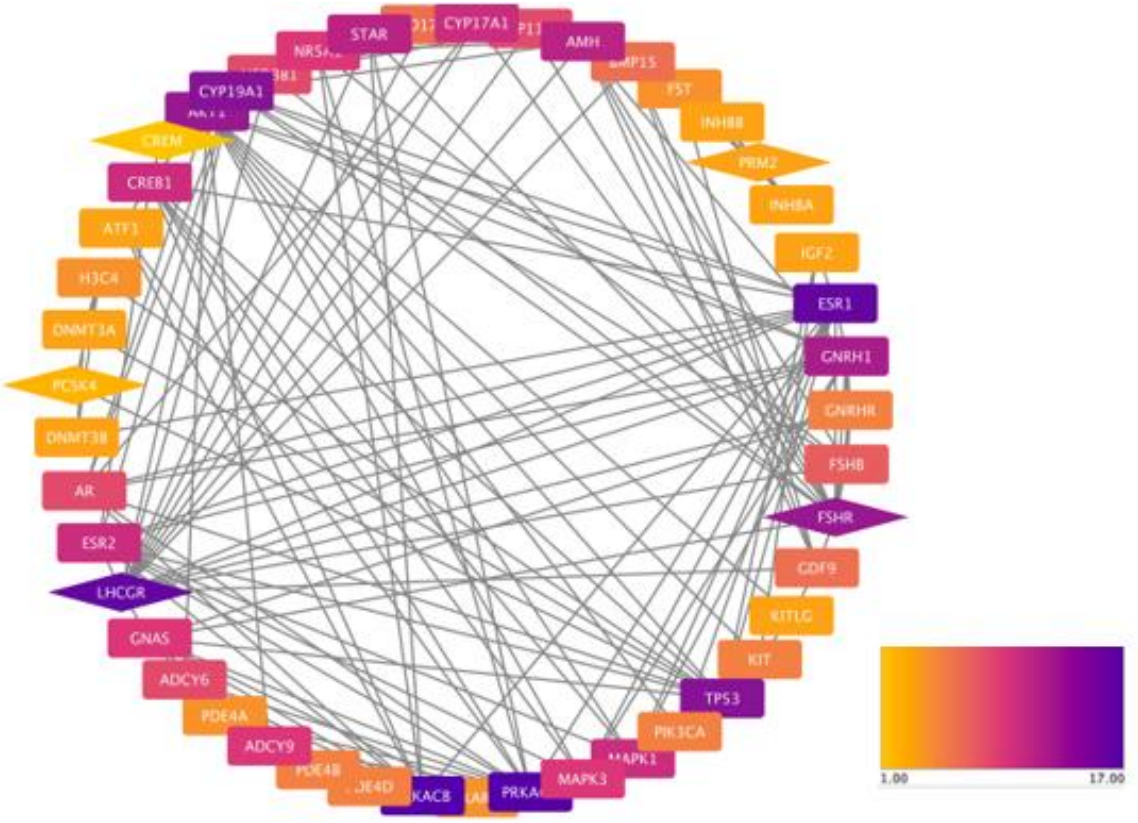


Figure 1. Network analysis of targets. The increasing purple color indicated higher degrees, while the increasingly yellow color signified lower degrees

The network shows a highly interconnected system with central nodes such as FSHR, LHCGR, estrogen receptor (*ESR1*), androgen receptor (*AR*), CYP19A1 aromatase (*CYP19A1*), and cAMP-responsive element modulator (*CREM*), which serve as major regulatory hubs. These genes are known to coordinate hormonal signaling between the hypothalamus–pituitary–gonadal (HPG) axis and local gonadal responses. For instance, *FSHR* and LHCGR (luteinizing hormone/choriogonadotropin receptor) are key G-protein-coupled receptors mediating folliculogenesis and steroidogenesis through cyclic AMP (cAMP) and protein kinase A (PKA) signaling cascades [21]. Meanwhile, *CYP19A1* (aromatase), *CYP17A1*, and *STAR* (steroidogenic acute regulatory protein) form the enzymatic core responsible for steroid biosynthesis, highlighting the tight coupling between receptor activation and downstream steroidogenic enzyme expression [22].

Network centrality analysis shows which genes play the most influential roles in the fertility-related regulatory network, which is likely derived from protein–protein interactions and gene co-expression patterns. The network structure reveals a tightly coordinated system in which hormone receptors and key signaling pathways, especially the cAMP/PKA and MAPK cascades, govern essential processes such as steroid hormone production, gamete development, and reproductive tissue differentiation. This organization reflects a system that is both stable and highly responsive, meaning that even small changes in major hub genes such as *ESR1* or *PRKACA* can have wide-ranging effects on reproductive function. Importantly, this network framework also helps explain how plant-derived compounds, such as

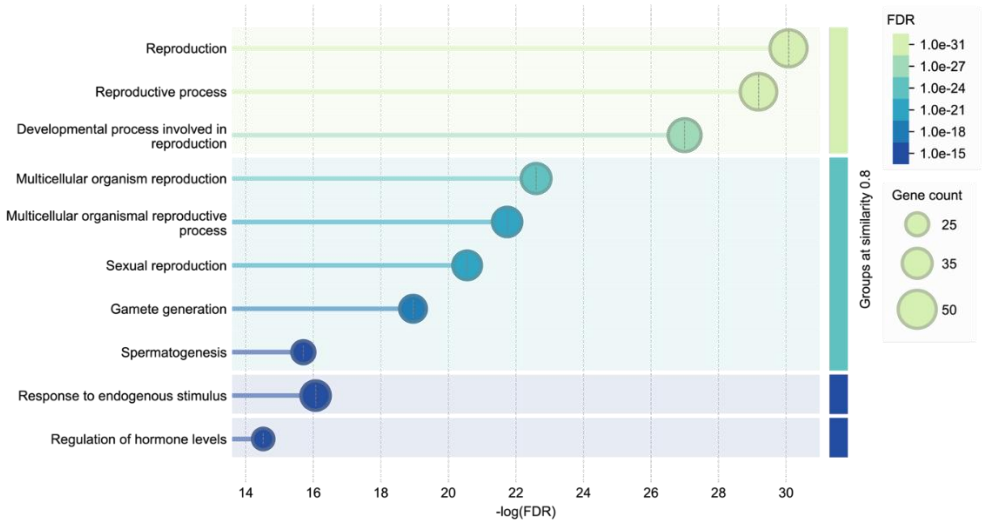
the bioactives from *M. koenigii*, may influence fertility by acting on central pathways involving FSHR, CREB1, and LHCGR.

Genes such as *CREM*, *PRM2*, and *PCSK4* are associated with spermatogenesis, sperm maturation, and capacitation (Table 2) [23]. Their interconnection with endocrine regulators (*e.g., AR* and *ESR1*) supports the hypothesis that the regulatory control of male reproductive function arises from multilayered cross-talk between hormonal, epigenetic, and transcriptional pathways. The dense interlinkage observed among *AR*, *ESR1*, *CREB1*, and MAPK family members (*MAPK1* and *MAPK3*) also suggests signal convergence between nuclear receptor-mediated transcription and kinase-driven post-translational regulation, consistent with previous findings that MAPK pathways can phosphorylate transcription factors and modulate steroidogenic gene expression [24]. Based on the network, the connection between each gene represents a complex, hormone-responsive gene architecture integrating transcriptional control, receptor activation, and second messenger signaling.

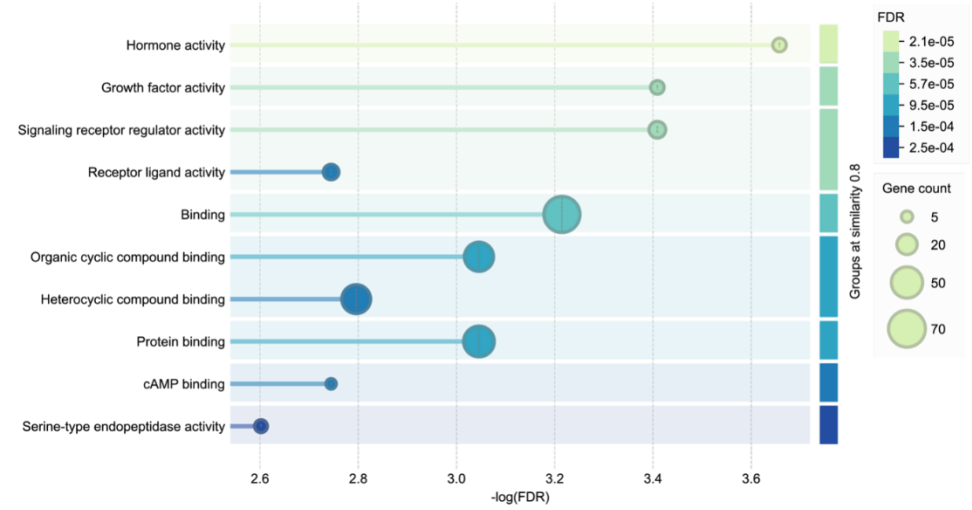
Table 2. List of genes with the highest degree from the network

Name	Description	Degree	Betweenness centrality	Closeness centrality	Clustering coefficient
PRKACB	cAMP-dependent protein kinase catalytic subunit beta	17	0.096	0.494	0.302
PRKACA	cAMP-dependent protein kinase catalytic subunit alpha	17	0.096	0.494	0.302
LHCGR	Luteinizing hormone/choriogonadotropin receptor	16	0.178	0.528	0.284
ESR1	Estrogen receptor alpha	16	0.161	0.561	0.375
CYP19A1	Cytochrome P450 family 19 subfamily A member 1 (aromatase)	14	0.048	0.494	0.451
TP53	Tumor protein p53	14	0.134	0.455	0.274
FSHR	FSHR	13	0.095	0.505	0.334
AKT1	AKT serine/threonine kinase 1	13	0.082	0.516	0.334
GNRH1	Gonadotropin-releasing hormone 1	12	0.038	0.484	0.454
STAR	Steroidogenic acute regulatory protein	11	0.042	0.46	0.563
AMH	Anti-Müllerian hormone	11	0.033	0.442	0.545
ESR2	Estrogen receptor beta	10	0.037	0.516	0.600
CYP17A1	Cytochrome P450 family 17 subfamily A member 1	10	0.008	0.425	0.645
CREB1	Cyclic AMP-responsive element-binding protein 1	10	0.058	0.451	0.556
MAPK1	Mitogen-activated protein kinase 1	10	0.013	0.465	0.667

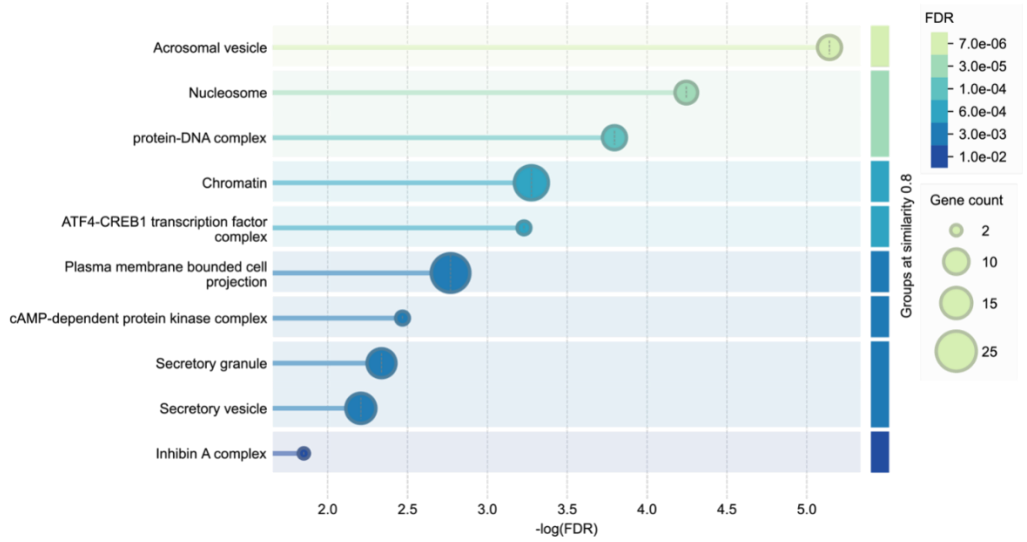
Gene Ontology (GO) enrichment analysis of the network (Figure 2) reveals (a) enriched biological processes, including the reproduction, reproductive processes, and developmental processes involved in reproduction; multicellular organism reproduction, multicellular organismal reproductive processes; sexual reproduction; gamete generation; spermatogenesis; response to endogenous stimuli; and regulation of hormone levels. (c) Enriched molecular functions, notably hormone activity, growth factor activity, signaling receptor regulator activity, receptor ligand activity, binding, organic cyclic compound binding, heterocyclic compound binding, protein binding, cAMP binding, and serine-type endopeptidase activity. (c) Enriched cellular components, such as acrosomal vesicles, nucleosomes, protein-DNA complexes, chromatin, ATF4-CREB1 transcription factor complexes, cAMP-dependent protein kinase complexes, secretory granules, secretory vesicles, and inhibin A complexes. (d) KEGG pathway enrichment analysis related to reproduction.



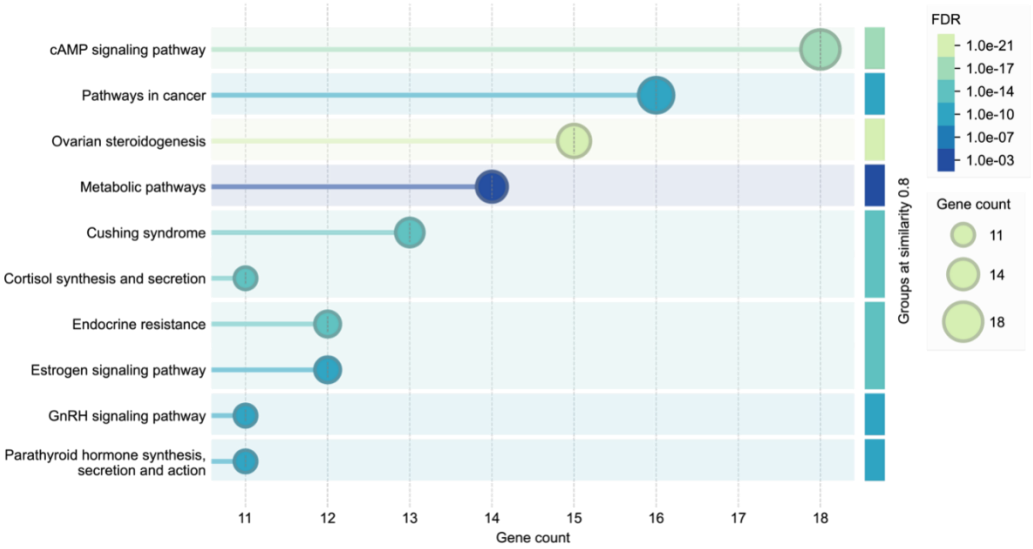
(a)



(b)



(c)



(d)

Figure 2. Enrichment of the gene ontology from the network (a) Biological Process (BP) enrichment; (b) Molecular Function (MF) enrichment; (c) Cellular Component (CC) enrichment; (d) Kyoto Encyclopedia Gene and Genomes (KEGG) Pathway

The analysis highlights significant enrichment in cAMP signaling pathway, the cancer pathway, ovarian steroidogenesis, metabolic pathway, Cushing's syndrome, cortisol synthesis and secretion, endocrine resistance, estrogen signaling pathway, GnRH signaling pathway, and parathyroid hormone synthesis, secretion, and action. Color intensity reflects the FDR, while bubble size corresponds to the number of associated genes. The STRING-derived interaction analysis revealed a set of associations among four proteins critically involved in male reproductive biology: CREM, gonadotropin-releasing hormone receptor (GNRHR), FSHR, and protamine 2

(PRM2). The network also highlights an association between FSHR and GNRHR, primarily supported by co-expression data. Both receptors are central to the hypothalamic–pituitary–gonadal (HPG) axis, with GNRHR mediating GnRH signals from the hypothalamus to the pituitary, thereby stimulating the secretion of FSH and LH, while FSHR is expressed in Sertoli cells, supporting spermatogenesis [25]. Although direct protein–protein binding is unlikely, functional coupling is evident: GnRH stimulation indirectly regulates FSHR signaling through gonadotropin secretion. The observed association reflects this physiological interplay, underscoring the importance of receptor crosstalk in maintaining male reproductive function. The gene ontology biological process (GO-BP) enrichment analysis revealed that the identified targets were significantly associated with biological processes fundamental to male reproductive function, including spermatogenesis, hormonal regulation, and the broader reproductive process. The enrichment in spermatogenesis indicates the involvement of genes essential for germ cell development, meiosis, and sperm maturation, which are critical for maintaining male fertility. The regulation of hormone secretion, particularly gonadotropins and androgens, further underscores the potential modulation of the hypothalamic–pituitary–gonadal (HPG) axis, a central regulatory system governing reproductive physiology [26].

In parallel, the KEGG pathway enrichment highlighted several key signaling cascades: the cyclic adenosine monophosphate (cAMP) signaling pathway, GnRH signaling pathway, steroidogenesis, estrogen signaling pathway, and chromatin organization. The cAMP pathway plays a pivotal role in mediating intracellular responses to hormonal stimuli, particularly in Leydig and Sertoli cells, thereby influencing testosterone biosynthesis and spermatogenic support [21]. The GnRH pathway regulates luteinizing hormone (LH) and FSH secretion, linking hypothalamic input to testicular function. Enrichment in steroidogenesis and estrogen signaling suggests modulation of enzymes and receptors that control sex hormone biosynthesis and feedback regulation, essential for maintaining hormonal homeostasis. Meanwhile, chromatin organization reflects transcriptional regulation and epigenetic remodeling required for sperm differentiation and chromatin condensation during spermatid maturation [27]. These enriched GO-BP terms and KEGG pathways collectively indicate that the investigated phytochemicals may exert their reproductive effects through multi-level regulation of hormonal signaling, gene expression, and spermatogenic processes, offering mechanistic insight into their potential as natural modulators of male fertility. FSH signaling in Sertoli cells promotes the expression of transcriptional regulators, including CREM, which in turn governs post-meiotic gene transcription such as PRM2 [28]. This cascade provides an indirect mechanistic bridge between the gonadotropin receptor signaling and chromatin remodeling machinery in sperm. Thus, although STRING assigns only modest confidence scores, existing biological evidence supports a hierarchical regulatory axis: FSHR → CREM → PRM2. Interrogating this network is especially relevant for evaluating *M. koenigii* phytochemicals as potential modulators of male fertility. Plant-derived ligands that engage FSHR or GNRHR could indirectly influence downstream CREM activity and ultimately PRM2 expression, thereby impacting sperm maturation. Previous studies have demonstrated that phytochemicals with phytoestrogenic or steroid-mimicking properties can interact with gonadotropin receptors or transcription factors, suggesting a plausible mechanism for botanical extracts in fertility modulation [29].

The docking results revealed that koenimbin exhibited the strongest overall binding across all five targets, particularly with LHR and PRM2, displaying binding energies as low as -8.9

kcal/mol (Figure 3). Girinimbine also demonstrated comparable affinities, suggesting strong receptor-ligand stability. These findings align with earlier studies in which carbazole alkaloids from *M. koenigii* induced apoptosis in cancer models and modulated mitochondrial pathways [30,31]. Phytol and caryophyllene oxide, while moderately less potent, still showed promising binding with CREM and PCSK4, reinforcing their reported roles in reducing oxidative stress and enhancing endocrine function. The data also indicated that alkaloid structures with aromatic rings and nitrogen heterocycles were particularly effective at forming strong interactions through hydrogen bonding and π–π stacking, which likely explains the higher binding affinities observed. In contrast, fatty acid esters and simpler hydrocarbons, although displaying weaker docking scores (around -4.0 to -6.0 kcal/mol), may serve as carriers or enhancers for drug delivery by improving solubility and bioavailability. The observed structure-activity relationship highlights the importance of molecular complexity and functional group positioning in driving receptor affinity.

Molecular docking of koenimbine and garinimbine against five male reproduction-related proteins, namely LHR, FSHR, proprotein convertase subtilisin/kexin type 4 (PCSK4), CREM, and protamine 2 (PRM2) revealed clear differences in binding affinity and interaction profiles (Table 3).

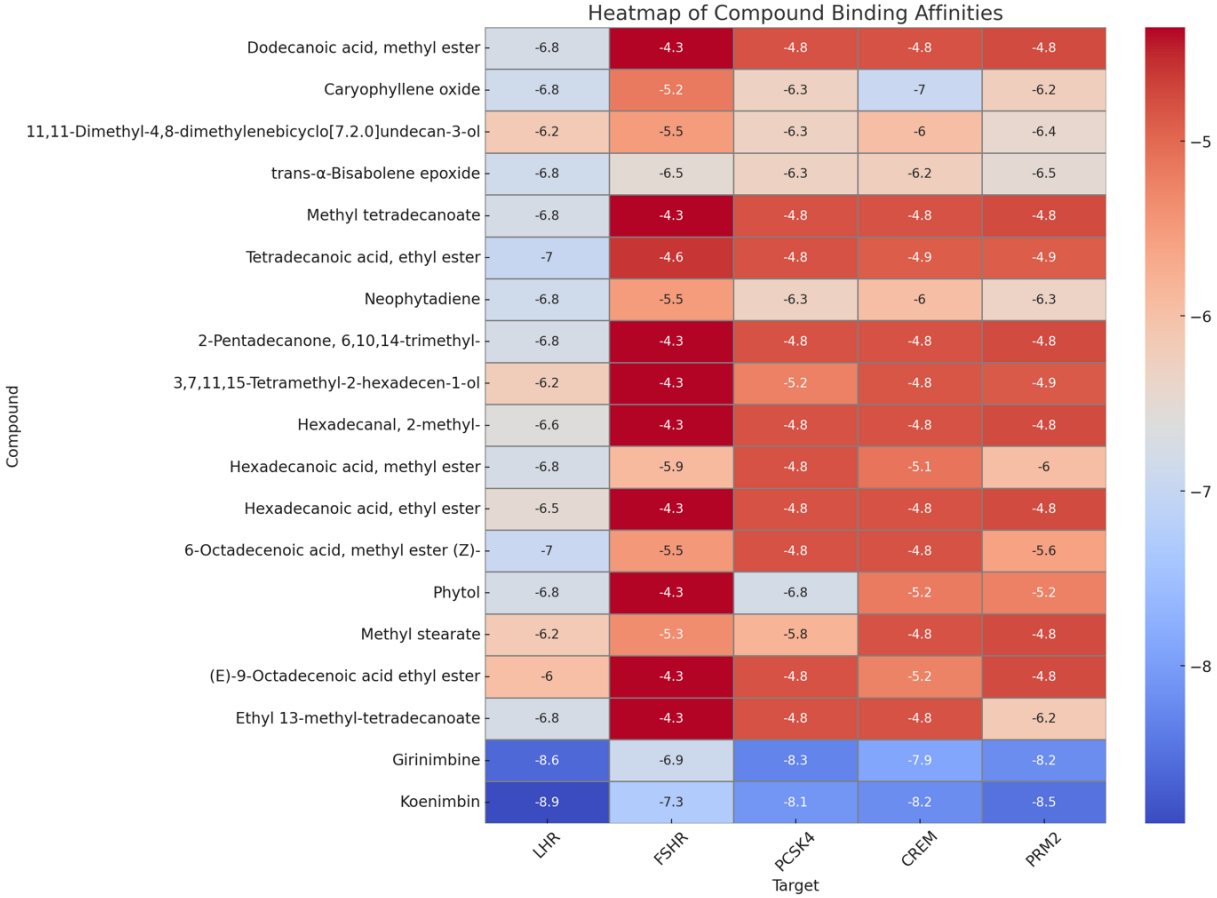
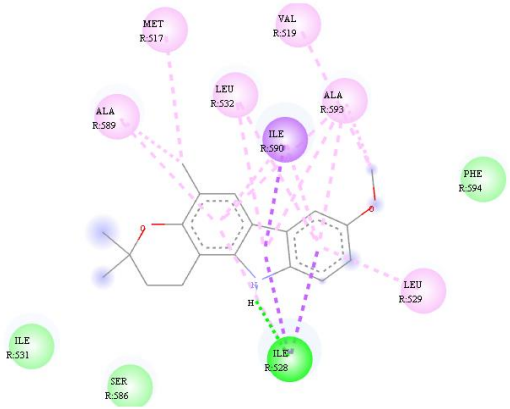
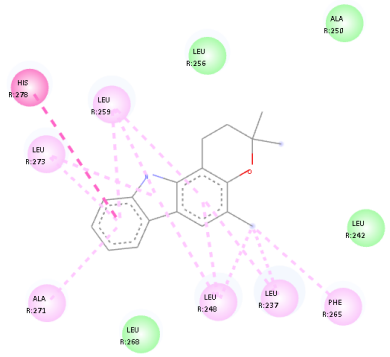
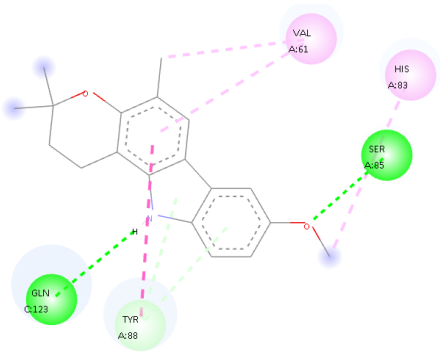
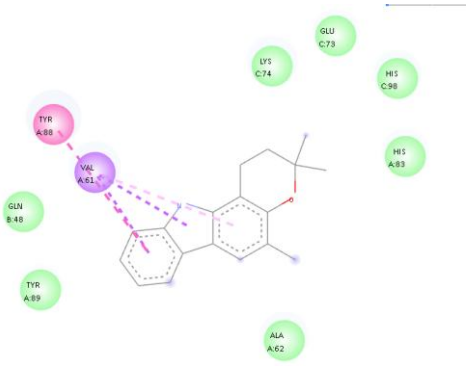
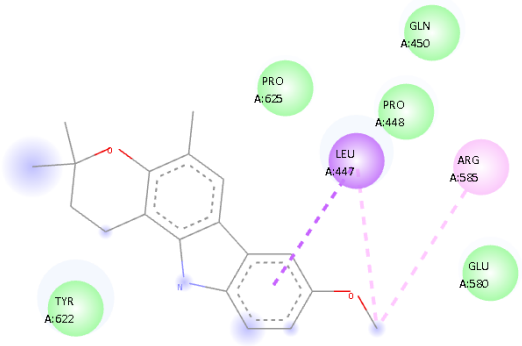
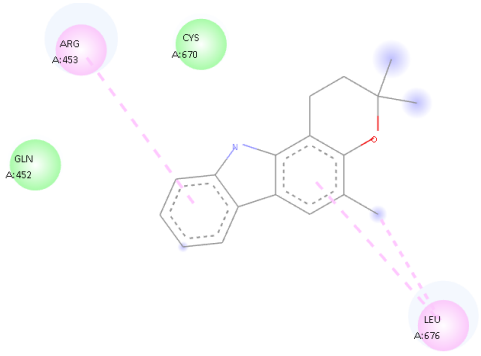
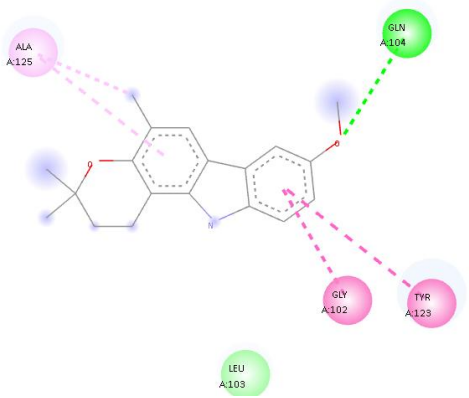
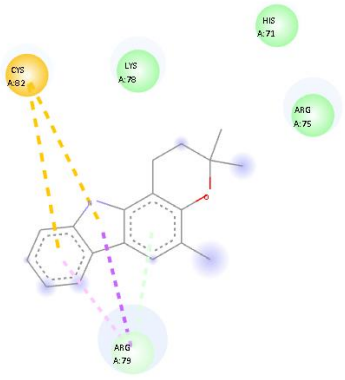
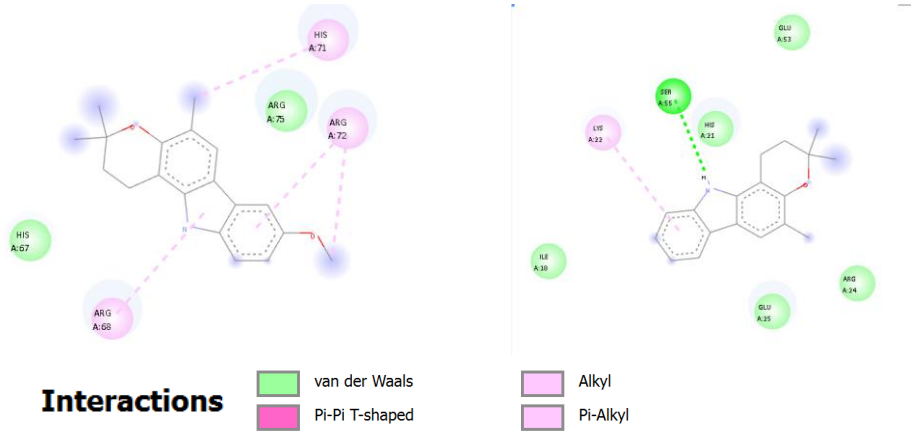


Figure 3. Heatmap of compound with binding free energy value (kcal/mol)

Table 3. 2D interactions of koenimbine and garinimbine with five receptors

Receptors	Koenimbine	Garinimbine
LHR		
FSHR		
PCSK4		
CREM		

PRM2



Koenimbine formed H-bonding and hydrophobic interactions with key residues in the putative ligand-binding domain, supporting a stable docking pose. The binding energy of –8.9 kcal/mol indicates a good affinity for the receptor. Garinimbine, in contrast, achieved a weaker binding score (–8.6 kcal/mol) and formed fewer stabilizing interactions. Given that LHR signaling regulates testosterone production and Leydig cell function, koenimbine's stronger interaction implies greater potential to modulate LH-mediated hormonal processes. The presence of both aromatic stacking and polar contacts likely contributes to its improved stabilization in the receptor pocket. FSHR plays a critical role in Sertoli cell activation and spermatogenesis. Koenimbine displayed the most favorable binding energy (–7.3 kcal/mol) among all protein–ligand pairs documented, accompanied by multiple van der Waals and π-interactions. Garinimbine again showed a lower affinity (–6.9 kcal/mol) with fewer hydrophobic anchors. The consistent superiority of koenimbine suggests its potential influence on FSH-driven maturation of germ cells. The predominantly hydrophobic architecture of the FSHR binding region appears better complemented by the planar aromatic system of koenimbine. PCSK4 is a sperm-specific protease essential for zona pellucida penetration and fertilization. Koenimbine bound PCSK4 with –8.1 kcal/mol, supported by hydrophobic interactions and at least one polar contact that stabilizes orientation within the catalytic cleft. Garinimbine has affinity (–8.3 kcal/mol) and showed interactions near the putative active site. The ability of koenimbine and garinimbine to engage residues lining the substrate recognition region may imply potential interference with PCSK4's proteolytic function, aligning with previously suggested contraceptive mechanisms targeting sperm proteases. CREM is a master transcription factor regulating post-meiotic spermatogenesis. Koenimbine again achieved stronger binding (–8.0 kcal/mol) than garinimbine (–7.2 kcal/mol), forming stabilizing contacts near residues implicated in DNA-binding regulation. Although transcription factors are structurally challenging drug targets, the docking pattern suggests that koenimbine may alter CREM conformation or impede co-factor interactions. This aligns with the broader hypothesis that CREB/CREM family modulation can suppress spermiogenesis-related gene expression. PRM2 is responsible for final chromatin condensation in sperm nuclei. Both ligands showed moderate affinities, but koenimbine (–8.5 kcal/mol) again outperformed garinimbine (–8.2 kcal/mol). The interactions were predominantly hydrophobic, consistent with the protein's basic, arginine-rich structure. Although PRM2 is not a traditional small-molecule target, the docking results suggest that koenimbine might interact with key residues involved in protamine cross-linking, potentially disrupting sperm chromatin stability. Overall,

koenimbine consistently demonstrated stronger binding energies and more extensive stabilizing contacts compared with garinimbine, suggesting that it may exert broader modulatory activity across hormonal regulation, sperm maturation, and chromatin compaction pathways. Across all evaluated proteins, koenimbine consistently demonstrated lower (more negative) binding energies and more extensive intermolecular interactions than garinimbine. This indicates a more stable ligand–protein complex and suggests broader bioactivity across hormonal signaling (LHR and FSHR), sperm maturation pathways (CREM), proteolytic regulation (PCSK4), and chromatin packaging (PRM2). The superiority of koenimbine may be attributed to its balanced combination of aromatic rings, hydrophobic surface area, and limited but well-positioned polar groups, which allow it to anchor effectively in diverse protein microenvironments.

The drug-likeness evaluation of koenimbine and girinimbine, two major carbazole alkaloids from *M. koenigii*, revealed that both compounds exhibit favorable physicochemical profiles consistent with orally active small molecules (Table 4). Their molecular weights (293.36 g/mol for koenimbine and 263.33 g/mol for girinimbine) fall well below the 500 Da threshold stipulated by Lipinski's Rule of Five, suggesting good membrane permeability and metabolic stability [32]. The presence of only two and one hydrogen-bond acceptors, respectively, and a single donor in each molecule further supports an optimal balance between polarity and hydrophobicity—an essential determinant for passive diffusion across biological membranes [33]. Both compounds displayed moderate lipophilicity, with Log P values around 4.1–4.2, indicating a balance between aqueous solubility and lipid affinity suitable for oral bioavailability. The high gastrointestinal absorption predicted for both molecules reinforces their suitability for oral administration, which is consistent with the lipophilic yet compact structure of carbazole alkaloids known to readily traverse biological membranes. Importantly, both compounds satisfy all Lipinski criteria, implying low risk of oral bioavailability issues and supporting their potential as lead scaffolds for further optimization.

The combination of gene–gene and compound–target network analyses reveals a multilayered signaling network linking steroid biosynthesis, cAMP/PKA signaling, MAPK cascades, and transcriptional regulation (Figure 4). This integrative topology underscores the systems-level mechanism by which koenimbine may exert its pharmacological effects on reproductive tissues by balancing hormonal output, modulating second messenger homeostasis, and influencing epigenetic regulation. The visualization demonstrates how a single bioactive compound from *M. koenigii* can interface with key reproductive signaling genes across multiple biological levels, reinforcing the polypharmacological nature of natural metabolites in modulating fertility-associated pathways.

Table 4. Druglike properties of selective compound from *M. koenigii*

No.	Parameter	Koenimbine	Garinimbine
1	Molecular formula	$C_{19}H_{19}NO_2$	$C_{18}H_{17}NO$
2	Molecular weight	293.36 g/mol	263.33 g/mol
3	Number H-bond acceptors	2	1
4	Number H-bond donors	1	1
5	Log $P_{o/w}$	4.15	4.17
6	Gastrointestinal absorption	High	High
7	Meet Lipinski Rules	Yes	Yes

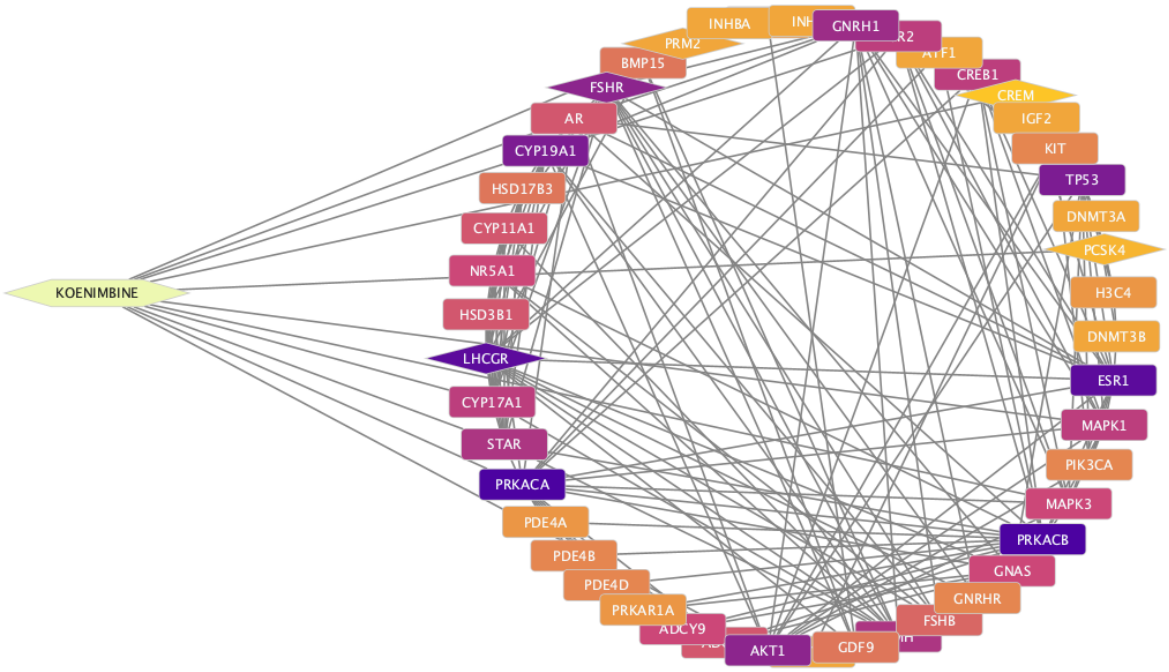


Figure 4. Integrative connection between the koenimbine compound and genes related to fertility

Notably, the target receptors examined in this study represent a comprehensive cross-section of male fertility mechanisms. LHR and FSHR are gonadotropin receptors essential for testosterone production and spermatogenic stimulation. PCSK4 is a testis-specific protease involved in the activation of proenzymes necessary for sperm maturation. CREM plays a pivotal role in regulating the expression of post-meiotic genes in spermatids, while PRM2 is integral to DNA condensation during spermiogenesis. The ability of *M. koenigii* compounds to bind with these diverse targets suggests their therapeutic potential spans multiple stages of the spermatogenic process. Moreover, the use of *in silico* methods such as molecular docking provides a cost-effective and high-throughput strategy for preclinical screening of herbal extracts. By predicting receptor-ligand binding affinities, such studies can prioritize the most promising candidates for downstream validation in cellular and animal models. These computational findings provide a strong rationale for further pharmacological investigation, including isolation of pure compounds, mechanistic studies, and toxicity profiling. Koenimbine, a carbazole alkaloid isolated from the ethanolic extract of *M. koenigii* (L.), has demonstrated remarkable cytotoxic and chemopreventive potential against various cancer cell lines. In breast cancer (MCF-7) cells, koenimbine induces apoptosis through the mitochondrial-mediated pathway by downregulating the anti-apoptotic protein Bcl-2 and upregulating the pro-apoptotic protein Bax, leading to cytochrome *c* release into the cytosol and consequent loss of mitochondrial membrane potential [34]. This apoptotic induction is accompanied by significant sub-G0 cell-cycle arrest ($p < 0.05$). In colon cancer models, koenimbine also exhibits cytotoxic activity and modulates gene expression within the Wnt/β-catenin signaling pathway, suggesting its broader role in regulating oncogenic transcriptional programs [35]. Furthermore, a semi-synthetic koenimbine derivative—8-methoxy-3,3,5-trimethylpyrano[3,2-a]carbazole-11(3H)-yl)(3-(trifluoromethyl)phenyl) methanone—has

shown potent anti-inflammatory activity in RAW 264.7 macrophages by inhibiting NF-κB and MAPK pathways, thereby reducing the production of pro-inflammatory mediators such as NO, IL-1β, TNF-α, LTB4, and IL-6 [36]. Similarly, girinimbine, another major carbazole alkaloid from *M. koenigii*, demonstrates dual chemopreventive and anti-inflammatory actions through apoptosis induction and suppression of inflammatory signaling [37]. Together, these findings highlight the therapeutic promise of *M. koenigii*–derived carbazole alkaloids, particularly koenimbine and girinimbine, as multitarget natural agents with potential applications in cancer prevention and early-stage cancer therapy.

Conclusion

GC-MS analysis of ethanol extract of *M. koenigii* leaf identified 19 distinct compounds, including alkaloids (koenimbin and girinimbine), terpenoids (phytol and caryophyllene oxide), and esters (methyl stearate and hexadecanoic acid derivatives). These compounds were subsequently docked against five male fertility-associated receptors to evaluate their binding affinity and therapeutic potential. The results demonstrate that *M. koenigii* harbors phytochemicals capable of engaging with critical male fertility–related proteins, particularly along the FSHR–CREM–PRM2 signaling axis. Such interactions suggest a plausible molecular basis for its ethnomedicinal use in reproductive health. While computational predictions provide a strong rationale, experimental validation is essential to confirm the pharmacological relevance of these interactions. Overall, this study establishes *M. koenigii* as a promising candidate for botanical drug discovery aimed at addressing male infertility and related reproductive disorders.

Disclosure Statement

No potential conflict of interest was reported by the authors.

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