

Molecular Docking and Pharmacokinetic Analysis of Antioxidant Content in Popular Indonesian Herbal Plants for Adjuvant Therapy in Cancer

Luqman Alwi¹, Irwan Budiono^{1,*}, Mahalul Azam¹,
Selamat Budijitno², Irena Intania¹ and Matheus Prayoga Claus³

¹Department of Public Health, Faculty of Medicine, Universitas Negeri Semarang, Jawa Tengah 50229, Indonesia

²Department of Oncology Surgery, Faculty of Medicine, Universitas Diponegoro, Jawa Tengah 50275, Indonesia

³Department of Pharmacy, Faculty of Medicine, Universitas Negeri Semarang, Jawa Tengah 50229, Indonesia

(*Corresponding author's e-mail: irwan.budiono@mail.unnes.ac.id)

Received: 12 November 2025, Revised: 10 December 2025, Accepted: 17 December 2025, Published: 20 February 2026

Abstract

Cancer remains a significant global health burden due to high rates of morbidity, mortality, and disease recurrence. Apoptosis has emerged as an essential therapeutic target in cancer, and natural antioxidants from Indonesian medicinal plants show promise as modulators of apoptotic pathways because of their relative safety and bioactivity. This study aimed to identify phytochemical candidates with potential for adjuvant cancer therapy by evaluating their interaction with key apoptosis-regulating proteins. Nine antioxidant compounds (chlorogenic acid, cinnamic acid, curcumin, eugenol, gallic acid, kaempferol, quercetin, rutin, and tanshinone) were subjected to molecular docking against ten proteins representing intrinsic and extrinsic apoptotic pathways using AutoDock Vina, with dasatinib as a reference inhibitor. Drug-likeness was assessed according to Lipinski's rules, while pharmacokinetics and toxicity predictions were performed using ADMETLab 3.0 and ProTox-II. The analysis showed that several compounds, particularly chlorogenic acid, curcumin, and quercetin, exhibited strong binding affinities to apoptosis-regulating proteins and interacted with critical amino acid residues associated with pro-apoptotic signaling. While rutin and tanshinone also demonstrated favorable docking scores, their pharmacokinetic limitations and predicted toxicity reduced their therapeutic potential. The combined evaluation of molecular binding, drug-likeness, and safety profiles supports chlorogenic acid, quercetin, and curcumin as the most viable candidates for further investigation. These findings indicate that specific plant-derived antioxidants may contribute to apoptosis-induced cancer inhibition and offer a rational basis for selecting lead compounds for future *in vitro* and *in vivo* validation. Overall, this study provides a systematic *in silico* framework to prioritize natural compounds as potential adjuvant therapeutic agents targeting apoptosis in cancer.

Keywords: Molecular docking, Natural antioxidants, Apoptosis, Adjuvant cancer therapy

Introduction

Cancer remains one of the leading causes of mortality worldwide, responsible for approximately 10 million deaths in 2020, thereby posing a major global health challenge [1]. Among all malignancies, lung cancer is the most frequently diagnosed and the deadliest, followed by colorectal, liver, stomach, and breast cancers, which collectively account for a significant proportion of cancer morbidity and mortality [1-3]. The economic burden of cancer treatment is

equally daunting, with global costs reaching hundreds of billions of dollars annually. This financial impact places substantial pressure on healthcare systems worldwide, particularly in low- and middle-income countries [2].

Although current treatment modalities, ranging from surgery, chemotherapy, and radiotherapy to immunotherapy and targeted molecular approaches, have improved patient outcomes, their overall effectiveness remains limited due to adverse side

effects, restricted selectivity, and the persistent challenge of tumor recurrence [4,5]. High relapse rates in cancers such as colorectal, lung, and breast further highlight the urgent need for innovative therapeutic strategies capable of eradicating residual malignant cells [1]. Within this context, apoptosis, or programmed cell death, has emerged as a promising therapeutic target. Apoptosis plays a central role in maintaining tissue homeostasis and suppressing tumor progression, and its dysregulation not only facilitates unchecked proliferation but also contributes to chemoresistance [6,7]. Apoptotic signaling is primarily governed by two canonical pathways: The intrinsic (mitochondrial-mediated) and extrinsic (death receptor-mediated) cascades, both converging on caspase activation that orchestrates cellular dismantling [8,9]. Beyond these, alternative mechanisms such as necroptosis, ferroptosis, and immunogenic cell death are increasingly investigated to overcome apoptotic resistance and enhance therapeutic efficacy [6]. Thus, a deeper understanding of apoptosis and its molecular regulators remains vital for developing novel cancer treatments with greater selectivity and reduced toxicity [10,11].

In pursuit of this goal, plant-derived natural compounds have gained increasing attention for their ability to selectively modulate apoptotic pathways in cancer cells [12,13]. These bioactive agents are valuable for their structural diversity, favorable safety profiles, and multifaceted biological activities. Several antioxidants, such as curcumin, resveratrol, quercetin, and chlorogenic acid, have promoted apoptosis in various cancer models while sparing normal cells [13]. Significantly, resistance to conventional therapies is often associated with oxidative stress and defective apoptosis, making antioxidant-rich phytochemicals promising candidates to restore cell death mechanisms [14].

Indonesia, recognized as one of the world's most biodiverse countries, provides a vast reservoir of natural products for drug discovery. With approximately 50,000 plant species, 7,500 of which contribute to traditional medicine, the nation harbors an immense pharmacopeia of bioactive compounds with untapped therapeutic potential [15,16]. Medicinal plants such as turmeric (*Curcuma longa*), ginger (*Zingiber officinale*), and temulawak (*Curcuma xanthorrhiza*) have long been applied for their anti-inflammatory and antioxidant

effects in Indonesian traditional medicine [17,18]. Many of these species are rich in secondary metabolites, including alkaloids, flavonoids, phenolics, saponins, and curcuminoids, which have demonstrated the capacity to modulate oxidative stress and apoptosis-related signaling pathways [19,20]. Bridging ethnomedicinal knowledge with molecular approaches may accelerate the discovery of novel anticancer agents [21,22]. To systematically evaluate these compounds, *in silico* methods such as molecular docking have become indispensable tools in modern drug discovery. Docking enables rapid and cost-effective screening of ligands against molecular targets, predicting binding affinities, identifying critical interacting residues, and prioritizing candidates for further validation [15,23]. Platforms such as AutoDock provide reliable computational insights that, when combined with phytochemical screening, significantly enhance the identification of promising drug-like molecules [12]. Moreover, integrating molecular docking with ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profiling offers a comprehensive strategy to evaluate both efficacy and pharmacokinetic feasibility, thereby increasing translational potential.

This study aims to investigate the molecular interactions between antioxidant compounds from selected Indonesian medicinal plants and apoptosis-regulating proteins using molecular docking, complemented by ADMET profiling. Combining ethnopharmacological resources with computational approaches, the research seeks to identify phytochemicals with significant anticancer potential and provide a rational foundation for their development as adjuvant therapeutic agents.

Materials and methods

Ligand selection

In this study, we selected nine antioxidant compounds which are chlorogenic acid (PubChem CID: 1794427), cinnamic acid (PubChem CID: 444539), curcumin (PubChem CID: 969516), eugenol (PubChem CID: 3314), gallic acid (PubChem CID: 370), kaempferol (PubChem CID: 5280863), quercetin (PubChem CID: 5280343), rutin (PubChem CID: 5280805), and tanshinone (PubChem CID: 164676) as ligands for molecular docking analysis. These compounds were selected based on their status as major

constituents of inexpensive herbal plants, widely available in local Indonesian markets, and long utilized in traditional medicine. Furthermore, these antioxidants are commonly consumed in daily diets through spices, herbs, or vegetables, making them culturally and nutritionally relevant. Their ease of isolation and abundant availability also ensure sufficient supply for further pharmacological investigation [23]. **Figure 1** presents a two-dimensional structural overview.

Receptor preparation

This study selected apoptosis as the central therapeutic mechanism due to its critical role in regulating programmed cell death and its relevance in overcoming cancer cell resistance. The investigation focuses on key molecular components involved in both the extrinsic (death receptor-mediated) and intrinsic

(mitochondria-mediated) apoptotic pathways, particularly TNFR1, Fas Receptor, Bcl-2 family protein, caspase-9, caspase-8, caspase-3 and endonuclease G. These targets were chosen because they represent crucial nodes in the initiation and execution of apoptosis: TNFR1 and the Fas Receptor initiates death signaling via death signaling complex (DISC) formation, caspase-8 activates downstream caspases (including caspase-9 and caspase-3) and connects extrinsic to intrinsic pathways through Bcl-2 family protein cleavage, and endonuclease G mediates DNA fragmentation following mitochondrial outer membrane leakage [24]. The study aims to identify phytochemicals capable of inducing apoptosis selectively in cancer cells by targeting these proteins, providing a mechanistic basis for developing more effective and less toxic adjuvant anticancer therapies.

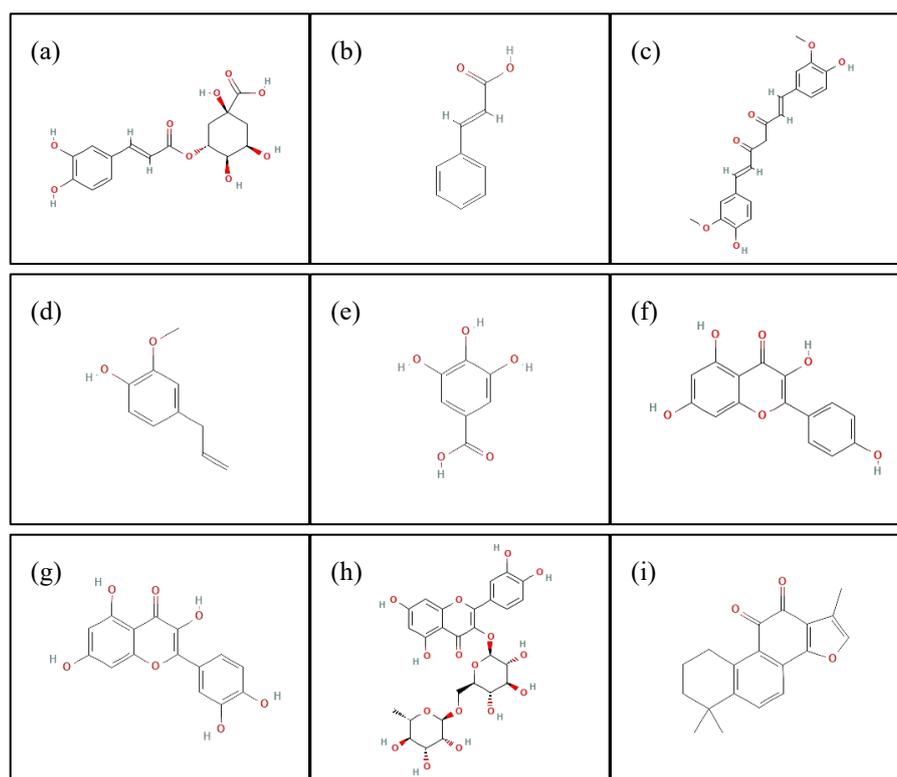


Figure 1 Two-dimensional structures of antioxidant compounds from Indonesian herbal plants: (a) chlorogenic acid, (b) cinnamic acid, (c) curcumin, (d) eugenol, (e) gallic acid, (f) kaempferol, (g) quercetin, (h) rutin, and (i) tanshinone.

The target proteins prepared for molecular docking included TNFR1 (PDB ID: 1NCF; resolution: 2.25 Å), Bid (PDB ID: 2BID), Bad (PDB ID: 2BZW chain A; resolution: 2.30 Å), Bax (PDB ID: 4BD6 chain A; resolution: 2.49 Å), Bak (PDB ID: 5FMI; resolution: 1.49 Å), FasR (PDB ID: 3EZQ chain A; resolution: 2.73

Å), EndoG (PDB ID: 6NJU chain D; resolution: 2.35 Å), caspase-8 (PDB ID: 7JKQ chain B; resolution: 3.30 Å), caspase-9 (PDB ID: 1NW9 chain B; resolution: 2.40 Å), and caspase-3 (PDB ID: 3H0E chain A and B; resolution: 2.00 Å), all retrieved from the Protein Data Bank (PDB, <https://www.rcsb.org/>). Before docking,

AutoDockTools optimized the receptors by removing water molecules, detaching native ligands, eliminating non-standard residues, and adding polar hydrogens and Gasteiger charges [25].

Binding site prediction

The prepared selected receptors are then predicted for their active site using CASTpFold [26]. The PDB file of the cleaned-up protein is uploaded to the server, and the results are downloaded to be thoroughly analysed in Chimera 1.19 [27]. The selected active sites have the highest molecular surface volume and pocket area within the range of binding site residues, determined as the binding location.

ADMET prediction

We obtained 2D structures in .sdf format and SMILES strings from PubChem database (<http://pubchem.ncbi.nlm.nih.gov>) and input it to ADMETLab 3.0 (<https://admetlab3.scbdd.com/>) to perform assessment for the compounds' Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) profile. Descriptors such as the number of hydrogen bond donors and acceptors, molecular weight, bioavailability, and compliance with Lipinski's Rule of Five guided the pharmacokinetic evaluation. The ProTox-II (<https://tox.charite.de/protox3/>) server generated toxicity predictions, following classifications aligned with the Globally Harmonised System (GHS).

Molecular docking simulation

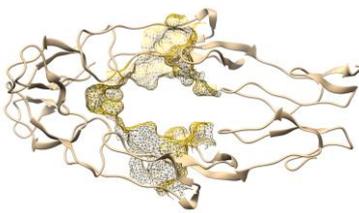
Ligand preparation employed BIOVIA Discovery Studio (<https://discover.3ds.com/discovery-studio-visualizer-download>). Ligand structures originated from the PubChem database (<http://pubchem.ncbi.nlm.nih.gov>), and PubChem stored them in Standard Data Format (SDF) for further analysis. Open Babel performed energy minimization of the ligands [28]. Molecular docking simulations evaluated the binding of nine antioxidant compounds from Indonesian medicinal plants to ten apoptosis-regulating proteins. Docking also included a comparative analysis with the reference drug Dasatinib (PubChem CID: 3062316), a leukemia therapy agent [29]. AutoDock Vina conducted the docking runs, while Discovery Studio Visualizer displayed the results [30]. We used exhaustiveness value of 8 and num modes of 8 as our docking parameters. All receptors received a grid box of 60×60×60 with 0.375 Å spacing.

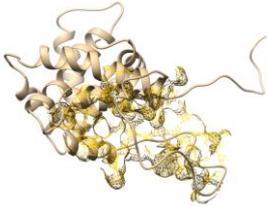
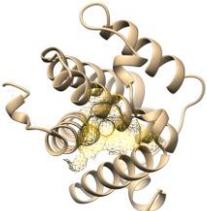
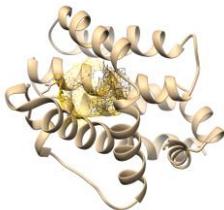
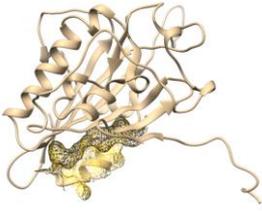
Results and discussion

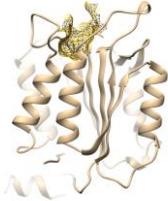
Binding site prediction

Binding site prediction was performed for each receptor using the CASTpFold server to identify potential ligand-binding pockets based on surface topology and geometric parameters. This step was essential to ensure that docking simulations were carried out within the most biologically relevant cavities, thereby improving the reliability of the predicted interactions. **Table 1** presents the identified binding sites and their structural characteristics.

Table 1 Active site mapping and grid box coordinates for molecular docking.

No	Protein	PDB ID	Active site visualization	Center Ref. Grid (Å) Coordinate
1	TNFR1	1NCF		X: 16; Y: 14; Z: 20

No	Protein	PDB ID	Active site visualization	Center Ref. Grid (Å) Coordinate
2	Bid	2BID		X: -13; Y: 0; Z: -2
3	Bad	2BZWa		X: 58; Y: 50; Z: 5
4	Bax	4BD6a		X: -2; Y: -26; Z: -15
5	Bak	5FMI		X: 0; Y: 7; Z: 24
6	FasR	3EZQa		X: -80; Y: 24; Z: -43
7	Endonuclease G	6NJUd		X: -15; Y: 39; Z: 30
8	Caspase-8	7JKQb		X: 169; Y: 160; Z: 218

No	Protein	PDB ID	Active site visualization	Center Ref. Grid (Å) Coordinate
9	Caspase-9	1NW9b		X: 51; Y: 10; Z: 90
10	Caspase-3	3H0E		X: 25; Y: 47; Z: 12

The predicted binding pockets correspond to key functional domains of apoptotic-regulating proteins. These results provide a reliable structural basis for subsequent docking simulations and ensure the assessment of ligand–receptor interactions within biologically relevant sites.

Drug-likeness evaluation

Assessing the drug-likeness of compounds is a vital preliminary phase in drug development, as it offers

critical insights into their potential efficacy as therapeutic agents [31]. Evaluating the physicochemical properties of these compounds against established criteria, such as Lipinski's Rule of Five, facilitates the identification of favorable pharmacokinetic profiles. **Table 2** presents the physicochemical analysis of the antioxidants and their compliance with Lipinski's criteria.

Table 2 Predicted physicochemical and drug-likeness properties of antioxidant compounds based on Lipinski's rule of five.

No	Ligand	Lipinski's rule of five			
		MW	logP ≤ 5	nHA	nHD
1	Chlorogenic Acid	354.1	1.036	9.0	6.0*
2	Cinnamic Acid	148.05	2.619	2.0	1.0
3	Curcumin	368.13	2.147	6.0	2.0
4	Eugenol	164.06	2.321	2.0	1.0
5	Gallic Acid	170.02	0.692	5.0	4.0
6	Kaempferol	286.05	1.965	6.0	4.0
7	Quercetin	302.04	1.448	7.0	5.0
8	Rutin	610.15*	0.986	16.0*	10.0*
9	Tanshinone	294.13	3.646	3.0	0.0

Nearly all of the tested antioxidant compounds complied with Lipinski's rule of five, which posits that orally active drug-like molecules typically possess a molecular weight ≤ 500 Da, $\log P \leq 5$, ≤ 5 hydrogen bond donors (nHD), and ≤ 10 hydrogen bond acceptors (nHA) [32]. Seven of the nine compounds, including cinnamic acid, curcumin, eugenol, gallic acid, kaempferol, quercetin, and tanshinone, satisfied all criteria, highlighting their favorable physicochemical properties and greater likelihood of exhibiting acceptable oral bioavailability.

Two compounds deviate from these criteria. Rutin exhibited three violations: a molecular weight of 610.15 Da, 16 hydrogen bond acceptors, and 10 hydrogen bond donors, which can significantly impair membrane permeability and intestinal absorption. These properties are consistent with pharmacokinetic studies reporting Rutin's poor oral bioavailability, attributed to its low solubility, poor stability, and limited permeability across the intestinal epithelium due to its bulky sugar moiety [32,33]. Despite these limitations, Rutin retains biological relevance because intestinal microbiota can hydrolyze it into aglycone metabolites such as quercetin, which remain bioactive and contribute to its pharmacological effects [35]. Chlorogenic acid displayed only one deviation (nHD = 6), slightly above the donor threshold, which may modestly limit membrane diffusion efficiency. However, its relatively low molecular weight (354.1 Da) and favorable $\log P$ suggest it still holds oral delivery potential [32].

From a broader perspective, compliance with Lipinski's criteria reinforces the potential of these

phytochemicals to progress as orally available therapeutic candidates. Still, the rule should serve as a guiding framework rather than an absolute standard. Natural products frequently deviate from Lipinski's thresholds yet remain pharmacologically active. For example, although Rutin have exceed the molecular weight limit of Lipinski's Rule, it may still be developed as a therapeutic agent when delivered intravenously, bypassing the oral absorption barrier and broadening its translational potential in anticancer adjuvant therapy [36].

ADMET prediction

In oncology drug discovery, *in silico* approaches such as ADMET prediction and molecular docking are widely applied to identify novel inhibitors with favorable pharmacokinetic and pharmacodynamic properties. ADMET evaluation, which encompasses absorption, distribution, metabolism, excretion, and toxicity, represents a critical step in early drug discovery for predicting the *in vivo* behavior of candidate compounds. It provides insight into oral bioavailability, membrane permeability, metabolic stability, and potential drug-drug interactions, thereby complementing molecular docking analyses and strengthening the interpretation of docking outcomes [37]. This study subjected nine antioxidant compounds to ADMET profiling to evaluate their pharmacokinetic suitability as therapeutic candidates. **Table 3** summarizes the predicted parameters of ADMET.

Table 3 Predicted ADMET properties of antioxidant compounds.

No	Ligand	Human		Caco-2 Permeability	BBB Permeant	CYP2D6 Substrate	CYP2D6 Inhibitor	CYP3A4 Substrate	CYP3A4 Inhibitor	T _{1/2}
		Intestinal Absorption (HIA)	F30%							
1	Chlorogenic Acid	0.106	0.996	-6.426	0	0	0	0	0	2.758
2	Cinnamic Acid	0.095	0.89	-4.441	0.025	0.086	0.006	0	0	1.882
3	Curcumin	0.017	0.479	-5.471	0.023	0.889	0.018	0	0.014	1.04
4	Eugenol	0.035	0.985	-4.57	0.106	0.999	0.342	0	0.998	1.464
5	Gallic Acid	0.017	0.355	-5.604	0.002	0	0	0	0.002	2.2
6	Kaempferol	0.015	0.716	-5.969	0.001	0.995	0.005	0.002	0.975	1.388

No	Ligand	Human Intestinal Absorption (HIA)	F30%	Caco-2 Permeability	BBB Permeant	CYP2D6 Substrate	CYP2D6 Inhibitor	CYP3A4 Substrate	CYP3A4 Inhibitor	T _{1/2}
7	Quercetin	0.134	0.991	-6.177	0	0.978	0	0	0.937	1.586
8	Rutin	0.64	1	-6.547	0	0	0	0	0.029	4.616
9	Tanshinone	0.763	0.664	-4.612	0	0.002	0.995	0.644	0.849	0.499

The ADMET analysis revealed distinct pharmacokinetic and metabolic properties among the nine antioxidant ligands examined. Human intestinal absorption (HIA) is one of critical determinant of oral bioavailability. It was evaluated using a 30% threshold, where compounds with HIA \geq 30% were classified as having good absorption and those with HIA $<$ 30% as having poor absorption, and based on this classification, rutin and tanshinone exhibited poor absorption potential, corresponding to an HIA rate of $<$ 30%. In contrast, curcumin and gallic acid demonstrated parameters consistent with higher oral bioavailability (HIA \geq 30%), suggesting a more favorable pharmacokinetic profile [38,39].

Analysis of Caco-2 permeability, which reflects intestinal epithelial transport, identified cinnamic acid, eugenol, and tanshinone as the most permeable compounds, with values above the threshold of -5.15 log units [40]. These findings indicate that these ligands possess adequate membrane permeability, an important determinant for oral drug absorption. Despite this, all ligands consistently showed negative blood-brain barrier (BBB) penetration (category 0), indicating minimal central nervous system exposure, which may reduce the risk of neurotoxicity but also limits potential applications for neurological cancers [41].

Metabolic profiling indicated that several ligands may interact with cytochrome P450 enzymes. Specifically, curcumin, eugenol, kaempferol, and quercetin emerged as substrates of CYP2D6, highlighting their susceptibility to rapid metabolism. Furthermore, eugenol, kaempferol, quercetin, and tanshinone were identified as potential inhibitors of CYP3A4, suggesting a risk of drug-drug interactions with other therapeutics metabolized by this isoform. Such interactions warrant careful consideration in future

drug development and clinical applications [42]. In terms of elimination, most compounds displayed moderate half-lives. However, tanshinone exhibited an exceptionally short half-life (0.499 h), which may compromise its therapeutic efficacy by limiting systemic exposure. Conversely, rutin showed the most extended half-life (4.616 h), but its poor intestinal absorption reduces its pharmacological relevance.

These findings suggest that among the nine antioxidants evaluated, curcumin and gallic acid present relatively favorable oral bioavailability, while cinnamic acid and eugenol exhibit superior permeability. However, potential liabilities include CYP-mediated interactions (particularly for eugenol, kaempferol, quercetin, and tanshinone) and poor absorption characteristics for rutin and tanshinone. These results underscore the importance of integrating ADMET profiling with docking analysis to prioritize candidates with strong binding affinities and acceptable pharmacokinetic properties.

Toxicity prediction

In addition to pharmacokinetic evaluation, toxicity assessment is essential in determining candidate compounds' safety and therapeutic feasibility. Computational prediction of toxicological endpoints provides early insight into potential adverse effects, reducing the likelihood of late-stage failures in drug development [43]. In this study, we analysed nine antioxidant compounds for toxicity analysis, including the estimation of acute toxicity (LD50 values and toxicity classes) as well as organ-specific toxicities such as hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity. The results of these predictions appear in **Tables 4** and **5**.

Table 4 Predicted acute toxicity profiles of the antioxidant compounds.

No.	Ligand	Predicted LD ₅₀ (mg/kg)	Predicted toxicity class	Average similarity (%)	Prediction accuracy (%)
1	Chlorogenic Acid	5,000	5	71.21	69.26
2	Cinnamic Acid	2,500	5	100	100
3	Curcumin	2,000	4	100	100
4	Eugenol	1,930	4	100	100
5	Gallic Acid	2,000	4	84.82	70.97
6	Kaempferol	3,919	5	82.46	70.97
7	Quercetin	159	3	100	100
8	Rutin	5,000	5	100	100
9	Tanshinone	1,655	4	48.84	54.26

Table 5 Predicted organ-specific toxicities of the antioxidant compounds.

No	Ligand	Organ toxicity				
		Hepatotoxicity (Acc. 93%)	Carcinogenicity (Acc. 81%)	Immunotoxicity (Acc. 75%)	Mutagenicity (Acc. 84%)	Cytotoxicity (Acc. 85%)
1	Chlorogenic Acid	Inactive	Inactive	Active	Inactive	Inactive
2	Cinnamic Acid	Active	Inactive	Inactive	Inactive	Inactive
3	Curcumin	Inactive	Inactive	Active	Inactive	Inactive
4	Eugenol	Inactive	Inactive	Inactive	Inactive	Inactive
5	Gallic Acid	Inactive	Active	Inactive	Inactive	Inactive
6	Kaempferol	Inactive	Inactive	Inactive	Inactive	Inactive
7	Quercetin	Inactive	Active	Inactive	Active	Inactive
8	Rutin	Inactive	Inactive	Active	Inactive	Inactive
9	Tanshinone	Inactive	Inactive	Inactive	Inactive	Inactive

The toxicological assessment of the nine antioxidant ligands revealed varying safety profiles, as indicated by their predicted LD₅₀ values, toxicity classes, and organ-specific effects. In terms of acute toxicity, most ligands fell within toxicity classes 4 - 5, suggesting relatively low toxicity at standard exposure levels. Compounds such as chlorogenic acid, cinnamic acid, kaempferol, and rutin showed high LD₅₀ values ($\geq 2,500$ mg/kg) and belonged to class 5, indicative of substances with limited acute toxicity. Curcumin, eugenol, gallic acid, and tanshinone occupied class 4, with LD₅₀ values ranging between 1,655 and 2,000 mg/kg, reflecting moderate safety but warranting careful dose consideration [44,45]. Quercetin displayed the lowest LD₅₀ (159 mg/kg), placing it in toxicity class 3, indicating its relatively higher acute toxicity compared

to the other ligands. This finding aligns with previous studies that report quercetin's higher toxicity due to its low LD₅₀ value and potential carcinogenic and mutagenic effects [46].

Predicted organ toxicity provided further insights into potential risks. Chlorogenic acid, curcumin, and rutin demonstrated immunotoxic activity which may reflect their immunomodulatory properties at higher concentrations. Cinnamic acid showed hepatotoxic potential, raising caution regarding possible liver-related adverse effects upon prolonged use. Among the flavonoids, quercetin exhibited both carcinogenicity and mutagenicity, suggesting that despite its well-documented antioxidant benefits, its therapeutic window requires careful evaluation to minimize risks of genotoxicity. Similarly, gallic acid displayed

carcinogenic potential, though other toxicity endpoints remained inactive. By contrast, kaempferol, eugenol, and tanshinone exhibited no activity across all assessed organ toxicity categories, underscoring their comparatively favorable safety profiles.

The toxicological profiling suggests that while most antioxidants investigated in this study exhibit acceptable safety margins, certain compounds, including quercetin and gallic acid, may pose risks related to mutagenicity and carcinogenicity. These findings emphasize the importance of dose optimization, structural modification, and comprehensive *in vivo* validation before advancing such compounds toward clinical applications. On the other hand, rutin, chlorogenic acid, and kaempferol stand out as relatively safe candidates given their high LD50 values and minimal predicted organ toxicity, reinforcing their suitability for further drug development efforts.

Molecular docking analysis

This analysis was performed to evaluate the binding affinities and interaction profiles of the nine selected antioxidant compounds against apoptosis-

regulating proteins, including TNFR1, Bax, Bak, Bid, Bad, Fas receptor, Endonuclease G, Caspase-8, Caspase-9, and Caspase-3. These proteins represent critical nodes within intrinsic and extrinsic apoptotic pathways, making them valuable targets for anticancer drug discovery. The docking simulations provided insights into the potential mechanisms by which natural antioxidants may modulate apoptotic signaling by stabilizing pro-apoptotic proteins or interfering with anti-apoptotic regulators. Binding energy values (kcal/mol) were used as a primary indicator of ligand-protein affinity, complemented by an analysis of hydrogen bonding, hydrophobic contacts, and π -type interactions with key amino acid residues [47,48]. Dasatinib, a clinically used small-molecule kinase inhibitor, was the reference compound to validate docking accuracy and allow comparative evaluation [49]. Collectively, this approach identifies antioxidant ligands with strong binding potential and favorable interaction networks, thereby supporting their candidacy as modulators of apoptosis in cancer therapy. The results of the interaction and energy evaluation between molecular bonds are presented in **Table 6**.

Table 6 Molecular docking interactions and binding energies of antioxidant ligands.

Molecular bond interaction	Chlorogenic acid	Cinnamic acid	Curcumin	Eugenol	Gallic acid	Kaempferol	Quercetin	Rutin	Tanshinone	Dasatinib
TNFR1										
H-Bond	HIS34							ASP91		
	GLU64				HIS34		HIS34	LYS35	GLU64	HIS34
	LYS35	ALA62	LYS35	-	GLU64	LYS35	LYS35	ALA62	LYS35	GLU64
	ALA62	LYS35	GLU64		SER63		SER63	HIS34		SER63
	ARG92						SER63			
π -Sigma π -Alkyl	-	LYS35	PHE60 ARG92 ALA62 ASP93 HIS34	HIS34 LYS35	LYS35	LYS35	SER63 LYS35	-	-	LYS35 LEU67
π -Cation π -Anion π -Sulphur	GLU64	GLU64	-	LYS35	-	GLU64	-	LYS35 GLU64	-	LYS35
Binding Energy (Kcal/mol)	-6.7	-5.3	-6	-5	-5.6	-6.3	-6.7	-6.8	-6.7	-6.4
Bid										
H-Bond	GLN60	THR61	GLN60	GLN60	GLU54	TRP53	GLN60	GLN60	THR61	TRP53
	LEU59	ASP57			LEU59 ASP57	GLN60	GLN52 THR61 LEU59	LEU59 ARG65	ASP57	THR61
π -Sigma π -Alkyl	-	TYR56	TYR56	TYR56	-	-	TYR56	-	TYR56	-

Molecular bond interaction	Chlorogenic acid	Cinnamic acid	Curcumin	Eugenol	Gallic acid	Kaempferol	Quercetin	Rutin	Tanshinone	Dasatinib
π -Cation π -Anion π -Sulphur	GLU54	-	-	-	-	-	-	-	-	GLU54
Binding Energy (Kcal/mol)	-5.5	-4.2	-4.8	-4	-4.2	-4.9	-5.1	-5.9	-6.2	-5.7
Bad										
H-Bond	PHE105 LYS20 GLN19 ARG102	-	ARG102 LYS20 SER106	PHE105	GLU153 SER145	ALA149 LEU99 PHE105 GLU98 GLU153	GLU98 LEU99 PHE105	LYS16 GLU98 SER106	-	ASP156 ASP95 GLU153 PHE105 ALA149 GLU98
π -Sigma π -Alkyl	LEU99	PHE105	-	LYS16 VAL152 ALA149 LYS20	ALA149	-	ALA149	VAL152 ALA149	LEU99 VAL152	VAL152 LYS16
π -Cation π -Anion π -Sulphur	-	-	-	-	-	-	-	LYS20 GLU153	-	LYS20
Binding Energy (Kcal/mol)	-7	-5.2	-7	-5.3	-5.3	-6.6	-6.6	-8.2	-8.3	-8
Bax										
H-Bond	ALA112 VAL83	-	ILE80	-	ILE80	-	-	MET79 MET99 LEU76	-	MET99 SER101
π -Sigma π -Alkyl	LEU76 ILE80	LEU76 ALA112	ALA112 LEU76 MET99 VAL95	MET99 ALA112 ILE80 VAL83 PHE116 LEU76	VAL95	VAL95 PHE116	LEU76 VAL95	VAL95 ALA112 VAL83 PHE116	LEU76 VAL95 PHE116 MET99 ALA112	LEU76 ILE80 ALA112
π -Cation π -Anion π -Sulphur	MET99	MET99	-	-	-	MET99	MET99	-	-	ASP102 ARG109
Binding Energy (Kcal/mol)	-6.2	-5.4	-6.8	-5.3	-4.3	-6.6	-6.6	-7.3	-7.7	-7
Bak										
H-Bond	GLN94 ARG137 ASP83 ARG42	-	ARG137 ARG42 ARG87 GLN45 ASP83	GLY82 ARG42	ASP83 ARG42 ARG137	SER91 GLN94 ASP83	GLN94 ARG137 ARG42	SER91 ASP90 ARG137 ASP83 ARG42	ARG42	ARG137 ARG42 GLN45
π -Sigma π -Alkyl	ARG87	ARG87	ALA49	ARG87	GLN45 ALA49	ARG87	ALA49	GLN45 ALA49	ARG87	ALA49 ILE80
π -Cation π -Anion π -Sulphur	ASP90	-	GLU50	ASP83 ASP90	-	-	ASP90 GLU46 GLU50	-	ASP 90 ARG137	ASP90 ASP83
Binding Energy (Kcal/mol)	-6.8	-4.7	-6.3	-5	-5.7	-6.5	-6.8	-7.6	-6.8	-7.1
Fas Receptor										
H-Bond	SER225 ASP228 THR235	LYS287 HIS282	HIS282	-	SER225 HIS282	HIS282	TYR232	SER225 TYR291	-	SER225

Molecular bond interaction	Chlorogenic acid	Cinnamic acid	Curcumin	Eugenol	Gallic acid	Kaempferol	Quercetin	Rutin	Tanshinone	Dasatinib
π -Sigma π -Alkyl	LEU 229	TYR232	ASP228 LEU229 TYR232	ALA290 TRP281 LEU294 LYS287	LEU229 ASP228	TYR232 ALA290 TYR291	TYR291 LYS287 ALA290 LEU229	TYR232 LYS287 ALA290	LEU229 TYR232	LEU298 TYR232 HIS282
π -Cation π -Anion π -Sulphur	-	-	-	HIS282	-	-	HIS282	HIS282	-	TYR291
Binding Energy (Kcal/mol)	-5.7	-4.5	-5.8	-4.2	-4	-6	-6	-6.6	-6.7	-6.6
Endonuclease G										
H-Bond	ARG95 ASP94 ALA97 GLU136 ARG105	ARG96 ASP94	ALA66 ARG95 ALA97 ASN128 ASN133	ASN133 ASN128	ALA102 ALA101 GLU136 ALA97	ALA97 ASN128	ALA101 ALA97 ARG95 ASP94	ASN128 ALA97 ALA101 ASN133 ASP65 ARG66 ARG106	ASN133 ARG105	ALA101 ALA102 ARG105 LEU60
π -Sigma π -Alkyl	ALA68	ALA97 ALA68 PHE71 ALA101	PHE71 ALA101 ALA100	ALA101 PHE115 PHE71 ALA97	ALA100	ALA101 ALA68	ALA68	ALA63	ALA102 ALA101 ALA68 PHE115	ALA97
π -Cation π -Anion π -Sulphur	-	-	-	-	-	ASP94	-	-	-	-
Binding Energy (Kcal/mol)	-7.4	-5.1	-7.7	-5.4	-6	-7.3	-7.7	-8.9	-7.8	-7
Caspase-8										
H-Bond	HIS326 ASP352 GLU366 HIS324 SER372	HIS252	SER193, SER372	-	ASP349 HIS252 PHE287	GLU366 ASP352 ASN369	SER372 ASP352	HIS326 ASP352 ASP349 TYR288	TYR323 SER372	HIS324 TYR323 PRO367
π -Sigma π -Alkyl	-	PHE287	ILE347 PHE287 TYR288	PHE287 TYR288 PRO251	THR195 PRO251	PHE370	ILE347 PHE370	ILE347 PHE287	PHE287 HIS324 ILE347 LEU368 PHE370	LEU256 PRO286 ILE347 HIS326
π -Cation π -Anion π -Sulphur	PHE370	-	HIS326	-	-	HIS326	HIS326	-	-	PHE287
Binding Energy (Kcal/mol)	-6.7	-6	-6.3	-5.6	-6.2	-6.2	-6.4	-7.5	-7.1	-7.2
Caspase-9										
H-Bond	THR181 ARG178 GLY176 HIS237 CYS287	ARG180 CYS287 ASP186 SER36	ARG178 TYR251	-	-	GLY176 THR181 HIS237	THR181 ARG178	GLY288 HIS237 CYS287 THR181 PRO357 THR179	THR181	CYS287 THR179 ARG180
π -Sigma π -Alkyl	-	-	PHE351 ARG180	ARG180 PHE351 HIS237	ARG180 PHE351	ARG180 PHE351	PRO357 ARG180 PHE351	ARG178	PRO357 PHE351 HIS237 ARG180 CYS287	LEU177 PHE351

Molecular bond interaction	Chlorogenic acid	Cinnamic acid	Curcumin	Eugenol	Gallic acid	Kaempferol	Quercetin	Rutin	Tanshinone	Dasatinib
π -Cation π -Anion π -Sulphur	ARG180	-	CYS287	CYS287	CYS287	-	-	-	-	HIS237
Binding Energy (Kcal/mol)	-6.7	-6	-6.6	-5.1	-5.2	-6.1	-6.3	-7	-7.3	-6.8
Caspase-3										
H-Bond	ARG207 TRP206	-	SER63 ARG207 SER209 ASN208 SER205	ARG207	TRP206	GLU123 CYS163 ARG207	SER205 GLU123 GLY122	SER209 ARG207 SER205	CYS163 HIS121 ARG207	ARG207
π -Sigma π -Alkyl	-	PHE256	-	PHE256 TRP206 HIS121 CYS163	-	-	TYR204	TYR204 PHE256	TYR204 TRP206 PHE256	MET61 TYR204 TRP206 PHE256
π -Cation π -Anion π -Sulphur	-	-	-	-	-	-	ARG207 CYS163	-	-	CYS163
Binding Energy (Kcal/mol)	-7.1	-5.3	-6.2	-5.2	-4.8	-6.5	-6.9	-7.6	-7.9	-7.3

TNFR1

The tumor necrosis factor receptor 1 (TNFR1) is a pivotal mediator of apoptotic and inflammatory signaling, making it an attractive target in cancer therapy. Molecular docking analysis revealed that rutin exhibited the strongest binding affinity (-6.8 kcal/mol), followed closely by quercetin, chlorogenic acid, and tanshinone (-6.7 kcal/mol each). Curcumin and kaempferol also demonstrated moderate affinity (-6.0 and -6.3 kcal/mol, respectively). Key binding interactions involved hydrogen bonds with residues such as LYS35, GLU64, and HIS34, underscoring their importance in ligand stabilization within the receptor pocket. Dasatinib, a reference inhibitor, displayed a binding energy of -6.4 kcal/mol and established a balanced network of hydrogen bonds and hydrophilic contacts, validating the docking protocol. Curcumin formed a diverse interaction profile, including hydrogen bonding with LYS35 and GLU64 and multiple π -type interactions with PHE60, ARG92, ASP93, ALA62, and HIS34. These combined electrostatic and hydrophobic interactions likely confer enhanced conformational stability despite its lower binding affinity than rutin. From a translational perspective, while rutin and quercetin demonstrated stronger affinities, their unfavorable pharmacokinetic properties, including poor absorption and bioavailability, may restrict therapeutic

application. Conversely, curcumin, with a slightly weaker binding score but superior drug-likeness, may represent a more viable candidate for further development.

Bid

Bid (BH3-interacting domain death agonist) is a pro-apoptotic Bcl-2 family protein that links the extrinsic and intrinsic apoptotic pathways. Based on previous research, the key residue that binds the ligand in Bid is GLN60 [50]. Docking analysis indicated that rutin (-5.9 kcal/mol) and tanshinone (-6.2 kcal/mol) showed the strongest binding affinities, surpassing other antioxidants but remaining slightly lower compared to the control dasatinib (-5.7 kcal/mol). Rutin exhibited multiple hydrogen bonds with GLN60, LEU59, ARG65, and π -alkyl stabilization with TYR56, suggesting favorable anchoring within the binding pocket. Curcumin (-4.8 kcal/mol) formed limited interactions, primarily with GLN60, while cinnamic acid and gallic acid demonstrated the weakest binding (-4.2 kcal/mol). Overall, rutin and tanshinone appear to be the most promising ligands for Bid, potentially enhancing Bid-mediated mitochondrial apoptosis through stable ligand-protein interactions. Although tanshinone binds more tightly to the receptors, it does not form hydrogen bonds with GLN60, which might reduce target specificity.

Quercetin, curcumin, and eugenol showed a similar bonding pattern to GLN60 and TYR56, which is considered the key binding pattern to the Bid Receptor.

Bad

Bad, or also commonly known to be Bcl-2-associated death promoter, is a BH3-only pro-apoptotic protein that antagonizes anti-apoptotic Bcl-2 family members, promoting apoptosis. Docking analysis showed that tanshinone (−8.3 kcal/mol) and rutin (−8.2 kcal/mol) exhibited the strongest binding affinities, surpassing dasatinib (−8.0 kcal/mol). Curcumin (−7.0 kcal/mol) and chlorogenic acid (−7.0 kcal/mol) followed with moderate affinities. The ligands consistently interacted with key residues, including GLU153, PHE105, GLU98, VAL152, and LYS20. These findings indicate that tanshinone and rutin possess strong potential to modulate Bad activity, thereby enhancing intrinsic apoptotic signalling.

Bax

Bax is a pro-apoptotic protein that is central to mitochondrial-mediated apoptosis by permeabilizing the outer mitochondrial membrane. Molecular docking against Bax resulted in tanshinone (−7.7 kcal/mol), rutin (−7.3 kcal/mol), and Curcumin (−6.8 kcal/mol) as top ligands with highly favorable binding energy similar to dasatinib (−7.0 kcal/mol). Residues that play important roles in the binding to Bax are ALA112, LEU76, MET99, and ILE80. Although tanshinone has a greater binding energy with Bax, it exhibits no hydrogen bonding to the protein. Despite slightly lower affinity, Curcumin exhibited multiple stabilizing interactions and binding patterns similar to dasatinib, making it a promising candidate for Bax receptor modulation.

Bak

Bak, a significant pro-apoptotic Bcl-2 family member, functions in synergy with Bax to mediate mitochondrial outer membrane permeabilization, a critical step in apoptosis. The binding energy from molecular docking toward the Bak receptor ranged from −4.7 to −7.6 kcal/mol. Rutin exhibited the strongest binding energy (−7.6 kcal/mol) among the tested compounds, surpassing dasatinib. Quercetin (−6.8 kcal/mol) and chlorogenic acid (−6.8 kcal/mol) also displayed high affinities comparable to dasatinib.

Curcumin also showed fine binding energy stabilized by multiple hydrogen bonds with residues similar to those of dasatinib, which are ARG137, GLN45, and ARG42. Although Kaempferol has a slightly better binding energy than curcumin, it does not exhibit the same binding model. Besides that, ASP83 and ALA49 also play important roles in the binding of ligands in the Bak receptor.

Fas receptor

The Fas receptor is an extrinsic apoptosis pathway regulator, and ligand binding can initiate caspase cascades. From the molecular docking results, the binding energies of the tested ligands ranged from −4.0 to −6.7 kcal/mol. Tanshinone (−6.7 kcal/mol) showed the strongest binding affinities, followed closely by rutin (−6.6 kcal/mol), similar to dasatinib (−6.6 kcal/mol). Quercetin (−6.0 kcal/mol), kaempferol (−6.0 kcal/mol), curcumin (−5.8 kcal/mol), and chlorogenic acid (−5.7 kcal/mol) showed almost similar binding affinities, whereas cinnamic acid and eugenol demonstrated weaker affinities (< −4.5 kcal/mol). Several residues commonly involved in ligand interactions are TYR291, HIS282, TYR232, and SER225, suggesting these are critical binding sites on the Fas receptor. Although Tanshinone showed the strongest binding, it still lacks hydrogen bonding, which could make the conformation unstable. These findings indicate that rutin and Tanshinone may effectively stabilize Fas receptor conformations, although their therapeutic potential remains dependent on pharmacokinetic optimization.

Endonuclease G (EndoG)

Endonuclease G is a mitochondria-localized nuclease involved in caspase-independent apoptosis. Binding energy from the molecular docking toward Endonuclease G ranged from −5.1 to −8.9 kcal/mol. Rutin exhibited the strongest binding (−8.9 kcal/mol), surpassing dasatinib. Rutin established multiple hydrogen bonds with ASN128, ALA97, and ARG106, alongside extensive hydrophobic contacts (PHE71, ALA101, ALA102). These interactions highlight rutin's ability to anchor deeply into the EndoG binding pocket; otherwise, the pattern differs from dasatinib. Tanshinone, curcumin, and quercetin also demonstrated high affinities around −7.7 kcal/mol. The three with substantial binding stability and overlapping residue

interactions with the reference drug emerge as a promising natural ligand of Endonuclease G-mediated apoptosis.

Caspase-8

Caspase-8 is a critical initiator caspase involved in the extrinsic apoptosis signalling pathway. Molecular docking results against the caspase-8 receptor resulted in binding energies ranging from -5.6 to -7.5 kcal/mol. Rutin exhibited the strongest binding affinity, followed by tanshinone, both of which have similar binding energy to Dasatinib. Chlorogenic acid, quercetin, and Curcumin also demonstrated favorable interactions toward caspase-8. The key residues in the caspase eight protein that bind to ligands are ILE347, HIS326, HIS324, TYR323, and PHE287. Compared with Dasatinib, rutin and tanshinone demonstrated nearly equivalent binding energies, suggesting their potential as potent natural caspase-8 modulators. While moderately strong, Curcumin displayed multiple hydrogen bonds and hydrophobic interactions overlapping with residues bound by Dasatinib (ILE347, PHE287, and HIS326), indicating a similar inhibitory mechanism despite a slightly weaker docking score.

Caspase-9

Caspase-9 is a crucial initiator in the intrinsic apoptotic pathway, activated via the apoptosome complex. It showed binding energies ranging from -5.1 to -7.3 kcal/mol, with tanshinone (-7.3 kcal/mol) and rutin (-7.0 kcal/mol) exhibiting the highest binding energy. Chlorogenic acid (-6.7 kcal/mol) and curcumin (-6.6 kcal/mol) showed similar binding energy with the reference drug dasatinib (-6.8 kcal/mol). Comparative analysis demonstrated that tanshinone and rutin had stronger binding energies than dasatinib, which appear to be promising ligands for caspase-9 activation, supporting the role of natural antioxidants in promoting mitochondrial apoptosis. The residues commonly binding to ligands are PHE351, CYS287, HIS237, ARG180, and THR179.

Caspase-3

Caspase-3 is a critical executioner caspase responsible for the final stages of apoptosis. The molecular docking results showed binding energies ranged from -4.8 to -7.9 kcal/mol, with tanshinone ($-$

7.9 kcal/mol) and rutin (-7.6 kcal/mol) exhibiting similar binding energies with dasatinib. Quercetin (-6.9 kcal/mol), kaempferol (-6.5 kcal/mol), and curcumin (-6.2 kcal/mol) showed moderate binding energy, indicating favorable interaction with caspase-3. The commonly similar residues that bind caspase 3 to ligands are PHE256, ARG207, TRP206, TYR204, and CYS163.

Candidate evaluation in apoptotic pathways

Among the tested antioxidants, chlorogenic acid demonstrated relatively strong binding affinities with apoptosis-regulating proteins, underlining its potential as a modulator of apoptotic signaling. Lipinski's evaluation showed hydrogen bond donor (nHD) parameter violation, which may limit its oral absorption and permeability [32]. Despite this, ADMET analysis indicated only two unfavorable pharmacokinetic scores, while toxicity profiling suggested no major safety concerns. This positions chlorogenic acid as a viable candidate for further exploration, especially if supported by formulation strategies aimed at improving its bioavailability. In order to mitigate this pharmacokinetic limitations, formulation approaches such as phospholipid-based nanophytosomes, phytosome complexes, liposomal carriers, and chitosan nanoparticles have been shown to enhance chlorogenic acid's lipid solubility, intestinal permeability, and systemic exposure [51].

Curcumin also exhibited notable molecular docking interactions, forming multiple hydrogen bonds and non-covalent contacts with key residues that reinforce binding stability. Although ADMET evaluation flagged two unfavorable pharmacokinetic properties, the overall profile remains acceptable. Furthermore, curcumin maintained a relatively safe toxicological profile, supporting its suitability as a therapeutic scaffold. The well-documented limitation of curcumin's poor systemic bioavailability could be overcome through nanocarrier delivery, combination therapy, or structural optimization, thereby enhancing its therapeutic efficacy in apoptosis regulation [52].

Quercetin and rutin displayed strong binding affinities toward apoptotic proteins but presented significant limitations in drug-likeness and safety profiles. Quercetin was predicted to have a relatively low LD50 and showed positive alerts for

carcinogenicity and mutagenicity, raising concerns about its potential risks in therapeutic applications despite its natural dietary abundance [44]. Conversely, rutin exhibited strong binding energy and failed three of Lipinski's rule criteria due to its high molecular weight and excessive hydrogen bonding potential, suggesting poor permeability and absorption [32]. ADMET predictions further highlighted unfavorable pharmacokinetics, undermining its development as a drug-like molecule. Therefore, while quercetin and rutin are biochemically active, their clinical translation may be constrained without substantial chemical modification or restriction to adjunctive, nutraceutical applications.

Tanshinone represents another strong-binding compound, yet its pharmacokinetic and toxicological characteristics pose significant hurdles. ADMET profiling indicated poor absorption and a short half-life, limiting systemic exposure. Moreover, toxicity analysis revealed a low predicted LD50, suggesting reduced safety margins compared to other tested antioxidants. Despite its molecular potential, these unfavorable properties substantially reduce tanshinone's feasibility as a direct therapeutic candidate. Strategies such as advanced drug delivery formulations may be required to harness its bioactivity while minimizing pharmacological and toxicological drawbacks [53].

In contrast to the compounds with stronger binding energies, cinnamic acid and gallic acid exhibited relatively weaker affinities toward apoptotic proteins. However, their pharmacokinetic evaluation revealed more favorable properties, with both compounds showing the least unfavorable ADMET scores. These results suggest that although their direct interaction strength with apoptosis-related targets may not be ideal, their pharmacokinetic stability and drug-likeness make them promising substrates for additional modification. Significantly, their smaller molecular size and favorable absorption potential could facilitate structural modification to improve binding interactions without substantially compromising pharmacokinetics, thus positioning them as promising lead molecules for rational drug design.

Meanwhile, kaempferol and eugenol demonstrated intermediate docking affinities and acceptable pharmacokinetic characteristics, with kaempferol showing susceptibility to CYP2D6

metabolism and eugenol acting as an inhibitor of CYP3A4. These features suggest potential drug-drug interactions that must be carefully considered in therapeutic contexts. Nevertheless, both compounds are widely consumed in dietary sources and are known to exert antioxidant and anti-inflammatory activities, supporting their biological relevance. Their moderate binding strength and relatively balanced ADMET profiles indicate that kaempferol and eugenol could serve as supportive therapeutic agents, potentially in synergistic formulations with stronger-binding molecules such as curcumin or chlorogenic acid.

Overall, the molecular docking analysis highlights that several antioxidant compounds, particularly chlorogenic acid, curcumin, quercetin, rutin, and tanshinone, exhibit strong binding affinities toward apoptosis-regulating proteins, suggesting potential roles in modulating apoptotic pathways. However, their pharmacokinetic and toxicological profiles vary considerably, with some compounds displaying limited drug-likeness or safety concerns. In contrast, cinnamic acid, gallic acid, kaempferol, and eugenol demonstrated more favorable ADMET characteristics despite lower docking affinities, indicating their promise as lead scaffolds for optimization. These findings collectively emphasize the importance of integrating docking results with pharmacokinetic and toxicological evaluations to identify antioxidant candidates with strong molecular interactions and feasible drug development potential.

Molecular interactions visualizations

This study focused on three compounds with consistently strong binding affinities to provide deeper insights into the molecular mechanisms underlying their pro-apoptotic potential. Two-dimensional and three-dimensional molecular docking visualizations were generated for chlorogenic acid (**Figure 2**), curcumin (**Figure 3**), and quercetin (**Figure 4**). Rutin and tanshinone were excluded from the visualization due to unfavorable ADMET profiles and poor drug-likeness scores. We selected ligand-protein interactions with binding energies lower than -6 kcal/mol, ensuring that the analysis emphasized biologically meaningful and energetically favorable complexes. These visualizations provide detailed insights into specific hydrogen bonds, hydrophobic contacts, and electrostatic interactions between the ligands and key amino acid residues within

the binding pockets. Both docking energy values with interaction mapping analysis strengthens the biological relevance of the predicted binding modes. It supports the

identification of potential lead compounds for future *in vitro* and *in vivo* validation.

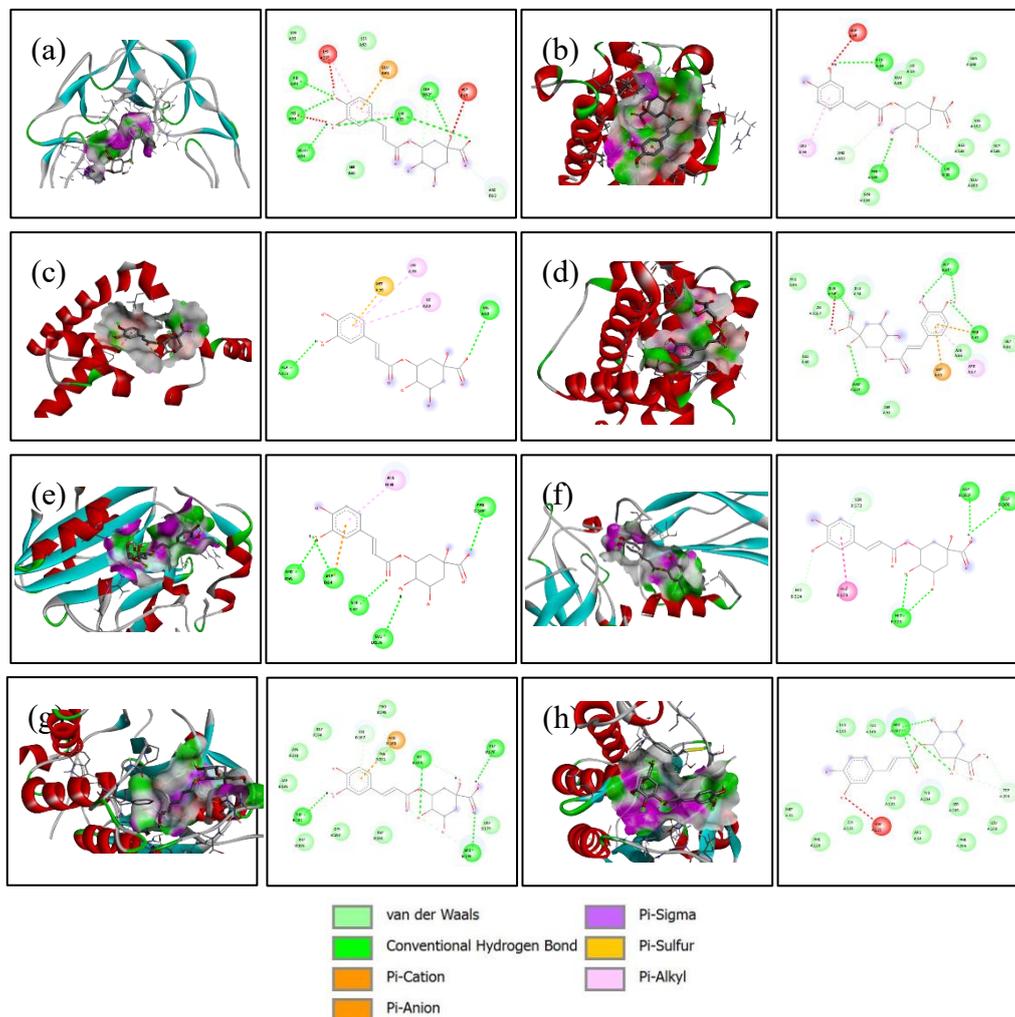


Figure 2 Visualization 3D and 2D of interactions of Chlorogenic Acid against the apoptotic-regulating protein. (a) TNFR1, (b) Bad, (c) Bax, (d) Bak, (e) Endonuclease G, (f) caspase-8, (g) caspase-9, and (h) caspase-3.

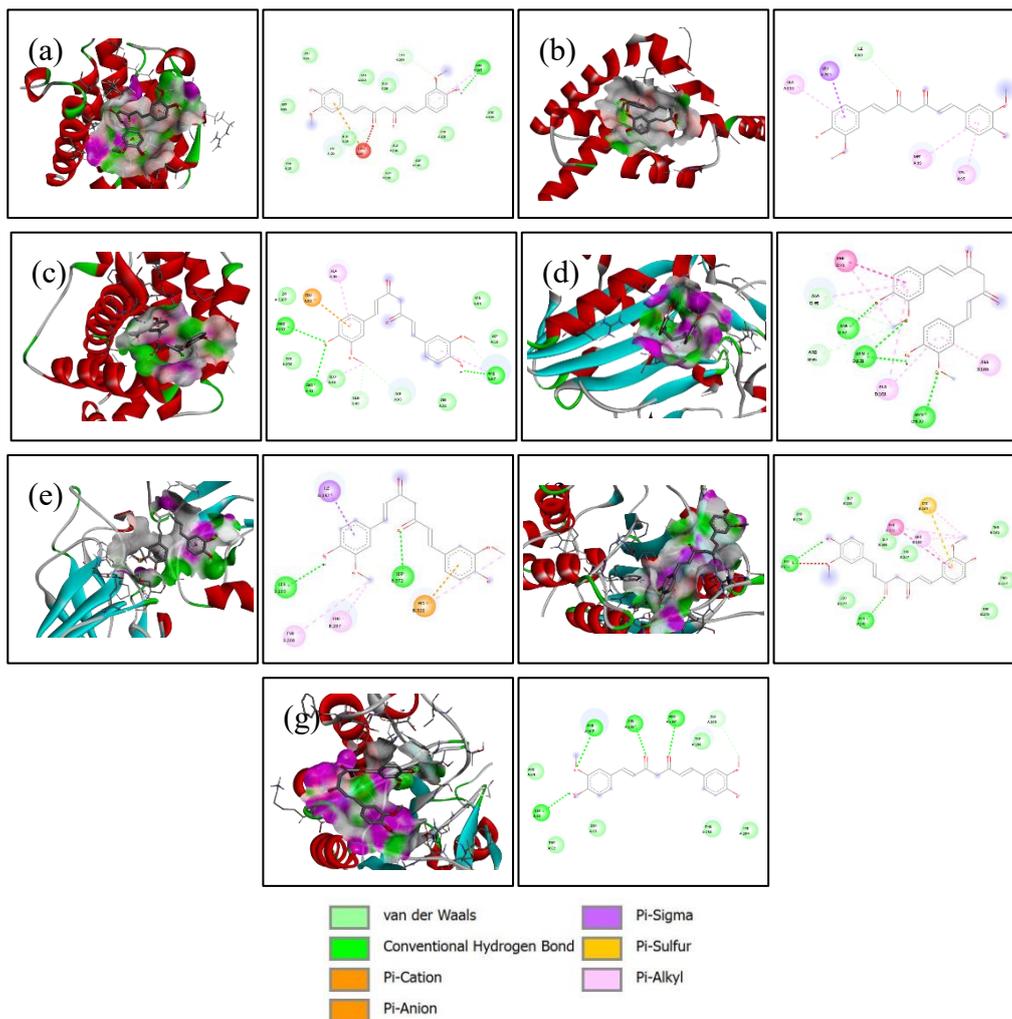


Figure 3 Visualization 3D and 2D of interactions of Curcumin against the apoptotic-regulating protein. (a) Bad, (b) Bax, (c) Bak, (d) Endonuclease G, (e) caspase-8, (f) caspase-9, and (g) caspase-3.

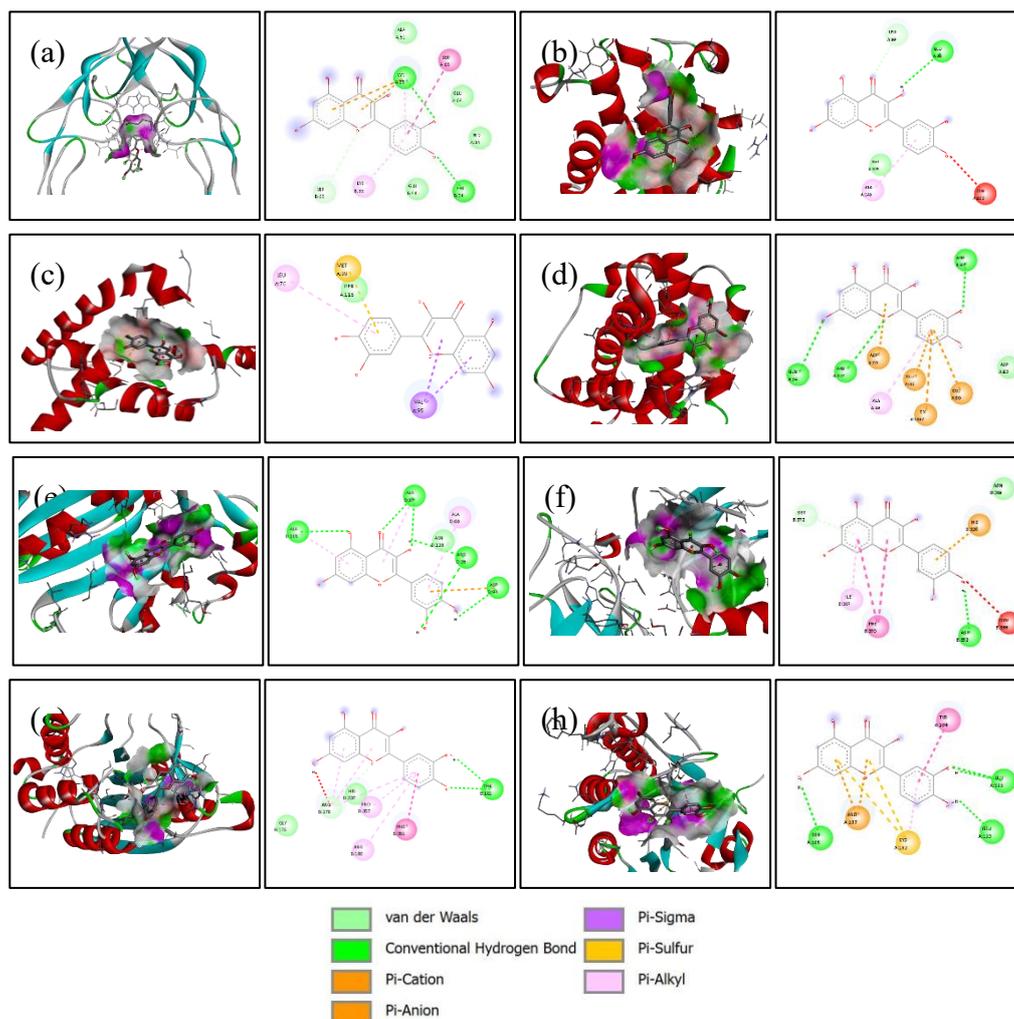


Figure 4 Visualization 3D and 2D of interactions of Quercetin against the apoptotic-regulating protein. (a) TNFR1, (b) Bad, (c) Bax, (d) Bak, (e) Endonuclease G, (f) caspase-8, (g) caspase-9, and (h) caspase-3.

Conclusions

This study comprehensively evaluated nine antioxidant compounds derived from Indonesian herbal plants through molecular docking, ADMET profiling, and toxicity prediction against key apoptotic-regulating proteins. This study revealed that several compounds, including chlorogenic acid, curcumin, quercetin, rutin, and tanshinone, exhibited strong binding affinities toward almost all apoptotic-regulating proteins, especially Bad, endonuclease G, caspase-8, and caspase-3 proteins. Suggesting their potential role in modulating apoptotic pathways. ADMET analysis indicated that most compounds possess favorable pharmacokinetic properties, although rutin and tanshinone showed limited absorption and bioavailability, kaempferol, and eugenol demonstrated possible CYP-mediated interactions. Toxicity

predictions further confirmed that most compounds fell into safe toxicity classes with low risk of organ-specific toxicities. These findings highlight the therapeutic promise of selected natural antioxidants as potential lead compounds in cancer adjuvant therapy, while also underscoring the need for further *in vitro* and *in vivo* validation to confirm their efficacy and safety. *In vitro* analysis could be conducted to search for activity and efficacy by using enzyme assays for each receptor that is tested. Furthermore, *in vivo* analysis also could be used to evaluate the compounds efficacy in living organisms while also seeing the safety and toxicity of the compounds itself.

This study is still limited to the exploration of possible interactions between the antioxidant compound we used as ligand with many receptors that could contribute to cell apoptosis by using molecular docking

simulations. There forewe didn't conduct molecular dynamics simulations as it is not part of the study and it is the limitation of this study.

Future studies should focus on validating these *in silico* findings through *in vitro* and *in vivo* experiments to establish the biological relevance of the predicted interactions. Isolation and standardized extraction of the identified antioxidants from Indonesian herbal plants could provide a sustainable source for further pharmacological testing. Moreover, structural optimization and formulation approaches, such as nanoparticle-based delivery systems, may help overcome limitations in solubility, bioavailability, and metabolic stability observed in some compounds, particularly rutin and tanshinone. Integration of advanced computational techniques, including molecular dynamics simulations and quantitative structure-activity relationship (QSAR) modeling, would also enhance the prediction accuracy of ligand-protein interactions and pharmacokinetic behaviors. Ultimately, these efforts may accelerate the translation of natural antioxidants into novel adjuvant therapeutic agents targeting apoptotic-regulating proteins for cancer treatment.

Acknowledgements

The authors would like to thank the Faculty of Medicine, Universitas Negeri Semarang, for providing academic support and research facilities. We also sincerely appreciate our colleagues for their technical assistance and for supporting this study, particularly through the provision of specialized research software that enabled the completion of the computational analyses.

Conflict of interest

The authors declare no conflict of interest.

Declaration of Generative AI in Scientific Writing

The authors acknowledge the use of generative AI tools (e.g., QuillBot and ChatGPT by OpenAI) in the preparation of this manuscript, specifically for language editing and grammar correction. AI performed no content generation or data interpretation. The authors take full responsibility for the content and conclusions of this work.

CRedit Author Statement

Luqman Alwi: Conceptualization, Methodology, Investigation. **Irwan Budiono:** Validation, Data Curation, Visualization. **Mahalul Azam:** Formal analysis, Resources. **Salamat Budijitno:** Writing – Review & Editing, Supervision. **Irena Intania:** Writing – Original Draft, Funding acquisition. **Matheus Prayoga:** Software, Project administration.

References

- [1] H Sung, J Ferlay, RL Siegel, M Laversanne, I Soerjomataram, A Jemal and F Bray. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 Cancers in 185 Countries. *CA: A Cancer Journal for Clinicians* 2021; **71(3)**, 209-249.
- [2] J Ferlay, M Colombet, I Soerjomataram, DM Parkin, M Piñeros, A Znaor and F Bray. Cancer statistics for the year 2020: An overview. *International Journal of Cancer* 2021; **149(4)**, 778-789.
- [3] RL Siegel, KD Miller and A Jemal. Cancer statistics, 2016. *CA: A Cancer Journal for Clinicians* 2016; **66(1)**, 7-30.
- [4] Z Si, J Ying and Y Zhou. A study on the global burden of non-melanoma skin cancer from 1990 to 2019. *Archives of Medical Science* 2024; **20(6)**, 1902-1908.
- [5] AN Hata, JA Engelman and AC Faber. The BCL2 family: Key mediators of the apoptotic response to targeted anticancer therapeutics. *Cancer Discovery* 2015; **5(5)**, 475-487.
- [6] F Pentimalli, S Grelli, ND Daniele, G Melino and I Amelio. Cell death pathologies: Targeting death pathways and the immune system for cancer therapy. *Genes and Immunity* 2019; **20**, 539-554.
- [7] CM Pfeffer and ATK Singh. Apoptosis: A target for anticancer therapy. *International Journal of Molecular* 2018; **19(2)**, 448.
- [8] MN Messmer, AG Snyder and A Oberst. Comparing the effects of different cell death programs in tumor progression and immunotherapy. *Cell Death & Differentiation* 2019; **26(1)**, 115-129.
- [9] S Baig, I Seevasant, J Mohamad, A Mukheem, HZ Huri and T Kamarul. Potential of apoptotic pathway-targeted cancer therapeutic research:

- Where do we stand? *Cell Death & Disease* 2016; **7(1)**, 2058.
- [10] A Yaghoubi, M Ghojzadeh, S Abolhasani, H Alikhah and F Khaki-Khatibi. Correlation of serum levels of vitronectin, malondialdehyde and Hs-CRP with disease severity in coronary artery disease. *Journal of Cardiovascular and Thoracic Research* 2015; **7(3)**, 113-117.
- [11] S Rajabi, M Maresca, AV Yumashev, R Choopani and H Hajimehdipoor. The most competent plant-derived natural products for targeting apoptosis in cancer therapy. *Biomolecules* 2021; **11(4)**, 534.
- [12] SR Lin, CH Chang, CF Hsu, MJ Tsai, H Cheng, MK Leong, PJ Sung, JC Chen and CF Weng. Natural compounds as potential adjuvants to cancer therapy: Preclinical evidence. *British Journal of Pharmacology* 2020; **177(6)**, 1409-1423.
- [13] ML Madhuri and RR Reddy. *In vitro* evaluation and molecular docking analysis of potential anticancer compounds from *Syzygium alternifolium*. *International Research Journal of Pure and Applied Chemistry* 2022; **23(6)**, 1-8.
- [14] JK Paul, M Azmal, ANMSNB Haque, OF Talukder, M Meem and A Ghosh. Phytochemical-mediated modulation of signaling pathways: A promising avenue for drug discovery. *Advances in Redox Research* 2024; **13**, 100113.
- [15] B Setiyono, MR Arif, QQ Aini, TH Soegianto, J Ohanna, RAF Gunawan and AP Rizkia. Identifikasi tanaman obat indonesia melalui citra daun menggunakan metode Convolutional Neural Network (CNN). *Jurnal Teknologi Informasi dan Ilmu Komputer* 2023; **10(2)**, 385-392.
- [16] M Apriana, RMS Toni, MC Huda, ZM Kamal, R Khoerunnisa, Allahuddin, RA Septiani, SR Ash-Shidiqi and F Anggraeni. Pengobatan penyakit kolestrol dengan menggunakan ekstrak herbal di indonesia - a review. *Jurnal Buana Farma* 2022; **2(2)**, 12-32.
- [17] MR Adiyasa and M Meiyanti. Pemanfaatan obat tradisional di Indonesia: Distribusi dan faktor demografis yang berpengaruh. *Jurnal Biomedika dan Kesehatan* 2021; **4(3)**, 130-138.
- [18] E Emelda, R Nugraeni and K Damayanti. Review: Exploration of Indonesian herbal plants for antiinflammatory. *INPHARNMED Journal (Indonesian Pharmacy and Natural Medicine Journal)* 2023; **6(2)**, 58.
- [19] YK Dewi and BA Riyandari. Potensi tanaman lokal sebagai tanaman obat dalam menghambat penyebaran COVID-19. *Jurnal Pharmascience* 2020; **7(2)**, 112-128.
- [20] A Sarasati, MH Syahrudin, A Nuryanti, ID Ana, A Barlian, CH Wijaya, D Ratnadewi, TDK Wungu and H Takemori. Plant-derived exosome-like nanoparticles for biomedical applications and regenerative therapy. *Biomedicines* 2023; **11(4)**, 1053.
- [21] M Alfarizi. Pengobatan komplementer alternatif lokal dan potensinya di Indonesia dalam perspektif kesehatan dan ekonomi: Kajian literatur sistematis. *Salus Cultura: Jurnal Pembangunan Manusia dan Kebudayaan* 2022, **2(2)**, 138-150.
- [22] M Devi, D Dahiya, N Sharma, A Soni, C Kumari and Yamini. *In silico* molecular docking study of some novel chalcone derivatives as anticancer agents. *Journal of Pharmaceutical Research International* 2023; **35(22)**, 54-65.
- [23] T Estiasih, JM Maligan, JE Witoyo, AAH Mu'Alim, K Ahmadi, T Mahatmanto and E Zubaidah. Indonesian traditional herbal drinks: Diversity, processing, and health benefits. *Journal of Ethnic Foods* 2025; **12**, 7.
- [24] V Shoshan-Barmatz, T Arif and A Shteinfe-Kuzmine. Apoptotic proteins with non-apoptotic activity: Expression and function in cancer. *Apoptosis* 2023; **28(5-6)**, 730-753.
- [25] GM Morris, H Ruth, W Lindstrom, MF Sanner, RK Belew, DS Goodsell and AJ Olson. Software news and updates AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of Computational Chemistry* 2009; **30(16)**, 2785-2791.
- [26] B Ye, W Tian, B Wang and J Liang. CASTpFold: Computed atlas of surface topography of the universe of protein folds. *Nucleic Acids Research* 2024; **52(W1)**, W194-W199.
- [27] EF Pettersen, TD Goddard, CC Huang, GS Couch, DM Greenblatt, EC Meng and TE Ferrin. UCSF Chimera - A visualization system for exploratory research and analysis. *Journal of Computational Chemistry* 2004; **25(13)**, 1605-1612.

- [28] NM O'Boyle, M Banck, CA James, C Morley, T Vandermeersch and GR Hutchison. Open babel: An open chemical toolbox. *Journal of Cheminformatics* 2011; **3**, 33.
- [29] PR Sankar, AB Sailu, MM Eswarudu, MN Satya, P Sreeja, P Roja and S Rijwana. Analytical methods for determination of different members of FDA approved tyrosine kinase inhibitors like Dasatinib, Lapatinib, Imatinib, Sorafenib, Nintedanib, Sunitinib and Pazopanib: A review. *Journal of Pharmaceutical Sciences and Research* 2021; **13(6)**, 313-318.
- [30] O Trott and AJ Olson. AutoDock vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry* 2010; **31(2)**, 455-461.
- [31] M Bitew, T Desalegn, TB Demissie, A Belayneh, M Endale and R Eswaramoorthy. Pharmacokinetics and drug-likeness of antidiabetic flavonoids: Molecular docking and DFT study. *PLoS One* 2021; **16(12)**, 0260853.
- [32] CA Lipinski, F Lombardo, BW Dominy and PJ Feeney. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews* 2012; **23(1-3)**, 3-25.
- [33] B Gullón, TA Lú-Chau, MT Moreira, JM Lema and G Eibes. Rutin: A review on extraction, identification and purification methods, biological activities and approaches to enhance its bioavailability. *Trends in Food Science & Technology* 2017; **67**, 220-235.
- [34] SR Akash, A Tabassum, LM Aditee, A Rahman, MI Hossain, MA Hannan and MJ Uddin. Pharmacological insight of rutin as a potential candidate against peptic ulcer. *Biomedicine & Pharmacotherapy* 2024; **177**, 116961.
- [35] J Terao. Potential role of quercetin glycosides as anti-atherosclerotic food-derived factors for human health. *Antioxidants* 2023; **12(2)**, 258.
- [36] T Przybylski, J Czerniel, J Dobrosielski and M Stawny. Flavonol technology: From the compounds' chemistry to clinical research. *Molecules* 2025; **30(15)**, 3113.
- [37] JPB Arango, DYM Rodriguez, SL Cruz and GT Ocampo. *In silico* evaluation of pharmacokinetic properties and molecular docking for the identification of potential anticancer compounds. *Computational Biology and Chemistry* 2026; **120(1)**, 108626.
- [38] LF Araujo, CHS de Pinto and LF Motta. *In silico* pharmacokinetic and toxicological study of cinnamic acid analogues. *Brazilian Journal of Development* 2022; **8(12)**, 80800-80817.
- [39] NN Wang, C Huang, J Dong, ZJ Yao, MF Zhu, ZK Deng, B Lv, AP Lu, AF Chenac and DS Cao. Predicting human intestinal absorption with modified random forest approach: A comprehensive evaluation of molecular representation, unbalanced data, and applicability domain issues. *RSC Advances* 2017; **7**, 19007-19018.
- [40] I Ahmad, AE Kuznetsov, AS Pirzada, KF Alsharif, M Daglia and H Khan. Computational pharmacology and computational chemistry of 4-hydroxyisoleucine: Physicochemical, pharmacokinetic, and DFT-based approaches. *Frontiers in Chemistry* 2023; **11**, 1145974.
- [41] R Kato, L Zhang, N Kinatukara, R Huang, A Asthana, C Weber, M Xia, X Xu and P Shah. Investigating blood-brain barrier penetration and neurotoxicity of natural products for central nervous system drug development. *Scientific Reports* 2025; **15**, 7431.
- [42] J Zhai, VH Man, B Ji, L Cai and J Wang. Comparison and summary of *in silico* prediction tools for CYP450-mediated drug metabolism. *Drug Discovery Today* 2023; **28(10)**, 103728.
- [43] AMB Amorim, LF Piochi, AT Gaspar, AJ Preto, N Rosário-Ferreira and IS Moreira. Advancing drug safety in drug development: Bridging computational predictions for enhanced toxicity prediction. *Chemical Research in Toxicology* 2024; **37(6)**, 827-849.
- [44] United Nations. *Globally Harmonized System of Classification and Labelling of Chemicals (GHS)*. 9th ed. United Nations, New York and Geneva, 2021.
- [45] CM Zwickl, JC Graham, RA Jolly, A Bassan, E Ahlberg, A Amberg, LT Anger, L Beilke, P Bellion, A Brigo, H Burleigh-Flayer, MTD

- Cronin, AA Devlin, T Fish, S Glowienke, K Gromek, AL Karmaus, R Kemper, S Kulkarni, ..., GJ Myatt. Principles and procedures for assessment of acute toxicity incorporating *in silico* methods. *Computational Toxicology* 2022; **24**, 100237.
- [46] R Chowdhury, MS Bhuia, A Islam Rakib, R Hasan, HDM Coutinho, IM Araújo, IRA de Menezes and MT Islam. Assessment of quercetin antiemetic properties: *In vivo* and *in silico* investigations on receptor binding affinity and synergistic effects. *Plants* 2023; **12(24)**, 4189.
- [47] APD Nurhayati, A Rihandoko, A Fadlan, SS Ghaisani, N Jadid and E Setiawan. Anti-cancer potency by induced apoptosis by molecular docking P53, caspase, cyclin D1, cytotoxicity analysis and phagocytosis activity of trisindoline 1,3 and 4. *Saudi Pharmaceutical Journal* 2022; **30(9)**, 1345-1359.
- [48] EM Terefe and A Ghosh. Molecular docking, validation, dynamics simulations, and pharmacokinetic prediction of phytochemicals isolated from croton dichogamus against the HIV-1 reverse transcriptase. *Bioinformatics and Biology Insights* 2022; **16**, 11779322221125605.
- [49] RK Tiwari, A Brown, N Sadeghiani, AN Shirazi, J Bolton, A Tse, G Verkhivker, K Parang and G Sun. Design, synthesis, and evaluation of dasatinib-amino acid and dasatinib-fatty acid conjugates as protein tyrosine kinase inhibitors. *ChemMedChem* 2017; **12(1)**, 86-99.
- [50] M Arya, A Tiwari, DB Singh and G Taj. Computational study of lactucine and its derivatives to investigate its anti-cancerous properties targeting apoptosis-inducing protein. *Letters in Drug Design & Discovery* 2023; **21(7)**, 1137-1147.
- [51] HR Trivedi and PK Puranik. Chlorogenic acid-optimized nanophytovesicles: A novel approach for enhanced permeability and oral bioavailability. *Future Journal of Pharmaceutical Sciences* 2023; **9**, 116.
- [52] Y Yan, Kulsoom, Y Sun, Y Li, Z Wang, L Xue and F Wang. Advancing cancer therapy: Nanomaterial-based encapsulation strategies for enhanced delivery and efficacy of curcumin. *Materials Today Bio* 2025; **33**, 101963.
- [53] L Xing, ZR Tan, JL Cheng, WH Huang, W Zhang, W Deng, CS Yuan and HH Zhou. Bioavailability and pharmacokinetic comparison of tanshinones between two formulations of *Salvia miltiorrhiza* in healthy volunteers. *Scientific Reports* 2017; **7**, 4709.