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Integrated Network Pharmacology, *In Silico*, and *In Vitro* Evaluation of Antioxidant Activity from the Methanol Extract of *Salacca zalacca* Skin

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Abstract

Salacca zalacca (SZ) is an indigenous tropical fruit from Indonesia, rich in phytochemicals with notable antioxidant potential. However, the antioxidant mechanisms of the methanol extract from SZ skin remain unclear. This study evaluated the antioxidant properties of the methanol extracts from SZ skin using an integrated approach combining network pharmacology, *in silico*, and *in vitro* analyses. The methanol extract of SZ skin was analyzed using targeted liquid chromatography-mass spectrometry (LC-MS), while the pharmacokinetic properties were assessed using the pkCSM and PASS Online databases. Protein-protein interactions were explored using NCBI and GeneCards. Molecular docking and dynamic simulations were performed with PyRx v8.0, PyMol, and YASARA. Targeted LC-MS identified 8 bioactive compounds: caffeic acid (C1), chlorogenic acid (C2), rutin (C3), stigmasterol (C4), gallic acid (C5), ferulic acid (C6), quercetin (C7), and β-sitosterol (C8). Pharmacokinetic analysis demonstrated that C1, C6, and C7 complied with Lipinski's Rule of Five. Biological activity predictions revealed that C3 had the highest antioxidant potential (Pa = 0.923) and free radical scavenging ability (Pa = 0.988). Molecular docking confirmed the strong binding affinity of C3 with KEAP1 (−9.3 kcal/mol), supported by molecular dynamics simulations showing stable ligand-receptor interactions and minimal residue fluctuations (< 3 Å). *In vitro* antioxidant assays showed that the methanol extract of SZ skin had stronger ABTS radical scavenging activity (EC_{50} = 87.83 µg/mL) than Trolox (EC_{50} = 325.1 µg/mL), although its DPPH activity was lower (EC_{50} = 496.0 vs. 61.18 µg/mL for Trolox). Overall, these findings suggest that the methanol extract of SZ skin is a promising natural source of antioxidants. However, further studies are needed to clarify the antioxidant mechanism.

Keywords: Methanol extract of *Salacca zalacca* skin, Oxidative stress, Molecular docking, Molecular dynamics, Natural compound, ABTS, DPPH

Introduction

Reactive oxygen species (ROS) are pivotal mediators in the development and progression of numerous chronic diseases, including cardiovascular diseases, diabetes mellitus, neurodegenerative diseases, and cancer [1]. Excessive ROS production disrupts the redox balance, leading to cellular damage, impairing cellular signaling, and loss of cellular homeostasis, thereby triggering the onset and progression of various pathological conditions [2].Targeting oxidative stress has become a key strategy in the development of therapeutic interventions, ranging from modulation of endogenous antioxidant systems, developing novel antioxidant compounds and targeting redox-sensitive signaling pathways. This approach holds promise in enhancing patient outcomes and developing more effective treatment strategies for a broad spectrum of chronic conditions [3].

Natural products, which represent a source of antioxidants, owing to their bioactivity and potential as an alternative treatment for oxidative stress. *Salacca zalacca* (SZ), or snake fruit, is a tropical plant native to Southeast Asia, with Indonesia recognized as the primary center of cultivation and diversity. Renowned for its unique scaly skin and sweet-tart flavor, SZ holds considerable cultural, economic, and nutritional value [4]. SZ, particularly in the skin, is rich in phytochemical compounds such as phenolic acids, flavonoids, and tannins [5]. Moreover, SZ exhibits strong antioxidant activity, including potent free-radical scavenging effects [6]. While the edible pulp is consumed, the skin is usually discarded. Research on SZ skin remains limited, and the mechanisms underlying its antioxidant potential have not been fully clarified. In this study, methanol was used as a solvent to explore the phytochemical compounds and potential antioxidant activities in SZ skin. Methanol is an efficient solvent for extracting diverse phytochemicals, making it an ideal solvent for the extraction of hydrophilic and lipophilic substances [7].

An *in silico* prediction method for drug discovery that predicts interactions between bioactive compounds and protein targets by quantifying binding affinities [8]. These methods have facilitated the identification of natural products as potential drug candidates. In this study, an integrated approach that combines phytochemical profiling, *in silico* predictions, and *in vitro* assays using DPPH and ABTS assays can provide a comprehensive understanding of the antioxidant activity of SZ skin extracts. This research also supports Sustainable Development Goals (SDGs) 12: Responsible Consumption and Production, highlights the pharmacological potential of SZ as a local plant and an underutilized agricultural byproduct, providing opportunities to advance human health (phytopharmaceuticals or health supplements), promote sustainable waste management, and reinforce the principles of a circular economy. The findings of this study contribute to establishing the value of the neglected SZ skin and address the existing knowledge gap by highlighting its potential as a novel source of antioxidant agents for managing oxidative stress-related diseases.

Materials and methods

Extraction of SZ using methanol solvent

The SZ skin samples were obtained from the local authorities and community in Tirtoyudo Village, Malang Regency, East Java, Indonesia. The specimens were taxonomically identified and verified at Materia Medica, Batu. These specimens belong to the kingdom *Plantae*, division *Magnoliophyte*, class *Liliopsida*, family *Arecaceae*, and genus *Salacca*. The objective of this collection was to document the specimens for future studies. Ninety-six-percent method used 96% methanol for maceration. One kg of fresh SZ *skin* was oven-dried to produce 820 g dried SZ skin. A total of 100 g of dried simplicial was soaked in 96% methanol at a 1:10 ratio (w/v) and stirred twice daily for 5 day. The extract was filtered, and the solvent was evaporated to obtain a thick extract, yielding 14.17 g [6].

Metabolomics profiling of methanol extract of SZ

Compounds in the methanol extract of SZ were analyzed using targeted liquid chromatography-mass spectrometry (LC-MS) at the Pharmacology Laboratory, Universitas Brawijaya. A 10 mg sample was dissolved in 10 mL of methanol, sonicated for 15 min, and filtered through a 0.22 µm PTFE membrane into a 2 µL vial for analysis. LC-MS analysis was performed on a Thermo Scientific Vanquish Autosampler HPLC system

equipped with solvent A (0.1% formic acid in water) and solvent B (90% acetonitrile in water). Chromatographic separation was achieved on a C18 Hypersil GOLD column (100×2.1×1.9 mm^3) at a flow rate of 300 µL/min, employing a 15-minute gradient elution [9,10]. Mass spectrometric detection was performed on a Waters UPLC-TQD system operated in positive ionization mode with data-dependent acquisition. Metabolite identification was carried out using Compound Discoverer software, with spectral matching against the Thermo Scientific MS/MS Library.

Drug-likeness, pharmacokinetic prediction, absorption, distribution, metabolism, excretion, and toxicity (ADMET) of the methanol extract of SZ skin compounds

The drug-likeness and pharmacokinetic properties of the methanol extract of SZ skin compounds were assessed using the SwissADME web server (http://www.swissadme.ch/). Canonical simplified molecular-input line-entry system (SMILES) structures from PubChem (https://pubchem.ncbi.nlm.nih.gov/) and submitted to predict absorption, distribution, metabolism, and excretion (ADME) characteristics. The analysis included predictions of blood-brain barrier (BBB) permeability, overall drug-likeness, and human intestinal absorption (HIA). Toxicological properties were further evaluated using the ProTox-II web tool, which estimates the predicted lethal dose (LD_{50}) and assigns toxicity classifications [11].

Prediction of the biological activity of the methanol extract of SZ skin compounds

The biological activities of the methanol extract of SZ skin compounds were predicted using the Prediction of Activity Spectra for Substances (PASS) online tool. Canonical SMILES were used as input, and predictions were expressed as probability of active (Pa) and probability of inactive (Pi) values. Compounds with Pa values higher than Pi were considered to possess significant biological activity, with higher Pa values indicating strong potential activity [12].

Protein-Protein Interaction (PPI) of the gene target analysis

Genes related to oxidative stress were collected from 2 databases: NCBI (https://www.ncbi.nlm.nih.gov/), and GeneCards (https://www.genecards.org/), using the keywords “oxidative stress” and “Homo sapiens”. Overlapping data were identified and merged using Venny 2.1.0 (https://bioinfogp.cnb.csic.es/tools/venny/), and hub gene targets were further analyzed using Jvenn (https://jvenn.toulouse.inra.fr/app/example.html). A protein-protein interaction (PPI) network was constructed by inputting the overlapping targets into the STRING database (https://string-db.org/) with the species set to *Homo sapiens* and a minimum interaction confidence score of > 0.4. The network was visualized in Cytoscape 3.8.2, and hub genes were identified based on degree, betweenness, stress, radiality, and closeness centrality parameters [13].

Molecular docking

Molecular docking was performed using AutoDock Vina in PyRx 0.8. The ligands, including Caffeic acid (C1), chlorogenic acid (C2), rutin (C3), stigmasterol (C4), gallic acid (C5), ferulic acid (C6), quercetin (C7), and β-sitosterol (C8), were obtained in SDF format and converted to PDB format using BIOVIA Discovery Studio 2024. The Keap1/Nrf2 complex structure (PDB ID: 2FLU) was retrieved from the Protein Data Bank [14]. PyMOL was used to preparation the Keap1 protein by removing water molecules and other non-essential ligands. A docking grid was created based on the active site residues identified in previous studies, including Tyr334, Asn382, His436, Tyr525, Tyr57, Arg380, Arg415, Arg483, Ser363, Ser508, Ser555, and Ser602 [15,16], as shown in **Table 1**. The result docking interactions were visualized using BIOVIA Discovery Studio 2024.

Table 1 Grid coordinates for specific docking.

	Center			Dimensions (Angstrom)		
Protein	**X**	**Y**	**Z**	**X**	**Y**	**Z**
2FLU	8.306	13.5217	1.4436	19.2760	27.5153	26.2072

Molecular dynamics simulation

Molecular dynamics (MD) simulations were carried out using YASARA software with the AMBER14 force field under physiological conditions (1 atm pressure, 0.9% NaCl, 37 °C, and pH 7.4). Following system equilibration, a 20 ns production simulation was conducted with a timestep of 50.000 fs. The structural stability of the ligand-protein complexes was assessed by calculating the root-mean-square deviation (RMSD) and root-mean-square fluctuation (RMSF) parameters using YASARA macros [14].

Antioxidant capacity of the methanol extract of SZ skin assays

The antioxidant activity of the methanol extract of *Salacca zalacca* (SZ) skin was evaluated using the ABTS assay. The ABTS assay was generated by mixing 2.4 mM potassium persulfate with 7 mM ABTS and incubating the mixture in the dark at room temperature for 12 - 16 h. The resulting ABTS solution (1 mL) was then diluted wth 60 mL of ethanol. Trolox served as the positive control, and both the extract and Trolox were tested at concentrations of 40, 80, 160, 320, 640, and 1,280 µg/mL. All assays were performed in triplicate, and absorbance was recorded at 734 nm [17]. The percentage of inhibition was calculated using the following formula:

$$\text{Scavenging activity (\%)} = \frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{reaction}}}{\text{Absorbance}_{\text{control}}} \times 100$$

The antioxidant activity of the methanol extract of SZ skin was also analyzed using the DPPH assay. Both the extracts and Trolox were initially dissolved in ethanol at a concentration of 1000 µg/mL and then diluted with 90 µM ethanolic DPPH solution to obtain final concentrations of 40, 80, 160, 320, 640, and 1,280 µg/mL. For the assay, 1 mL of the methanol extract of SZ skin at each concentration was transferred into a 96-well plate, followed by the addition of a 0.3 mM DPPH solution. The mixtures were incubated in the dark at 25 °C, and absorbance was measured at 517 nm using a microplate reader [17]. The percentage of radical scavenging activity was then determined using the following formula:

$$\text{Scavenging activity (\%)} = \frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{reaction}}}{\text{Absorbance}_{\text{control}}} \times 100$$

Results and discussion

Metabolic profiling of SZ skin using LC-MS analysis

Targeted LC-MS analysis revealed that the methanol extract of SZ skin contained 6 compounds, such as caffeic acid (C1), chlorogenic acid (C2), rutin (C3), stigmasterol (C4), gallic acid (C5), ferulic acid (C6), quercetin (C7), and β-sitosterol (C8), as listed in Table 2. All of these compounds were further analyzed for their potential antioxidants and free radical scavenging activities. These findings are consistent with previous studies showing that SZ skin contains phenolic acids, flavonoids, and tannins [5]. Previous study also reported that SZ skin contains various bioactive compounds, including rutin, gallic acid, ferulic acid, chlorogenic acid, caffeic acid, stigmasterol, linoleic acid, quercetin, and rosmarinic acid [18,19]. Furthermore, both the skin and fruit of SZ have been reported to exhibit anticancer, antioxidant, anti-inflammatory, and antidiabetic activities [20]. These bioactive compounds are essential for preventing of various diseases, particularly oxidative stress and inflammation-related diseases, supporting their potential as promising natural antioxidant agents.

Table 2 List of the methanol extract of *Salacca zalacca* skin compounds from targeted LC-MS analysis.

Compounds ID	Compounds Name	PubChem ID	Molecular Formula	Smile
C 1	Caffeic Acid	689043	$C_9H_8O_4$	C1=CC(=C(C=C1C=CC(=O)O)O)O
C 2	Chlorogenic Acid	1794427	$C_{16}H_{18}O_9$	C1C(C(C(CC1(C(=O)O)O)OC(=O)C=CC2=CC(=C(C=C2)O)O)O)O

Compounds ID	Compounds Name	PubChem ID	Molecular Formula	Smile
C 3	Rutin	5280805	$C_{27}H_{30}O_{16}$	CC1C(C(C(C(O1)OCC2C(C(C(C(O2)OC3=C(OC4=CC(=CC(=C4C3=O)O)O)C5=CC(=C(C=C5)O)O)O)O)O)O)O)O
C 4	Stigmasterol	5280794	$C_{29}H_{48}O$	CCC(C=CC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C)C(C)C
C 5	Gallic acid	370	$C_7H_6O_5$	C1=C(C=C(C(=C1O)O)O)C(=O)O
C 6	Ferulic acid	445858	$C_{10}H_{10}O_4$	COC1=C(C=CC(=C1)C=CC(=O)O)O
C 7	Quercetin	5280343	$C_{15}H_{10}O_7$	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O)O
C 8	Beta-sitosterol	222284	$C_{29}H_{50}O$	CCC(CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C)C(C)C

Absorption, distribution, metabolism, excretion, and toxicity (ADMET) and druglikeness of SZ skin

ADMET and drug-likeness analyses were conducted to evaluate the pharmacokinetic profiles of SZ skin compounds and predict their potential as drug candidates (**Table 3**). Based on these analyses, 3 compounds, caffeic acid (C1), ferulic acid (C6), and quercetin (C7) complied with Lipinski's Rule of Five (RoF), indicating favorable drug-likeness characteristics and good oral bioavailability. In contrast, chlorogenic acid (C2), rutin (C3), stigmasterol (C4), gallic acid (C5), and β-sitosterol (C8) did not meet the RoF criteria due to their higher molecular weights and excessive numbers of hydrogen bond donors and acceptors, exceeding the optimal thresholds.

Toxicity analyses using Pro-Tox II showed that caffeic acid, chlorogenic acid, and rutin were classified as toxicity class 5, while stigmasterol, gallic acid, ferulic acid, and beta-sitosterol were classified as toxicity class 4. Quercetin was classified as toxicity class 3 (**Table 3**), indicating a minor oral risk but not severe toxicity, though careful dose consideration is warranted in therapeutic applications. Furthermore, none of the SZ skin compounds tested positive in the AMES assay, and all were predicted to be non-hepatotoxic and unlikely to cause skin irritation. However, rutin (C3), stigmasterol (C4), and beta-sitosterol (C8) were identified as potential HERG II inhibitors (**Table S1**), suggesting a possible risk of cardiac side effects [21].

Table 3 Pharmacokinetic, Drug likeness, and toxicity computation analysis of the methanol extract of *Salacca zalacca* skin compounds.

SZ compounds	Pharmacokinetic Test			Drug likeness (Lipinski Rules)*					Toxicity Computation Analysis**		
	Water Solubility	Human intestinal absorption ion (HIAi)	Penetrate Blood-Brain Barrier (BBB)	Molecular Weight	Acceptor Hydrogen	Donor Hydrogen	LogP	Molar Refractivity	Categories	Predicted LD_{50} (mg/kg)	Toxicity class
C 1	Low	Medium	Low	180.159	3	3	1.1956	47.1	Accepted	2980	5
C 2	Low	Medium	Low	354.311	8	6	-0.6459	83.5	Rejected	5000	5
C 3	Low	Medium	Low	610.521	16	10	-1.6871	141.38	Rejected	5000	5
C 4	Low	High	High	412.702	1	1	7.8008	132.75	Rejected	890	4
C 5	Low	Medium	Low	170.12	4	4	0.5016	39.47	Rejected	2000	4

SZ compounds	Pharmacokinetic Test			Drug likeness (Lipinski Rules)*						Toxicity Computation Analysis**	
	Water Solubility	Human intestinal absorption ion (HIAi)	Penetrate Blood-Brain Barrier (BBB)	Molecular Weight	Acceptor Hydrogen	Donor Hydrogen	LogP	Molar Refractivity	Categories	Predicted LD_{50} (mg/kg)	Toxicity class
C 6	Low	High	Low	194.186	3	2	1.4986	51.63	Accepted	1772	4
C 7	Low	High	Low	302.238	7	5	1.988	78.03	Accepted	159	3
C 8	Low	High	High	414.718	1	1	8.0248	133.23	Rejected	890	4

*SWISS ADME, **Pro-Tox II

Biological activity prediction

Online PASS analysis revealed that several SZ skin compounds, particularly rutin, exhibited the highest predicted antioxidant and free radical-scavenging activities. Rutin showed the highest scores in both categories (Pa = 0.923 for antioxidants and Pa = 0.988 for free radical scavenging). Other compounds scored as follows: Quercetin (0.872), chlorogenic acid (0.785), caffeic acid (0.0603), ferulic acid (0.540), gallic acid (0.520), stigmasterol (0.215), and beta-sitosterol (0.178) for antioxidants prediction and chlorogenic acid (0.856), quercetin (0.811), ferulic acid (0.731), caffeic acid (0.647), gallic acid (0.570), stigmasterol (0.008), and beta-sitosterol (0.007), as shown in **Figure 1**. These results are consistent with previous studies demonstrating that rutin has numerous pharmacological effects, including anti-inflammatory, diabetes management, blood vessel protection, antimicrobial, anticancer, and most importantly, antioxidant properties [22].

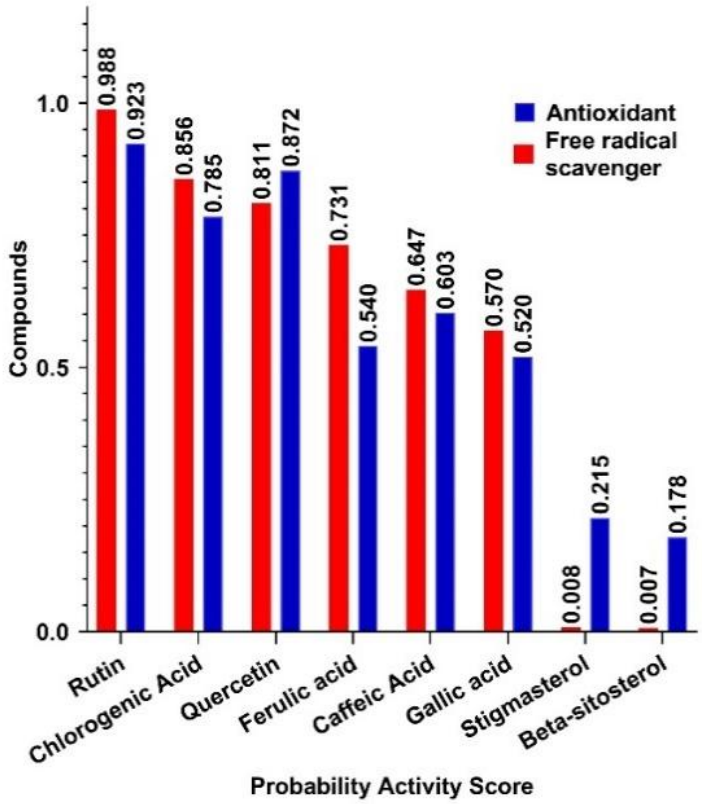

Figure 1 Prediction of the characteristic the methanol extract of *Salacca zalacca* skin compounds. Biological activity the methanol extract of *Salacca zalacca* skin compounds prediction.

Protein-Protein Interaction (PPI) and pathway analysis

A total of 64,700 oxidative stress-related genes were identified from the NCBI database, of which 519 were selected from Homo sapiens. Similarly, 14.221 oxidative stress-related genes were identified in GeneCard, with 519 selected based on a relevance score above 20. A Venn diagram revealed 106 overlapping gene targets between the NCBI and GeneCard databases (**Figure 2(A)**). The overlapping targets were analyzed for network centrality based on degree parameters (**Figure 2(B)**), with nodes arranged in an elliptical layout and color-coded from dark red to orange-yellow according to their degree values. Prominent antioxidant-related targets included SOD2 (degree = 65), SOD1 (degree = 64), KEAP1 (degree = 56), and SOD3 (degree = 23), represented as larger, darker-red nodes, indicating high interaction levels. Network topology was further examined in Cytoscape using 5 parameters: Degree, betweenness centrality, closeness centrality, radiality, and stress. A total of 72 hub targets were identified and visualized using a Venn diagram (Figure 2C), with corresponding gene names provided in Supplementary **Table 2**. This analysis highlighted the NRF2-KEAP1 pathway, with particular focus on KEAP1 (degree = 56), an oxidative stress-related gene. KEAP1 functions as an intracellular ROS sensor and acts as a Cullin3 adaptor protein, serving as a molecular "switch" to regulate Nrf2 degradation. Under physiological conditions, KEAP1 binds Nrf2 and promotes its degradation via the ubiquitin-proteasome pathway, thereby maintaining redox homeostasis [23].

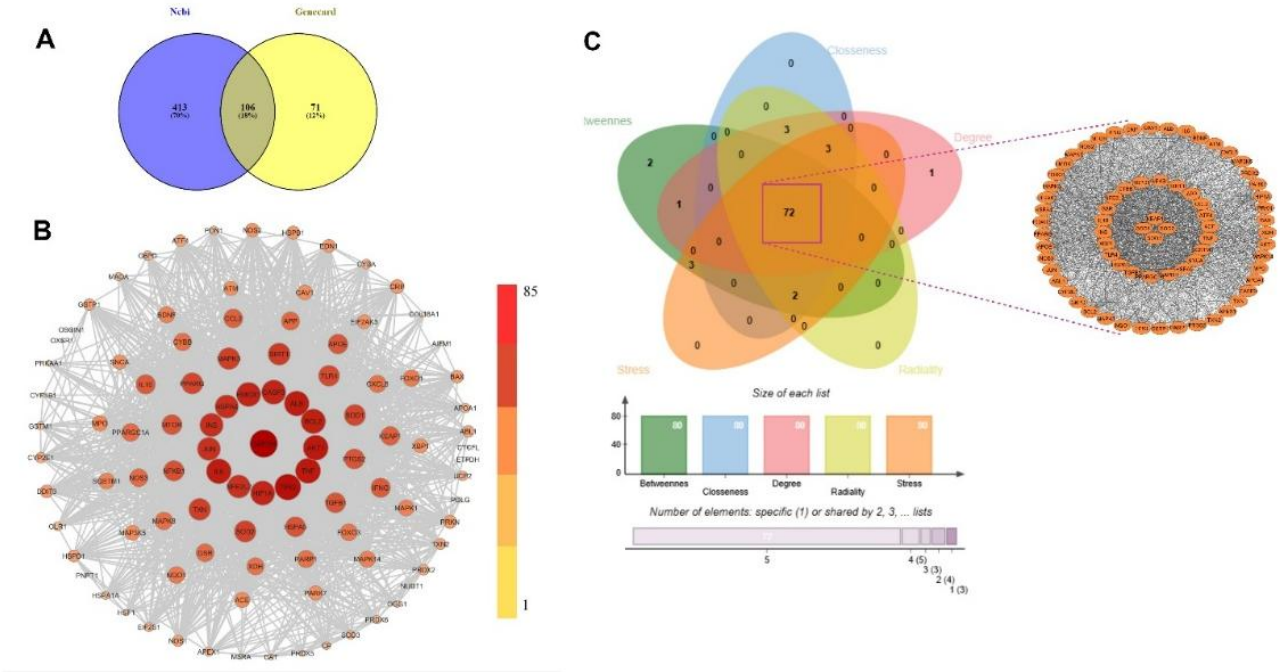

Figure 2 Visual analysis of the pharmacological network of oxidative stress genes. (A) Venn diagram illustrating the overlap of oxidative stress-related gene targets obtained from the NCBI and GeneCards databases. (B) Protein-protein interaction (PPI) network of 106 overlapping oxidative stress target genes, larger node size and darker coloration represent higher degree values within the network. (C) Identification of 72 hub targets with the highest degree, betweenness centrality, closeness centrality, stress, and radiality values, visualized using a Venn diagram.

Molecular docking

To validate the interactions between SZ skin compounds and hub targets identified from the PPI network, the KEAP1-Nrf2 pathway was selected as a representative endogenous antioxidant system. Molecular docking analysis revealed that rutin exhibited the strongest binding affinity (−9.0 kcal/mol), followed by chlorogenic acid (−8.2 kcal/mol), quercetin (−7.7 kcal/mol), stigmasterol (−7.7 kcal/mol), β-sitosterol (−7.4 kcal/mol), gallic acid (−6.4 kcal/mol), caffeic acid (−6.2 kcal/mol), and ferulic acid (−6.0 kcal/mol), as shown in **Figure 3**. Two-dimensional visualizations of

amino acid interactions were generated using BIOVIA Discovery Studio, as shown in **Figure 4**. Detailed interactions of the methanol extract of SZ skin with the KEAP1-Nrf2 active site are detailed in **Table S3**.

Rutin binds to KEAP1 through hydrogen bonds at SER 363, TYR 572, SER 555, and GLN 530; hydrophobic interactions at GLY 574, ALA 556, ARG 483, SER 602, GLY 364, PHE 577, ASN 382, SER 338, ARG 415, GLY 603, and ARG 380; and Pi-alkyl interactions at TYR 525. Stigmasterol interacts with KEAP1 via hydrophobic interactions at GLY 603, GLN 530, GLN 528, ASP 529, GLY 574, TYR 334, SER 602, SER 363, GLY 364, ARG 415, and GLN 530, along with Pi-alkyl interactions at TYR 572, ALA 556, and TYR 525. Beta-sitosterol binds to KEAP1 through hydrophobic interactions at GLY 574, GLN 528, ASP 529, SER 602, TYR 334, SER 363, GLY 603, GLY 364, ARG 415, and GLN 530, and Pi-alkyl interactions at TYR 572, ALA 556, and TYR 525. Chlorogenic acid binds to KEAP1 via hydrogen bonds at ARG 483, ASN 414, and SER 363; hydrophobic interactions at TYR 525, GLN 530, ARG 415, SER 555, GLY 509, TYR 334, ALA 556, ASN 382, SER 383, GLY 364, GLY 603, SER 602, TYR 572, and PHE 577; and π-alkyl interactions at ARG 380.

Gallic acid binds to KEAP1 through hydrogen bonds at SER 602, SER 363, ASN 414, and ARG 380; hydrophobic interactions at TYR 572, PHE 577, TYR 334, SER 338, GLY 364, ARG 415, and GLY 603; and unfavorable donor-donor interactions at ASN 382. Ferulic acid binds to KEAP1 via hydrogen bonds at SER 363 and ASN 382; hydrophobic interactions at GLY 603, TYR 334, SER 338, GLY 364, and PHE 577; and PI-PI-stacked and PI-PI-shaped interactions at TYR 572 and TYR 334. Quercetin binds to KEAP1 through hydrogen bonds at SER 602 and SER 555; hydrophobic interactions at TYR 525, SER 363, GLN 530, GLY 509, ASN 414, ARG 415, ARG 380, GLY 364, GLY 603, and TYR 334; PI-PI-shaped interactions at TYR 572; and Pi-alkyl interactions at ALA 556. Caffeic acid binds to KEAP1 via hydrogen bonds at ARG 380, SER 363, and ASN 414; hydrophobic interactions at TYR 572, SER 602, GLY 603, GLY 364, ASN 382, SER 338, TYR 334, and PHE 577; and unfavorable donor-donor interactions at ARG 380. The visualization of amino acid residues for each compound in the SZ skin methanol extract is shown in Figure 4, and detailed amino acid interactions are provided in Supplementary **Table 3**.

Previous studies have identified key KEAP1–NRF2 compleactive site residues, including alanine residues at His436, Tyr572, Tyr334, Asn382, and Tyr525; arginine residues at Arg415, Arg380, and Arg483; and serine residues at Ser363, Ser555, Ser602, and Ser508. Modifications at these residues markedly reduce the ability of KEAP1 to bind Nrf2, thereby diminishing its capacity to repress Nrf2-dependent gene expression [15,16]. These findings are similar to those of previous studies, demonstrating that the skin SZ may bind to the active site of KEAP1.

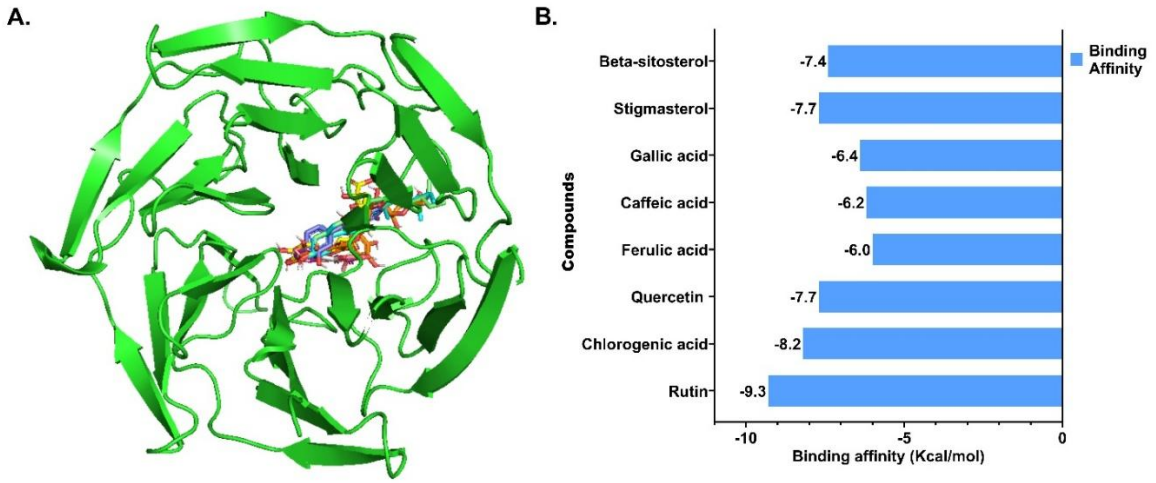

Figure 3 Molecular docking results showing the binding affinity of SZ skin compounds for KEAP1 (A) Superimposition of 2FLU with its ligand. (B) Binding affinity scores from the molecular docking results of SZ compounds against KEAP1.

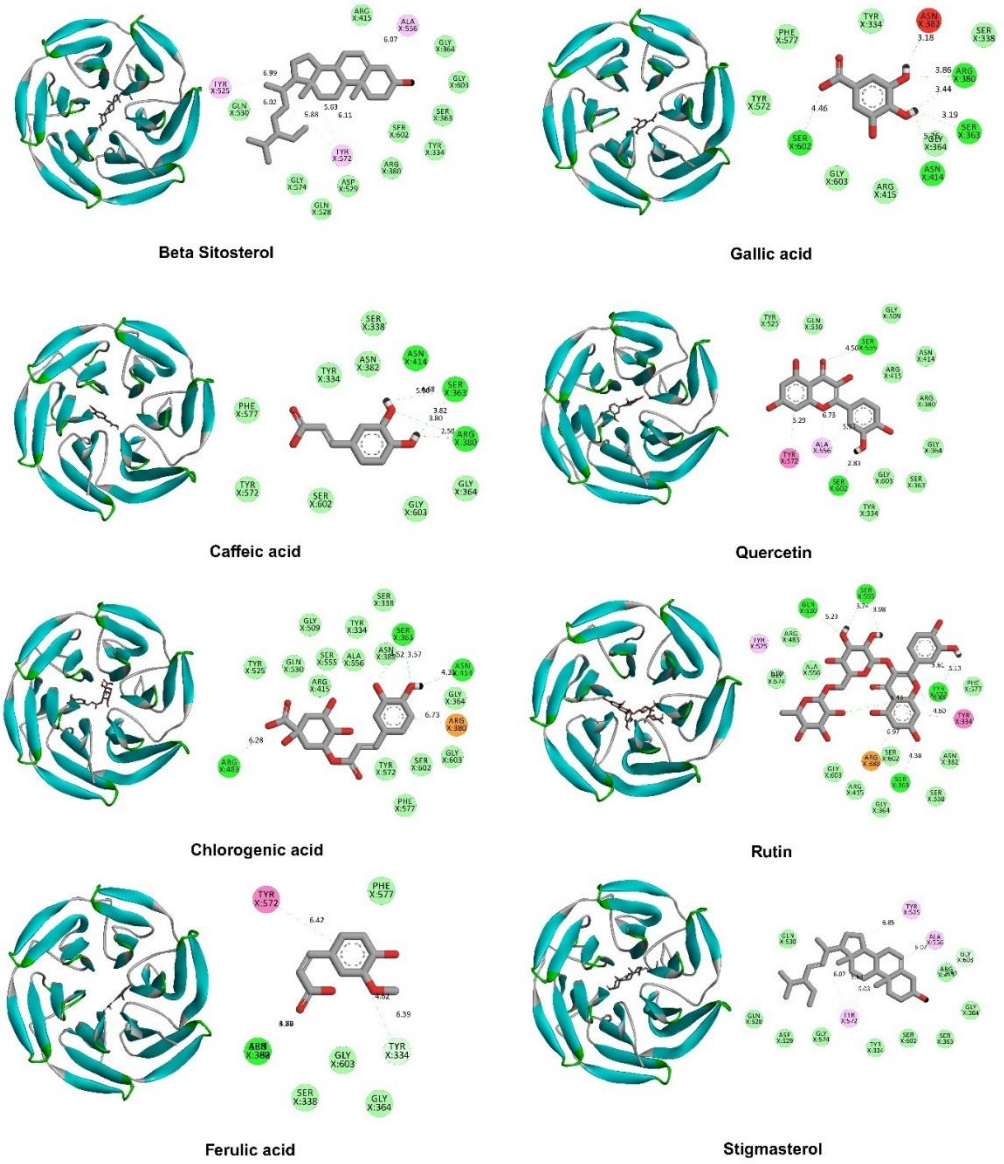

Figure 4 Visualization of 2D interactions between beta-sitosterol, gallic acid, caffeic acid, quercetin, chlorogenic acid, rutin, ferulic acid, and stigmasterol against KEAP1.

Molecular dynamics analysis

The RMSD analysis was used to evaluate the stability of the receptor-ligand complexes during a 20 ns molecular dynamics simulation. Caffeic acid, ferulic acid, and quercetin showed the greatest stability, with RMSDAll values below 3 Å, as shown in **Figure 5**, and RMSD ligand movement, as shown in **Figure 6**. This indicates that when the compound interacts with the target protein, the interaction is stable. These findings were consistent and confirmed by the RMSD value of ligand mobility, which measures ligand movement during simulation. The caffeic acid compound showed prominent fluctuations in the period of 0 - 5 ns at the beginning of the simulation, but after that, the ligand moved again to a stable state until the simulation was completed. The same phenomenon was observed for the ferulic acid compound, which showed fluctuations in the period of 0 - 5 ns at the beginning of the simulation; however, after that, the ligand moved again to a stable state until the simulation was completed. For the quercetin compound, the results of the ligand movement analysis showed several fluctuations during the simulation, especially in the time span of 5 - 15 ns, which showed that ligand movement fluctuated most often (**Figure 6**). RMSD analysis helps track the extent to which a simulated system's structural changes are

compared with its original or experimental form. By observing RMSD over time, we can evaluate the stability of the system and pinpoint the moments of balance or fluctuation. Higher RMSD values suggest that the structure deviated more significantly from the reference, which may indicate greater flexibility or notable conformational shifts [24].

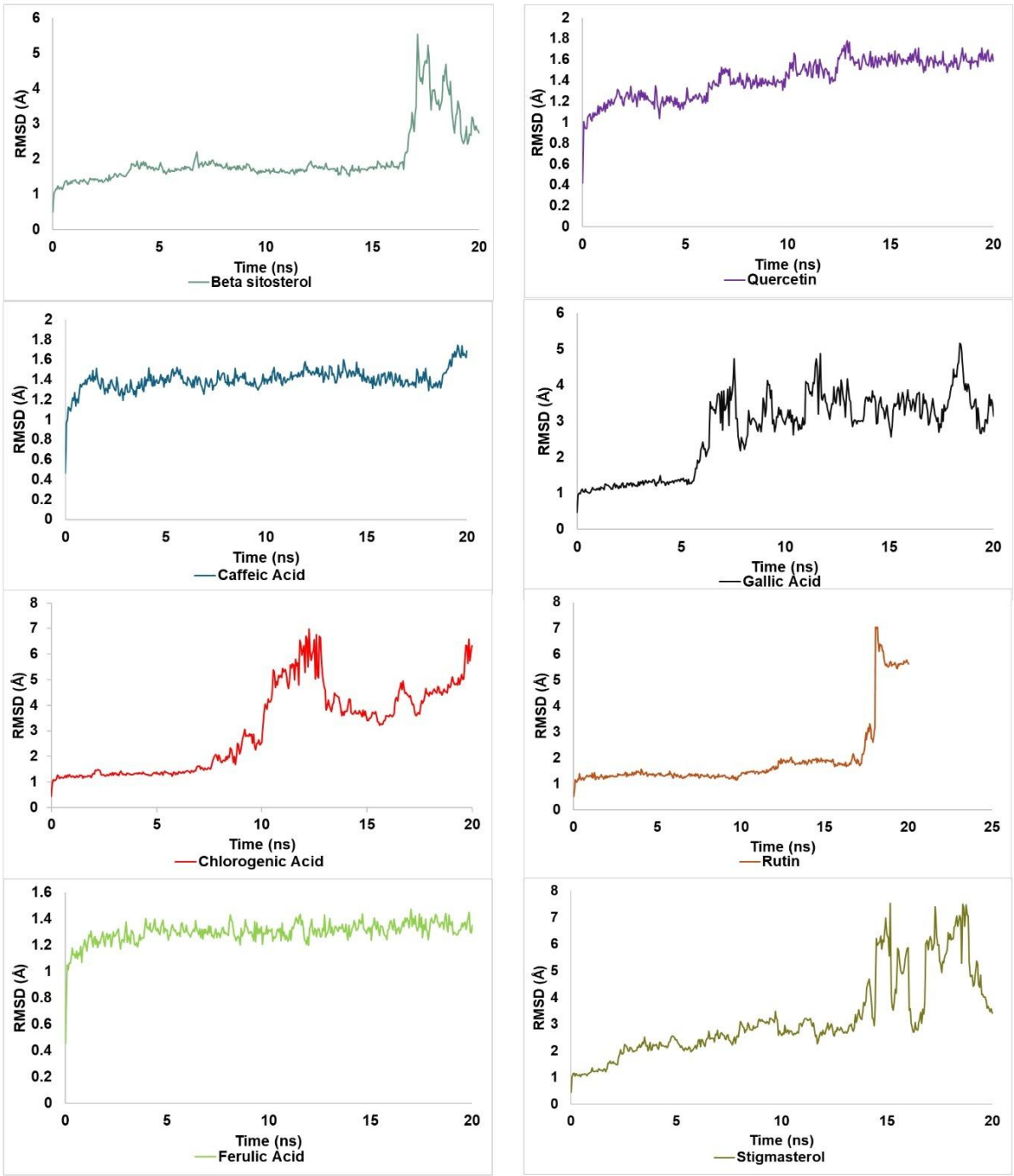

Figure 5 RMSDAll from the methanol extract of *Salacca zalacca* skin compounds.

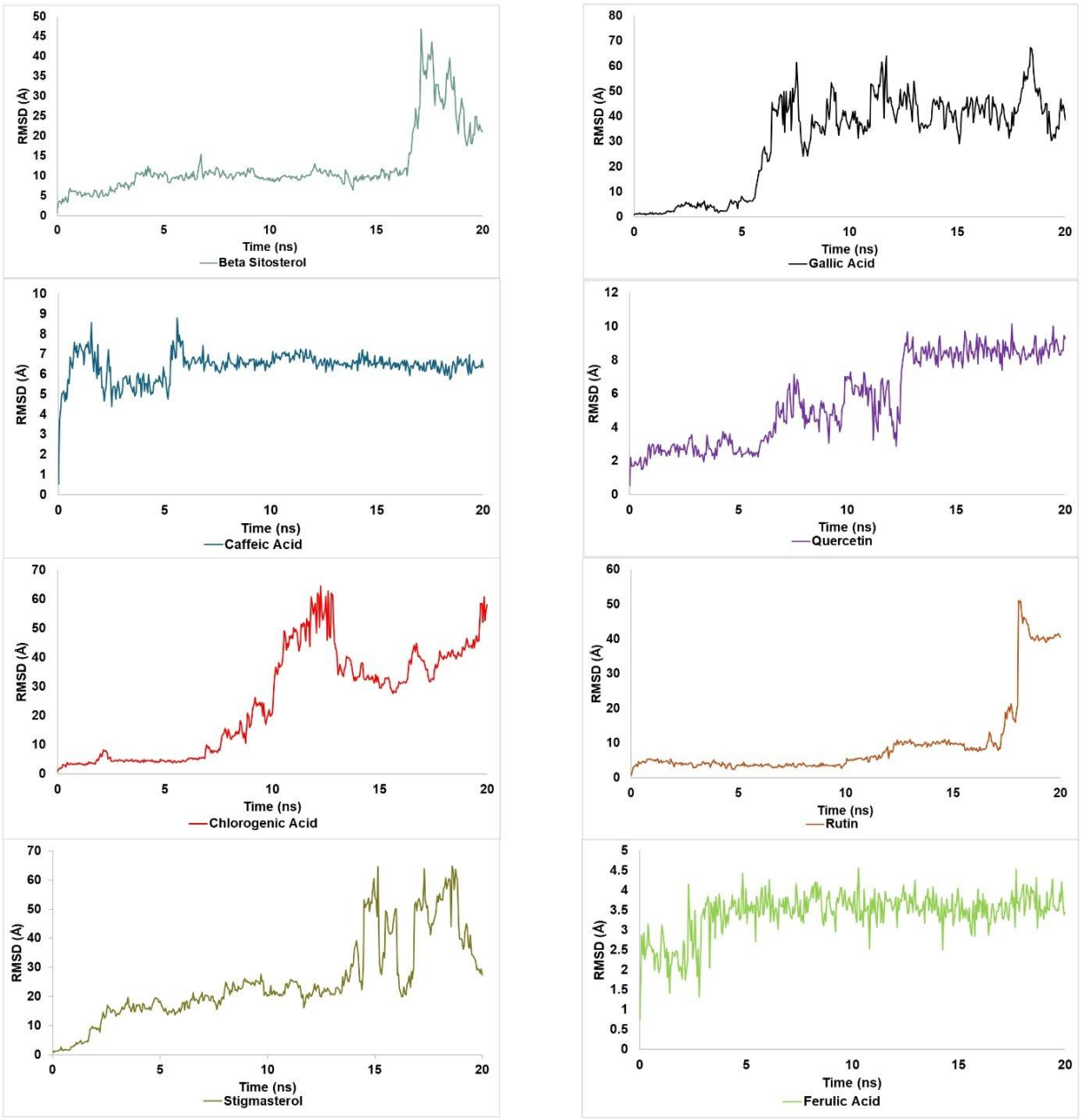

Figure 6 RMSDLigMove the methanol extract of *Salacca zalacca* skin compounds.

In addition to RMSD, RMSF analysis was used to determine the stability of all amino acid residues present on the interacting ligand. RMSF is a way to measure the extent to which individual atoms or residues in a biomolecule move or shift during an MD simulation. This helps to identify parts of the molecule that are highly flexible or experience significant changes in their shape over time. The atoms with low RMSF values were stable and did not fluctuate significantly, whereas those with high RMSF values were more flexible and dynamic. This insight can reveal which areas of the molecule are rigid and more adaptable [24]. From all the compounds analyzed, it was generally seen that all compounds showed stable RMSF values with an average value below 3 Å, except for chlorogenic acid and gallic acid, which appeared to have quite high fluctuations in several amino acid residues. When viewed on the amino acid residues considered as the active site of the KEAP1-NRF2 protein, the finding of the MD analysis showed that all active site amino acid residues had low fluctuation values (below 3 Å), which explains why the ligand compound was able to form strong and stable interactions (**Figure 7**).

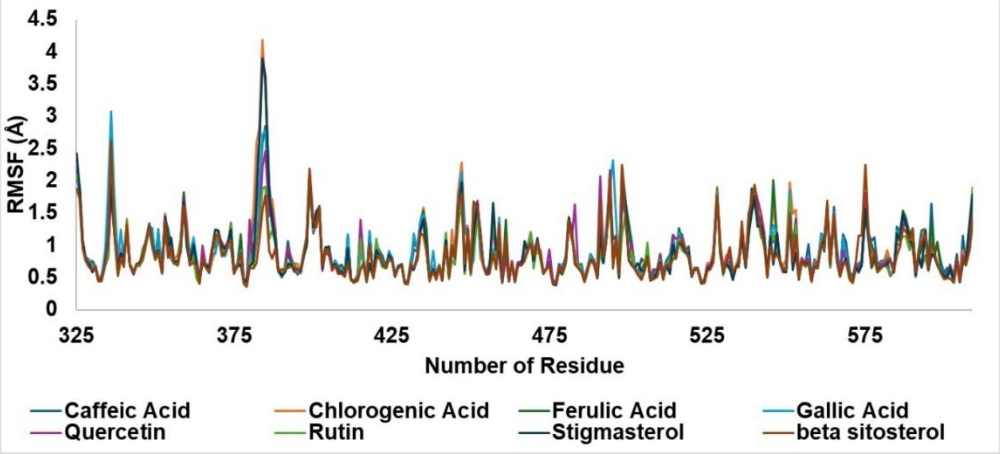

Figure 7 RMSF the methanol extract of *Salacca zalacca* skin compounds compounds.

Antioxidant capacity of the methanol extract of SZ skin

The radical scavenging capacity of the methanol extract of SZ skin was evaluated using the ABTS inhibition assay. The extract demonstrated greater effectiveness than the control (Trolox) with EC_{50} values of 87.83 µg/mL for the extract and 325.1 µg/mL for Trolox, indicating stronger radical scavenging activity and greater effectiveness than Trolox. The ABTS radical scavenging activity of the methanol extract of SZ skin increased in a concentration-dependent manner (**Figure 8(A)** and **Table S4**), showing that higher extract concentrations were associated with greater ABTS radical scavenging capacity.

The ABTS assay is versatile because ABTS radicals are soluble in water and organic solvents, allowing evaluation of the antioxidant capacity of both lipophilic and hydrophilic molecules. Homogeneous solutions of lipophilic substances, such as carotenoids and tocopherols, were used to assess the protective effects on lipid. The ABTS assay is also cost-effective and simple to perform. Previous studies reported that ethanol extracts from SZ fruit, skin, and seeds exhibit antioxidant activity in both DPPH and ABTS assays. The antioxidant potential of the SZ seed extract is likely attributed to its hydrogen-donating ability, derived from its phenolic constituents [25,26]. For comparison, other tropical fruits also exhibit significant antioxidant activity, such as avocado skin extract, which exhibited high antioxidant activity by the *in vitro* tests of ABTS (1,004.5 ± 52.0 µmol/g) [27]. Among banana cultivars, DPPH radical scavenging activity was highest in ripe Ducasse pulp (1.68 mg AAE/g) and ripe Ladyfinger peel (2.85 mg AAE/g), whereas ripe Plantain pulp (0.069 mg AAE/g), ripe Red Dacca pulp (0.36 mg AAE/g), and ripe Monkey peel (0.59 mg AAE/g) showed the lowest activity [28].

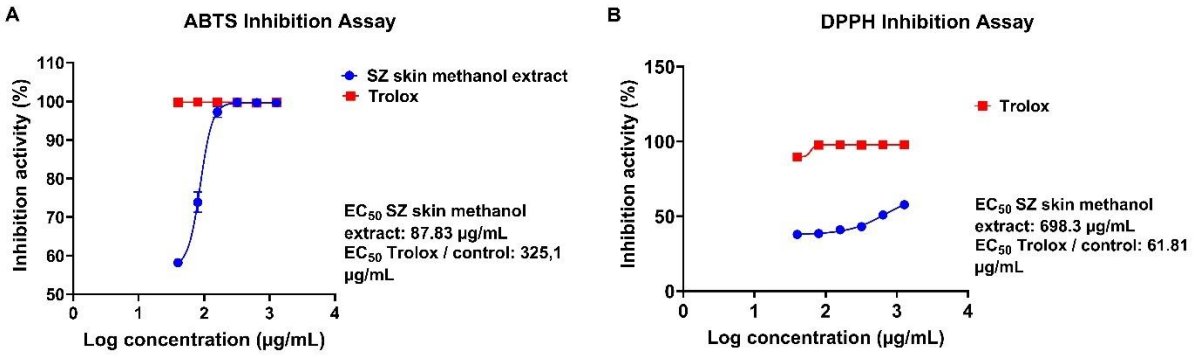

Figure 8 *In vitro* assays for evaluating the antioxidant activity of the methanol extract of SZ skin. (A) ABTS radical inhibition assay of the methanol extract of SZ skin. (B) DPPH radical inhibition assay of the methanol extract of SZ skin.

In contrast to the ABTS assay, the DPPH free radical assay revealed that the methanol extract of SZ skin was less effective, with EC_{50} values of 496.0 µg/mL for the extract and 61.18 µg/mL for Trolox (**Figure 8(B)** and **Table S4**). Although the methanol extract of SZ skin exhibited significant dose-dependent antioxidant activity, its free radical-scavenging capacity was lower than that of Trolox. This result aligns with the characteristics of natural antioxidants, whose free radical-scavenging ability generally increases with the concentration. comparative studies have shown variability in antioxidant potential among natural products, particularly comparative analyses of DPPH activity between snake fruit and mangosteen, indicating that snake fruit exhibits significantly higher antioxidant activity, with a DPPH activity (EC_{50} = 110.4 µg/mL) than mangosteen [29]. Similarly, among tropical fruits, antioxidant activity has been reported in the order guava > mango > papaya > lemon, with guava exhibiting the highest activity [30]. In the DPPH method, the antioxidant capacity of Tainong 1 (2,930 ± 18 µM) was stronger, and Guifei (461 + 22 µM) was weaker than that of the other genotypes of mango fruit [31].

Limitations

This study has several limitations in establishing the efficacy of the methanol extract of SZ skin as a treatment for oxidative stress-related diseases. The study was limited to computational analyses and *in vitro* antioxidant assays (ABTS and DPPH), necessitating further validation through cell-based assays and *in vivo* models. In addition, comprehensive toxicity evaluations are required to establish the safety profile of the methanol extract of SZ skin. Future research should focus on both the mechanism and translational studies to support the potential use of the methanol extract of SZ as a therapeutic agent for oxidative stress-related diseases in Indonesia.

Conclusions

In conclusion, targeted LC-MS analysis identified 8 bioactive compounds of the methanol extract of SZ skin, such as caffeic acid, chlorogenic acid, rutin, stigmasterol, gallic acid, ferulic acid, quercetin, and β-sitosterol. The results of *in silico* prediction and *in vitro* assays (DPPH and ABTS) revealed that the methanol extract of SZ skin possesses notable free radical-scavenging capacity. These findings indicate that the methanol extract of SZ skin has promising antioxidant potential and can be employed as a valuable Indonesian natural product with potential therapeutic candidate for oxidative stress-related diseases.

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Declaration of generative AI in scientific writing

No use of generative AI in scientific writing.

CRediT author statement

Sri Utami: Conceptualization; Methodology; Validation; Original draft preparation; **Yuyun Yueniwati**: Resources; Data curation; Writing - Original draft preparation; Funding acquisition. **Mokhamad Fahmi Rizki Syaban**: Visualization; software; Investigation. **Diana Yuswanti Putri**: Data curation; investigation, data analysis. **Nirmala Halid**: Software; Validation. **Happy Kurnia Permatasari**: Resources; Writing - Reviewing and Editing. **Sharida Fakurazi**: software; Writing - Reviewing and Editing. **Eko Arisetijono Marhaendraputro**: Data curation; investigation; Validation.

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