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Unveiling anticancer mechanisms of *Withania somnifera*: An integrated computational study targeting ERBB2

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Withania somnifera (Ashwagandha) is a widely used traditional medicinal plant with reported anticancer activity. However, the precise molecular mechanisms underlying its anticancer effects in breast cancer remain unclear. This study employed an integrated network pharmacology and molecular docking approach to investigate the potential therapeutic targets and pathways of phytoconstituents from *W. somnifera*. Thirty-five bioactive compounds were identified from literature and phytochemical databases, and their protein targets were predicted using DIGEP-Pred. Breast cancer-related genes were retrieved from GeneCards, and overlapping targets were identified using Venny. Protein–protein interaction networks were constructed using STRING, analysed in Cytoscape, and functional modules were identified using MCODE. Gene Ontology and KEGG enrichment analyses revealed involvement in apoptosis regulation, hormone receptor signaling, and key pathways, including PI3K-Akt, MAPK, FoxO, and p53. An integrated compound–target–pathway network highlighted Esculetin, FOXO1, and Pathways in Cancer as central nodes. Molecular docking against ERBB2 (PDB ID: 3PP0) using Schrödinger Glide XP identified Withasomnine and Scopoletin as top binders, forming key hydrogen bonds with MET801, comparable to established inhibitors. These findings suggest that *W. somnifera* may exert anti-breast cancer effects via multi-target, multi-pathway modulation, including the regulation of apoptosis and hormone receptor signaling. The identification of Withasomnine and Scopoletin as potential ERBB2 modulators supports further *in vitro and in vivo* studies and their prospective application in complementary anticancer strategies.

Keywords: Anticancer, ERBB2, MCODE, Network pharmacology, Protein–protein interaction, *Withania somnifera*

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Introduction

Breast cancer is a major global health challenge and the most frequently diagnosed malignancy among women, with approximately 2.3 million new cases and 6,85,000 deaths reported worldwide in 2020, according to the World Health Organisation[1]. Despite significant advances in early detection and the development of chemotherapy, hormone therapy, targeted therapy, and immunotherapy, clinical outcomes are often compromised by severe adverse effects, drug resistance, and disease recurrence, which limit therapeutic success and reduce quality of life[2,3]. A distinct and clinically aggressive subset of breast cancers is characterised by amplification or overexpression of the human epidermal growth factor receptor 2 (HER2/ERBB2), a ligand-independent receptor tyrosine kinase belonging to the ErbB family[4,5]. ERBB2 activation predominantly occurs through heterodimerization with other ErbB family members, initiating autophosphorylation of the intracellular domain and recruitment of signaling complexes[6]. This activation drives oncogenic programs via the PI3K–AKT–mTOR axis[7], which promotes cell survival, metabolic reprogramming, and resistance to apoptosis, and the RAS–RAF–MEK–ERK cascade, which regulates proliferation and cell-cycle progression[8]. In addition, ERBB2 signaling intersects with the tumour microenvironment, influencing angiogenesis, immune modulation, and extracellular matrix remodeling, while also engaging DNA damage response pathways and anti-apoptotic regulators such as the BCL-2 family[9,10]. These interactions collectively enhance tumour aggressiveness, metastatic potential, and resistance to therapy.

HER2-targeted agents, including monoclonal antibodies, such as trastuzumab and pertuzumab[11],

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antibody–drug conjugates such as trastuzumab and emtansine[12], and tyrosine kinase inhibitors such as lapatinib, neratinib, and tucatinib[13], have significantly improved progression-free and overall survival in early-stage and metastatic HER2-positive breast cancer. Mechanisms of resistance include compensatory activation of alternative receptor tyrosine kinases, reactivation of downstream signaling due to mutations in PI3K or loss of PTEN, and spatial and temporal heterogeneity of HER2 expression within tumours[14,15]. Furthermore, the occurrence of toxicities, especially cardiotoxicity linked to certain monoclonal antibodies and tyrosine kinase inhibitors, limits their prolonged administration and use in combination regimens[16,17]. In addition, high costs and limited accessibility, especially in resource-constrained healthcare settings, further hinder the widespread application of these targeted therapies.

The need for novel therapeutic strategies that are effective, safe, affordable, and capable of modulating multiple oncogenic pathways has renewed interest in plant-derived natural products[18]. Historically, natural products such as paclitaxel and vincristine have provided the foundation for major chemotherapeutic agents, partly due to their inherent ability to interact with diverse molecular targets and cellular processes, potentially mitigating the risks associated with pathway redundancy and adaptive resistance[19].

Withania somnifera (L.) Dunal, commonly known as Ashwagandha, belongs to the family Solanaceae and is considered a cornerstone of traditional Ayurvedic medicine. It contains a diverse array of bioactive secondary metabolites, including steroidal lactones (withanolides such as withaferin A), alkaloids, and other phytoconstituents, which have been reported to exhibit anticancer, anti-inflammatory, and immunomodulatory activities[20]. However, the therapeutic potential of ERBB2-positive breast cancer remains largely unexplored. In this study, we employed an integrated network pharmacology approach to systematically evaluate the anti-breast cancer potential of *W. somnifera* against ERBB2-driven disease. This methodology combines phytochemical target prediction, gene–disease association mapping, protein–protein interaction (PPI) network construction, and Molecular Complex Detection (MCODE) cluster analysis to identify hub proteins, enriched biological processes, and key signaling pathways. Through this systems-level investigation, we aim to elucidate the molecular basis of the multi-target, multi-pathway actions of *W. somnifera*, thereby providing a mechanistic rationale for its potential development as a plant-derived therapeutic or adjuvant for HER2-positive breast cancer treatment.

Materials and Methods

Identification and collection of phytoconstituents of *W. somnifera*

A comprehensive collection of phytoconstituents present in *W. somnifera* was undertaken through a systematic review of the scientific literature and phytochemical databases. Peer-reviewed research articles were retrieved using keyword combinations such as *W. somnifera* phytochemicals, *W. somnifera* bioactive compounds, and ethnomedicinal uses of *W. somnifera* from established electronic databases, including Scopus and Google Scholar. Additionally, structured data were mined from specialised phytochemical and ethnobotanical databases such as Dr. Duke's Phytochemical Database and the Indian Medicinal Plants Phytochemistry[21] and Therapeutics (IMPPAT) database[22]. The chemical identities of the bioactive constituents were confirmed using the PubChem database[23], and their SMILES (Simplified Molecular Input Line Entry System) notations were retrieved to facilitate downstream *in silico* target prediction analysis.

Target prediction of *W. somnifera* phytoconstituents and cancer-associated geness

The biological targets of each identified phytoconstituent were predicted using the DIGEP-Pred database[24], a web-based tool that utilises 2D and 3D molecular similarity to predict probable protein targets in *Homo sapiens*. Only those targets with a probability score >0.5 were retained for further analysis to enhance prediction specificity. For disease relevance, cancer-associated genes were compiled using the GeneCards database[25]. The keyword "cancer" was used to screen for relevant oncogenes and tumour suppressor genes. In the GeneCards database, a relevance score cutoff of >17.50 was applied to filter genes with strong associations with cancer-related pathologies. The overlapping targets between the predicted phytoconstituent targets and the cancer-related genes were identified using Venny 2.1.0,[26] a Venn diagram-based tool for intersection analysis. These common targets were subsequently selected for protein-protein interaction (PPI) network construction and clustering analysis[26].

Construction of compound–target interaction network

The intersection of *W. somnifera* compound targets and cancer-associated genes served as the basis for the compound–target interaction network. This network was visualised and analysed using Cytoscape version 3.10.3. Each node represented a compound or a gene/protein target, and edges denoted the interactions or associations between them. Topological parameters, such as degree centrality (number of connections per node) and betweenness centrality (influence of a node over the flow of information), were calculated using the NetworkAnalyzer tool integrated into Cytoscape to identify key bioactive compounds and potential hub targets[27].

Construction of the Protein–Protein Interaction (PPI) network

To investigate the molecular interactions among the shared target genes, a PPI network was constructed using the STRING database[28]. The species was limited to *Homo sapiens*, and a minimum required interaction score of 0.400 (medium confidence) was applied. The resulting network was exported in tab-delimited format and imported into Cytoscape version 3.10.3 for advanced visualisation and analysis[29]. For the identification of highly interconnected regions within the PPI network, the Molecular Complex Detection (MCODE) plugin in Cytoscape was employed[30]. These clusters, or molecular complexes, often represent functionally significant modules or signaling hubs in biological systems. The MCODE analysis was performed using the following optimised parameters: Degree Cutoff: 2, Node Score Cutoff: 0.2, K-Core: 2, and Max Depth: 100. The top-scoring clusters were selected for further enrichment analysis to identify the most functionally relevant gene modules involved in cancer pathways[30].

Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis

The core targets derived from the PPI network and significant MCODE clusters were subjected to functional enrichment analysis using the KEGG database and GO annotations. GO enrichment was conducted for three major categories: Biological Process (BP), Cellular Component (CC), and Molecular Function (MF). KEGG pathway analysis was performed to uncover key signalling cascades and biological pathways implicated in cancer progression and therapy. Only pathways and GO terms with p-values <0.05 were considered statistically significant. For graphical representation and easier biological interpretation, a Scatter Ratio (SR) plot was constructed to visualise enriched pathways and GO terms. These visualisations provided insights into the functional roles of the target genes and the mechanistic pathways modulated by the phytoconstituents of *W. somnifera* in the context of cancer[31].

Construction of a network

To construct the interaction networks, two distinct files were prepared: (i) a compound-target file, containing the names of bioactive compounds from *W. somnifera* and their corresponding predicted protein targets, and (ii) a pathway-target file, listing enriched KEGG pathways and the associated genes involved. These files were imported into Cytoscape (v3.10.3) for network visualisation and analysis. Both networks were customised using the "Style" panel to apply specific visual mappings based on node attributes such as node type, size (based on degree centrality), and colour (based on function or classification). The "Merge Networks" feature in Cytoscape was optionally used to integrate both networks into a comprehensive compound–target–pathway network, providing a systems-level view of how *O. corniculata* phytoconstituents potentially modulate cancer-related pathways. Topological parameters such as degree, closeness, and betweenness centrality were analysed using the NetworkAnalyzer plugin to identify key regulatory nodes (hub genes or major compounds), thereby offering mechanistic insights into the multi-target and multi-pathway interactions underlying the anticancer potential of *W. somnifera*[32].

Molecular docking studies

Molecular docking studies were performed using Schrödinger's Glide software in Extra Precision (XP) mode to investigate the binding orientations and molecular interactions of selected inhibitors within the active site of the ERBB2 receptor[33]. The XP mode employs an advanced scoring function that accounts for steric clashes and desolvation effects, along with enhanced conformational sampling to improve prediction accuracy and minimise false positives. Top-ranked docking poses were analysed based on key molecular interactions, including hydrogen bonding, hydrophobic contacts, and π–π stacking interactions.

Ligands were preprocessed using the LigPrep module, which included energy minimisation and the

generation of low-energy conformers with appropriate protonation states. Torsional flexibility was incorporated using the OPLS4 force field to ensure optimised geometry and accurate electronic properties[34]. The crystal structure of ERBB2 (PDB ID: 3PP0), co-crystallised with the inhibitor 03Q [2-{2-[4-({5-chloro-6-[3-(trifluoromethyl) phenoxy] pyri dine-3-yl}amino)-5H-pyrrolo[3,2-d]pyrimidin-5-yl]et hoxy}ethanol], was retrieved from the Protein Data Bank. The key amino acid residues present in the protein binding site included ALA706, PRO707, ASN708, GLN709, ALA710, LEU711, ARG713, ILE714, LEU715, LYS716, GLU717, THR718, GLU719, LEU720, VAL723, GLY727, SER728, GLY729, ALA730, PHE731, GLY732, THR733, VAL734, and TRP739. The protein structure was prepared using Schrödinger's Protein Preparation Wizard, which involved removing water molecules, assigning proper bond orders, optimising hydrogen-bonding networks, predicting pKa values at physiological pH (7 ± 2), and performing energy minimisation using the OPLS4 force field. The receptor grid for docking was generated from the position of the co-crystallised ligand to accurately define the binding pocket. All selected compounds were subsequently docked into this active site to evaluate their potential as dual inhibitors of the ERBB2 receptor[35].

Results and Discussion

Identification of phytoconstituents from *W. somnifera*

Through a literature survey and mining of phytochemical databases, a total of 35 bioactive compounds were identified in *W. somnifera*. These included major classes such as flavonoids, phenolic acids, terpenoids, and sterols. The chemical structures and SMILES notations of these compounds were retrieved from the PubChem database for subsequent target prediction analysis.

Prediction of potential targets for phytoconstituents and identification of cancer-associated genes

Using the DIGEP-Pred database[36], approximately 692 unique human protein targets were predicted for the 35 phytochemicals, with each compound associated with multiple targets. After filtering with a probability threshold of >0.5, only the high-confidence targets were retained. Simultaneously, a total of 913 breast cancer-associated genes were retrieved from GeneCards and filtered to retain those with a relevance score >17.50 to ensure strong association with cancer. By comparing the compound-related targets with the cancer-associated gene set using Venny 2.1, 87 overlapping targets were identified. These common targets were considered potential mediators of the anticancer effects of *W. somnifera* and were selected for subsequent network and enrichment analyses.

Protein–Protein Interaction (PPI) Network and MCODE cluster analysis

The 87 overlapping targets were submitted to the STRING database to construct the PPI network, which comprised 87 nodes and 643 edges (expected number of edges: 242; average node degree: 14.8, and average local clustering coefficient: 0.504). The network was visualised and analysed in Cytoscape 3.10.3, revealing dense interconnectivity among key proteins involved in cancer-related signalling (Fig. 1). MCODE (Molecular Complex Detection) cluster analysis was used to identify densely connected regions within the protein-protein interaction (PPI) network, which often represent functional modules or molecular complexes. In breast cancer, MCODE analysis revealed five significant clusters involving key regulatory genes. Cluster 1 includes central oncogenes and transcription factors such as KRAS, CTNNB1, EP300, and ERBB2, which drive tumour proliferation, angiogenesis, and metastasis through pathways including PI3K/AKT, Wnt/β-catenin, and MAPK signaling. Cluster 2 comprises genes including CASP8, MMP2, and MET, which play roles in apoptosis evasion, extracellular matrix remodeling, and tumour invasiveness. Cluster 3, featuring AURKA, CHEK1, and MDM2, is associated with cell cycle dysregulation, DNA damage response, and p53 inhibition-key hallmarks of breast cancer progression. Clusters 4 and 5 include DNA repair genes such as ERCC2 and ERCC3, as well as tumour suppressors such as MSMB and RNASEL, indicating the disruption of genomic stability. Collectively, these MCODE clusters highlight interconnected gene modules that orchestrate breast cancer development, providing insights into potential therapeutic targets for multi-gene modulation strategies. The results are summarised in Table 1 and illustrated in Fig. 2.

GO and KEGG pathway enrichment analysis

The integrated GO enrichment analysis, encompassing biological processes, cellular components, and molecular functions, provides a comprehensive mechanistic landscape of breast

Table 1 — MCODE (Molecular Complex Detection) analysis

Cluster	Score	Nodes	Edges	Targets
1	10.909	23	120	AURKB, CCL5, CD44, CTNNB1, DNMT1, ELAVL1, EP300, ERBB2, ESR2, FLT1, FOXO1, KLK3, KRAS, KRT18, NFE2L2, PGR, PLAU, PRKCA, RARA, TNFRSF10B, TOP2A, VIM, WT1
2	6.2	11	31	AR, CASP8, CCND2, CDKN2B, ETS1, MCL1, MET, MMP2, NOTCH1, NPM1, STAT5A
3	3.6	6	9	AURKA, CHEK1, FANCI, MAPK8, MDM2, NR3C1
4	3	3	3	ERCC2, OCG1, ERCC3
5	3	3	3	ELAC2, MSMB, RNASEL

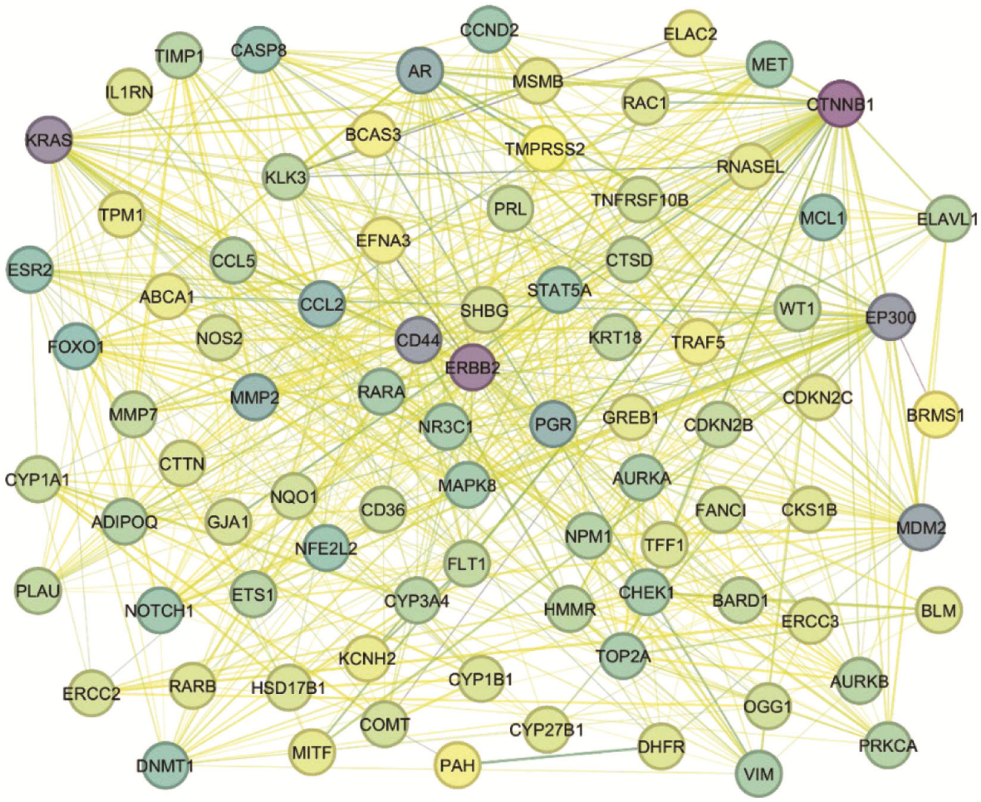

Fig. 1 — Protein–protein interaction (PPI) network of overlapping targets associated with *Withania somnifera* phytoconstituents and ERBB2-related breast cancer. The network was generated using the STRING database and visualised in Cytoscape. Nodes represent proteins, while edges indicate protein–protein interactions. Highly connected nodes denote key hub proteins involved in anticancer activity.

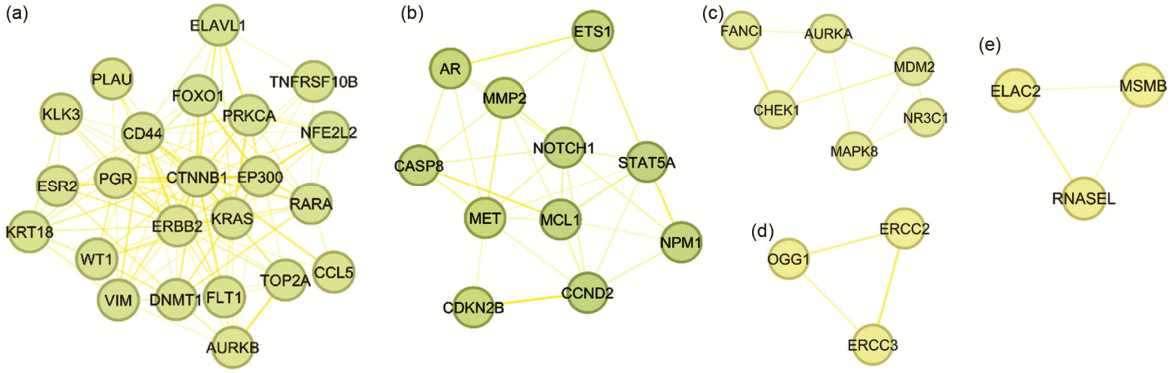

Fig. 2 — Molecular Complex Detection (MCODE) analysis of the protein–protein interaction network. MCODE analysis was performed using Cytoscape to identify highly interconnected clusters and significant hub modules within the network associated with *Withania somnifera* phytoconstituents and ERBB2-related breast cancer.

cancer progression and potential therapeutic intervention. Key biological processes such as the regulation of programmed cell death (GO:0043067), apoptotic process (GO:0042981), and regulation of cell death (GO:0010941), underscoring the pivotal role of apoptosis and cell survival in determining tumour response to therapy, are mediated by genes like AURKA, MDM2, FOXP1, and CTNNB1. Simultaneously, pathways involved in the positive and negative regulation of biological (GO:0048518/GO:0048519) and cellular processes (GO:0048522/GO:0048523) indicate the dual modulatory effects on tumour proliferation and drug resistance. From the cellular component perspective, enriched locations such as the nuclear lumen (GO:0031981), nucleoplasm (GO:0005654), and chromatin (GO:0000785) underscore the importance of transcriptional regulation, DNA repair, and epigenetic control by factors including NR3C1, RARA, DNMT1, and EP300. Moreover, cytosol (GO:0005829) and focal adhesion sites (GO:0005925) contribute to signal transduction, stress response, and metastatic behaviour via KRAS, NFE2L2, and VIM. In terms of molecular function, the enrichment of protein binding (GO:0005515), enzyme binding (GO:0019899), and steroid binding (GO:0005496) points to the functional significance of protein-protein interactions and hormone receptor signaling in breast cancer, involving key targets like ERBB2, ESR2, AR, and PGR. Additionally, nuclear receptor activity (GO:0004879) and transcription coregulator binding (GO:0001221) reflect the transcriptional and hormonal dynamics driving breast cancer subtypes. Metal ion-binding functions such as zinc ion binding (GO:0008270) and transition metal ion binding (GO:0046914) also highlight the involvement of metalloproteins (MMP2, DNMT1) in invasion, redox homeostasis, and ECM remodeling. Collectively, these GO terms and associated genes provide a multi-dimensional view of breast cancer biology, offering vital insights into cellular localization, functional dynamics, and actionable therapeutic targets. The results are shown in Fig. 3.

KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analysis

The KEGG pathway enrichment analysis highlights several critical signaling and regulatory pathways implicated in breast cancer development, progression, and treatment. Notably, the pathways in cancer (hsa05200) and microRNAs in cancer (hsa05206) are significantly enriched, involving key oncogenes and

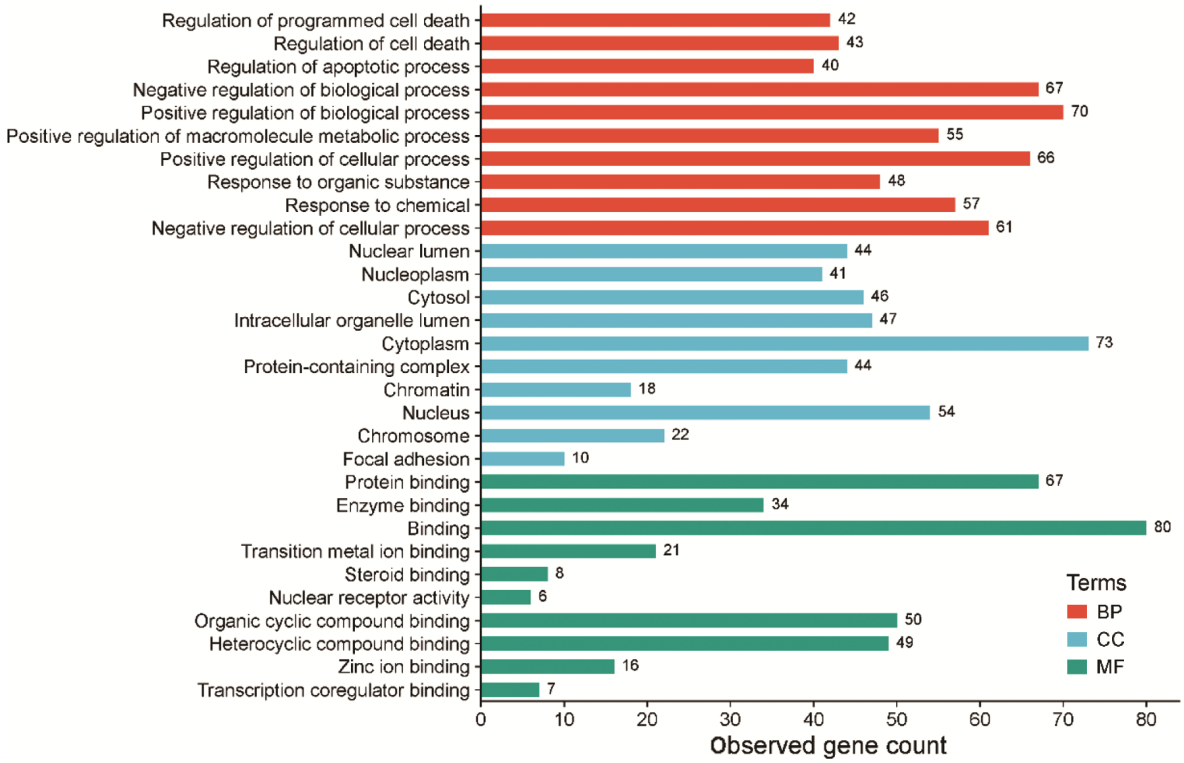

Fig. 3 — GO enrichment analysis of *Withania somnifera* for the treatment of cancer.

tumour suppressors such as KRAS, MDM2, ERBB2, CCND2, and NOTCH1, which regulate proliferation, apoptosis, and metastasis. The PI3K-Akt (hsa04151), MAPK (hsa04010), and FoxO (hsa04068) signaling pathways play vital roles in cell survival, growth, and stress response, representing common targets for therapeutic intervention. The Estrogen signaling pathway (hsa04915) and the Breast cancer pathway (hsa05224) include hormone-responsive genes such as PGR, ESR2, and CTNNB1, reinforcing the importance of endocrine therapy in estrogen receptor-positive subtypes. The p53 (hsa04115) and cell cycle (hsa04110) pathways involve regulators like MDM2, CDKN2B, and CHEK1, which are essential for genomic stability and chemotherapy sensitivity. Pathways including Wnt (hsa04310), ErbB (hsa04012), and EGFR resistance (hsa01521) are associated with tumour aggressiveness and drug resistance mechanisms, involving ERBB2, MAPK8, and PRKCA. Additionally, Apoptosis (hsa04210) and HIF-1 signaling (hsa04066) pathways highlight genes involved in hypoxic response and programmed cell death, such as CASP8, NOS2, and TIMP1. The JAK-STAT (hsa04630) and AMPK (hsa04152) pathways further reveal metabolic and immune-related regulation in tumour suppression and drug response. Collectively, these enriched pathways emphasise the multifaceted molecular basis of breast cancer and offer diverse therapeutic targets for personalised treatment strategies. The results are illustrated in Fig. 4.

Integrated compound–target–pathway network analysis

To gain a comprehensive understanding of the mechanistic interplay between phytoconstituents, molecular targets, and pathways, an integrated compound–target–pathway (C–T–P) network was constructed using Cytoscape software by merging the

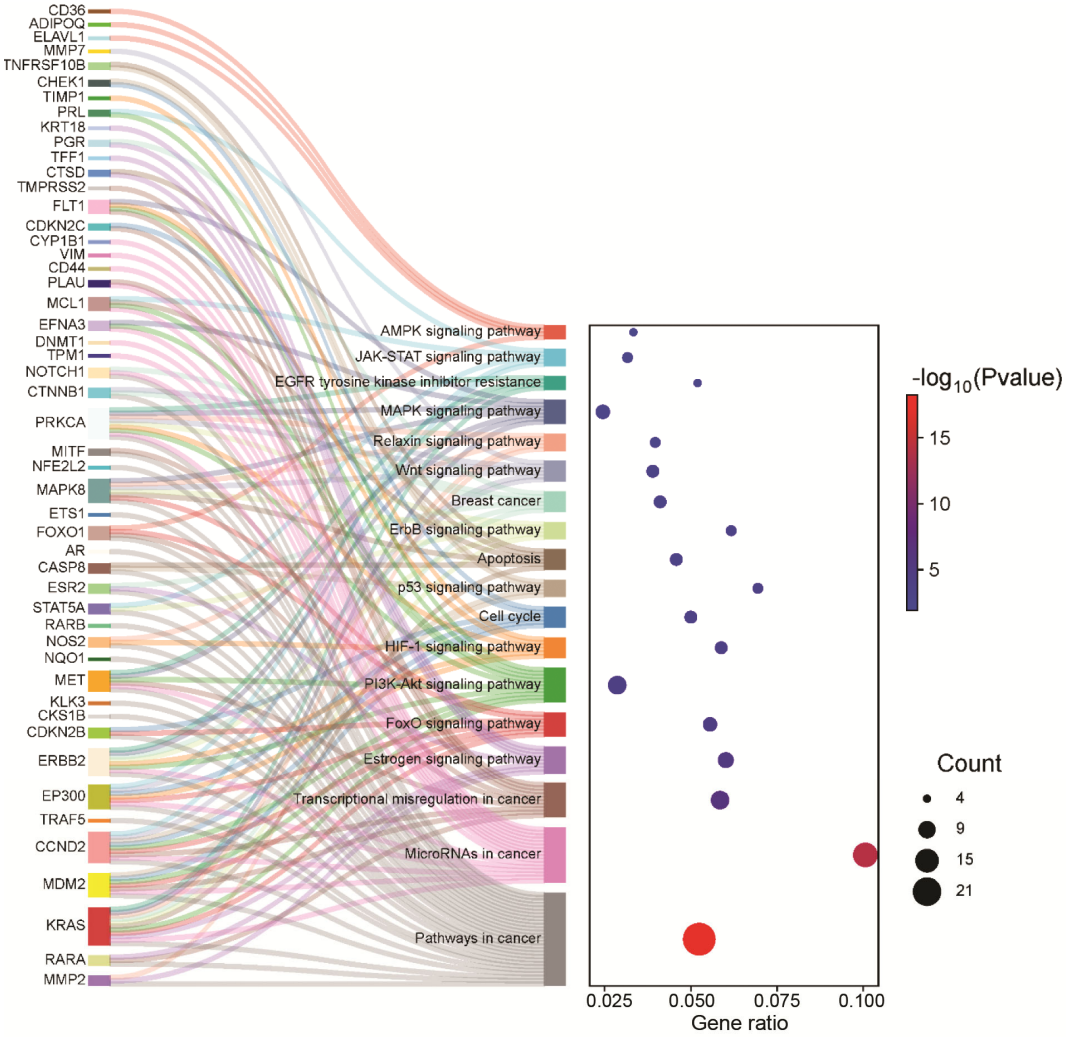

Fig. 4 — KEGG pathway analysis of selected signaling pathways involved in the treatment of lung cancer.

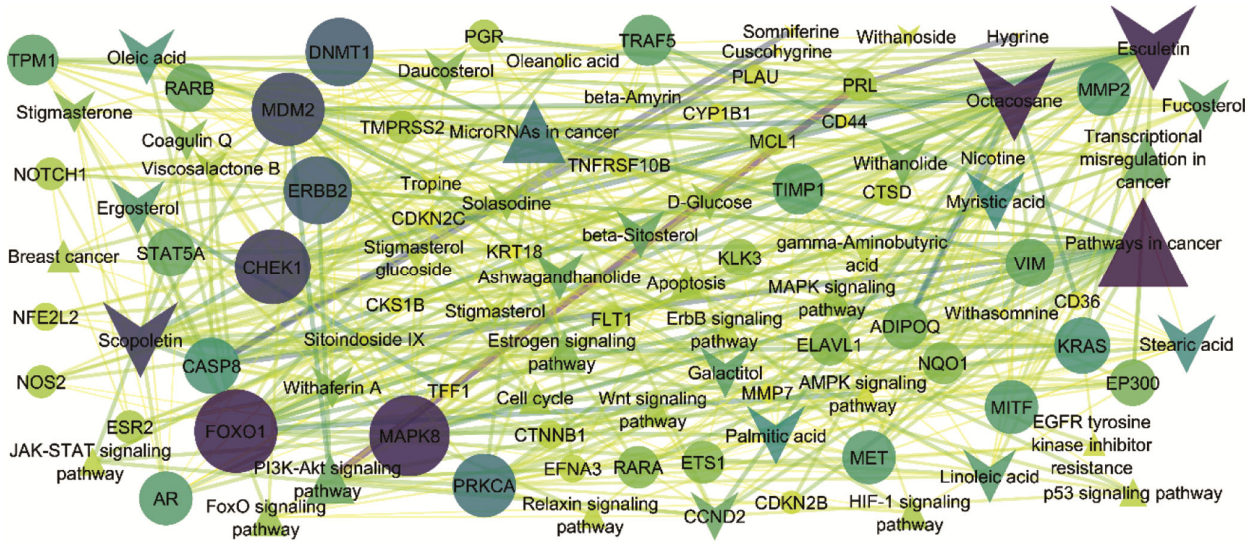

Fig. 5 — Network analysis of *Withania somnifera* involved in the treatment of cancer.

individual compound–target and pathway–target interaction datasets (Fig. 5). The resultant network comprised 103 nodes, including bioactive compounds, target proteins, and KEGG pathways and 443 edges, illustrating a systems-level interaction map through which *W. somnifera* phytochemicals potentially exert anticancer effects.

Network topology analysis revealed *Esculetin, FOXO1,* and the "Pathways in cancer" module as the most central and influential nodes, indicating their pivotal roles in mediating therapeutic responses. Notably, *Esculetin* exhibited extensive polyphar macological interactions by targeting 25 key cancer-associated proteins: *CHEK1, ERBB2, CASP8, KRAS, MITF, AR, MET, MMP2, TIMP1, TPM1, RARB, EP300, NQO1, KLK3, NOTCH1, NOS2, CTSD, CTNNB1, NFE2L2, TNFRSF10B, PLAU, MMP7, CDKN2C, CYP1B1*, and *CKS1B*. Further, hub node analysis performed using the Cytohubba plugin identified Octacosane, FOXO1, MAPK8, Pathways in cancer, ERBB2, CHEK1, DNMT1, Esculetin, Scopoletin, and MDM2 as key regulatory nodes (Fig. 6). These central elements likely serve as major contributors to the observed multi-targeted anticancer potential of *W. somnifera*, underscoring the therapeutic relevance of its phytoconstituents in modulating diverse oncogenic signaling networks. The results are summarised in Table 2.

Molecular docking analysis of identified compounds with ERBB2

Molecular docking studies were performed using the Schrödinger Suite 2020-1 to evaluate the binding affinities of key phytoconstituents from *W. somnifera*

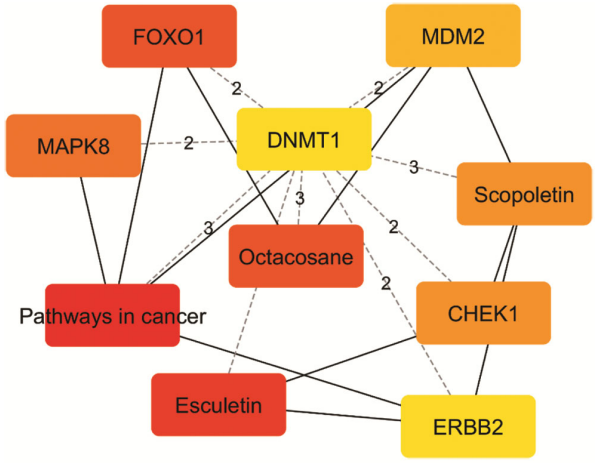

Fig. 6 — Cytohubba analysis of the merged network of *Withania somnifera*.

against the ERBB2 protein target (PDB ID: 3PP0), an oncogene modulated in breast cancer. The docking simulations were carried out in Glide Extra Precision (XP) mode, and the resulting interaction profiles were analysed and visualised in the Maestro interface.

The docking results, including docking scores, Glide energies, and receptor–ligand interactions, are summarised in Table 3. Among the tested compounds, Withasomnine exhibited the highest binding affinity with a docking score of –8.45 and a Glide energy of –32.34 kcal/mol, forming a hydrogen bond with residue MET801 (Fig. 7a). Scopoletin also demonstrated significant binding (docking score: –7.37; Glide energy: –30.41 kcal/mol), engaging MET801 through hydrogen bonding. Stigmasterone showed a docking score of –6.65, forming hydrogen

Table 2 — KEGG pathway analysis of selected pathways involved in the breast cancer

Term ID	Description	Gene Ratio	p-value	Gene ID	Gene count
hsa05200	Pathways in cancer	0.052427	5.85E-19	MMP2, RARA, KRAS, MDM2, CCND2, TRAF5, EP300, ERBB2, CDKN2B, CKS1B, KLK3, MET, NQO1, NOS2, RARB, STAT5A, ESR2, CASP8, AR, FOXO1, ETS1, MAPK8, NFE2L2, MITF, PRKCA, CTNNB1, NOTCH1	27
hsa05206	MicroRNAs in cancer	0.100629	8.07E-15	KRAS, MDM2, CCND2, EP300, ERBB2, MET, TPM1, DNMT1, EFNA3, MCL1, PLAU, CD44, PRKCA, VIM, CYP1B1, NOTCH1	16
hsa05202	Transcriptional misregulation in cancer	0.058480	5.85E-07	RARA, MDM2, CCND2, CDKN2C, FLT1, MET, PLAU, FOXO1, TMPRSS2, MITF	10
hsa04915	Estrogen signaling pathway	0.060150	8.32E-06	MMP2, CTSD, RARA, KRAS, TFF1, PGR, ESR2, KRT18	8
hsa04068	FoxO signaling pathway	0.055556	4.43E-05	KRAS, MDM2, CCND2, EP300, CDKN2B, FOXO1, MAPK8	7
hsa04151	PI3K-Akt signaling pathway	0.028653	8.30E-05	KRAS, MDM2, CCND2, ERBB2, FLT1, PRL, MET, EFNA3, MCL1, PRKCA	10
hsa04066	HIF-1 signaling pathway	0.058824	1.40E-04	TIMP1, EP300, ERBB2, FLT1, NOS2, PRKCA	6
hsa04110	Cell cycle	0.050000	3.10E-04	MDM2, CCND2, CDKN2C, EP300, CDKN2B, CHEK1	6
hsa04115	p53 signaling pathway	0.069444	3.40E-04	MDM2, CCND2, TNFRSF10B, CASP8, CHEK1	5
hsa04210	Apoptosis	0.045802	4.10E-04	CTSD, KRAS, TNFRSF10B, CASP8, MCL1, MAPK8	6
hsa04012	ErbB signaling pathway	0.061728	4.80E-04	KRAS, ERBB2, STAT5A, MAPK8, PRKCA	5
hsa05224	Breast cancer	0.041096	6.50E-04	KRAS, ERBB2, PGR, ESR2, CTNNB1, NOTCH1	6
hsa04310	Wnt signaling pathway	0.038961	7.50E-04	MMP7, CCND2, EP300, MAPK8, PRKCA, CTNNB1	6
hsa04926	Relaxin signaling pathway	0.039683	2.10E-03	MMP2, KRAS, NOS2, MAPK8, PRKCA	5
hsa04010	MAPK signaling pathway	0.024476	2.20E-03	KRAS, ERBB2, FLT1, MET, EFNA3, MAPK8, PRKCA	7
hsa01521	EGFR tyrosine kinase inhibitor resistance	0.051948	2.90E-03	KRAS, ERBB2, MET, PRKCA	4
hsa04630	JAK-STAT signaling pathway	0.031646	4.60E-03	CCND2, EP300, PRL, STAT5A, MCL1	5
hsa04152	AMPK signaling pathway	0.033333	1.17E-02	FOXO1, ELAVL1, ADIPOQ, CD36	4

Table 3 — Binding affinity and interactions of identified compounds from the extract of *Valeriana wallichii* using the Glide module of Schrödinger's

Title	Docking score	Glide_gscore	Glide energy	Residue interactions
Lapatinib	-12.8086	-12.9073	-70.9837	Hydrogen bond with ARG 811, MET 801, pi-pi stacking PHE 864, Halogen bond LYS 753
Co-crystal	-11.0264	-11.0264	-68.2294	Halogen bond LEU 796
Neratinib	-9.31295	-9.51055	-62.6248	Halogen bond SER 728
Withasomnine	-8.4509	-8.4509	-32.3416	Hydrogen bond MET 801
Scopoletin	-7.37104	-7.38404	-30.4183	Hydrogen bond MET 801
Stigmasterone	-6.65213	-6.65213	-16.3972	Hydrogen bond LYS 724
Pelletierine	-6.37431	-6.37461	-22.2502	Hydrogen bond ASP 863
Ergosterol	-6.36658	-6.36658	-28.2708	No interaction
Esculetin	-6.28879	-6.30439	-29.8893	Hydrogen bond ASP 808
Hygrine	-5.27585	-5.28135	-21.4441	Hydrogen bond SER 728 and Salt bridge ASP 863
Withaferin A	-4.94788	-4.96228	-31.1352	Hydrogen bond SER 728, ASP 808, CYS 805, ASP 863
Galactitol	-3.75215	-3.75215	-29.359	Hydrogen bond SER 783, ASP 863, THR 862
Linoleic acid	-3.57634	-3.58114	-40.2523	Hydrogen bond MET 801
Oleanolic acid	-3.48306	-3.49106	-26.2825	Salt bridge ARG 811
Oleic acid	-2.29606	-2.30096	-35.0563	Hydrogen bond LYS 724, Salt bridge LYS 736
Myristic acid	-2.06657	-2.07147	-31.5532	Hydrogen bond MET 801
Stearic acid	-2.03366	-2.03856	-34.4054	Hydrogen bond LYS 724
Palmitic acid	-1.31068	-1.31558	-34.5596	Hydrogen bond CYS 805

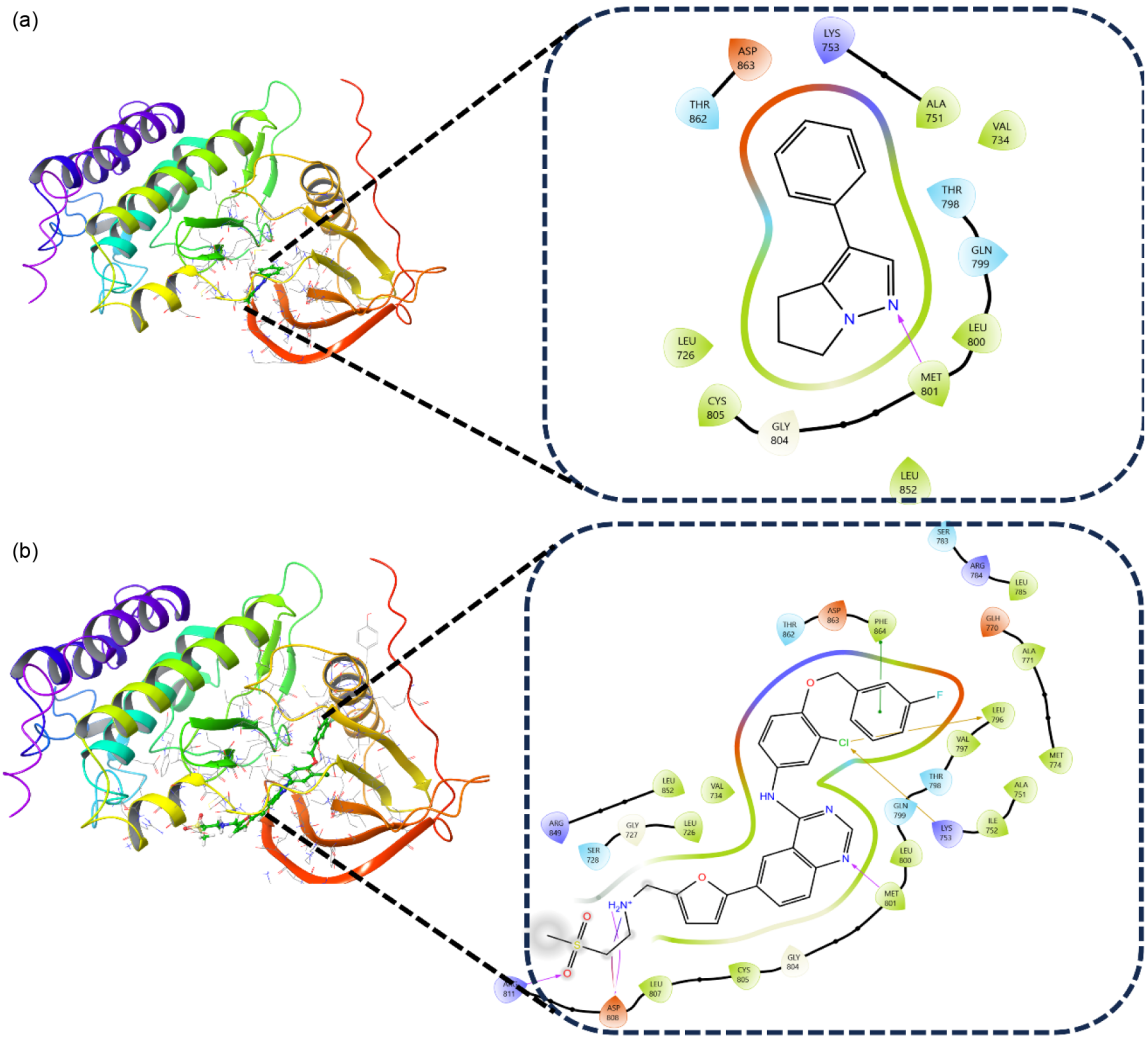

Fig. 7 — Orientation of ligands within the ERBB2 binding pocket crystal structure (PDB ID: 3PP0): (a) Withasomnine, and (b) Lapatinib.

\bonds with residue LYS724. Other noteworthy compounds included Pelletierine (–6.37), Ergosterol (–6.36), Esculetin (–6.28), and Hygrine (–5.27), all of which established hydrogen bonds with key residues within the active site of ERBB2.

As a standard reference, Lapatinib, an FDA-approved ERBB2 inhibitor, exhibited a markedly higher docking score of –12.80 and a Glide energy of –70.98 kcal/mol, validating its strong binding affinity through interactions with ARG811 and MET801 (Fig. 7b). These results indicate that selected phytoconstituents from *W. somnifera* may serve as potential modulators of ERBB2 activity.

Conclusion

This integrative network pharmacology and molecular docking study demonstrates that *W. somnifera* possesses a diverse array of phytoconstituents capable of modulating multiple molecular targets involved in breast cancer progression. The study identified 87 overlapping therapeutic targets, notably associated with apoptosis regulation, hormone-dependent signaling, and critical cancer pathways including PI3K-Akt, MAPK, and p53. Protein–protein interaction (PPI) and MCODE analyses revealed interconnected oncogenic and tumour suppressor modules, while compound–target–pathway (C–T–P) network evaluation highlighted the plant's polypharmacological potential. Molecular docking identified Withasomnine (binding score: -8.45) and Scopoletin (binding score: -7.37) as promising ERBB2 modulators, providing a rationale for further pharmacological investigation. Overall, *W. somnifera* emerges as a promising multi-targeted

candidate for breast cancer therapy, bridging traditional medicinal knowledge with modern molecular evidence.

Conflict of interest

The authors declare no conflict of interest in relation to the present research paper.

AI use disclosure

The authors declare that no artificial intelligence (AI) tools were used for data generation, analysis, interpretation, or preparation of scientific conclusions in this manuscript. AI-assisted tools were used only for language improvement and grammatical editing purposes.

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