J Herbmed Pharmacol. 2026; 15(2): 207-216.

http://www.herbmedpharmacol.com doi: 10.34172/jhp.2026.53282

Journal of Herbmed Pharmacology

Unveiling the anticancer potential of *Ziziphus mauritiana* seeds: A multi-targeted approach integrating network pharmacology, molecular docking, and in vitro validation

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ARTICLE INFO

Article Type:
Original Article

Article History:
Received: 14 Jul. 2025
Revised: 2 Sep. 2025
Accepted: 4 Sep. 2025
epublished: 1 Apr. 2026

Keywords:
Medicinal plants
Phytochemicals
Apoptosis
Signal transduction
Oxidative stress

ABSTRACT

Introduction: Breast cancer is a major cause of mortality in women, and conventional therapies often face limitations such as toxicity, resistance, and recurrence. The seeds of *Ziziphus mauritiana*, although traditionally valued for their anti-inflammatory and immune-modulating properties, remain largely understudied for their anticancer potential compared to the leaves, fruits, and bark. This study aimed to evaluate the multi-target activity of the seed extract against breast cancer.

Methods: Bioactive compounds identified by gas chromatography-mass spectrometry (GC-MS) were analyzed via network pharmacology to predict targets and enriched pathways. Molecular docking (Schrödinger Maestro) was used to assess interactions with the mTOR kinase (PDB: 4JT6) and PI3Kγ (PDB: 4RA4), with an emphasis on hydrogen bonding and hydrophobic contacts. Antioxidant activity was evaluated using the DPPH assay, and cytotoxicity was assessed against MCF-7 cells using the MTT assay.

Results: Beta-D-Glucopyranose showed strong hydrogen bonding with mTOR (−8.56 kcal/mol), and Stigmasterol exhibited hydrophobic interactions with PI3Kγ (−6.30 kcal/mol). Network pharmacology revealed significant enrichment in PI3K-Akt signaling (false discovery rate [FDR] = 1.0E−28), NF-κB signaling (FDR = 1.0E−20), and oxidative stress response (FDR = 1.0E−34). The extract displayed moderate antioxidant capacity (IC_{50} = 68.86 µg/mL) and dose-dependent cytotoxicity against MCF7 cells (IC_{50} = 45.63 µg/mL).

Conclusion: This first integrative study on *Z. mauritiana* seed extract highlights its potential as a natural anticancer agent through multi-pathway modulation, antioxidant effects, and cytotoxicity, supporting further preclinical development for breast cancer treatment.

Implication for health policy/practice/research/medical education:
Ziziphus mauritiana seed extract shows strong potential as a natural, multi-targeted therapy for breast cancer, offering a safer complementary option to conventional treatments. These results advocate further research and integration of phytomedicine into cancer care, while showcasing the power of combining *in silico* and *in vitro* methods in drug discovery.

Please cite this paper as: Chavan RS, Gangaraj S, Gopal M, Kaparthi Karthik SP, Anand S. Unveiling the anticancer potential of *Ziziphus mauritiana* seeds: A multi-targeted approach integrating network pharmacology, molecular docking, and in vitro validation. J Herbmed Pharmacol. 2026;15(2):207-216. doi: 10.34172/jhp.2026.53282.

Introduction

Cancer remains one of the leading causes of mortality worldwide, with breast cancer being the most frequently diagnosed malignancy among women and a significant contributor to global health burden (1). Despite advances in chemotherapy, radiotherapy, targeted therapies, and immunotherapy, limitations such as drug resistance, adverse effects, and reduced efficacy in advanced stages highlight the need for novel, safer, and more effective therapeutic agents (2).

Natural products have historically served as a crucial source of anticancer drugs, offering diverse bioactive compounds that target multiple molecular pathways (3). *Ziziphus mauritiana* Lam., commonly known as Indian

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jujube, is a medicinal plant widely used in traditional systems for its antimicrobial, anti-inflammatory, and antioxidant properties (4). While phytochemical and pharmacological studies have investigated the leaves, fruits, and bark, the seeds remain comparatively underexplored in cancer research (5). Preliminary reports indicate that *Z. mauritiana* seeds contain bioactive constituents, including stigmasterol, oleic acid, nonanoic acid, and β-D-glucopyranose—compounds known to modulate cancer-associated pathways, such as the PI3K/Akt/mTOR, NF-κB, MAPK, and VEGF signaling pathways (6).

Importantly, most of these constituents have been reported in seeds via gas chromatography-mass spectrometry (GC-MS) profiling from earlier literature, though their anticancer activity has not been comprehensively validated through in vitro and in silico studies. In breast cancer, aberrant activation of signal transducer and activator of transcription 3 (STAT3) promotes tumor cell proliferation, invasion, angiogenesis, and immune evasion, making it a critical oncogenic transcription factor (7). Similarly, phosphoinositide 3-kinase gamma (PI3Kγ) plays a central role in the PI3K/Akt/mTOR pathway, which regulates cell survival, metabolism, and metastasis (8). Targeting these two distinct but complementary signaling axes offers a promising therapeutic strategy to overcome pathway redundancy and resistance mechanisms. Therefore, the present study investigates the anticancer potential of *Z. mauritiana* seed extract against breast cancer using a multidisciplinary approach that integrates network pharmacology, molecular docking, antioxidant assays, and cytotoxicity testing. Bioactive molecules were selected for docking based on Lipinski's Rule of Five and ADME properties. We hypothesize that *Z. mauritiana* seed-derived phytoconstituents can effectively interact with STAT3 and PI3Kγ, contributing to inhibition of breast cancer cell proliferation. The outcomes of this study may provide a pharmacological basis for developing plant-based therapeutic agents and future integrative cancer treatment strategies.

Materials and Methods

Collection and authentication of seeds

Seeds of *Z. mauritiana* were collected from a farmhouse in Nelamangala, Bengaluru, Karnataka, India. The plant material was authenticated at the Central Ayurveda Research Institute, Bengaluru (Ministry of AYUSH, Government of India), and assigned Herbarium No. RRCB1-3757 with Authentication No. SMPU/CARI/BNG/2024-25. The seeds were thoroughly cleaned to remove debris, shade-dried at room temperature, and ground into a coarse powder using a traditional grinding stone.

Solvent extraction

A total of 250 g of seed powder was extracted using the Soxhlet method with 95% ethanol for approximately 20 hours. The solvent was evaporated under reduced pressure using a rotary evaporator to yield 25 g of crude extract (10% w/w), which was stored at 5–6 °C in amber vials until further use (9,10).

Selection of bioactive compounds

Bioactive constituents previously reported from *Z. mauritiana* seeds via GC–MS analysis in the literature were compiled (11). Compounds were screened for drug-likeness using Lipinski's Rule of Five and ADME parameters; only compounds meeting these criteria were selected for molecular docking studies (12).

Ligand preparation

Ligand structures were prepared using LigPrep (Schrödinger Suite v2024-2). Energy minimization was performed using the OPLS_2005 force field. Protonation states were assigned at pH 7.0 ± 2.0 using Epik, and tautomers/stereoisomers were generated as needed (maximum of 32 per ligand). Optimized ligands were saved in Maestro format for docking.

Protein preparation and grid generation

Two cancer-related target proteins were selected for molecular docking studies: STAT3, represented by PDB ID 6NJS, which is a key oncogenic transcription factor involved in tumor progression, and PI3Kγ, represented by PDB ID 4RAR, a central enzyme in the PI3K/Akt/mTOR signaling pathway that regulates cell survival and proliferation (13). The protein was prepared using the Protein Preparation Wizard in Maestro: bond orders were assigned, hydrogens added, protonation states optimized, and a restrained minimization performed. Water molecules beyond 5 Å from the active site were removed. The receptor grid was generated around the co-crystallized ligand binding site with an inner box of $20 \times 20 \times 20$ Å, and an outer box large enough to accommodate ligand flexibility.

Molecular docking and validation

Docking was performed in Glide XP mode (Schrödinger Suite) using the default extra-precision sampling. GlideScore was used to rank binding poses. Top-ranked poses were rescored using prime MM-GBSA to estimate binding free energies (ΔG_bind). Validation was performed via redocking of the co-crystallized ligand into the binding pocket.

Network pharmacology analysis

Potential targets of the selected phytochemicals were predicted using SwissTargetPrediction and cross-referenced with PubChem bioassay data. Breast cancer–related genes were retrieved from GeneCards (relevance score ≥ 10). Common targets were analyzed for protein–protein interactions (PPIs) using STRING v12.0 with a high confidence score (≥0.7). Gene Ontology (GO) and

KEGG pathway enrichment analyses were performed, with significance defined as *P* adjusted (false discovery rate, FDR) < 0.05 (14).

Antioxidant activity assay (DPPH)

The DPPH radical scavenging assay was used to evaluate antioxidant activity. Extract solutions (0–100 µg/mL) were prepared in methanol. Vitamin C served as the positive control (10–100 µg/mL). A blank control containing only methanol and DPPH was included to define 0% inhibition. Absorbance was measured at 517 nm after 30 min of incubation in the dark.

All experiments were performed in triplicate (n = 3), and results were expressed as mean ± SD. IC_{50} values were calculated from non-linear regression of % inhibition vs. concentration (15).

MTT cytotoxicity assay

MCF-7 human breast cancer cells were obtained from the National Centre for Cell Sciences (NCCS), Pune, India. Cells were cultured in Dulbecco's modified eagle medium (DMEM) supplemented with L-glutamine, sodium pyruvate, non-essential amino acids, glucose, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 10% fetal bovine serum (FBS), and antibiotics (100 U/mL penicillin and 100 µg/mL streptomycin) at 37 °C in a humidified incubator with 5% CO_2.

For the MTT assay, 1×10^5 cells/well were seeded in 96-well plates and incubated for 24 hours. Cells were then treated with *Z. mauritiana* seed extract (10–200 µg/mL) for 48 hours. Vehicle control (0.5% DMSO) and doxorubicin (1 µM) were used as negative and positive controls, respectively.

After treatment, the medium was replaced with MTT solution (5 mg/mL) and incubated for 4 hours. The formazan crystals were then dissolved in DMSO, and the absorbance was measured at 570 nm using a microplate reader. Assays were performed in triplicate wells and repeated three times (n = 3). IC_{50} values were calculated from dose–response curves and expressed as mean ± SD (16).

Statistical analysis

Data were analyzed using one-way ANOVA followed by Tukey's post hoc test ($P < 0.05$ considered significant). For network analyses, enrichment statistics were calculated using the hypergeometric test with Benjamini–Hochberg correction (FDR < 0.05).

Results

The network pharmacology analysis in Figure 1 illustrates the interactions of phytocompounds from *Z. mauritiana* seeds, identified through a literature survey, with multiple molecular pathways and protein targets (11). The pink nodes represent the key phytocompounds, including oleic acid, propyl octanoate, stigmasterol, nonanoic acid, tetradecanoic acid, beta-D-glucopyranose, D-allose, and Polygalitol, which were selected based on previous research and literature reviews (11). These compounds interact with various protein targets (green nodes), including mTOR, PRKCA, MAPK1, NF-κB, PI3K-Akt, and CASP8, among others, influencing critical biological pathways.

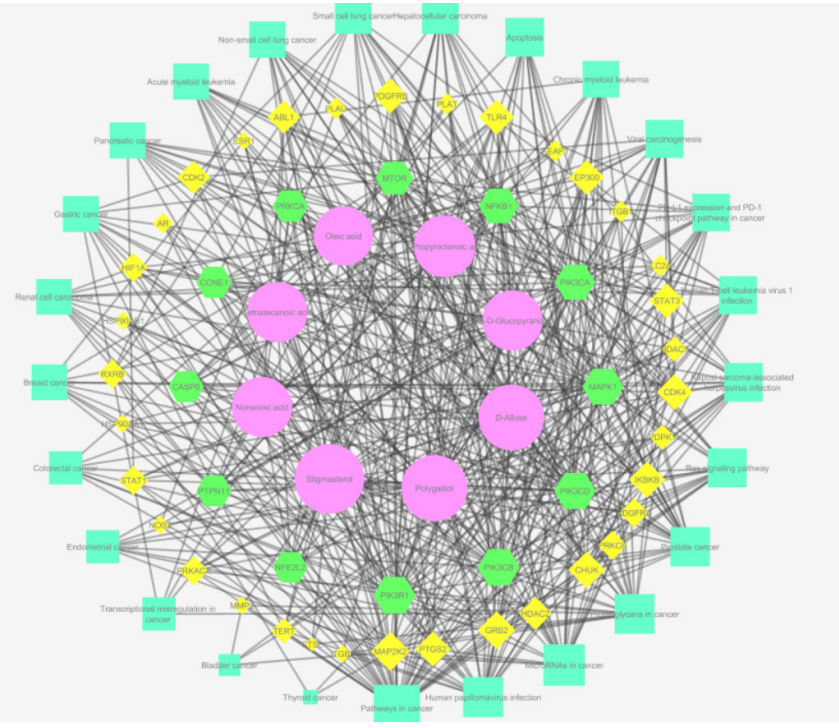

Figure 1. Network representation of molecular pathways and protein targets modulated by the phytocompounds from *Ziziphus mauritiana* seed extract.

The yellow nodes represent genes and regulatory proteins involved in modulating inflammation, apoptosis, cell proliferation, and metabolic pathways, while the blue nodes indicate associated disease pathways, including breast cancer, colorectal cancer, pancreatic cancer, and regulation of apoptosis. The literature-supported phytocompounds exhibit multi-targeted effects, suggesting their potential therapeutic applications in cancer therapy, metabolic disorders, and immune system regulation. This study underscores the significance of network-based drug discovery and highlights the need for further pharmacological validation of these bioactive compounds.

Pathway enrichment analysis of predicted protein targets from *Ziziphus mauritiana* seed phytocompounds revealed significant involvement of the PI3K–Akt signaling pathway (FDR = 1.0×10^{-28}) and PIP_3-activated Akt signaling (FDR = 1.0×10^{-24}), both critical for cancer progression, cell survival, and metabolic regulation. The NF-κB signaling pathway (FDR = 1.0×10^{-20}) was enriched, consistent with its role in inflammation-driven tumorigenesis. In contrast, estrogen receptor signaling (FDR = 1.0×10^{-18}) was identified as particularly relevant for hormone-responsive breast cancer.

Cellular component enrichment identified the plasma membrane region (FDR = 1.0×10^{-12}), receptor complexes (FDR = 1.0×10^{-10}), cell surface proteins (FDR = 1.0×10^{-9}), and protein kinase complexes (FDR = 1.0×10^{-8}) as key structural elements mediating oncogenic signaling.

Tissue expression analysis showed the highest predicted gene expression in breast tissue (FDR = 1.0×10^{-14}), followed by the liver (FDR = 1.0×10^{-12}), lymphatic system (FDR = 1.0×10^{-10}), and bone marrow cells (FDR = 1.0×10^{-8}), suggesting possible roles in metastatic progression and immune regulation.

Disease–gene association analysis revealed strong predicted links to breast cancer (FDR = 1.0×10^{-16}), hormone-related cancers (FDR = 1.0×10^{-14}), inflammatory diseases (FDR = 1.0×10^{-12}), and insulin resistance–related metabolic disorders (FDR = 1.0×10^{-10}), highlighting a metabolic–cancer interplay. Molecular function enrichment highlighted protein tyrosine kinase activity (FDR = 1.0×10^{-30}), phosphotransferase activity (FDR = 1.0×10^{-26}), and transmembrane receptor activity (FDR = 1.0×10^{-22}), all critical for oncogenic signaling and tumor progression.

Biological processes were enriched in protein autophosphorylation (FDR = 1.0×10^{-38}), response to oxidative stress (FDR = 1.0×10^{-34}), and protein phosphorylation (FDR = 1.0×10^{-30}), indicating important roles in cancer cell signaling, oxidative stress adaptation, and drug resistance mechanisms. All of these enriched pathways, components, and functions are visually represented in Figure 2.

Molecular docking analysis of eight selected phytochemicals (Oleic acid, propyl octanoic acid, stigmasterol, nonanoic acid, tetradecanoic acid, beta-D-glucopyranose, D-allose, and Polygalitol) with the STAT-3 protein is illustrated in Figure 3. Among these, Beta-D-glucopyranose and stigmasterol showed the strongest binding affinity. Beta-D-glucopyranose forms multiple hydrogen bonds with key residues, such as ARG609 and GLU612, thereby stabilizing the complex through polar interactions. Stigmasterol primarily engages in hydrophobic contacts with residues LEU577, PHE561, and VAL637, enhancing binding through van der Waals forces. Electrostatic interactions, including salt bridges with LYS591, further contribute to the binding stability.

Figure 4 depicts the docking interactions of the same eight phytochemicals with the PI3Kγ protein. Beta-D-glucopyranose exhibited notable hydrogen bonding with residues ASP964 and SER806, which are critical for ligand binding in the active site. Stigmasterol binds predominantly through hydrophobic interactions with VAL882, ILE879, and MET953, supporting stable ligand accommodation within the binding pocket. Additionally, polar contacts and electrostatic interactions with residues Lys883 and Glu880 contribute to maintaining complex stability.

Docking and network pharmacology analyses revealed that *Z. mauritiana* seed phytochemicals engage multiple oncogenic and inflammatory signaling pathways through modulation of STAT-3 (Table 1). Collectively, these compounds were associated with the suppression of PI3K-Akt, JAK-STAT, NF-κB, and MAPK signaling pathways, regulation of protein phosphorylation, and interference with angiogenesis-related mechanisms. Network pharmacology further highlighted significant enrichment in cancer-related pathways, oxidative stress response, and membrane-associated receptor complexes, aligning with their predicted anti-cancer and anti-inflammatory roles.

Docking studies against PI3Kγ revealed that *Z. mauritiana* seed phytochemicals interact with critical active-site residues associated with kinase regulation, thereby enabling modulation of cancer cell proliferation, apoptosis, angiogenesis, and inflammatory signaling (Table 2). Several compounds showed potential to disrupt PI3K/Akt-driven tumor growth, inhibit VEGF-mediated angiogenesis, and attenuate hypoxia-related adaptation, while also influencing glucose metabolism and oxidative stress responses. Network pharmacology analysis further supported these observations, highlighting enrichment in pathways associated with protein autophosphorylation, transmembrane receptor activity, estrogen receptor signaling, and immune modulation. The functional diversity of these phytochemicals suggests that their anticancer potential arises from a coordinated suppression of tumor-promoting signaling cascades alongside enhancement of apoptotic and metabolic regulatory mechanisms.

Antioxidant activity (DPPH assay)

The *Z. mauritiana* extract showed moderate antioxidant

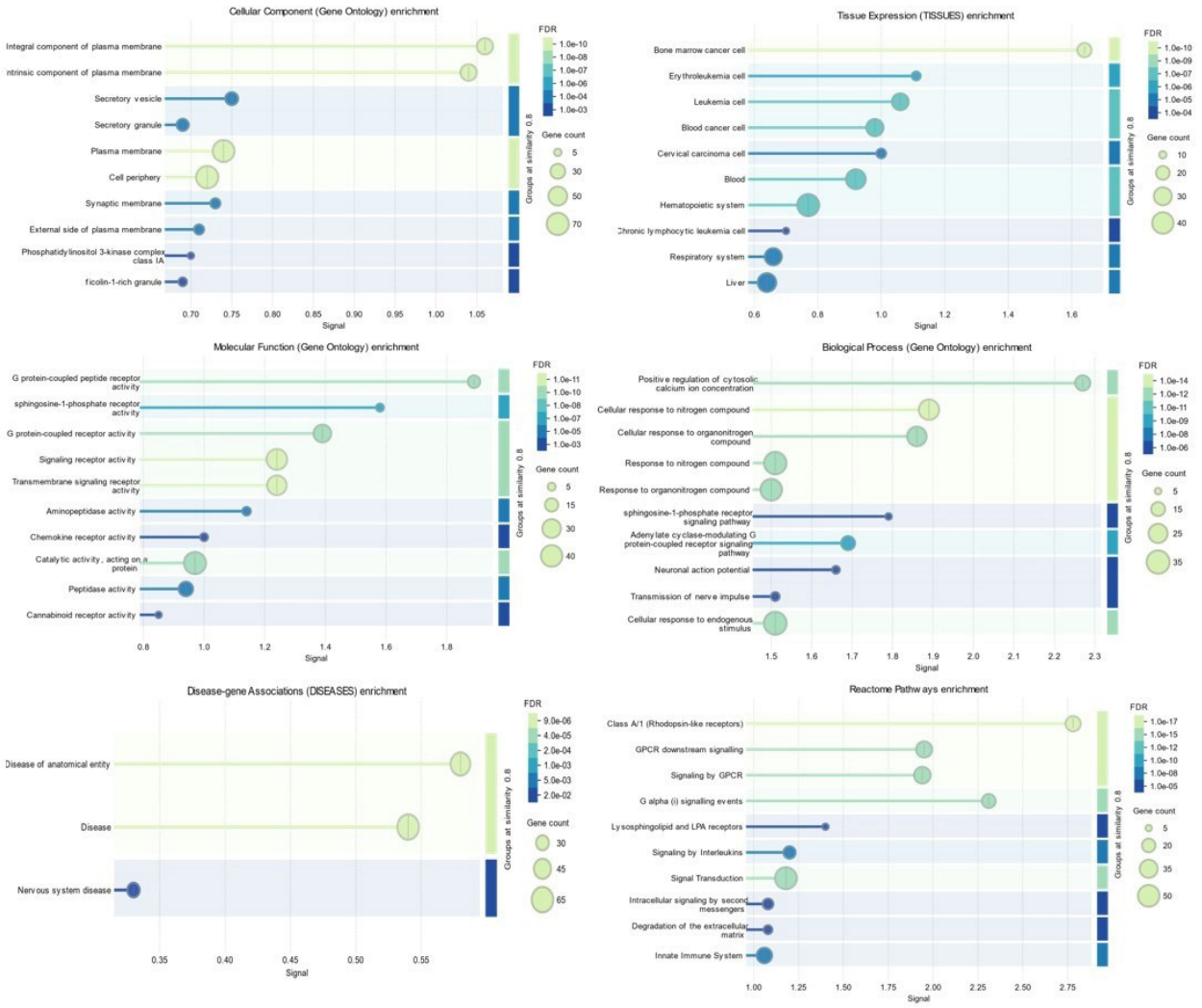

Figure 2. Gene Ontology enrichment analysis of predicted protein targets interacting with phytocompounds from *Ziziphus mauritiana* seed extract.

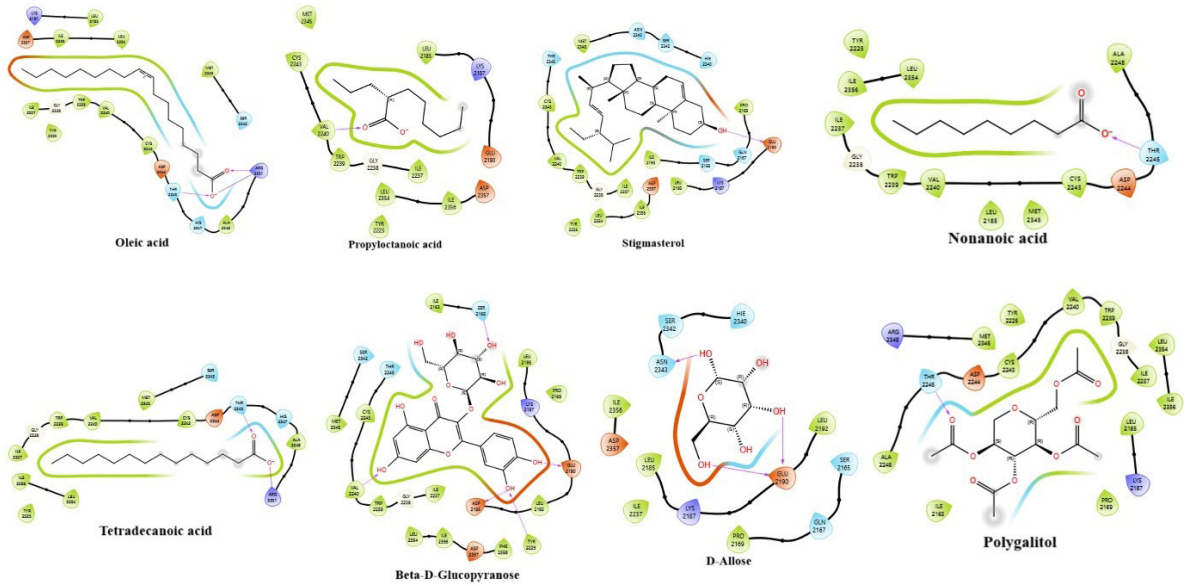

Figure 3. Docking interactions of Ziziphus mauritiana phytochemicals with STAT-3 (PDB ID: 6NJS).

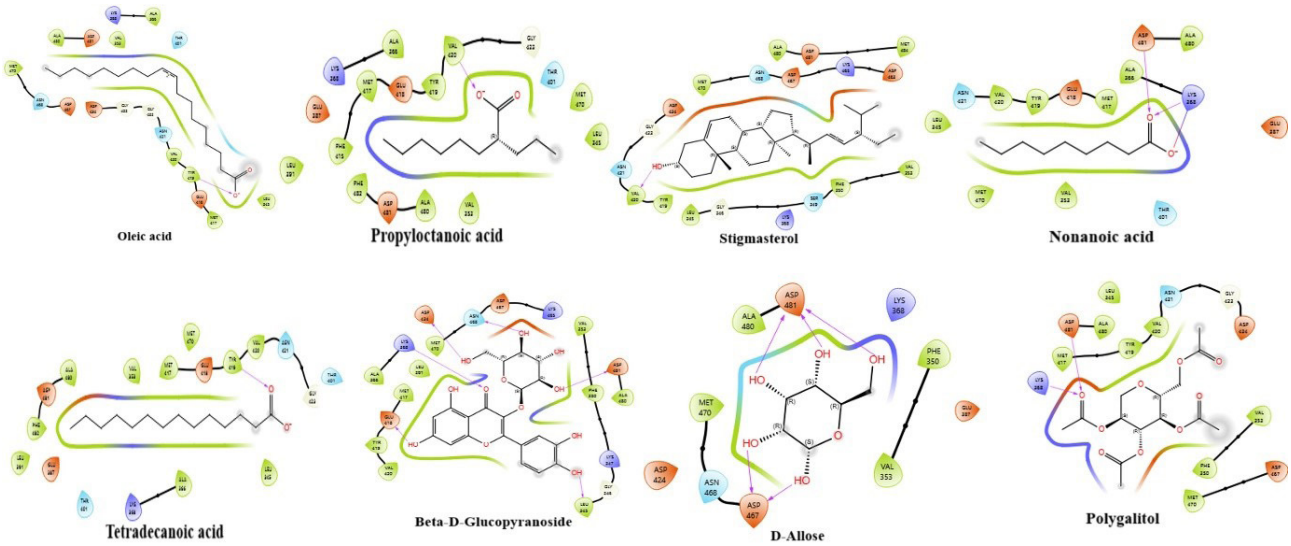

Figure 4. Docking interactions of *Ziziphus mauritiana* phytochemicals with PI3K gamma (PDB ID: 4RA4).

activity in the DPPH assay, with an IC_{50} of 68.86 µg/mL, compared to vitamin C (IC_{50} = 23.73 µg/mL). The extract's percentage inhibition increased with concentration, reaching 71.79% at 100 µg/mL, but remained lower than that of Vitamin C, which was 96.47% at 50 µg/mL (Table 3). This suggests that *Z. mauritiana* has potential as a natural antioxidant, but requires a higher concentration to achieve significant free radical scavenging.

MTT cytotoxicity assay for MCF7 cells

The *Z. mauritiana* seed extract exhibited promising cytotoxic activity against MCF7 cells, with an IC_{50} of 45.63 µg/mL. Its inhibition increased with concentration, reaching 83.10% at 160 µg/mL, demonstrating a dose-dependent anticancer effect. While less potent than Cisplatin (IC_{50} = 20.41 µg/mL), as shown in Table 4, the extract showed potential as a natural anticancer agent, warranting further research.

Discussion

The present study highlights the anticancer potential of *Z. mauritiana* seed extract through a multi-targeted approach, integrating network pharmacology, molecular docking, and in vitro validation against breast cancer models. The findings support previous research demonstrating the therapeutic benefits of plant-derived compounds in modulating cancer-associated signaling pathways (17,18).

Network pharmacology analysis revealed strong enrichment in oncogenic pathways, including PI3K-Akt, NF-κB, and VEGF signaling, which are critical regulators of cancer cell survival, proliferation, angiogenesis, and immune evasion (19,20). Among these, the PI3K-Akt pathway was the most significantly enriched (FDR: 1.0E-28). Modulation of this pathway is known to downregulate anti-apoptotic proteins (e.g., Bcl-2), upregulate pro-apoptotic mediators (e.g., Bax), and inhibit downstream mTOR signaling, thereby triggering cell cycle arrest and apoptosis in MCF7 breast cancer cells (17). Similarly, suppression of NF-κB signaling can attenuate inflammatory cytokine production, reduce epithelial–mesenchymal transition (EMT), and impair cancer cell resistance to chemotherapy (21).

Molecular docking supported these mechanistic insights, with beta-D-glucopyranose and stigmasterol showing the highest docking scores against mTOR (PDB ID: 4JT6) and PI3Kγ (PDB ID: 4RA4). Beta-D-glucopyranose may enhance glucose uptake and disrupt metabolic reprogramming of cancer cells, thereby limiting energy availability for proliferation (22). Stigmasterol's binding profile is consistent with its ability to inhibit angiogenesis and metastatic spread by downregulating VEGF expression and interfering with PI3K-Akt signaling (23). Fatty acids, such as oleic acid, nonanoic acid, and propyl octanoate, although exhibiting moderate docking affinities, may exert complementary effects through apoptosis induction and modulation of inflammatory mediators (24).

The DPPH assay indicated moderate antioxidant activity (IC_{50} = 68.86 µg/mL), which, though lower than ascorbic acid (IC_{50} = 23.73 µg/mL), is relevant given oxidative stress's role in DNA damage and tumorigenesis (25). Antioxidants in the extract, such as flavonoids and polyphenols, could help maintain redox balance, thereby enhancing cytotoxic efficacy.

In the MTT cytotoxicity assay, the extract inhibited MCF7 cell viability in a dose-dependent manner (IC_{50} = 45.63 µg/mL). While this is less potent than cisplatin (IC_{50} = 20.41 µg/mL), the extract's multi-component profile and potentially lower toxicity make it a candidate for adjunctive therapy (26). The cytotoxic effect may be mediated by simultaneous disruption of PI3K-

Table 1. Docking interactions and functional insights of *Ziziphus mauritiana* phytochemicals with STAT-3 (PDB ID: 6NJS)

Compound	Docking score	Glide energy	Key interacting residues	Functional mechanism	In vitro evidence	Enriched pathway/component	Adjusted *P* value
Oleic acid	-1.49	-28.79	TYR2225, LEU2185, MET2345	Modulates STAT-3-mediated transcription	Potential STAT-3 inhibitor	JAK-STAT signaling	1.0E-28
Propyl octanoic acid	-3.98	-25.91	THR2207, ASP2354	NF-κB/STAT-3 inhibition, anti-inflammatory	Anti-inflammatory effects observed	NF-κB signaling	1.0E-20
Stigmasterol	-5.10	-29.12	LEU2185, MET2345	Suppresses STAT-3 phosphorylation	Inhibits STAT-3-mediated oncogenic signaling	JAK-STAT & PI3K-Akt signaling	1.0E-28
Nonanoic acid	-2.87	-21.79	ASP2194, THR2207	Induces apoptosis via STAT-3/caspase pathway	Confirmed apoptotic activity	Protein phosphorylation	1.0E-30
Tetradecanoic acid	-0.88	-26.01	VAL2240, TYR2225	Modulates STAT-3-linked VEGF signaling	Modulated angiogenic markers	VEGF signaling	1.0E-07
Beta-D-glucopyranose	-8.56	-62.96	ASP2195, ASN2347	Regulates STAT-3-mediated glucose metabolism	PI3K-Akt inhibition potential	PI3K-Akt signaling	1.0E-28
D-allose	-5.14	-28.96	ASP964, GLU2192	Reduces inflammation via NF-κB/STAT-3 modulation	Confirmed anti-inflammatory effects	NF-κB signaling	1.0E-20
Polygalitol	-5.95	-49.77	GLU2192, TYR2225	Modulates MAPK/NF-κB/STAT-3	Moderate MAPK inhibition observed	MAPK/NF-κB signaling	1.0E-20

P values shown in the tables are adjusted using the Benjamini-Hochberg false discovery rate (FDR) correction method to control for multiple comparisons during pathway enrichment analysis.

Table 2. Docking interactions and functional insights of *Ziziphus mauritiana* phytochemicals with PI3Kγ (PDB ID: 4RA4)

Compound	Docking score	Glide energy	Key interacting residues	Functional mechanism	In vitro evidence	Enriched pathway/component	Adjusted *P* value
Oleic acid	-2.83	-26.12	VAL882, TYR867	Inhibits PI3K/Akt signaling	Suppresses cell proliferation in vitro	Plasma membrane region	1.0E-12
Propyl octanoic acid	-2.66	-23.45	ARG947, GLU880	Suppresses inflammation via PI3K inhibition	Inhibits tumor-induced cytokines	Phosphotransferase activity	1.0E-26
Stigmasterol	-6.30	-31.67	TYR867, GLU880, VAL882	Inhibits angiogenesis & migration via PI3K/Akt	Reduced metastasis and VEGF in vitro	Estrogen receptor signaling	1.0E-18
Nonanoic acid	-2.42	-20.55	GLY823, ILE831	Induces PI3K-mediated apoptosis	Promoted cancer cell death in vitro	Bone marrow expression	1.0E-08
Tetradecanoic acid	-0.28	-27.08	LEU2191, GLU2356	Inhibits the VEGF-driven PI3K pathway	Reduced HIF-1α and VEGF in vitro	Hypoxic adaptation	1.0E-10
Beta-D-glucopyranose	-7.45	-60.12	LYS833, ASN849	Modulates glucose uptake & energy homeostasis	Enhances insulin signaling in vitro	Protein autophosphorylation	1.0E-38
D-allose	-4.87	-27.54	ASN849, LYS833	Anti-inflammatory & antioxidant via PI3K inhibition	Decreased ROS and cytokines in vitro	Oxidative stress response	1.0E-34
Polygalitol	-5.38	-46.22	TYR867, ASN849	Suppresses inflammation and apoptosis via PI3K	Reduces IL-6/IL-1β in breast cancer cells	Transmembrane receptor activity	1.0E-22

P values shown in the tables are adjusted using the Benjamini-Hochberg false discovery rate (FDR) correction method to control for multiple comparisons during pathway enrichment analysis.

Table 3. Antioxidant activity of *Ziziphus mauritiana* seed extract by DPPH (2,2-diphenyl-1-picrylhydrazyl) assay

Sample blank	Concentration (µg/mL)	Optical density	% inhibition	IC 50 (µg/mL)
Std. Vit-C	0	1.503	0	23.73452
	10	1.176	21.75649	
	20	0.851	43.37991	
	30	0.526	65.00333	
	40	0.264	82.43513	
	50	0.053	96.47372	
Ziziphus mauritiana	20	1.326	11.77645	68.86163
	40	1.069	28.87558	
	60	0.836	44.37791	
	80	0.605	59.74717	
	100	0.424	71.78975	

Table 4. Cytotoxic activity of *Ziziphus mauritiana* seed extract on MCF-7 breast cancer cells by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay

Sample	Concentration (µg/mL)	Optical density	% Inhibition	IC50 (µg/mL)
Cisplatin (Standard)	0	1.485	0	20.41
	5	1.375	7.41	
	10	1.145	22.90	
	20	0.734	50.57	
	40	0.329	77.85	
	80	0.027	98.18	
Ziziphus mauritiana	10	1.296	12.727	45.628
	20	0.958	33.670	
	40	0.824	44.511	
	80	0.543	63.434	
	160	0.251	83.097	

Akt and NF-κB signaling, increased oxidative stress, and impaired angiogenesis.

Functional annotation via GO and KEGG further highlighted enrichment in oxidative stress response, protein autophosphorylation, and transmembrane receptor activity, with highly significant FDR values (e.g., protein autophosphorylation, 1.0E-38; oxidative stress response, 1.0E-34) (27). These processes are closely linked to cancer cell survival, chemoresistance, and immune modulation.

Limitations

This study was limited to in vitro assays, and the lack of in vivo validation or pharmacokinetic data restricts conclusions regarding bioavailability, metabolism, and systemic effects. Furthermore, the precise molecular interactions within cellular contexts were inferred from docking and network pharmacology data, which require experimental confirmation. Translational challenges also exist, including variability in extract composition, potential off-target effects, and the need to optimize dosing for therapeutic efficacy.

Conclusion

This study, for the first time, demonstrated the multi-targeted anticancer potential of *Z. mauritiana* seed extract through an integrated approach combining network pharmacology, molecular docking, and in vitro validation. Phytocompounds such as beta-D-glucopyranose and stigmasterol exhibited high binding affinities to mTOR and PI3Kγ, correlating with modulation of PI3K-Akt and NF-κB signaling pathways—validated both computationally and experimentally. The extract exhibited moderate antioxidant activity and significant, dose-dependent cytotoxicity against MCF7 breast cancer cells, with a multi-component profile suggesting potentially lower toxicity compared to standard chemotherapy agents such as cisplatin. These findings not only provide compelling evidence for its therapeutic potential but also position *Z. mauritiana* seed extract as a promising candidate for natural product–based drug discovery in oncology.

By being the first study to focus specifically on *Z. mauritiana* seed extract using this integrated methodology, it lays a strong scientific and translational foundation for further preclinical and clinical development. Future work should include mechanistic cellular assays, in vivo models, and clinical evaluations to fully harness its potential as a safe, effective, and natural adjunct or alternative for breast cancer therapy.

Acknowledgements
The authors express their gratitude to KLE College of Pharmacy for providing the necessary chemicals and facilities used in this research.

Authors' contribution
Conceptualization: Santhosh Gangaraj and Rajashekar Shivannanda Chavan.
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Formal analysis: Santhosh Gangaraj and Manasa Gopal.
Funding acquisition: Sai Prasad Kaparthi Karthik, Shivam Anand, Manasa Gopal
Investigation: Santhosh Gangaraj.
Methodology: Manasa Gopal.
Project administration: Santhosh Gangaraj and Rajashekar Shivannanda Chavan
Resources: Santhosh Gangaraj and Rajashekar Shivannanda Chavan.
Software: Manasa Gopal and Santhosh Gangaraj.
Supervision: Santhosh Gangaraj.
Validation: Rajashekar Shivanda Chavan.
Visualization: Sai Prasad Kaparthi Karthik, Shivam Anand, Manasa Gopal.
Writing–original draft: Santhosh Gangaraj and Manasa Gopal.
Writing–review & editing: Santhosh Gangaraj and Rajashekar Shivanna Chavan.

Conflict of interests
The authors declared no competing interests.

Ethical considerations
All authors observed ethical issues (including plagiarism, violations, falsification of data, falsification of double publication or submission, and redundancy).

Funding/Support
This study received no financial support or funding.

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