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Original Article

Analysis of Bioactive Compounds Using LC-HRMS and Antioxidant Potential Test of *Rhizophora mucronata* Leaves: *in vitro*, Bioinformatic, and Molecular Docking Studies

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Abstract

Increased concentration of free radical exposure in the body can cause various serious health problems, such as cardiovascular disease, cancer, tumors, and so on. Free radicals at low concentrations have a physiological role in various cellular activities in the body. Therefore, the condition of free radical concentration needs to be controlled with antioxidants. Antioxidants have an important role in maintaining and protecting the body from free radical exposure. Antioxidants are divided into two: exogenous and endogenous. Endogenous is obtained from the results of enzymatic reactions in the body, while exogenous is obtained from outside the body through food sources (synthetic and natural). Exogenous antioxidants from synthetic sources such as BHT, BHA, and others are considered unsafe, and long-term use is very dangerous for the body. Therefore, natural resources are a safe choice to use. One plant that can be explored for its potential antioxidant activity is *Rhizophoramucronata* (*R. mucronata*) leaves. The results of the water extract investigation contain phenolic and flavonoid groups and provide antioxidant activity in the moderate category. The results of the LC-HRMS analysis showed that the compound methyl α-D-mannoside had the highest content of 23.20%. A bioinformatics study using six of the highest concentrations of bioactive compounds targets the TP-binding cassette (ABC) protein receptor subfamily C member 9 (ABCC9). Regulation of this protein's expression plays a crucial role in the development and prevention of various diseases. In vitro antioxidant activity testing and bioinformatics studies revealed that this plant leaf component has potential for further investigation.

Keywords: *Antioxidant activity; bioinformatics; free radicals: LC-HRMS; Rhizophora mucronata.*

Introduction

Free radicals are molecules or atoms that contain unpaired electrons in their orbitals. Free radicals in low concentrations have beneficial effects and are involved in various physiological processes such as mitogenic responses, redox regulation, cellular signaling pathways, and immune function. While high concentrations will cause uncontrolled oxidative reactions and cause various health problems in the body (Chaudhary *et al.*, 2023; Sinaga *et al.*, 2023). Radical species are generally in the form of reactive oxygen species (ROS) and reactive nitrogen species (RNS). Uncontrolled oxidative reactions in the body cause various health problems such as cancer, tumors, dermatitis, cataracts, stroke, asthma, and various degenerative diseases (Gurning & Haryadi, 2022; Tumilaar *et al.*, 2024).

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Sources of increased free radicals can occur in the body both endogenously and exogenously. Endogenous sources are produced from mitochondrial respiration and immune cell activation, while exogenous sources are through exposure to radiation, pollution, and smoking (Chandimali *et al.*, 2025).

The effect of increasing free radicals in the body can be minimized, and its balance maintained, by consuming functional foods rich in antioxidants. Antioxidants can be divided into two, namely synthetic and natural ingredients: synthetic sources such as butylhydroxytoluene (BHT), butylhydroxyanisole (BHA), tert-butyl hydroxyquinone (TBHQ), and propyl gallate, and natural antioxidant sources such as vitamin C, vitamin E, and secondary metabolite compounds such as flavonoids, phenolics, and polyphenols. Long-term use of synthetic antioxidants causes toxic and carcinogenic effects, so antioxidants from natural sources are considered safer and are abundant in nature (Viana da Silva *et al.*, 2022). One of the plants whose antioxidant potential needs to be studied is mangrove *Rhizophora mucronata* (*R. mucronata*) leaf extract.

R. mucronata (Figure 1) is a plant that grows abundantly in tropical and subtropical regions, rich in secondary metabolites such as terpenoids, tannins, steroids, flavonoids, glucosides, glycosides, and alkaloids. These metabolite compounds exhibit pharmacological effects such as antiviral, antibacterial, antifungal, antioxidant, and anticancer activities (Sivaperumal *et al.*, 2023; Umashankari *et al.*, 2012). Increasing the application of various secondary metabolites from plants is often done through biosynthesis by producing nanoparticles. The biosynthesis reaction that produces many nanoparticles is silver nanoparticles (AgNPs). AgNPs have many advantages and are widely applied in bionanotechnology, medicine, the food industry, agriculture, and so on (Abdi *et al.*, 2019). Therefore, this study aims to analyze the secondary metabolite groups from *R. mucronata* leaf extract using liquid chromatography-high resolution mass spectrometry (LC-HRMS) and to test the potential antioxidant activity *in vitro*, as well as to conduct molecular docking studies on the main target proteins from the bioinformatics approach.

Figure 1: *Rhizophora mucronata* (*R. mucronata*); (a trees, and b leaves).

Materials and Methods

Materials

Ascorbic acid (Merck), gallic acid (Merck), quercetin (Merck), methanol (p.a), acubides, potassium persulfate (Merck), 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS; Merck), 2,2-diphenyl-1-picryl-hydrazyl (DPPH; Merck), alkaloid reagents (dragendrof, mayer, and wagner), $FeCl_3$ (Merck), $AlCl_3$ (Merck), Mg (Merck), and HCl (Merck).

Sample preparation and extraction

The *R. mucronata* leaf samples used were in good and fresh condition, taken from the coastal forest of Senggarang in Tanjungpinang, Riau Islands. The coordinates of the sampling location were 0°5' – 0°59' N and 104°23' – 104°34' E. The samples obtained were cleaned under running water, dried, and after drying were pollinated using a magnetic blender to expand the contact surface of the sample to the solvent to be used. The samples used were also subjected to taxonomic tests at the Laboratory of Biological Plant Systematics, Faculty of Biology, Universitas Gadjah Mada (No. 0417/S.Tb./VIII/2023).

The powdered samples were macerated using aquabidest solvent by heating at 60°C for 30 minutes and filtered using Whatman filter paper no. 1. The liquid extract obtained was concentrated using a press dryer to obtain a thick-dry extract. The concentrated extract obtained was placed in a glass container and closed, then placed in a sample storage cabinet before further use.

Screening and determination of phytochemical groups

Phytochemical screening

R. mucronata leaf extract was dissolved sufficiently with distilled water and continued with screening of phytochemical compound groups using standard methods referring to previous studies (Gurning *et al.*, 2020; Syahrina *et al.*, 2020). The compound components analyzed included phenolics and polyphenol groups, flavonoids, alkaloids, saponins, and tannins.

Determination of total phytochemical content

Determination of total phytochemical content was carried out using a colorimetric method using a UV-Vis spectrometer. The total phytochemical content determined included the phenolic group using the Folin-Ciocalteu reagent with gallic acid as a standard (Lubis *et al.*, 2023), the flavonoid group using 10% $AlCl_3$ reagent with quercetin as a standard, and the determination of the tannin group using the Folin-Ciocalteu reagent and saturated Na_2CO_3 with tannic acid as a standard (Gurning & Sastrohamidjojo,, *et al.*, 2021). The content of the determined phytochemical groups was expressed in mg equivalents of standard compounds per gram of dry sample weight used (mg ES/g d.w extract).

Analysis of R. mucronata leaf extract using LC-HRMS

Analysis of bioactive compounds from *R. mucronata* leaves extract was carried out by following the steps of previous studies (Anggraeni *et al.*, 2025; Gurning *et al.*, 2024). A brief description of the procedure for analyzing bioactive compounds of samples using the LC-HRMS method: 50 mg of dry extract of *R. mucronata* leaves were placed in a 2 mL centrifuge tube plus 1 mL of methanol (LC-MS grade) and then vortexed for 1 minute. The mixture was centrifuged at 5000 rpm for 10 minutes. Analysis using liquid chromatography and high-resolution orbitrap spectrometry with a Thermo Scientific™ Accucore™ C-18 100 mm analytical column, 2.1 mm ID, 2.6 m. The mobile phase used a gradient technique consisting of water (A) and methanol (B) with 0.1% formic acid at a flow rate of 0.3 mL/minute. The initial mobile phase (A) was 95% and gradually reduced to 5% over a period of 16 minutes. In condition A 5%, and B 95% were maintained for 4 minutes. Positive ionization polarity and full MS/dd-MS2 collection mode were used for targetless screening.

In vitro Antioxidant Activity Testing

Determination by DPPH method

Testing the potential antioxidant activity of *R. mucronata* leaves extract using concentration ranges of 50 to 250 ppm, while the positive control compounds used ascorbic acid (AA), gallic acid (GA), and quercetin (Q) with concentration ranges of 5 to 25 ppm. Each extract and positive control compound was dissolved using methanol solvent. The procedure for testing antioxidant activity followed the previous research method with slight modifications (Gurning, Haryadi, *et al.*, 2021). 500 µL of DPPH• 0.4 mM was put into a 5 mL volumetric flask, 1 mL of each sample was added, methanol was added to the limit mark, and it was incubated for 30 minutes at room temperature in the dark (avoid light). After the incubation time was reached, each mixture was measured at a wavelength of 516 nm using a UV-Vis spectrometer. The same steps were repeated three times.

Determination using the ABTS method

Antioxidant activity testing of extracts and positive controls (AA, GA, and Q) using the ABTS method followed previous research with slight modifications (Chaves *et al.*, 2020). ABTS•+ radicals were produced by mixing 5 mL of 7 mM ABTS and 5 mL of 4.95 mM potassium persulfate, then stored in a dark place and left for 16 hours at room temperature. Then, the mixture was diluted with methanol to reach 25 mL. The concentration of *R. mucronata* leaf extract used ranges of 50 to 250 ppm, while the

concentration ranges used were 5 to 25 ppm. 1 mL of each sample was added with 1 mL of ABTS•+ dilution and made up with methanol to 5 mL. Absorbance was measured at 734 nm using a UV-Vis spectrophotometer. Blanks were made with 1 mL of ABTS•+ with 4 mL of methanol. All measurements were performed with 3 repetitions.

Bioinformatics and molecular docking studies of antioxidant receptors

Bioinformatics and molecular docking studies identified and investigated various selected bioactive compounds from *R. mucronata* leaves extract as antioxidant agents. The molecular structures of the bioactive compounds were optimized using the density functional theory (DFT) method with the B3LYP hybrid functional and 6–31 G basis sets (Kurniawan *et al.*, 2025). The SMILES codes of the bioactive compounds were uploaded to the Swiss Target Prediction database (https://www.swisstargetprediction.ch). Protein receptors involved as antioxidants were obtained from the GeneCards database and the National Center for Biotechnology Information (NCBI) with the keyword "antioxidant." The main target proteins were filtered from the intercept results of bioactive compound receptors with antioxidant receptors using a Venn diagram (https://bioinfogp.cnb.csic.es/tools/venny/). Protein-protein interactions were constructed using the STRING database (https://string-db.org/) and further analyzed with Cytoscape 3.10.2 software to obtain the main proteins that act as target receptors. After identifying the main target protein as a receptor, it was downloaded from the Protein Data Bank (https://www.rcsb.org/) and prepared using Chimera 1.13.1 before conducting molecular docking studies. Molecular docking was declared valid if the root means square deviation (RMSD) was less than 2 Å (Gurning *et al.*, 2025). Docking was then carried out by replacing the original ligand with the selected bioactive compound with a grid box setting of 50 *x* 50 *x* 50 Å (Gurning *et al.*, 2025). The complex compounds formed were analyzed, and their interactions were visualized using the Biovia Discovery study. ADMET predictions of each selected bioactive compound were based on the pkCSM database (https://biosig.lab.uq.edu.au/pkcsm/) and ProTox 3.0 (https://tox.charite.de/protox3/).

Statistical analysis

Data for determining the total content of phytochemical groups and antioxidant activity are expressed as mean ± standard deviation (mean ± SD). Statistical analysis was performed on antioxidant activity data using GraphPad Prism 10.0.1 software; significant differences in the average antioxidant activity between sample extracts and standard reference compounds using analysis of variance (ANOVA) were considered statistically significant with a significance level of $p < 0.05$.

Result

Preparation, screening, and determination of phytochemical groups

The sample used is based on the results of taxonomic tests at the Biological Plant Systematics Laboratory, Faculty of Biology, Universitas Gadjah Mada, with the genus *Rhizophora* and species named *Rhizophora mucronata* Lamk. (*R. mucronata*). Samples were obtained from the Senggarang coastal forest in Tanjungpinang, Riau Islands. The results of phytochemical screening using various standard reagents showed positive content of flavonoids, phenolics, triterpenoids, and tannins. These qualitative results are also supported by quantitative data from the total phenolic content obtained at 1.24 ± 0.17 mg GAE/g d.w. extract and the total flavonoid content of 1.09 ± 0.03 mg QE/g d.w. extract.

Analysis of water extract of R. mucronata leaves using LC-HRMS

Liquid chromatography-high resolution mass spectrometry (LC-HRMS) is a cutting-edge method for analyzing various bioactive compounds from natural materials. The results of the analysis of the compound content of water extracts from *R. mucronata* leaves are shown in Figure 2.

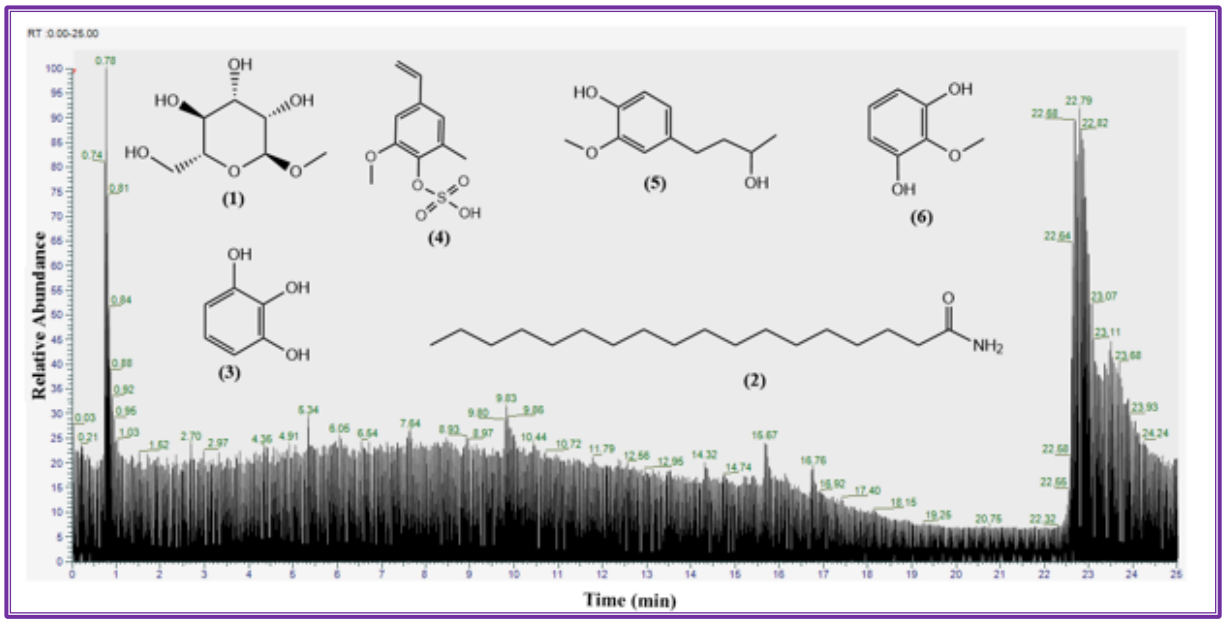

Figure 2: LC-HRMS chromatogram of water extract of *R. mucronata* leaves with six main compounds in the extract

The results of the analysis of bioactive compounds using LC-HRMS obtained 6 compounds with the highest peak areas, namely (1) methyl α-D-mannoside (23.20%), (2) stearamide (8.77%), (3) pyrogallol (4.99%), (4) 2-hydroxy-6-methoxy-4-vinylphenyl hydrogen sulfate (4.24%), (5) zingerol (2.92%), and (6) 2-methoxyresorcinol (1.34%), and a more complete description is shown in Table 1. The bioactive compounds identified for the stearamide compound are included in the alkaloid group; the pyrogallol, zingerol, 2-hydroxy-6-methoxy-4-vinylphenyl hydrogen sulfate, and 2-methoxyresorcinol compounds are included in the phenolic group; and the methyl α-D-mannoside compound is included in the glycoside group. The results of qualitative analysis using standard reagents and quantitative analysis using UV-Vis spectrometer and LC-HRMS instruments showed a mutually supportive correlation in the identification and analysis of bioactive compounds contained in the water extract of *R. mucronata* leaves.

Table 1: *LC-HRMS of R. mucronata leaves extract*

Kode	R_T (minute)	Compound names	Formula	Molecular weight (g/mol)		Annot. Δ Mass (ppm)	Area max (a.u.)	area%
				Calc.	Theory			
1.	0.78	Methyl α-D-mannoside	$C_7H_{14}O_6$	194.08	194.18	-1.64	2331604007.05	23.20
2.	15.67	Stearamide	$C_{18}H_{37}NO$	283.28	283.50	-1.49	881071025.85	8.77
3.	0.81	Pyrogallol	$C_6H_6O_3$	126.03	126.11	0.33	502348523.18	4.99
4.	4.35	2-Hydroxy-6-methoxy-4-vinylphenyl hydrogen sulfate	$C_9H_{10}O_6S$	246.02	246.23	-1.26	426164247.95	4.24
5	6.05	Zingerol	$C_{11}H_{16}O_3$	196.11	196.24	-0.36	293378290.81	2.92
6	0.79	2-methoxyresorcinol	$C_7H_8O_3$	140.05	140.14	-1.27	135001423.47	1.34

Analysis of antioxidant activity of water extract of R. mucronata leaves

The antioxidant activity of the samples was compared with standard compounds, namely ascorbic acid, quercetin, and gallic acid, which showed very strong natural antioxidant activity. The results of the antioxidant activity test are shown in Figure 3.

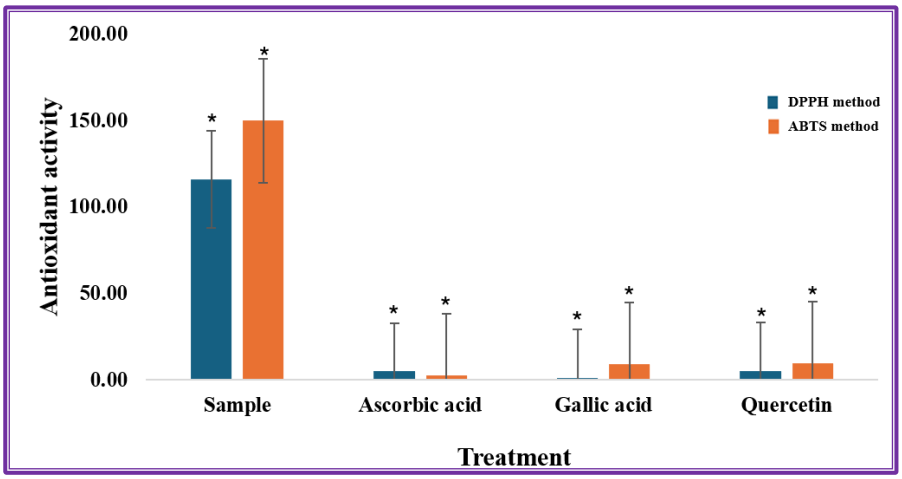

Figure 3: *Testing of antioxidant activity of water extract of R. mucronata leaves compared with standard*

The test results showed that the antioxidant activity of *R. mucronata* leaves extract was in the moderate category with both methods used, namely activity (IC_{50}) of 115.80 ± 0.01 ppm (DPPH) and 149.82 ± 0.06 ppm (ABTS), while for the standard compounds of ascorbic acid, quercetin, and gallic acid, respectively, it was 4.71 ± 0.01; 4.90 ± 0.01; 0.90 ± 0.01 ppm with the DPPH method and 2.48 ± 0.01; 9.33 ± 0.01; 9.03 ± 0.01 ppm with the ABTS method. Based on both methods of testing antioxidant activity, the water extract of *R. mucronata* leaves showed a moderate category and was still weaker than the activity of the three standard compounds, which showed a very strong category as antioxidants.

Bioinformatic approaches and molecular docking of antioxidant receptors

Determination of protein receptors that are the main targets of various compounds was predicted by their mode of action through bioinformatics. The six bioactive compounds collected protein receptor data through the Swiss Target Prediction database by uploading the SMILES code of each compound. The total protein receptors of all bioactive compounds were 241 targets, the predicted antioxidant protein receptors were 5055 targets, and the total intersecting protein receptors were 111 potential targets. The intersecting protein receptors were constructed as protein-protein interactions (PPI) using STRING data with a confidence level setting (p = 0.04). The statistical values of the STING data obtained included the number of nodes (111), the number of edges (299), the average node degree (5.39), the average local clustering coefficient (0.517), the expected number of edges (94), and the PPI enrichment p-value $<1.0e^{-16}$. The STING data obtained were further analyzed using Cytoscape 2.10.2 software (Figure 4).

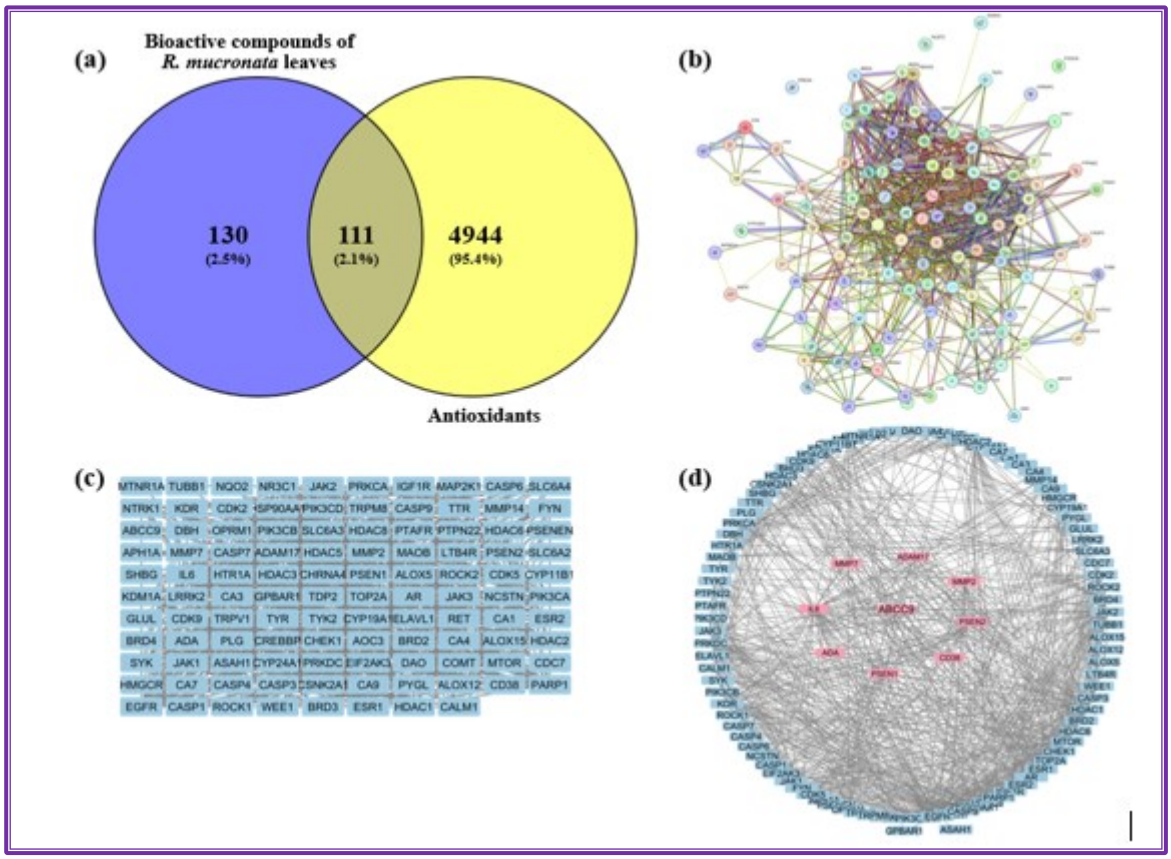

Figure 4: *Determination of major receptor proteins based on bioinformatics. (a) Venn diagram; (b) PPI data using STRING; (c) PPI using Cytoscape 2.10.2; and (d) Top 10 receptors and major receptor proteins in the middle*.

The primary target protein obtained was then subjected to molecular docking studies of bioactive compounds against the target protein receptor (ABCC9). Each bioactive compound and standard compound was optimized and ADMET predicted (Table 2). Simultaneously, the ABCC9 protein was downloaded (PDB ID: 7VLR) and prepared, then the redocking process was continued as a basis for the molecular docking study.

Table 2: *Optimization, drug-likeness, and ADMET prediction of bioactive compounds of R. mucronata leaves.*

Parameters	Compounds								
	(1)	(2)	(3)	(4)	(5)	(6)	AA	Q	GA
Molecular optimization									
Energy (kcal/mol)	-772.46	-829.62	-455.33	-1041.98	-650.81	-494.40	-680.91	-1097.92	-642.84
Dipole moment (D)	3.58	3.42	3.70	4.12	2.13	3.76	5.11	4.00	3.75
Physicochemical Properties									
Molecular weight (g/mol)	194.18	283.50	126.11	244.27	196.25	140.14	176.12	302.24	170.12
H-bond acceptors	6	1	3	4	3	3	6	7	4
H-bond donors	4	1	3	1	2	2	4	5	4
Molar Refractivity (cm^3/mol)	40.47	91.55	32.51	60.21	55.51	36.98	35.12	70.03	39.47
TPSA ($Å^2$)	75.33	43.09	60.69	81.21	49.69	49.69	107.22	111.13	97.99
Absorption									
Solubility (log mol/L) in water	0.23	-6.30	-0.10	-2.26	-1.52	-1.45	0.12	-2.93	-2.56
Caco-2 permeability (cm/s)	-0.26	1.57	1.17	0.90	1.25	1.16	-0.36	-0.23	-0.08
HIA (%)	53.16	92.03	65.75	95.43	94.49	90.48	59.45	77.21	43.37

Parameters	Compounds								
	(1)	(2)	(3)	(4)	(5)	(6)	AA	Q	GA
Distribution									
VDss for human	-0.49	-	-0.41	-1.20	-0.18	0.13	-0.54	1.56	-1.86
CNS permeability	-9.99	-	-2.45	-2.67	-2.53	-2.45	-0.38	-3.07	-3.74
log BBB permeability	-0.84	-	-0.34	-0.57	0.07	-0.31	-1.05	-1.10	-1.10
Metabolism									
CYP2E1	No	No	No	No	No	No	No	No	No
CYP3A4	No	No	No	No	No	No	No	No	No
CYP2C19	No	No	No	No	No	No	No	No	No
CYP2D6	No	No	No	No	No	No	No	No	No
CYP1A2	No	Yes	No	Yes	Yes	No	No	Yes	No
Excretion									
Total clearance (mL/min/kg)	0.66	-	0.13	0.25	0.26	0.17	0.60	0.41	0.52
Renal OCT2 substrate	No	-	No	No	No	No	No	No	No
Toxicity									
Hepatotoxicity	No	-	No	No	No	No	No	No	No
LD_{50} (mol/kg)	23000	1000	300	2500	2000	550	3367	159	2000
Class	6	4	3	5	4	4	5	3	4

Based on the study of molecular docking of bioactive compounds and reference compounds along with native ligands, it shows that all compounds—bioactive compounds, reference compounds, and native ligands—show negative binding affinity, indicating that the complex bond formed between each compound and the main target protein has good stability (Table 3). The molecular docking process was declared valid with a re-docking RMSD value of 1.1 Å. The best re-docking results and complex molecular interactions in molecular docking are shown in Figure 5.

Table 3: *Molecular docking results of potential compounds against antioxidant protein receptors*

Compounds	Antioxidants (PDB ID 7Y1J)			
	Binding affinity (kcal/mol)	Interaction		
		H – bond	van der Waals – bond	Other-bond
(1)	-5.4	Asn1212; Arg1213	-	-
(2)	-5.7	Ser1209	-	Ile374; Tyr370; Trp423; Leu427; Leu584
(3)	-5.1	Asn430	Leu427	Tyr370; Arg304
(4)	-6.8	Arg1263; Arg1213; Asn1212	-	Leu427; Phe426; Prp423; Ile374; Arg1213
(5)	-6.4	Arg1263; Asn430	Leu427	Trp1260; Tyr370; Asn430
(6)	-5.0	-	Leu427	Leu427; Tyr370
AA	-5.5	Arg363; Gln362; Gln437; Asp308	-	-
Q	-8.6	Gly1345	-	Ser1350; Tyr938; Tyr1317;
GA	-6.1	Glu1216; Arg1213; Asn430	-	Tyr370; Asn430
Native (CID: 65981)	-8.2	Lys527	Trp1055	Leu531; Leu10051; Ala530; Lys527; Glu1054; Leu523; Ala530; Leu1058

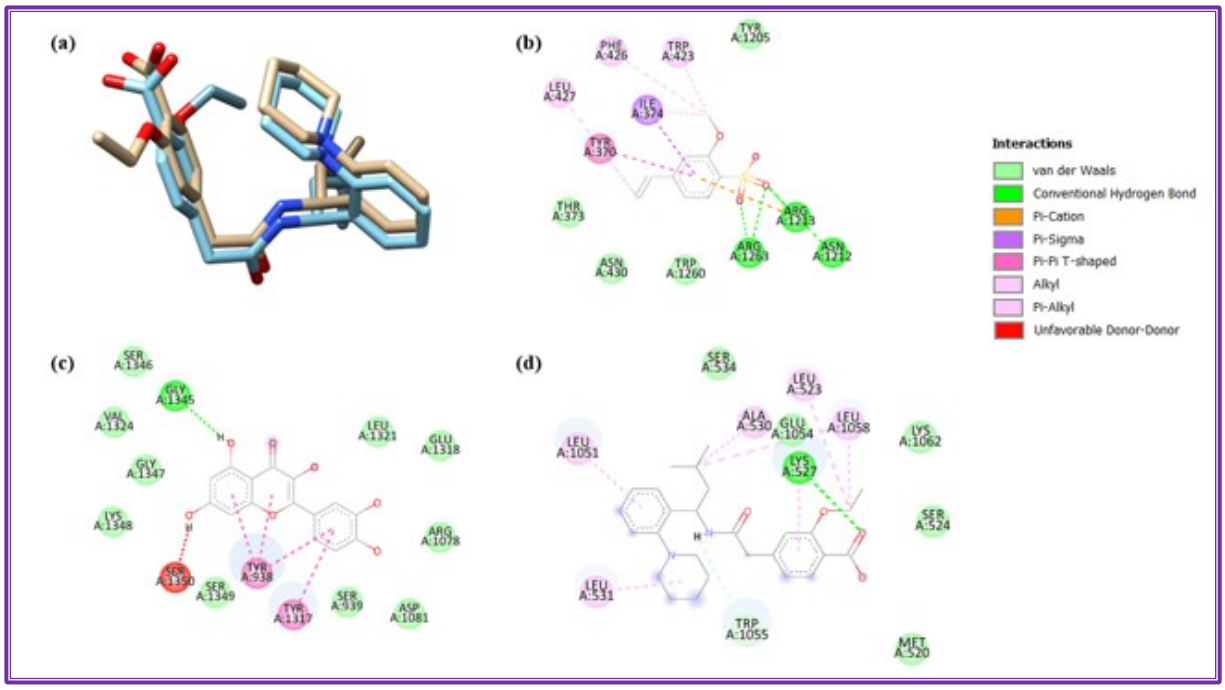

Figure 5:*(a) Superimposition of redocking, which is the basis of arrangement in molecular docking; (b) Interaction of potential bioactive compounds; (c) Interaction of reference compounds; and (d) Interaction of native ligand with the main target protein.*

Discussion

The water extract of *R. mucronata* leaves exhibited a variety of bioactive compounds and moderate antioxidant activity. Extraction of bioactive compounds from medicinal plants using water is a highly sustainable, safe, and cost-effective method (green extraction) and minimizes the use of hazardous organic solvents. Water acts as a powerful solvent for extracting polar bioactive compounds (phenolics and flavonoids) that confer antioxidant activity, offering high selectivity, low cost, and no toxic residues for applications in the pharmaceutical and food industries (Abubakar & Haque, 2020; Ncama *et al.*, 2025). According to the literature, phenolic and flavonoid compounds found in various plants exhibit antioxidants and other biological activities (Chelliah *et al.*, 2023; Vittaya *et al.*, 2022). The results of the analysis of bioactive compound content using the LC-HRMS instrument showed that the methyl α-D-mannoside compound is a glycoside group and has also been reported to exhibit antioxidant activity (V. Gupta *et al.*, 2025; Liu *et al.*, 2018). The compounds pyrogallol (A. Gupta *et al.*, 2021; Hamed *et al.*, 2024) , 2-hydroxy-6-methoxy-4-vinylphenyl hydrogen sulfate (Jiménez-Salcedo *et al.*, 2025), zingerol (Alharbi *et al.*, 2022; Chuljerm *et al.*, 2023), and 2-methoxyresorcinol (Ding *et al.*, 2025) are phenolic derivatives reported to exhibit antioxidant activity. Analysis of bioactive compounds with LC-HRMS instruments has advantages and is one of the latest and most sophisticated, offering higher sensitivity and efficiency than previous instruments (Aydoğan, 2020; Chelliah *et al.*, 2023).

The water extract of *R. mucronata* leaves was determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) method and the ABTS (2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) method. Determination of antioxidant activity by the DPPH method is based on the single electron of the nitrogen atom in the DPPH radical molecule (purple), which is reduced to hydrazine (purple-yellow color degradation) by the transfer of a hydrogen atom from the antioxidant compound, thus producing a stable compound (Gulcin & Alwasel, 2023). Antioxidant testing by the ABTS method is based on measuring the antioxidant capacity in neutralizing the stable ABTS •+ (radical cation), which is characterized by a blue-greenish color degradation measured at a maximum wavelength of 734 nm. The decrease in ABTS •+ intensity in the presence of strong antioxidants. The measured decrease in

absorbance is highly dependent on the intrinsic antioxidant activity, reaction duration, and sample concentration (Munteanu & Apetrei, 2021).

Further analysis results found the main protein receptor, namely TP-binding cassette (ABC) subfamily C member 9 (ABCC9). This protein is expressed in heart tissue, which functions as an important regulatory subunit of ATP-sensitive potassium channels. Regulation and suppression of ABCC9 expression are considered capable of inhibiting the development of cardiovascular disease (Peng *et al.*, 2026), and cardiovascular disease is a major cause of morbidity and mortality worldwide, including heart attacks, heart failure, stroke, arrhythmia, and so on. The emergence of these various diseases is inseparable from the free radical oxidation reaction of polyunsaturated fatty acids (PUFA) in lipoproteins or cell membranes, called lipid peroxidation (LPO), which plays an important role in atherosclerosis (clogged and ruptured blood vessels) (Chen *et al.*, 2021).

The results of molecular optimization for bioactive compounds and compounds as a standard comparison of antioxidant activity show negative values, indicating that they have a stable molecular structure. The bioactive compound that has the most stable bond stability is compound (4) 2-hydroxy-6-methoxy-4-vinylphenyl hydrogen sulfate (-1041.98 kcal/mol); this is possible due to the stability of electron delocalization in the benzene ring and stable electron resonance in the sulfate group. The stable standard compound is found in quercetin (1097.92 kcal/mol); this is greatly influenced by the long electron delocalization in both benzene rings bridged by C3, a characteristic of the flavonoid compound group. In addition, the dipole moment of each compound bond is polar (the dipole moment is not zero). This shows that the higher the dipole moment value of a compound, the more polar the type of bond formed in the molecule will be (Targema *et al.*, 2013). In accordance with Lipinski's five rules as a basis for developing oral drugs, it shows that all bioactive compounds and comparison compounds as antioxidants are fulfilled. Based on the ADMET prediction data from bioactive compounds and comparison compounds (positive controls), it also shows a relatively good system for further development, except for the bioactive compound (2) due to the limitations of the existing database in revealing distribution, excretion, and hepatotoxicity predictions.

Bioactive compound (4), namely 2-hydroxy-6-methoxy-4-vinylphenyl hydrogen sulfate, shows better complex stability (-6.8 kcal/mol) compared to other bioactive compounds. This is stabilized by the presence of three hydrogen bonds on the amino acid residues Arg1263, Arg1213, and Asn1212. Meanwhile, the more stable reference compound is the quercetin compound with a binding affinity value of -8.6 kcal/mol, which is stabilized by one hydrogen bond with the amino acid residue Gly1345. While the stability of the native ligand complex is 8.2 kcal/mol with one hydrogen bond stabilized by the amino acid residue Lys527.

Limitations & Future Scope

These findings have limitations in the initial screening of bioactive compound components using standard reagents and determining the total levels of phenolic and flavonoid groups; analysis of bioactive compounds using sophisticated instruments, namely LC-HRMS; *in vitro* antioxidant activity testing with DPPH and ABTS methods; and conducting molecular docking studies with a bioinformatics approach. These findings are an important first step in the development of natural-based antioxidants and also confirm that the bioactive compound content of *R. mucronata* leaves shows potential as an antioxidant.

Conclusion

Traditional A study of the bioactive compounds contained in the water extract of *Rhizophora mucronata* (*R. mucronata*) leaves revealed diverse bioactive compounds and demonstrated potential antioxidant activity in vitro. A bioinformatics study of a combination of six bioactive compounds targeted the major protein receptor TP-binding cassette (ABC) subfamily C member 9 (ABCC9). Regulation of this protein expression plays a crucial role in the prevention and treatment of various cardiovascular diseases. Therefore, this preliminary study provides an overview for further

development of the use of bioactive compounds from this plant in the fields of functional food management, pharmaceuticals, and health.

Conflict of Interests

There is no conflict of interest, according to the authors.

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