RESEARCH ARTICLE

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Exploring the Therapeutic Potential of *Ginkgo biloba*: Phytochemical Composition, Antibacterial Activity Against Multidrug-Resistant Pathogens, and Modulation of Cancer Signaling

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Abstract

Objective: Evaluate the phytochemical composition of *Ginkgo biloba* methanolic extract and investigate its anticancer and antibacterial activity, along with exploring its potential molecular mechanism of action through molecular docking analysis. **Methods:** Phytochemical protocols including phenols, flavonoids and saponins were qualitatively evaluated. The anti-bacterial activity was tested versus G+ and G- bacteria using agar well diffusion method. Cytotoxic activity was investigated using the MTT assay on HepG2 (liver cancer), A549 (ling cancer) and WRL-68 (normal) cell lines. Additionally, molecular docking analysis was performed to evaluate the interaction between selected bioactive compounds and the Akt1 protein (PDB ID:2C6T). **Results:** The extract demonstrated high levels of total phenols (309.47 ± 4.21 mg GAE/g), total flavonoids (299.78 ± 14.81 mg/mL), and total saponins (137.76 ± 1.72 g/100 g). A significant antibacterial activity was observed in a concentration-dependent manner; the strongest effect was against Escherichia coli, showing inhibition zones ranging from 10 to 27 mm. The MTT assay showed a dose-dependent reduction in the viability of cancer cells, where HepG2 viability decreased from 92.28 ± 1.88% to 45.48 ± 2.61% (IC_{50} = 144.8 µg/mL), and A549 viability decreased from 93.17 ± 0.41% to 56.16 ± 3.56% (IC_{50} = 54.33 µg/mL). Meanwhile, lower cytotoxicity was observed in normal WRL-68 cells (IC_{50} = 145 µg/mL). Molecular docking analysis revealed favorable binding of Ginkgo phytochemicals to the Akt1 active site, with ginkgolide B showing the highest binding affinity (−7.19 kcal/mol), followed by isorhamnetin (−6.79 kcal/mol). **Conclusion:** *Ginkgo biloba* methanolic extract demonstrates significant antibacterial, antioxidant, and anticancer activities. These biological activities mostly arise from its rich phytochemical composition, particularly the bioactive constituents that may target key signaling pathways such as PI3K/Akt. Consistently, molecular docking analysis suggests a synergistic multi-compound mode of action, supporting the idea that *Ginkgo biloba* has the potential to serve as a valuable source for multi-target therapeutic development.

Keywords: Phytochemical profiling, multidrug-resistant bacteria, cytotoxicity, cancer signaling pathways

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Introduction

Over the last few decades, infectious disease and cancer have continuously placed a global burden on health sector, representing the leading causes of illness and death worldwide. A major driver of growing infectious diseases is the rise of multi-drug resistance (MDR) bacteria, mainly driven by the abuse and overuse of antibiotics, thereby reduced the effectiveness of conventional antimicrobial therapies [1, 2]. At the same time, cancer treatment outcome remains remain challenging because of development of resistance, systemic toxicity of chemotherapeutic drugs, and biological heterogeneity of tumors. These limitations highlight the urgent need for alternative therapeutic options; multi-target therapeutic strategies that are effective, safer and capable of acting via multiple targets. In this context, medicinal plants have received increasing attention because they represent rich sources of bioactive compounds with diverse pharmacological properties [3, 4]. Numerous plant-derived molecules have revealed a significant antibacterial effect through different mechanisms of action, including destroying bacterial cell membranes, inhibiting essential enzymes and interference with nucleic acid synthesis [5, 6].

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Similarly, plant constituents have demonstrated anticancer potential activity by modulating key cellular processes, including apoptosis induction, cell cycle arrest, inhibition of angiogenesis and suppression of metastasis [7–10]. Beyond these side effects, their antioxidant properties can also contribute to the lowering of oxidative stress, a critical factor involved in cancer development and progression [11]. The therapeutic value of medicinal plants is closely linked to their rich secondary metabolites. These metabolites include major chemical classes such as phenolic compounds, flavonoids, terpenoids, and saponins, each of which contributing specific biological function [3, 5]. Phenolics and flavonoids are widely recognized for their strong antioxidant capacity and ability to scavenge reactive oxygen species (ROS), whereas terpenoids are often associated with antimicrobial and anticancer activities through modulation of pathways signaling and cellular targets [8, 11]. Importantly, plants extracts may act through additive or synergistic interactions among their constituents, which can enhance overall therapeutic efficacy of plant extracts [12–14]. Among these medicinal plants, *Ginkgo biloba* represents one of the oldest living tree species, which has been used for centuries in both traditional and modern medicinal practices [6, 7]. The plant contains a complex mixture of flavonoids such as quercetin and kaempferol as well as terpenoids including ginkgolides, which are widely regarded as major contributors to its biological activity [10, 11]. Prior studies have reported that these compounds possess potent antioxidant properties, antimicrobial activity against various pathogenic bacteria, and significant anticancer activity [9, 12, 13]. Mechanistically, *Gingo biloba* constituents have been implicated in suppressing tumor cell growth, promoting apoptosis, and reducing angiogenesis, thereby limiting tumor growth and metastasis [12–15]. Furthermore, several constituents have been suggested to interact with signaling pathways central to cancer cell survival, such as PI3K/Akt [16]. Despite these promising findings, comprehensive investigations that integrate phytochemical profiling, antibacterial assessment, cytotoxic assessment, and molecular mechanism exploration of *Gingo biloba* extracts remain limited. Specifically, the molecular mechanism underlying its anticancer activity is not yet fully clarified. Therefore, the present study was designed to (i) evaluate the phytochemical composition of *Gingo biloba* methanolic extract, (ii) investigate its antibacterial effect against selected pathogenic bacteria, (iii) assess its cytotoxic effects on cancer cell lines, and (iv) investigate the potential molecular mechanisms of action through molecular docking analysis. Together, these aims provide more integrated understanding of the therapeutic potential of *Gingo biloba* as a natural source of bioactive compounds for addressing both multidrug-resistant bacterial infection and cancer.

Materials and Methods

Plant collection

Ginkgo biloba aerial parts were collected from cultivated sources in Erbil city, Kurdistan Region, Iraq (36.1911° N, 44.0093° E) during (October, 2025). The plant material was taxonomically authenticated by a qualified botanist at the Department of Biology, College of Science, Baghdad University.

Preparation of Ginkgo biloba methanolic extract

Approximately 50 g of dried aerial parts of *Ginkgo biloba* were washed with distilled water, air-dried at room temperature, and ground into a fine powder. The powdered material was extracted with 80% methanol (1 L) at 40°C for 24 hrs under continuous agitation using a shaking incubator. The extract was filtered through cheesecloth followed by Whatman No. 1 filter paper to remove insoluble residues. The filtrate was concentrated under reduced pressure using a rotary evaporator and dried to obtain a crude extract, which was stored at 4°C until further use [17, 18].

Determination of Phytochemical Contents

Determination of total flavonoids content

Total flavonoid content was determined using a colorimetric according to Sakanaka et al. [19] method Briefly, 3.2 g of *G. biloba* methanolic extract was initially dissolved in 5 mL of 50% methanol (methanol: distilled water). Subsequently, 1 mL of 5% sodium nitrite ($NaNO_2$) solution was added. After 6 mins, 1 mL of 10% aluminum chloride ($AlCl_3$) solution was added. Following an additional 5 min, 10 mL of 10% sodium hydroxide (NaOH) solution was added to terminate the reaction, and the final volume was adjusted to 50 mL with distilled water. The reaction mixture was allowed to stand for 15 mins. at room temperature, after which the absorbance was measured at 450 nm using a UV–Vis spectrophotometer. All measurements were performed in triplicate. A calibration curve was constructed using rutin standard solutions under the same conditions, and the total flavonoid content was calculated using the following regression equation:

$y = 0.0012x - 0.1109$ ($R^2 = 0.9317$). Results were expressed as milligrams of rutin equivalents per gram of extract (mg RE/g).

Determination of total phenols content

The total phenolic content of *G. biloba* methanolic extract was determined using the Folin–Ciocalteu method [20, 21]. 1 mL of the extract solution was mixed with 2.5 mL of 10% (w/v) Folin–Ciocalteu reagent. After 5 min, 2.0 mL of 7.5% sodium carbonate (Na_2CO_3) solution was added. The mixture was incubated at 50°C for 10 min with intermittent mixing and then allowed to cool to room temperature. The absorbance was measured at 765 nm using a UV–Vis spectrophotometer (Shimadzu UV-1800, Japan) against a reagent blank. All measurements were performed in triplicate. A calibration curve was constructed using gallic acid as a standard, and the total phenolic content was calculated using the following regression equation:

$y = 9.35x - 0.13$ ($R^2 = 0.996$). Results were expressed as milligrams of gallic acid equivalents per gram of dry

extract (mg GAE/g).

Determination of total saponin content

Total saponin content was determined spectrophotometrically according to the method described by Lim et al. [22] . Briefly, 0.25 mL of the extract solution was mixed with 1 mL of a reagent mixture consisting of glacial acetic acid and sulfuric acid (1:1, v/v). The mixture was vortexed and incubated in a water bath at 60°C for 30 min, followed by cooling to room temperature. The absorbance was measured at 527 nm using a UV–Vis spectrophotometer. All measurements were carried out in triplicate. A standard calibration curve was prepared using oleanolic acid (0–1,000 µg/mL), and the total saponin content was calculated using the regression equation:

$y = 3.99x - 0.2097$ ($R^2 = 0.993$). Results were expressed as grams of oleanolic acid equivalents per 100 g of extract (g/100 g).

Bacterial strains and culture conditions

The antibacterial activity of *Ginkgo biloba* extract was evaluated against Gram-positive bacteria (Staphylococcus aureus, Streptococcus pyogenes) and Gram-negative bacteria (*Escherichia coli*, Pseudomonas aeruginosa). Bacterial strains were obtained from the Microbiology Laboratory, College of Biotechnology, Al-Nahrain University, Iraq. All isolates were sub-cultured on Mueller–Hinton agar and incubated at 37°C for 18–24 h prior to testing to ensure viability and purity.

Antibacterial assay

Antibacterial activity was evaluated using the agar well diffusion method as described by Santurio et al. [23]. Bacterial suspensions were standardized to 0.5 McFarland turbidity (1.5×10^8 CFU/mL) and uniformly inoculated onto Mueller–Hinton agar plates using sterile cotton swabs. Wells (6 mm in diameter) were aseptically punched into the agar, and 50 µL of *G. biloba* extract at concentrations of (100, 75, 50, and 25%) were added. Plates were incubated at 37°C for 18–24 h. Antibacterial activity was evaluated by measuring the diameter of the inhibition zone (mm), defined as the clear region surrounding the well where visible bacterial growth was completely suppressed. All measurements were performed in triplicate, and mean values were calculated.

Cytotoxicity assay

The cytotoxic activity of *G. biloba* extract was evaluated using the MTT assay on human liver cancer cell line (HepG2), lung cancer cell line (A549), and normal human liver cell line (WRL-68). Cell lines were obtained from the Cell Culture Unit, College of Biotechnology, Al-Nahrain University, Baghdad, Iraq,and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and 1% antibiotics under standard conditions (37°C, 5% CO_2). Cells were seeded in 96-well plates and treated with different concentrations of the extract (25, 50, 100, 200, and 400 µg/mL) for 24 h. Following incubation, MTT reagent was added and incubated for an additional 4 h. The resulting formazan crystals were dissolved using dimethyl sulfoxide (DMSO), and absorbance was measured at 575 nm using an ELISA plate reader. Cell viability (%) was calculated relative to untreated control cells. The half-maximal inhibitory concentration (IC_{50}) was determined for each cell line. Comparative cytotoxicity was assessed by evaluating the selectivity of the extract toward cancer cell lines relative to normal cells (WRL-68) [24 , 25].

Molecular docking simulation

To assess how some active substances from *Ginkgo biloba* might interact with the ATP-binding site (e.g., the active site), they were docked with the three-dimensional human Akt1 kinase domain structure (PDB: 2C6T) extracted from the protein data bank [26]. The receptor was refined by deleting water molecules, adding hydrogens and relaxing the structure to a stable state in MOE software (2024, Chemical Computing Group) [27]. The structures for selected potential bioactive compounds (flavonoids: quercetin, kaempferol and isorhamnetin, terpene: ginkgolide B) were built and energy minimized. Docking of ligands was centered on the binding site region, based on the position of the existing ligand in the protein crystal structure. 100 poses for each ligand were generated to visualize binding modes. The most promising poses from each run were docked with induced fit to allow the receptor flexibility. Binding energy score (kcal/mol) and the types of interactions (hydrogen bonds, hydrophobic and π-π interactions) between residues and each pose were recorded and the highest binding energy score (i.e., lowest energy, most negative binding) in the absence of a specific active site binding amino acid within reasonable proximity to any ligand molecule was chosen for the ligand structure analysis. Based on this analysis, we were able to find promising interactions that involved the residues from the catalytic domain of Akt1, which might play a role in inhibition.

Statistical analysis

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation (SD). Statistical analysis was carried out using GraphPad Prism version (11.0.0.0) (GraphPad Software, USA) or SPSS version (IBM Corp., USA). Differences between groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. A p-value ≤ 0.05 was considered statistically significant.

Results

The results of the present study demonstrated that the methanolic extract of *Ginkgo biloba* possesses a rich phytochemical profile, characterized by high levels of flavonoids (299.78 ± 14.81 mg RE/g), phenolic compounds (309.47 ± 4.21 mg GAE/g), and saponins (137.76 ± 1.72 g/100 g). The extract exhibited significant antibacterial activity against both Gram-positive and Gram-negative bacteria, with the highest inhibition observed against *Escherichia coli*. Furthermore, cytotoxicity analysis revealed a concentration-dependent reduction in cancer

cell viability in HepG2 and A549 cell lines, with IC_{50} values of 144.8 µg/mL and 54.33 µg/mL, respectively, while showing lower toxicity toward normal cells.

Estimation the total flavonoids, phenol, and saponin contents

Contents of total flavonoids, saponin, and phenol in *Ginkgo biloba* were assessed. The methanolic extract showed high levels of total flavonoids (299.78 ± 14.81 mg RE/g), total phenols (309.47 ± 4.21 mg GAE/g), and total saponins (137.76 ± 1.72 g/100 g). The phytochemical profile of the *Gingo biloba* extract is summarized in Table (1), revealing comparatively high levels of Phenolic compounds, flavonoids and saponins.

Evaluation of anti-bacterial potential of Ginkgo biloba

The methanolic extract of *Gingo biloba* showed anti-bacterial effect across different concentrations. At 25%, it produced moderate activity against *Escherichia coli* with an inhibition zone measuring 10 mm. In contrast no inhibitory activity was detected against the other tested bacteria. At higher concentrations (50%, 75%, and 100%), the inhibition zones ranged from 28 to 15 mm for *S. aureus*, 25 to 11 mm for S. pyogenes, 27 to 15 mm for *E. coli*, and 26 to 18 mm for *P. aeruginosa*. As shown in Table 2, the antibacterial activity increased in a concentration-dependent manner, with *Escherichia coli* demonstrating the highest sensitivity. The inhibition of bacterial growth at different concentrations is illustrated in Figure 1.

In vitro evaluation of the cytotoxic effect of Ginkgo biloba methanolic extract on liver (HepG2) and lung (A549) cancer cell lines

The cytotoxic effect of *Ginkgo biloba* extract against liver (HepG2) and lung (A549) cancer cell lines, in comparison to the normal cell line (WRL-68), was

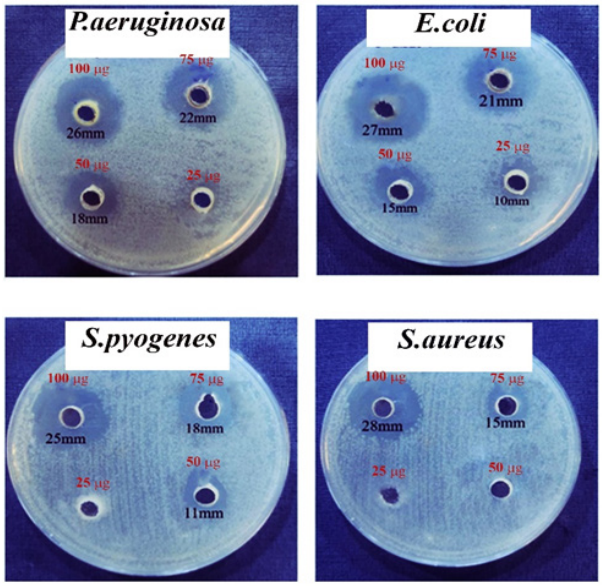

Figure 1. Antibacterial Activity of *Ginkgo biloba* methanolic extract against Pseudomonas aeruginosa, Escherichia coli, Streptococcus pyogenes, and Staphylococcus aureus.

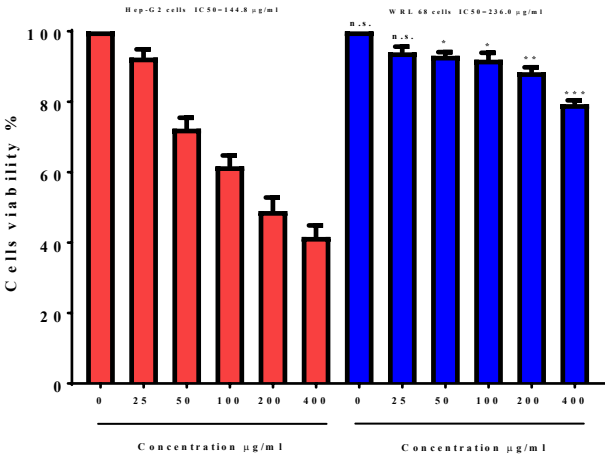

Figure 2. Cytotoxic Effect of *G. biloba* Extract on Liver Cancer Cell Line

evaluated using the MTT assay at different concentrations (400, 200, 100, 50, and 25 µg/mL).

As shown in Table 3 and Figure 2, cell viability of HepG2 cells decreased in a concentration-dependent manner following treatment with *Ginkgo biloba* extract. The maximum inhibition was observed at 400 µg/mL 45.48 ± 2.61%, whereas cell viability reached 92.28 ± 1.88% at 25 µg/mL, with an IC_{50} value of 144.8 µg/mL. In contrast, the normal cell line (WRL-68) exhibited lower sensitivity to the extract, with an IC_{50} value of 236.0 µg/mL and cell viability ranging from 76.19 ± 3.44% to

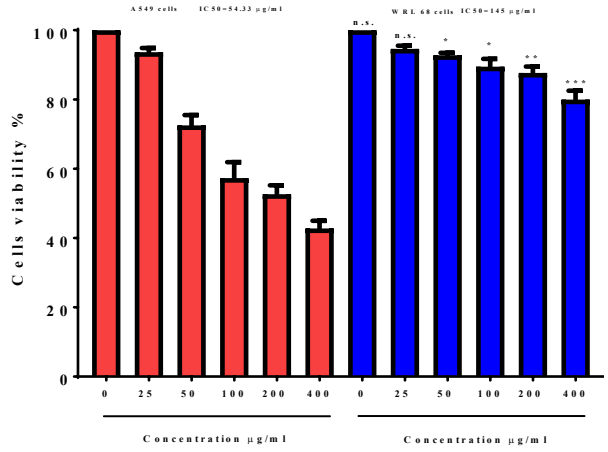

Figure 3. Cytotoxic Effect of *G. biloba* Extract on the Lung-Cancer Cell Line

Table 1. Quantitative Phytochemical Constituents of *Ginkgo biloba* Extract

Phytochemical Component	Indication	Results	Mean ± S.D.
Total flavonoid (mg/ml)	Yellow-ppt	Positive	299.78 ± 14.81
Total phenol (Mg GAE/G)	Yellowish-white precipitate	Positive	309.47 ± 4.21
Total saponin (g $100g^{-1}$)	Foam	Positive	137.76 ± 1.72

Where values are expressed as mean ± SD (n = 3)

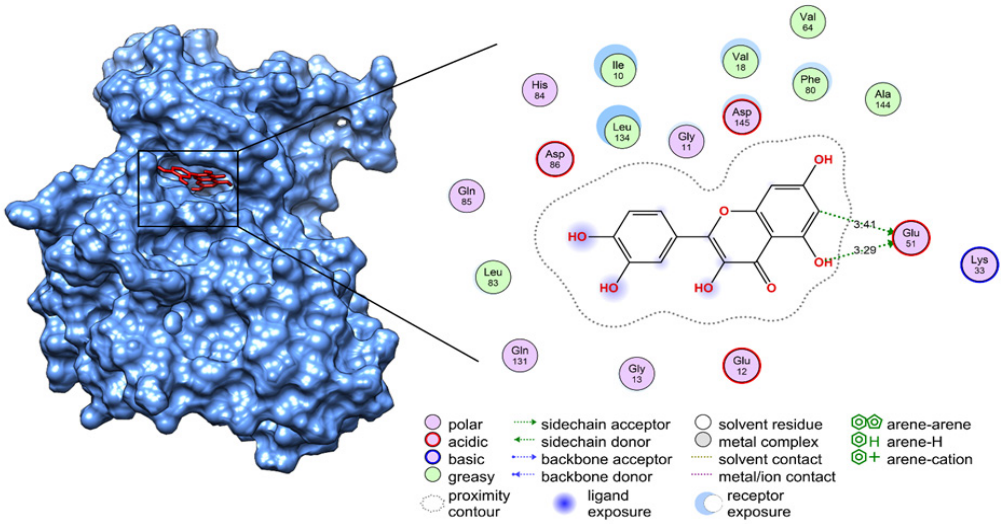

Figure 4. The Analysis of the Best Docked Complex between Quercetin and PDB ID: 2C6T

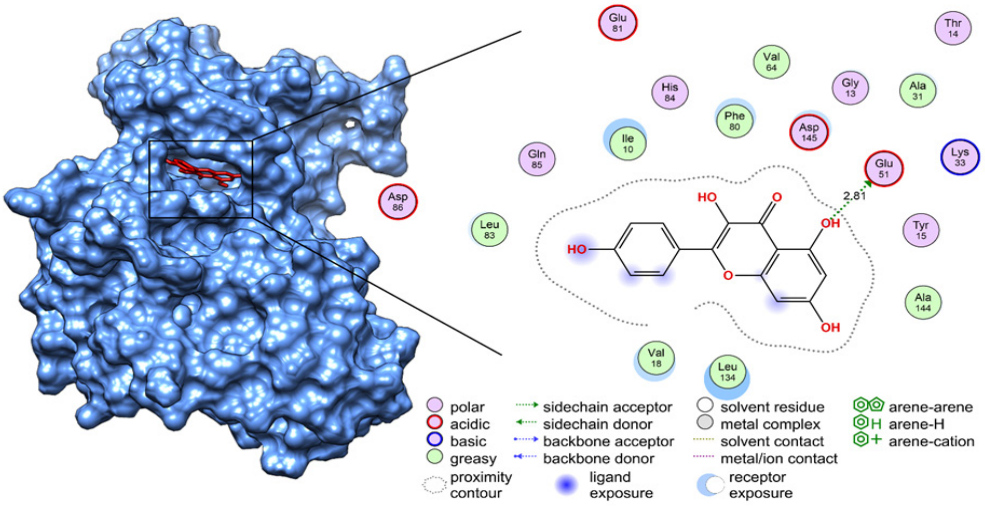

Figure 5. The Analysis of the Best Docked Complex between Kaempferol and PDB ID: 2C6T

95.33 ± 1.47% at concentrations of 400 to 25 µg/mL, respectively.

Similarly, Table 4 demonstrates the cytotoxic activity of the extract against A549 cells, confirming its potential anticancer effect. Which indicated the effect of *G. biloba* on lung cancer cell line explained that cell viability reduced from 93.17 ± 0.41% to 56.16 ± 3.56% for 25 to 400 µg/ml within IC_{50} value of 54.33 µg/ml however an IC_{50} value of 145 µg/ml was obtained from the effect of *G. biloba* on lung cancer cell line (see Figure 3 shows the cytotoxic effect of the extract on A549 cells)

Table 2. Antibacterial Activity of *Ginkgo biloba* Extract against Selected Bacterial Strains

Isolate bacteria	Inhibition zone in (mm)			
	100 (µg/mL)	75 (µg/mL)	50 (µg/mL)	25 (µg/mL)
Staphylococcus aureus	28	15	---	---
Streptococcus mutans	25	18	11	---
Escherichia coli	27	21	15	10
Pseudomonas aeruginosa	26	22	18	---

Molecular docking simulation

To simulate molecular interactions of select bioactive components of *Ginkgo biloba* extract with the human

Table 3. Cytotoxic Effect of *Ginkgo biloba* against Liver Cancer Cell Line HepG2 and Natural Cell Line WRL-68 (24 hrs. 37°C)

Ginkgo biloba extract Conc. (µg/ ml)	WRL68 Mean ± SD	HepG2 Mean ± SD	P value
400	76.19±3.44	45.48±2.61	***
200	86.95±4.90	55.82±7.99	**
100	93.86±1.50	65.50±2.86	*
50	94.17±0.74	77.19±2.08	*
25	95.33±1.47	92.28±1.88	ns

Where values are expressed as mean ± SD (n = 3), $p \leq 0.05$ *, $p \leq 0.01$ **, $p \leq 0.001$ ***, NS nonsignificant

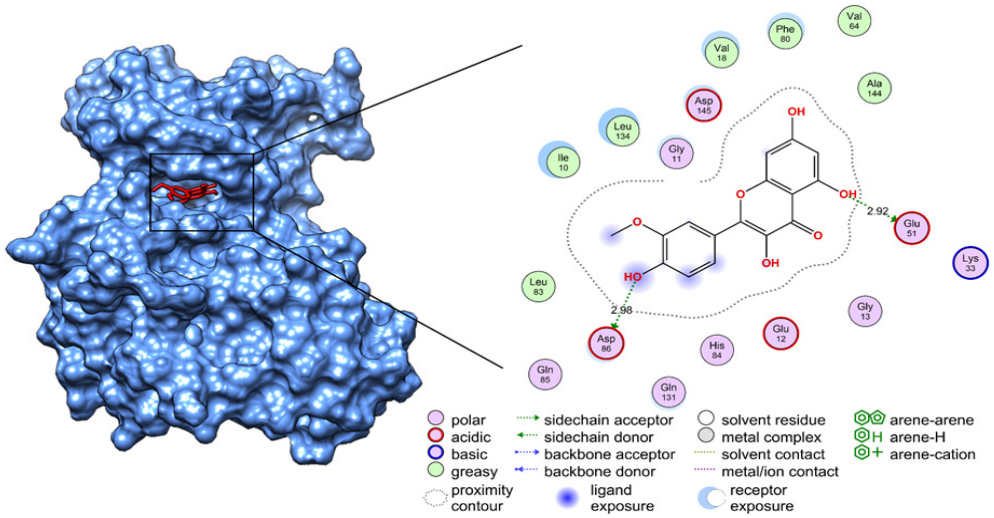

Figure 6. The Analysis of the Best Docked Complex between Isorhamnetin and PDB ID: 2C6T

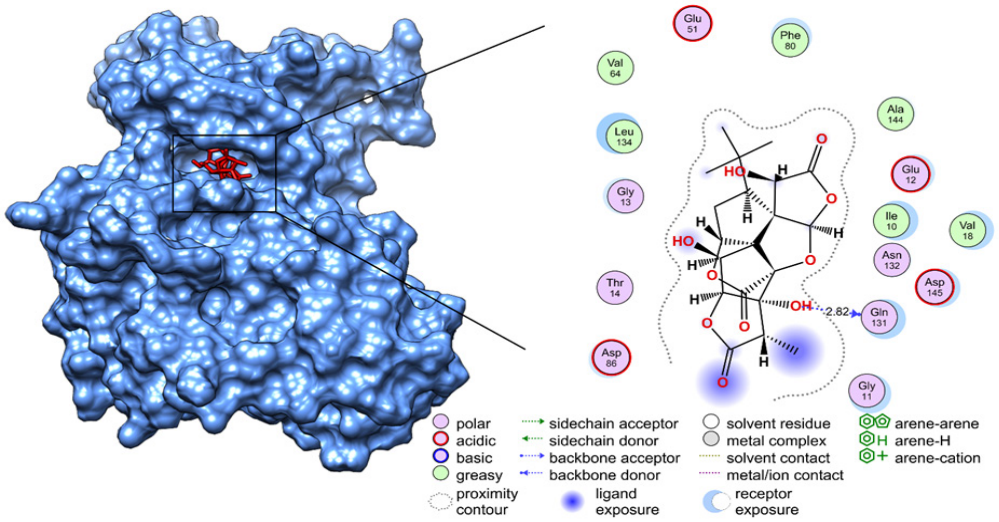

Figure 7. The Analysis of the Best Docked Complex between Ginkgolide B and PDB ID: 2C6T

Akt1 kinase domain, we carried out molecular docking. We used the active site of human Akt1 protein (PDB ID: 2C6T) as receptor. The docking scores (binding energy and RMSE value) of quercetin, kaempferol, isorhamnetin and ginkgolide B are given in Table 5. All bioactive compounds showed good binding into the active pocket of Akt1 and all resulted negative free energy values.

Table 4. Cytotoxic Effect of *Ginkgo Biloba* against Lung Cancer Cell Line A549 and Natural Cell Line WRL-68 (24 hrs. 37°C)

Ginkgo biloba extract Conc. (µg/ ml)	WRL68 Mean ± SD	A549 Mean ± SD	P value
400	68.57±1.12	56.16±3.56	***
200	74.83±1.02	63.50±2.60	**
100	86.35±0.47	72.45±2.40	*
50	91.37±2.04	83.06±1.33	*
25	94.05±0.17	93.17±0.41	ns

Where values are expressed as mean ± SD (n = 3), $p \leq 0.05$ *, $p \leq 0.01$ **, $p \leq 0.001$ ***, NS, nonsignificant.

These negative free energy values prove spontaneous formation and stable docking. Ginkgolide B showed strongest binding affinity (highest favorable binding energy of −7.193 kcal/mol, RMSE: 2.28 Å). Among the studied flavonoids, isorhamnetin presented most favorable binding (score of −6.790 kcal/mol, RMSD: 1.33 Å), followed by quercetin (−6.491 kcal/mol, RMSD: 1.81 Å) and kaempferol (−6.250 kcal/mol, RMSD: 1.13 Å). The values of RMSE that were≤2.28 Å of top docked poses confirm the validity and confidence of conformational stabilities attained from induced-fit dock.

Table 5. Binding Energy Scores of the Top Selected *Ginkgo biloba* Compounds Docked with Target Protein (2C6T)

Ligand	Binding Energy (kcal/mol)	RMSD
Quercetin	-6.49155	1.816129
Kaempferol	-6.25034	1.139131
Isorhamnetin	-6.79044	1.334959
Ginkgolide B	-7.19345	2.282999

To elaborate, 2D interactions in the docked models (Figures 4-7) show that these compounds stably docked in the Akt1 binding site via multiple interactions, such as arene-cation or pi-pi hydrophobic interactions among flavonoid rings and lipophilic amino acid residues, alongside polar contacts involving hydroxyl and carbonyl groups of phytochemicals and backbone and side chains of active sites (Glu and Asp, among others). These varied non-covalent forces were the drivers of ligand stability and Akt1-inhibitory effects.

Discussion

The findings of the present study are consistent with previous reports indicating that *Ginkgo biloba* is rich in flavonoids and phenolic compounds, which contribute to its strong antioxidant capacity [24]. These bioactive constituents play a critical role in reducing oxidative stress and protecting cells from damage associated with cancer development through the regulation of ROS levels .

Recent studies have further demonstrated that *G. biloba* extracts exert their biological effects via multiple antioxidant mechanisms, including modulation of intracellular ROS and reduction of chronic inflammation, a key factor in cancer progression [27, 28]. In consistency with these observations, the present study demonstrated a significant reduction in HepG2 cell viability, alongside morphological and biological features characteristic of apoptosis [29].

The anticancer potential of *Gingo biloba* is thought to be driven largely by its rich and diverse phytochemical composition, specifically flavonoids and terpenoids (including ginkgolides), which are known to influence essential cellular signaling pathways [30] , additionally, interaction between *Gingo biloba* extract and conventional antibiotics and chemotherapeutic agents warrant careful consideration, because such combinations may either enhance therapeutic efficacy through synergistic effects or, alternatively, increase the risk of adverse interactions [30, 31].

Notably, many of these bioactive compounds have been reported to modulate the PI3K/Akt pathway, thereby suppressing of cancer cell proliferation and promoting programmed cell death (apoptosis) [32, 33]. Moreover, secondary metabolites from medicinal plants, including *Gingo biloba*, may triger apoptosis via mitochondrial dysfunction and reactive oxygen species (ROS) generation, and can further contribute to cell cycle arrest [34].

In addition to its anticancer potential, *G. biloba* extract exhibited notable antibacterial activity against both Gram-positive and Gram-negative bacteria. Similar findings have been reported in previous studies, particularly against *E. coli* and *S. aureus* [35–37]. This antibacterial effect may be attributed to the presence of phytochemicals capable of disrupting bacterial cell membranes, inhibiting essential enzymes, and interfering with microbial metabolic processes.

Overall, these findings highlight the therapeutic potential of *G. biloba* as a natural source of bioactive compounds with both antibacterial and anticancer properties. However, further in vivo studies and clinical investigations are required to validate its mechanisms of action and confirm its safety and efficacy [38].

The PI3K/Akt pathway is involved in cancer cell proliferation, resistance to apoptosis and overall survival, making Akt1 a clinically important therapeutic target. The in-silico docking results confirm the mechanism responsible for the substantial cytotoxicity displayed in both the HepG2 and A549 cancer cell lines. High calculated binding energies between quercetin, kaempferol, isorhamnetin, ginkgolide B and the ATP binding site of Akt1 suggest competitive inhibitory mechanisms. In particular, Ginkgolide B, the most unique terpenoid in the *G. biloba* extract, is predicted to be the strongest inhibitor of the kinase.

This inhibition can be attributed to its unique, bulky, cage-like structure containing several functional oxygen groups, enabling it to occupy and rigidly block the catalytic pocket of Akt1 through substantial hydrophobic contacts and stable hydrogen bonding. Additionally, the observed stability of the flavonoids results mainly from the favorable orientation of the aromatic rings, which promotes arene-mediated and pi stacking interactions with the kinase amino acids, while the presence of amino acids in the catalytic site suggests these molecules might be able to stabilize the enzyme in an inactive state or outcompete ATP binding.

We noted that as many as 10 compounds in the *G. biloba* methanolic extract demonstrated very strong affinity toward Akt1, indicating that the extract was effective through a "multi-target/multi-compound" mechanism. Thus, distinct classes of molecules in the extract are suggested to work synergistically to cause more powerful inhibition of the PI3K/Akt survival signaling pathway, leading to the rapid inhibition of cancer cell cycle growth and apoptosis, as suggested by the substantial loss in cell viability seen in our MTT assay.

These predicted computational results confirm the findings reported in recent studies that demonstrate the anti-cancer and apoptotic effects of natural flavonoids and terpenoids, indicating that G. biloba's strong biological effect is mediated via disruption of survival signaling pathways. *G. biloba* thus may prove effective in developing "multi-action" chemopreventive as well as adjuvant therapies against cancer [39].

In conclusion, this work effectively showcases synergistic effects with antibiotic therapy and anticancer activities by using extracts of the methanolic extracts from the leaves of *Ginkgo biloba*. The immense bioactivity presented throughout this investigation was the result of the presence of secondary metabolites such as high yield of phenols, flavonoids and saponins in the extract. In addition, biological study confirmed dose-dependent inhibition of Gram-positive and Gram-negative multiple antibiotic-resistant multidrug resistant (MDR) bacteria with significant potency against *Escherichia coli* and was parallel with dose-dependent death of HepG2 (liver) and A549 (lung) cancer cell lines at low toxicity for WRL-68 normal liver cells. Docking studies further confirm mechanisms involved, among those molecules investigated, specific chemical constituents of the *Ginkgo*

biloba have the strongest molecular docking with a good affinity toward Akt1 kinase pocket, with Ginkgolide B showing best binding affinity and docked positions within the binding pocket. Results are strongly suggestive that anticancer effects are associated with blocking tumor-survival axis via the disruption of the PI3K/Akt signal transduction pathway by a multi-target-oriented chemotherapeutic agent. This finding supports *Ginkgo biloba* as a potent alternative therapeutic strategy in overcoming common public health burdens like antibiotic resistance and diverse human cancers and future studies require in vivo mammalian models to determine metabolic stability and bioavailability before incorporation into Complementary Oncology settings in cancer management.

Author Contribution Statement

S. B. I., N. D. H., M. E. A., N. B. A., R. H. Y., and M. S. J.: Writing Original draft, Methodology, Investigation and Formal analysis. S. B. I., and M. S. J.: Main Concept, Data interpretation, and supervision. S. B. I., N. D. H., M. E. A., N. B. A., R. H. Y., and M. S. J.: Writing-review and editing, Visualization, and Data curation. All authors reviewed the manuscript.

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Conflict of Interest

The authors declare no competing interests.

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