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Research Article

Integrative Multi-Omics and Experimental Approaches Identify SRC as a Central Target of *Garcinia atroviridis* Flavonoids in Lung Cancer

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Abstract
Garcinia atroviridis (GA) is widely used in Southeast Asia, but its anticancer potential is less studied than that of other *Garcinia* species. This work aimed to investigate the flavonoid constituents of GA and their molecular mechanisms against lung cancer using an integrated systems pharmacology and experimental approach. Phytochemical profiling was performed by LC–HRMS, and flavonoid-associated targets were predicted using SwissTargetPrediction. Lung cancer–related genes were collected from GSE19188 and GeneCards, with overlapping targets identified through Venn analysis. Protein–protein interaction (PPI) analysis defined hub genes, while molecular docking and dynamics simulations assessed ligand–target interactions. Finally, cytotoxicity of the GA ethanolic extract was evaluated in A549, H460, and BEAS-2B cells after 72 h treatment. Our investigation revealed the presence of 5 major flavonoids, including icariin. Network analysis revealed 142 overlapping targets, with SRC, HSP90AA1, CTNNB1, PIK3R1, and AKT1 emerging as hubs. Docking showed strong binding affinities, particularly between quercetin-3β-D-glucoside and SRC, which was confirmed by stable molecular dynamics simulations. *In vitro*, GA extract reduced the viability of A549 and H460 cells with lower IC_{50} values than in BEAS-2B. GA exerts selective anticancer effects against lung cancer primarily through targeting SRC, with supportive modulation of HSP90AA1, CTNNB1, PIK3R1, and AKT1. These findings position SRC as the key mechanistic anchor and highlight GA as a promising source for multi-targeted cancer therapy.

Keywords: anticancer, flavonoids, *Garcinia atroviridis*, lung cancer, network pharmacology

1. INTRODUCTION

Lung cancer continues to be one of the most lethal malignancies and is responsible for a significant proportion of global cancer-related deaths annually [1]. The high mortality rate is largely attributable to late diagnosis, rapid disease progression, and the limited efficacy of current therapeutic regimens [2]. Although conventional chemotherapy, targeted therapy, and immunotherapy have improved survival in some patients, the overall prognosis remains dismal [2] [3]. Treatment resistance, severe side effects, and tumor heterogeneity pose major challenges, leaving a pressing need for novel strategies that can provide safer, more durable, and effective treatment options [3].

Natural products have historically played a central role in the development of anticancer drugs, offering structurally diverse compounds that act on multiple signaling pathways simultaneously [4]. This multi-target capacity is particularly advantageous in complex diseases such as lung cancer, where single-target agents often fail to produce sustained clinical benefits [5]. Among natural product classes, flavonoids have attracted considerable attention due to their broad pharmacological activities, including antioxidant, anti-inflammatory, and anticancer effects [6]. Several flavonoids have been shown to interfere with cancer cell proliferation, survival, and metastasis through modulation of pathways such as PI3K/AKT, MAPK, and STAT3, positioning them as promising candidates for cancer therapy [3][7] [8].

Garcinia atroviridis (GA), a tropical plant commonly found in Southeast Asia, has long been used in traditional medicine and as a dietary ingredient [9]. Phytochemical studies on the *Garcinia* genus have reported a rich composition of bioactive compounds, including xanthones, benzophenones, and flavonoids, many of which display significant pharmacological effects [10]. However, while certain *Garcinia* species such as *Garcinia mangostana* have been extensively

Table 1. Annotated flavonoids identified from the ethanolic extract of GA fruit by LC-HRMS.

Checked	Name	Formula	Annot. Delta Mass [ppm]	Calc. MW	m/z	RT [min]	Reference Ion	Area	Compound Classification
TRUE	Icaritin	$C_{21}H_{20}O_6$	1.83	368.1267	369.1339	9.367	[M+H]+1	126235132.7	Flavonoid
TRUE	Paratocarpin G	$C_{25}H_{28}O_6$	1.50	424.1892	425.1965	11.394	[M+H]+1	1170461117.7	Flavonoid
TRUE	Quercetin-3β-D-glucoside	$C_{21}H_{20}O_{12}$	0.75	464.0958	465.1031	4.101	[M+H]+1	7857172.01	Flavonoid
TRUE	Quercetin	$C_{15}H_{10}O_7$	0.90	302.0429	303.0502	4.103	[M+H]+1	19021537.62	Flavonoid
TRUE	Spinochalcone A	$C_{25}H_{28}O_3$	0.81	376.2042	377.2114	16.326	[M+H]+1	2253602.842	Flavonoid

investigated for anticancer properties [11], the potential of GA, particularly its flavonoid constituents remains underexplored. To date, no comprehensive study has integrated chemical profiling with mechanistic and biological validation of GA flavonoids in the context of lung cancer.

Recent advances in bioinformatics provide new opportunities to investigate such underexplored natural products [12]. Network pharmacology, in particular, allows systematic prediction of compound–target interactions and their integration into protein–protein interaction networks and signaling pathways [13]. This approach highlights the poly-pharmacological nature of phytochemicals and can reveal key molecular hubs relevant to cancer progression [12][14][15]. Applying this strategy to GA flavonoids may therefore provide valuable mechanistic insights and guide subsequent experimental validation. In this study, we employed LC-HRMS to identify flavonoid compounds from the ethanolic extract of GA. Identified compounds were investigated using network pharmacology to predict lung cancer-related targets, followed by molecular docking and dynamics simulations to examine binding affinities and stability. Finally, cytotoxicity assays were conducted in A549 and H460 lung cancer cells and the non-tumorigenic BEAS-2B cell line. To the best of our knowledge, this is the first integrative investigation combining LC-HRMS, network pharmacology, molecular docking/dynamics, and experimental validation of *G. atroviridis* flavonoids against lung cancer.

2. MATERIALS AND METHODS

2.1. Plant Materials and Extraction

Fruits of GA were collected from Karo Regency, North Sumatera, Indonesia. Botanical identification was carried out at the Herbarium Medanense (MEDA), Universitas Sumatera Utara, and a voucher specimen (No. 145/MEDA/2025) was deposited. The fruits were dried at 50 °C for 5–7 days and subsequently ground into powder, which was stored at room temperature until use. A total of 500 g of GA powder was extracted with 5 L of 70% ethanol using maceration, and the filtrates were concentrated under reduced pressure with a rotary evaporator to obtain the crude extract.

2.2. Methods

2.2.1. LC–HRMS Analysis

A 10 mg powdered sample was extracted with 1.0 mL HPLC-grade methanol, vortexed for 1 min, and filtered through a 0.20 µm nylon syringe filter. Chromatographic separation was performed on a Vanquish Horizon UHPLC system (Thermo Fisher Scientific, Waltham, MA, USA) with an Accucore Phenyl-Hexyl column (100 × 2.1 mm, 2.6 µm) at 40 °C, using water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B) as mobile phases. The gradient was 5% B at 0 min, raised to 90% at 16 min, held for 4 min, and returned to 5% until 25 min at 0.3 mL/min, with a 5 µL injection. Mass spectrometry was conducted on an Orbitrap Exploris 240 (Thermo Fisher Scientific, Waltham, MA, USA) in Full MS/dd-MS² positive ion mode, with scan range m/z 70–1000 at 90,000 resolution, dd-MS² at 22,500 resolution, and stepped collision energies of 30, 50, and 70. Source conditions were spray voltage +3,500 V, sheath gas 35 AU, auxiliary gas 7 AU, sweep gas 1 AU, ion transfer tube 300 °C, and vaporizer 320 °C. Data were processed in Compound Discoverer 3.3, and metabolites were annotated against mzCloud, ChemSpider, and curated mass lists including the Arita Lab Flavonoid Structure Database, endogenous metabolites, and LipidMaps. LC–HRMS was chosen because it offers high sensitivity and mass accuracy for detecting flavonoids and other semi-polar metabolites in crude plant extracts.

2.2.2. Differential Gene Expression (DGE) Analysis

Transcriptomic data for lung cancer were obtained from the publicly available GEO dataset GSE19188 [16]. Differential expression analysis between tumor and normal samples was conducted using the limma package in R [17]. Genes with $|\log_2$ fold change $\geq 1|$ and adjusted p-value < 0.05 were considered significantly dysregulated. From the GSE19188 differential expression analysis, the top 100 upregulated and top 100 downregulated genes were selected, for subsequent integration with other datasets.

2.2.3. Target Identification

Lung cancer–related targets were retrieved from the GeneCards (https://www.genecards.org/) [16] database using “lung cancer” and top data obtained from GSE19188. Potential protein targets of the five flavonoids identified from LC–HRMS were predicted using SwissTargetPrediction (http://www.swisstargetprediction.ch/) [17]. Overlapping targets between flavonoid targets and lung cancer–related genes were identified using a Venn diagram constructed with the Venny 2.1 tool.

2.2.4. Protein-Protein Interaction (PPI) and KEGG Pathway Analysis

The overlapping targets were imported into the STRING database (version 12.0) to construct the

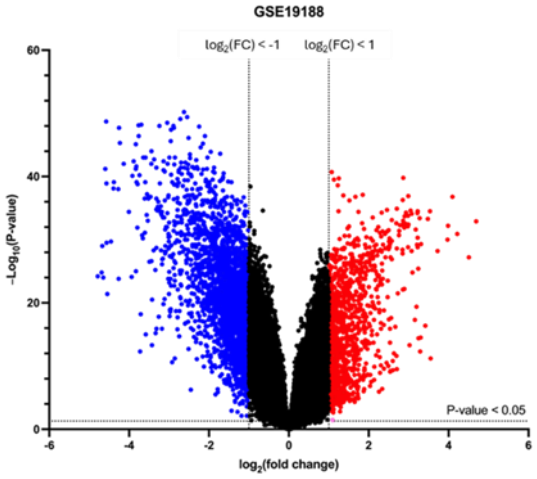


Figure 1. Volcano plot of differentially expressed genes (DEGs) from the GSE19188 lung cancer dataset (GPL570 platform). Analysis was performed using the limma package with adjusted $p < 0.05$ and $|\log_2FC| \geq 1$. Red = upregulated genes ($\log_2FC \geq 1$), blue = downregulated genes ($\log_2FC \leq -1$), black = non-significant genes.

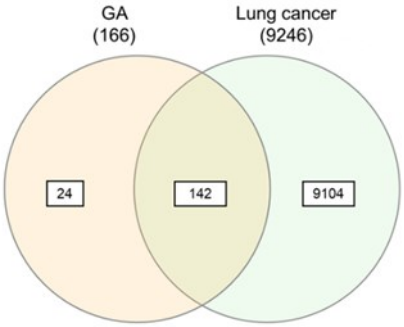


Figure 2. Venn diagram showing the overlap between lung cancer–related genes (GeneCards + GSE19188) and predicted flavonoid targets from GA. A total of 142 shared targets were identified.

protein–protein interaction network. The analysis was performed using the *Homo sapiens* organism setting, with an interaction confidence score cutoff of 0.9. The resulting STRING network was exported as a TSV file and visualized using Cytoscape (version 3.10.1). To identify key regulatory nodes, network topology analysis was performed in Cytoscape using the cytoHubba NetworkAnalyzer plug-in. Degree centrality was used as the primary hub-gene metric, and genes with the highest degree values were defined as hub targets. For the KEGG pathway analysis, the 142 overlapping targets were analyzed using the clusterProfiler package in R with Benjamini–Hochberg correction. Significantly enriched pathways were identified using an FDR-adjusted p-value threshold of $q < 0.05$, and the top pathways were visualized using dot plots.

2.2.5. Molecular Docking Investigation

The crystal structures of SRC (PDB ID: 2h8h), HSP90AA1 (4bqg), CTNNB1 (7afw), PIK3R1 (8exo), and AKT1 (4ejn) were obtained from the RCSB Protein Data Bank (https://www.rcsb.org/). Receptor preparation involved removal of water molecules, ligands, and ions, followed by protonation state assignment with PROPKA and conversion to PDBQT format. Ligand structures were generated with the ChemAxon pKa calculator, retaining conformers with >20% population at pH 7.0, and optimized using ORCA 6.0 at the B3LYP/def2-TZVP level. Docking was carried out with AutoDock Vina 1.2.5 using grid boxes centered on the co-crystallized binding sites [18], with exhaustiveness set to 56 and 20 poses generated. The best-scoring pose for each ligand was selected for further analysis.

2.2.6. Molecular Dynamic Simulation

The dynamic simulation was carried out using GROMACS version 2021.7 with the CHARMM36 force field applied to the protein. Ligand topologies were generated through the CGenFF server, using the optimized geometry obtained from ORCA calculations. Each protein–ligand complex was solvated in a cubic box containing TIP3P water molecules, and counterions were added to neutralize the system. Energy minimization was conducted with the steepest descent algorithm until the maximum force was reduced below 1000 kJ/mol·nm. Equilibration was performed in two stages: first under constant volume and temperature (NVT) for 100 ps at 300 K, followed by constant pressure and temperature (NPT) for 100 ps at 1 bar using the Parrinello–Rahman barostat. Production simulations were then run for 100 ns with a 2 fs time step. Bond lengths were constrained with the LINCS algorithm, while long-range electrostatics were treated using the Particle Mesh Ewald (PME) method. Trajectories were analyzed for root mean square deviation (RMSD) backbone and ligand using GROMACS built-in utilities which is commonly used to evaluate the structural stability of protein–ligand complexes, where stable or plateauing RMSD values indicate minimal conformational fluctuation and sustained binding throughout the MD trajectory

2.2.7. Cell Culture

Human lung cancer cell lines A549 and H460, and non-tumorigenic lung epithelial BEAS-2B cells, were obtained from ATCC (Manassas, VA, USA). The A549 and BEAS-2B cells were cultured in Dulbecco's Modified Eagle Medium (DMEM),

while H460 cells were maintained in RPMI-1640. All media were supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin. Cells were incubated at 37 °C with 5% CO_2 in a humidified atmosphere. All media and supplements were purchased from GIBCO (Grand Island, NY, USA).

2.2.8. Cytotoxicity Assay

Cell viability was assessed using the MTT assay. Cells were seeded in 96-well plates at a density of 2 × 10^3 cells per well and allowed to adhere overnight. GA ethanolic extract and cisplatin (Sigma-Aldrich, St. Louis, MO, USA) were applied different concentrations ranging, followed by 72 h incubation. After treatment, MTT (Sigma-Aldrich, St. Louis, MO, USA) solution (0.5 mg/mL) was added and incubated for 4 h, and the resulting formazan crystals were dissolved in DMSO (Sigma -Aldrich, St. Louis, MO, USA). Absorbance was recorded at 570 nm with a microplate reader (VICTOR3/Wallac 1420, Perkin Elmer, Waltham, MA, USA). Half-maximal inhibitory concentration (IC_{50}) values were determined by nonlinear regression, and the selectivity index (SI) was calculated as the ratio of IC_{50} in BEAS-2B cells to that in cancer cell lines.

2.2.9. Statistical Analysis

Data are expressed as mean ± SEM from at least three independent experiments. Statistical analyses were performed using GraphPad Prism version 8 (GraphPad Software, San Diego, CA, USA). One-way ANOVA followed by Tukey's multiple comparison test was used to evaluate differences between control and treatment groups, and values of $*p < 0.05$ were considered statistically significant.

3. RESULTS AND DISCUSSIONS

3.1. LC-HRMS Profiling of GA

The LC–HRMS analysis of the GA ethanolic extract revealed a highly complex chromatographic profile (Figure S1), characterized by numerous peaks corresponding to diverse classes of secondary metabolites. This chemical diversity reflects the richness of the *Garcinia* genus, which is known to produce phenolic acids, xanthones, benzophenones, and flavonoids [9]. Among these, flavonoids were prioritized in the present study given their well-documented anticancer properties and multi-target pharmacological activities [9][19]. From the LC–HRMS dataset, 5 flavonoids were confidently identified and selected for downstream analyses (Table 1). Identification was established based on accurate mass, retention time, and MS/MS fragmentation patterns compared with reference databases. The detected compounds included icariin, paratocarpin G, quercetin-3β-D-glucoside, quercetin, and spinochalone A. These flavonoids

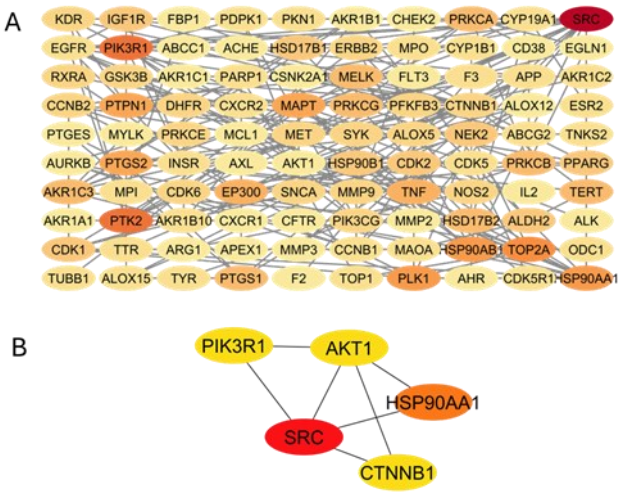


Figure 3. PPI network constructed from the intersected targets of *G. atroviridis* flavonoids and lung cancer DEGs. (A) Main cluster of the PPI network. (B) Top five hub genes ranked by degree value, highlighting their central role in the network. Color from light yellow to dark red) indicates increasing degree connectivity.

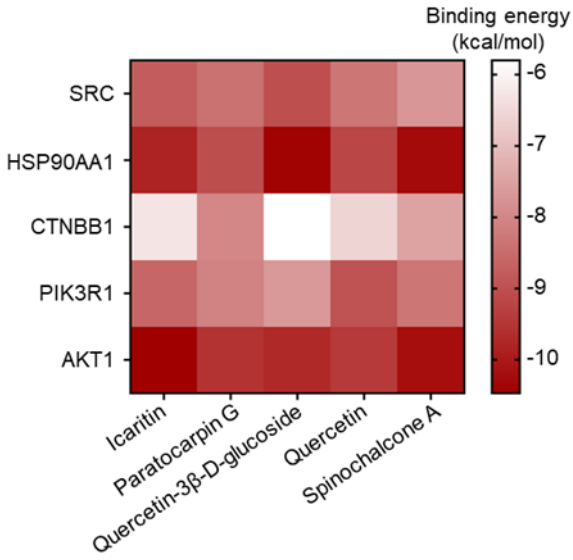


Figure 4. Heatmap representation of molecular docking scores between the top five hub targets and the selected flavonoids from *G. atroviridis*. Color gradients indicate relative binding affinities, with darker shades representing stronger predicted interactions.

have been previously reported in related *Garcinia* species and are associated with antioxidant and anticancer activities, supporting the pharmacological relevance of GA as a reservoir of bioactive metabolites [11][19]-[22]. This study provides a comprehensive investigation into the anticancer potential of GA, combining chemical profiling, computational modeling, and experimental validation into a single framework. While several species within the *Garcinia* genus, such as *G. mangostana*, have been widely studied for their xanthones and benzophenones [11], GA remains underexplored despite its longstanding use in traditional medicine. Our findings expand the phytochemical knowledge of this plant by demonstrating that GA ethanolic extract contains a diverse array of flavonoids, several of which have been previously reported in related *Garcinia* species but have not been systematically linked to cancer mechanisms [9][19][22]. By identifying five major flavonoids from GA fruit and subjecting them to integrated bioinformatics and biological analyses, we provide new evidence that this plant represents a valuable reservoir of anticancer metabolites.

3.2. Identification of Overlapping Targets between GA Flavonoids and Lung Cancer

To identify lung cancer–associated targets, transcriptomic data were retrieved from the GSE19188 dataset. Differential expression analysis (Table S1) revealed many genes that were significantly upregulated or downregulated in lung cancer compared to normal tissues (Figure 1). From this dataset, the top 100 upregulated and top 100 downregulated genes were selected as representative disease-associated targets (Table S2) and then combined with lung cancer–associated genes retrieved from GeneCards to create a comprehensive disease-related gene list before performing target intersection analysis.

In parallel, the potential protein targets of the five flavonoids identified from GA were predicted using SwissTargetPrediction. This process generated 166 putative targets for the flavonoid dataset. When compared with the integrated lung cancer target set (9,246 genes), a total of 142 overlapping targets were identified (Figure 2). These shared targets represent the potential molecular interface through which GA flavonoids may exert therapeutic activity against lung cancer. The advantage of applying bioinformatics lies in its ability to capture the complexity of phytochemical interactions with disease-related targets [23]. Unlike conventional “one drug–one target” strategies, network pharmacology emphasizes the multi-target and multi-pathway nature of natural compounds

[12][24].

3.3. PPI Network and Hub Target Identification

To further characterize the biological significance of the overlapping targets, a PPI network was constructed using STRING and visualized in Cytoscape. The resulting network (Figure 3(a)) revealed a highly interconnected cluster of proteins, indicating that the shared targets of GA flavonoids and lung cancer genes are functionally associated rather than randomly distributed. Topological analysis of the PPI network was performed to identify hub proteins, which may serve as key regulatory nodes. Based on degree values, the top five hub targets were identified as Proto-oncogene tyrosine-protein kinase Src (SRC), Heat shock protein 90 alpha family class A member 1 (HSP90AA1), Catenin beta-1 (CTNNB1), Phosphoinositide-3-kinase regulatory subunit 1 (PIK3R1), and protein kinase B (AKT1) (Figure 3 (b)).

Among these targets, proteins such as SRC, HSP90AA1, CTNNB1, PIK3R1, and AKT1 stood out as hubs within the protein–protein interaction network. These nodes are well-established drivers of lung cancer, with SRC and AKT1 promoting proliferation and survival [3][25], CTNNB1 maintaining stemness and epithelial–mesenchymal transition [26][27], PIK3R1 contributing to PI3K/AKT signaling and regulates cancer aggressiveness [3], and HSP90AA1 stabilizing multiple oncogenic proteins simultaneously [14][28]. KEGG pathway analysis of the 142 overlapping targets revealed significant enrichment (FDR < 0.05) in cancer-related pathways, including PI3K–Akt signaling, proteoglycans in cancer, HIF-1 signaling, EGFR-TKI resistance, focal adhesion, and non-small cell lung cancer pathways. A KEGG dot plot summarizing the top enriched pathways is shown in Figure S2. The ability of GA flavonoids to converge on these nodes suggests that their pharmacological activity may arise from simultaneous interference with multiple oncogenic pathways, a property that could potentially circumvent the limitations of single-target therapies that often succumb to resistance.

3.4. Molecular Docking and Dynamics Validation of Flavonoid–target Interactions

To evaluate the binding potential of the five selected flavonoids toward the core hub proteins, molecular docking analysis was performed. The docking results are summarized as a heatmap (Figure 4), which illustrates the binding energies between each ligand–target pair. All compounds exhibited favorable binding affinities (−6.0 to −10.0 kcal/mol), indicating stable interactions with the hub proteins. Among them, quercetin-3β-D-glucoside displayed the strongest binding affinity toward SRC, suggesting this flavonoid–target pair as a promising candidate for further study.

To further validate the stability of this interaction, molecular dynamics simulations were conducted on the SRC–quercetin-3β-D-glucoside complex. The 100 ns trajectory analysis showed that both the protein backbone and the ligand maintained stable conformations throughout the simulation (Figure 5). The RMSD of the ligand

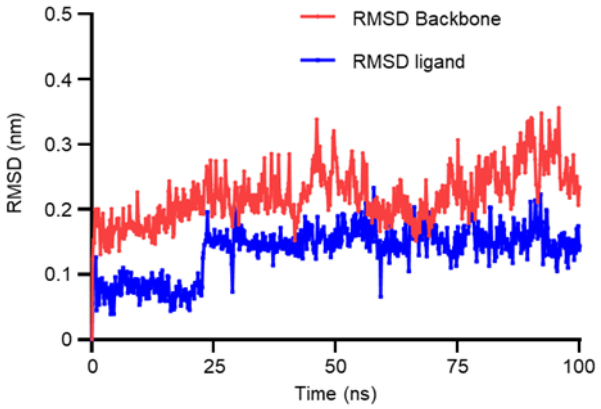
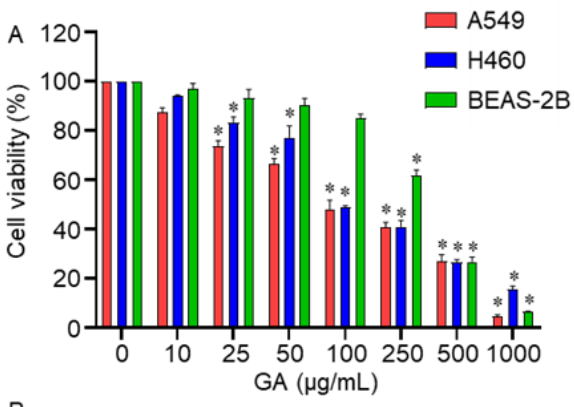


Figure 5. Molecular dynamics simulation of the SRC–quercetin-3β-D-glucoside complex.



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Cells	IC_{50} (µg/mL)	SI
A549	95.33 ± 6.45	2.80
H460	92.93 ± 8.86	2.87
BEAS-2B	266.93 ± 6.55	

Figure 6. Anticancer activity of the ethanolic extract of GA in A549, H460, and BEAS-2B cells after 72 h treatment. (a) Cell viability curves across increasing GA concentrations. (b) Calculated IC_{50} values and selectivity index (SI) for each cell line. Data represent mean ± SEM of three independent biological replicates (n = 3), each performed with three technical replicates. Statistical significance was assessed using one-way ANOVA followed by Tukey's post-hoc test, with $*p < 0.05$ versus control.

remained below 0.2 nm, while the protein backbone fluctuated within 0.2–0.3 nm, confirming that the complex achieved equilibrium without significant structural disruption.

Computational analyses provided additional mechanistic plausibility to these predictions [23]. Molecular docking demonstrated that the identified flavonoids could form stable interactions with the hub proteins, and molecular dynamics simulations further confirmed the stability of representative complexes. The interaction between quercetin derivatives and SRC is particularly notable, given existing evidence that flavonoids can attenuate SRC -mediated signaling cascades involved in cancer growth [29]. The persistence of this interaction throughout simulation suggests that the binding is not only energetically favorable but also dynamically stable, reinforcing the idea that GA flavonoids are capable of exerting sustained molecular interference under physiological conditions. These computational findings complement the network pharmacology predictions by moving beyond statistical associations and offering structural explanations for how GA flavonoids may modulate oncogenic targets.

SRC is a well-established oncogenic driver in non-small cell lung cancer, and numerous studies have demonstrated its involvement in multiple malignant phenotypes. Activated SRC enhances cell proliferation and survival through downstream signaling involving PI3K/AKT, STAT3, and RAS/ RAF/MEK pathways [30], while also promoting epithelial–mesenchymal transition (EMT), cytoskeletal remodeling, invasion, and metastatic spread [31]. Clinically, elevated SRC expression or activation has been associated with aggressive tumor behavior and poorer overall survival in lung cancer patients [32] and other types of cancer [33]. Prior reports also show that SRC confers resistance to chemotherapy and targeted agents, indicating that SRC-driven signaling is a critical contributor to treatment failure [34]. Therefore, inhibition of SRC has emerged as an attractive therapeutic strategy, and several SRC inhibitors have demonstrated antitumor effects in preclinical lung cancer models. In this study, quercetin-3β-D-glucoside showed the strongest predicted interaction with SRC, and MD simulations confirmed stable binding, suggesting that GA-derived flavonoids may suppress SRC-mediated oncogenic signaling.

3.5. In Vitro Anticancer Validation of GA Fruit Extract

To validate the anticancer potential predicted

from network pharmacology and docking analyses, the GA ethanolic extract was evaluated *in vitro* using lung cancer cell lines (A549 and H460) and normal lung epithelial cells (BEAS-2B). After 72 h of treatment, GA induced a concentration-dependent reduction in cell viability (Figure 6(a)). The cytotoxic effect was more pronounced in the cancer cell lines compared with BEAS-2B, indicating selective activity toward malignant cells. The calculated IC_{50} values further confirmed this selective cytotoxicity (Figure 6(b)). GA exhibited lower IC_{50} values in A549 and H460 cells relative to BEAS-2B, suggesting higher sensitivity of cancer cells to treatment. The SI defined as the ratio of IC_{50} in BEAS-2B to IC_{50} in cancer cells, exceeded the threshold of 2.0, indicating preferential toxicity against cancer cells over normal lung cells. This property is critical in anticancer development, as lack of selectivity has long hindered the translation of many natural compounds into clinical practice [35]. To place these findings in context, cisplatin was included as a positive control (Figure S3). As expected, cisplatin produced potent, concentration-dependent growth inhibition in both A549 and H460 cells, with IC_{50} values of 3.57 ± 0.26 μM and 4.36 ± 0.26 μM, respectively. However, this strong cytotoxicity extended to normal BEAS-2B cells as well (IC_{50} = 3.14 ± 0.35 μM), resulting in SI values below 1.0 (0.88 for A549 and 0.72 for H460). These results highlight the lack of selectivity of cisplatin between malignant and normal cells, in stark contrast to GA extract, which demonstrated preferential toxicity against cancer cells. The observed selectivity index above the conventional threshold suggests that GA metabolites exploit vulnerabilities unique to malignant cells. Such preferential cytotoxicity is consistent with prior reports on flavonoids, which have been shown to induce apoptosis, cell cycle arrest, and suppression of metastasis more effectively in tumor cells than in normal tissues [8] [36]-[38].

Nevertheless, certain limitations must be acknowledged. The LC–HRMS analysis, while comprehensive, was limited by the availability of spectral references, and it is possible that other active constituents remain unidentified. The biological assays employed crude GA ethanolic extract rather than isolated flavonoids, making it difficult to attribute activity to specific compounds or to assess potential synergistic effects. Computational predictions, though validated by docking and dynamics, require further experimental confirmation. These limitations do not diminish the validity of our findings but highlight important directions for future work. The identified hub proteins may also serve as biomarkers to stratify patients who are most likely to respond to flavonoid -based interventions, especially those with tumors driven by SRC. Integration of omics technologies, such as proteomics and metabolomics, with *in vivo* efficacy studies could further unravel the systemic impact of GA metabolites.

4. CONCLUSIONS

This study provides the first integrated evidence linking flavonoids from GA to potential anticancer mechanisms against lung cancer. Using LC–HRMS profiling, five major flavonoids (icariin, paratocarpin G, quercetin-3β-D-glucoside, quercetin, and spinochalone A) were identified as prominent constituents. Network pharmacology analysis revealed strong associations between these compounds and lung cancer–related targets, with protein–protein interaction analysis highlighting SRC, HSP90AA1, CTNNB1, PIK3R1, and AKT1 as central hubs. Molecular docking and dynamics simulations confirmed stable interactions, particularly between Quercetin-3β-D-glucoside and SRC, suggesting a plausible molecular basis for therapeutic action. *In vitro* validation further demonstrated selective cytotoxicity of the GA ethanolic extract against A549 and H460 lung cancer cells while sparing normal BEAS-2B cells, underscoring the translational relevance of these findings. Together, these results suggest that GA flavonoids may act through multi-target modulation of oncogenic signaling pathways, offering potential advantages over single-target strategies in overcoming resistance and improving therapeutic outcomes.

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Conflicts of Interest

The authors declare no conflict of interest.

SUPPORTING INFORMATION

Supplementary data associated with this article can be found in the online version at doi: 10.47352/jmans.2774-3047.344

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DECLARATION OF GENERATIVE AI

Not applicable.

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