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Ethnomedicinal bioprospecting of *Rhizophora apiculata* leaves through in silico and in vitro approaches as antioxidant, α-glucosidase inhibitor and anticancer

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Abstract. *Sibero MT, Pribadi R, Ambariyanto A, Haryanti D, Kharisma VD, Dewi AS, Patantis G, Zilda DS, Murwani R. 2022. Ethnomedicinal bioprospecting of* Rhizophora apiculata *leaves through in silico and in vitro approaches as antioxidant, α-glucosidase inhibitor and anticancer. Biodiversitas 23: 6437-6447.* Investigating marine natural products for biopharmaceutical development leads to a massive study of mangrove metabolites. *Rhizophora apiculata* is utilized as a traditional medicine by the local community in Indonesia. However, only a few studies reported the lead compounds. The aim of this study was to discover the biological properties of *R. apiculata* metabolites from the leaves as an antioxidant, α-glucosidase inhibitor, and anticancer agent through *in vitro* and *in silico* approaches. The leaves of *R. apiculata* were extracted and then fractionated using the silica-OCC method. All fractions were screened for antioxidant, α-glucosidase inhibitors, and cytotoxicity assays. Then the bioactive compounds in prospective fractions were identified using LC-HRMS. The selected compounds with ppm error <10 were applied for in silico analysis. The result of biological properties screening indicated Fr. 5 and Fr. 6 as the most potent sources of antioxidants, α-glucosidase inhibitors, and cytotoxic compounds. In total, 19 compounds were selected from two prospective fractions. The drug-likeness and bioavailability prediction results indicated that all selected compounds act as drug-like molecules. In addition, 17 were predicted to have antioxidant activity, and 15 compounds had antidiabetic activity. Moreover, 15 compounds had a more negative affinity binding than cytarabine. Molecular docking analysis showed that the mechanism of cytotoxicity against P388 Murine Leukaemia Cells was through the interaction of apigenin with protein tyrosine kinase (c-Kit) with a stronger inhibitory activity than the control drug (Cytarabine) and had the same binding site as the control (Cytarabine).

Keywords: Antidiabetic, antioxidant, cytotoxic, molecular docking, *Rhizophora apiculata*

INTRODUCTION

Unhealthy lifestyles in the past years have induced various health problems in society. The overconsumption of alcohol and tobacco, low intake of fruit and vegetable, high intake of sugar and instant food, and a lack of physical activities increase the possibility of developing obesity, cancer, and diabetes in the body (WHO, 2018, 2019a, b). The International Agency for Research on Cancer WHO put Indonesia number two for cancer prevalence in South-East Asia (WHO, 2022). In addition, the data on adults with diabetes from the International Diabetes Federation places Indonesia in the 5th position globally (IDF, 2021). However, 30-62% of people seem to have barriers to changing their unhealthy lifestyle to prevent disease development in their bodies (Nielsen et al. 2017). Hence, the consumption of drugs and supplement are more desirable. This practice raises the exploration of natural remedies to treat diseases. Recent studies stated that marine environments are very promising as the source of bioactive compounds for drug development (Carroll et al. 2020; Shinde et al. 2019).

The massive investigation for future biopharmaceutical products from marine environments leads to plenty of interesting bioactive compounds from mangroves (Mitra et al. 2021). As a salt-tolerant plant that grows in tropical and subtropical countries, the mangrove has interesting mechanisms and physiology to live that impact the production of its phytochemical substances (Ravi et al. 2020). Tomlinson (1986) categorized three mangrove groups, namely, major mangrove, minor mangrove, and mangrove associates. The major mangrove has essential roles in establishing the community structure due to its ability to form a pure stand, specialized morphology and

mechanisms for gas exchange and salt secretion, and restricted salinity from 17 to 36.6 ppm. Thus far, several genera of major mangroves have been reported from Indonesia, such as *Aegiceras*, *Avicennia*, *Bruguiera*, *Ceriops*, *Excoariai*, *Lumnitzera*, *Rhizophora*, and *Sonneratia*, with more than 43 species (Nugraha et al. 2022; Sidik et al. 2018; van Oudenhoven et al. 2015; Widyastuti et al. 2018; Sibero et al. 2020a).

The major mangrove has become one of the most profiled among other coastal plants. It was triggered by the ethnomedical application of these plants by local communities worldwide. Bibi et al. (2019) reported that some Asia countries utilize various mangroves to treat pathogens infections, malaria, inflammatory diseases, diarrhea, and many more. In addition, some mangrove species are also utilized as ethnomedicine by the local community in Indonesia. These mangroves are applied to cure skin diseases, poison neutralizers, inflammation, tumor, and fever (Purnobasuki 2019; Purwanti 2016; Tamalene et al. 2021). Among all species, *Rhizophora apiculata* is one of the reported species that is used as ethnomedicine in Indonesia. Purnobasuki (2019) stated that the leaf of this mangrove is used to treat hepatitis by the local community. Then, a further study proves that the leaf of this mangrove has a hepatoprotective effect on a dysfunctional liver (Zhang et al. 2019). Other studies showed the outstanding potential of *R. apiculata* as an antioxidant and antidiabetic (Ramalingam & Rajaram 2018; Selvaraj et al. 2016). Our previous study also found the anticancer potential of *R. apiculata* collected from Rembang, Central Java (Sibero et al. 2020[b]). Unfortunately, this study did not report the bioactive compounds from *R. apiculata* and their antioxidant or antidiabetic activity.

Moreover, for several years *in silico* approach has been widely applied to understand the drugs-likeness compound and their molecular mechanisms through computational modeling (Padmi et al. 2022). In the case of mangrove metabolites, our previous study applied *in silico* molecular docking to understand the molecular mechanism of (-)-Epicatechi of mangrove *Aegiceras corniculatum* to inhibit the growth of several periodontal pathogens (Nugraha et al. 2022). In addition, Illian et al. (2022) also successfully applied *in silico* approach to discover several potential anticancer agents. Therefore, the objectives of our current study were to discover the biological properties of *R. apiculata* metabolites from the leaves as an antioxidant, α-glucosidase inhibitor, and anticancer agent through *in vitro* and *in silico* approaches. As a result, we expect to obtain several compounds from *R. apiculata* that are the potential for future biomedicine to treat cancer and diabetes.

MATERIALS AND METHODS

Sample handling and metabolite extraction

The sample was collected from a mangrove forest in Rembang District, Central Java, Indonesia. Healthy leaves with no defects were taken from the *R. apiculata* tree and then kept in a plastic bag. The leaves were resized and then dried using an oven for three days at 40°C. In total, 75 gr of dried sample was extracted using methanol with a ratio of 1:4. Further, the organic solvent was separated from the sample and evaporated at 30-35°C. After that, the crude extract was stored at -20°C for further analysis (Sibero et al. 2020a).

Fractionation of *Rhizophora apiculata* metabolite

The crude extract was fractionated using open-column chromatography (OCC). Silica powder was used as a stationary phase and sample absorbent. As a stationary phase, the amount was the total silica that filled the column at 15 cm height. While, as the sample absorbent, the silica amount was three times the sample weight. The sample was prepared by dissolving the crude extract in $CHCl_3$ inside a rotary evaporation flask. Then the silica absorbent was added to absorb the sample solution. After that, the wet silica was evaporated to obtain dry silica containing the sample. Clean silica powder was packed with $CHCl_3$ as the first solvent inside the column. The silica sample was sown evenly on the top of the silica column. A gradient ratio of chloroform ($CHCl_3$) and methanol (MeOH) was applied to run the fractionation. The ratio of $CHCl_3$:MeOH were 1:0 (Fr. 1); 20:1 (Fr. 2); 10:1 (Fr. 3); 4:1 (Fr. 4); 2:1 (Fr. 5); 1:1 (Fr. 6); and 0:1 (Fr. 7) (v/v). All fractions were collected for evaporation at 30-35 °C (Sibero et al. 2019; Sibero et al. 2020c). The extraction gave 2.75 g of crude extract. The yield of each fraction in the sequence was 0.09 g (Fr. 1), 0.13 g (Fr. 2), 0.22 g (Fr. 3), 0.65 g (Fr. 4), 0.41 g (Fr. 5), 0.45 g (Fr. 6), and 0.30 g (Fr. 7).

Bioassay

Antioxidant activity

The antioxidant activity of R. apiculata fractions was determined according to Salazar-Aranda et al. (2011) by the 2,2-diphenyl-1-pikrilhidrazil (DPPH) method. First, the screening was done by testing all fractions at a concentration of 500 ppm, prepared by dissolving a 5 mg sample in 10 mL ethanol. Next, the DPPH stock was prepared by dissolving 2.5 mg DPPH in 50 mL of ethanol to reach a concentration of 125 μM. Finally, to screen the percentage of radical scavenging activity of each fraction, 2 mL of fraction solution was added to 2 mL of DPPH solution and kept in the dark chamber for 30 min. The absorbance was observed with a Spectrophotometer at 517 nm and then calculated with the following formula:

$$Radical\ scavening\ activity\ (\%RSA) = \frac{(A-B)}{A} \times 100$$

Where, A was the absorbance of the DPPH solution as a blank (DPPH in ethanol), while B was the absorbance of the sample and DPPH after incubation. The fractions with RSA higher than 50% were continued to determine the IC_{50} value. Hence, the fractions were diluted in DMSO to reach the following concentrations: 500 ppm, 250 ppm, 125 ppm, 62.5 ppm, 31.25 ppm, 15.625 ppm, and 7.812 ppm in a 96-microwell plate. In total, 100 μL of each fraction and 100 μL of DPPH (125 μM) were transferred into the 96-microwell plate, incubated in a dark chamber for 30 mins,

then the absorbance was measured at 517 nm. Finally, the IC_{50} value was calculated by plotting the concentration and its RSA percentage in a linear regression calculation.

α-glucosidase inhibitory activity

Evaluation of α-glucosidase inhibitory activity was only conducted on the fractions with RSA higher than 50%. This assay was conducted according to (Sancheti et al. 2009). For this assay, several reagents were prepared, such as 0.1 M phosphate buffer (pH 7.0), 0.5 mM 4-nitrophenyl α-D-glucopyranoside in 0.1 M phosphate buffer (pH 7.0), and α-glucosidase enzyme solution. The enzyme solution was prepared by dissolving the enzyme in a 0.01 M phosphate buffer (pH 7.0) to reach a concentration of 0.04 units/mL. Mangrove fractions were prepared by dissolving the sample in DMSO to reach particular concentrations by serial dilution. The concentrations for this assay were the same as the antioxidant assay.

Further, the reaction mixture was prepared by adding 50 µL of 0.1 phosphate buffer (pH 7.0), 25 µL of 0.5 mM 4-nitrophenyl α-D-glucopyranoside, 25 µL of glucosidase solution, and 10 µL of test fraction into a 96-well plate. The plate was incubated at 37°C for 30 mins to start the enzymatic reaction. Then, 100 µL of 0.2 M sodium carbonate was added to stop the reaction. The ability of each fraction to inhibit the enzymatic reaction was measured at 410 nm. Each concentration was prepared in two wells. Then, the IC_{50} value was analyzed using linear regression by plotting the concentration and the percentage inhibition.

Cytotoxicity assay

Cytotoxicity assay was performed by testing all fractions on P388 Murine Leukemia Cells. Cell revival and maintenance were conducted according to (Sharma et al. 2019). All fractions were screened with a concentration of 1 mg/mL. The calculation of cell viability was conducted with the following formula:

$$Cell\ Viability\ (\%) = \frac{A}{B} \times 100\%$$

Where: A was the absorbance of cells without any treatment, while B was the absorbance of cells with the addition of fraction. The fraction with more than 50% cell viability was continued for IC_{50} determination. Determination of the IC_{50} value was conducted according to our previous work with the XTT method (Sibero et al. 2022). The selected fraction was diluted in DMSO to reach the following concentration: 0.0002 µg/mL; 0.002 µg/mL; 0.02 µg/mL; 0.2 µg/mL; and 2 µg/mL. The IC_{50} value was determined using linear regression.

LC-HRMS analysis

Metabolite analysis using LC-HRMS was conducted according to Riyadi et al. (2021). First, the sample was prepared by dissolving the selected fractions in DMSO to reach a 2 mg/mL concentration and then filtered using a nanopore filter. Then a total of 10 µL sample solution was injected into HPLC (Thermo Scientific Dionex Ultimate 3000 RSLCnano), which was connected to HRMS (Thermo Scientific Q Exactive). The column that was applied in this system was Hypersil GOLD aQ (50 × 1 mm × 1.9 µm particle size, pore diameter 175 Å), flow rate 40 µL/min, and analysis time for 30 mins. Analysis was done using a gradient system with 0.1% Formic acid in water (A) and 0.1% Formic acid in Acetonitrile (B) as the mobile phase with the following condition: 5% B for 0-2 mins, 5-60% B for 2-15 mins, 60-95% B for 15-22 mins, 95% B for 22-25 mins and 95-5% for 25-30 mins. The mass was analyzed at 70,000 resolution for 30 mins and then processed using Compound Discoverer with mzCloud MS/MS Library. Further, only compounds with an error (ppm) of less than 10 were used in this study.

In silico analysis

Sample retrieval

Canonical 3D, CID, and SMILE structures for the selected compounds were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Energy minimization in compounds was performed through the OpenBabel v2.3.1 software to increase the flexibility of the bonds between the atoms that make up the molecule (Kharisma et al. 2021). C-kit tyrosine kinase acting as the target was revealed from the Protein Databank (https://www.rcsb.org/). The sterilization of water molecules on the target protein using PyMol v2.5.2 software (Schrödinger, Inc., USA) to increase the ligand binding ability (Fadholly et al. 2021). In addition, cytarabine as a leukemia-control drug was also used in this study (Di Francia et al. 2021).

Drug-likeness and bioavailability identification

The probability of similarity with drug molecules and absorption of chemical compounds from *R. apiculata* leaf extract by the human body was predicted by the Lipinski Rule of Five (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp) and SwissAdme (http://www.swissadme.ch/). Chemical compounds referred to as drug-like molecules must follow at least one Lipinski Rule of Five; the parameter rules consist of molecular mass, LogP, hydrogen bond acceptors, donors, and molar refractivity (Ansori et al. 2021a). In addition, the candidate compound must have a bioavailability value of at least 0.10-0.17, enabling it as a drug-like molecule that can be used orally (Ansori et al. 2021b).

Bioactivity prediction

Antioxidant and antidiabetic probabilities of chemical compounds from leaves of *R. apiculata* were predicted through PASS Online (http://way2drug.com/PassOnline/). The obtained bioactivity ability is calculated by the value of probability activation (Pa) and the inhibitory activity through probability inhibition (Pi). The prediction of a potential bioactive compound with scientific evidence only through a theoretical approach or the value of Pa >0.3 indicates medium confidence. The type of prediction Pa >0.3 is used for the identification of potential as antioxidants and antidiabetics in chemical compounds from

leaves extract of *R. apiculata* to strengthen the results of in vitro analysis (Husen et al. 2019).

Docking simulation

Molecular docking aims to simulate the interaction of ligand-protein molecules and measure ligand binding activity through binding affinity (kcal/mol) (Ansori et al. 2021c). Binding affinity is the free energy formed in the ligand-protein molecular complex; binding affinity has a negative value and a ligand activity indicator (Proboningrat et al. 2022). Molecular docking simulations in this study were performed using PyRx 0.9.9 software (Scripps Research, USA) and aimed to compare the bonding activity of chemical compounds from *R. apiculata* leaves with cytarabine. The 3D structure of molecular complexes is displayed with the structure of cartoons, surfaces, and sticks using PyMol v2.5.2 software (Schrödinger, Inc., USA) with structural selection and coloring methods (Fahmi et al. 2021). Molecular interactions in ligand-protein complexes were displayed via Discovery Studio Visualizer™ v.16.1 (Dassault SystèmesSE, France) to identify weak bonds such as hydrogen, hydrophobic, pi, electrostatic, and van der Waals (Kharisma et al. 2022).

RESULTS AND DISCUSSION

Antioxidant activity

Antioxidants are chemical substances that inhibit the free radical reaction in the cells. Hence, these compounds can prevent or delay cell damage (Nimse and Pal 2015). There are two types of antioxidant agents due to their source, namely synthetic and natural antioxidants. Synthetic antioxidants are substances produced by chemical synthesis, while natural antioxidants are produced by living organisms, especially plants and fruits. Several examples of synthetic antioxidants that are commonly used in industry due to their strong ability to prevent food deterioration are butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and propyl gallate (PG) (Alexandre et al. 2022; Nimse and Pal 2015). However, the negative impact of synthetic antioxidants on human DNA and enzyme systems increases the demand for natural antioxidants (Neha et al. 2019).

Among other antioxidant assays, DPPH free radical scavenging is the most popular performed method from crude extract to pure compounds (Johari and Khong 2019; Neha et al. 2019; Pandurangan et al. 2021; Salazar-Aranda et al. 2011; Sethi et al. 2020). This method measures the scavenging capacity of samples on the DPPH reagent. The instability of the free radical DPPH due to its odd electron on the nitrogen atom is neutralized by the addition of a hydrogen atom from the antioxidant agent (Kedare and Singh 2011; Xiao et al. 2020). Many studies successfully discovered antioxidant compounds from mangroves using the DPPH method (Purwaningsih et al. 2013; Sadeer and Rocchetti et al. 2019; Sudirman et al. 2014); therefore, this method was also applied in this study. Our current study explores the antioxidant activity of *R. apiculata* leave fractions. The RSA percentage for each fraction is shown in Figure 1.

The results of antioxidant screening showed that there were three fractions noted as prospective candidates due to the RSA values being more than 50%, namely Fr. 4 (62.74 ± 4.29%), Fr. 5 (91.05 ± 1.82%), and Fr. 6 (88.71 ± 0.71%). Furthermore, these three fractions continued to be analyzed to determine the IC_{50} value. The IC_{50} value of the prospective fractions is presented in Figure 2.

The determination of the IC_{50} value of the prospective fractions designated that Fr. 5 had the most potent antioxidant activity (IC_{50} value of 58.89 ± 0.86 ppm), followed by Fr. 6 (IC_{50} value of 66.72 ± 0.01 ppm), then the weakest activity showed by Fr. 4 (IC_{50} value of 491.70 ± 4.30 ppm). It is noted that fractions with IC_{50} values of < 50 ppm a very strong, 50-100 ppm as strong, 100-200 ppm as moderate, and > 200 ppm as a weak antioxidant agent (Sudirman et al. 2014). Therefore, Fr. 5 and Fr. 6 were proposed as strong antioxidant agents. Subsequently, these prospective fractions were further investigated for antidiabetic potential.

α-glucosidase inhibitory activity

The antidiabetic potential was investigated by understanding the fraction's ability to inhibit the α-glucosidase activity. This enzyme has an essential role in carbohydrate digestion in the intestine. Therefore, inhibiting this enzyme reduces sugar production in the intestine and lowers the sugar intake by the blood (Alam et al. 2019). The result of α-glucosidase inhibitory activity assay is presented in Figure 3.

According to Figure 3, it was highlighted that Fr. 6 had the most potent antidiabetic prospect with an IC_{50} value was 540.05 ± 6.91 ppm; Fr.5 owned a similar activity with an IC_{50} value of 545.07 ± 19.20 ppm, while the weakest antidiabetic potential owned by Fr. 4 with an IC_{50} value of 1353.01 ± 12.27 ppm.

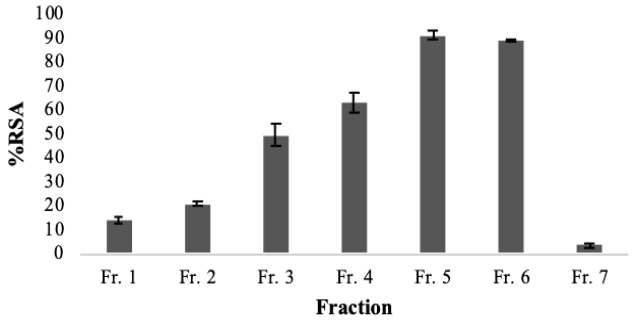


Figure 1. The value of radical scavenging activity (% RSA) leaves fractions of *Rhizophora apiculata*

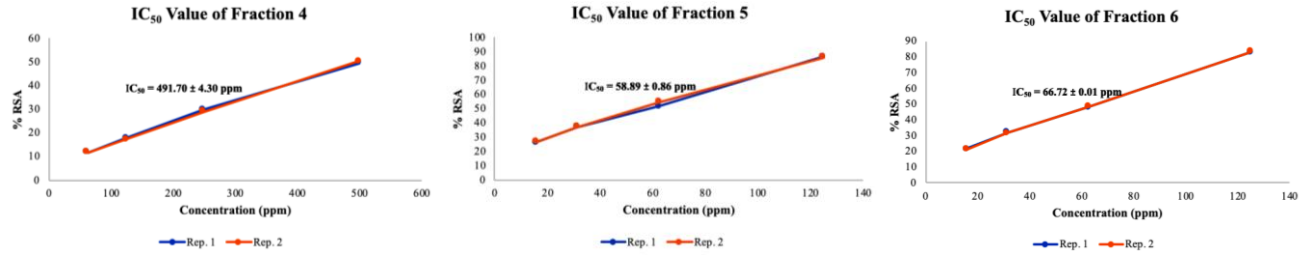


Figure 2. Antioxidant activity of leaves fraction of *Rhizophora apiculata*

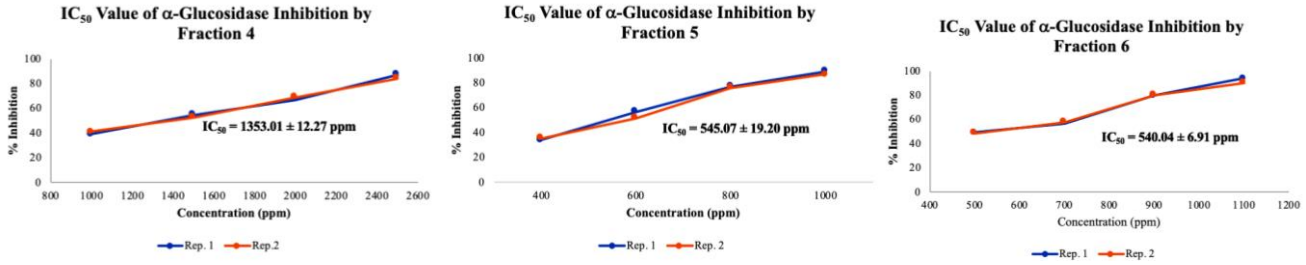


Figure 3. The IC_{50} value of α-glucosidase inhibition of leaves fraction of *Rhizophora apiculata*

Cytotoxicity

The previous study reported that the IC50 for cytotoxicity of *R. apiculata*'s crude extract was 0.0323 mg/mL against P388 Murine Leukemia cells (Sibero et al. 2020b). Therefore, our current study tried to discover lead compounds by investigating the active fractions. The percentage of cell viability after being treated by each fraction is shown in Figure 4.

Figure 4 shows that only Fr. 5 and Fr. 6 could inhibit the growth of P388 Murine Leukemia Cells up to < 50%. Hence, only these two fractions were continued for IC_{50} determination, shown in Figure 5.

It was found that Fr. 5 was the most potent candidate for an anticancer agent against P388 Murine Leukemia Cells with an IC_{50} value of 0.015 mg/mL, then followed by Fr. 6 (IC_{50} value of 0.021 mg/mL), while the lowest anticancer potential exhibited by Fr. 4 (IC_{50} value of 0.105 mg/mL). Therefore, according to all bioassays, we suggested that Fr. 5 and Fr. 6 were the most potential samples. Hence, metabolite characterization using LC-HRMS was performed only for these samples.

Metabolites from prospective fractions

Bioassay for antioxidant, α-glucosidase inhibitory, and cytotoxicity for the three prospective fractions lead to Fr. 5 and Fr. 6 as the most prospective candidate for bioactive compound profiling. The result of metabolite identification using LC-HRMS from Fr. 5 and Fr. 6 is presented in Table 1.

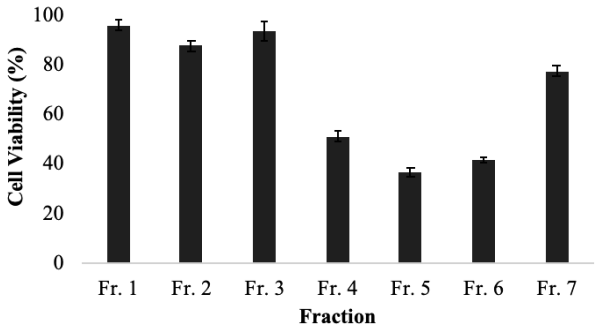


Figure 4. Cell viability (%) of P388 Murine Leukemia Cells after being treated by fractions of *Rhizophora apiculata* leaves

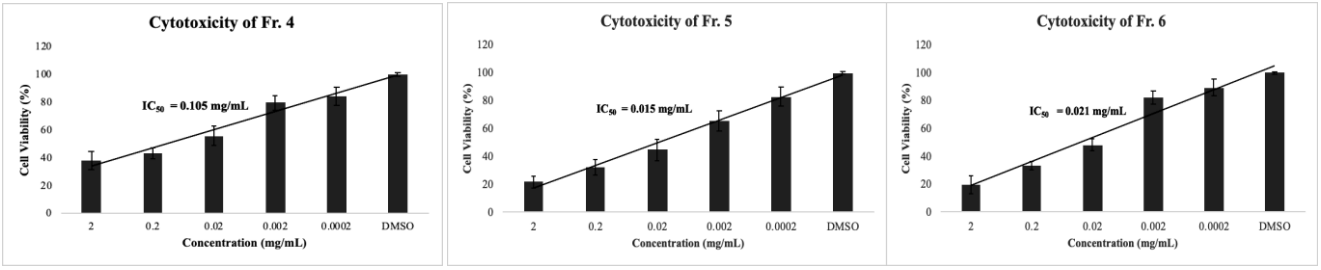


Figure 5. Cytotoxicity of leaves fractions of *Rhizophora apiculata* against P388 murine leukemia cells

Table 1. Metabolite profile of fractions 5 and 6 using LC-HRMS

Fraction	Compound	Retention time (Min.)	Experimental *m/z*	Theoretical *m/z*	Mass Error (ppm)
Fr. 5	Acetovanillone	0.863	166.06194	166.06299	-6.3
	3-Hydroxyphenylacetic acid	2.245	152.04637	152.04734	-6.4
	N-Butylbenzene sulfonamide	5.003	213.08162	213.08235	3.4
	Cuminaldehyde	6.244	148.08792	148.08882	-6.1
	3',4'-Dimethoxyacetophenone	6.377	180.07757	180.07864	-5.9
	4-Indolecarbaldehyde	7.666	145.05182	145.05276	-6.5
	Jasmone	8.998	164.11904	164.12012	-6.6
	Carvone	9.325	150.10349	150.10447	-6.5
	Apigenin	10.629	270.05331	270.05282	1.8
	Decanamide	12.588	171.16113	171.16231	-6.9
	2-(Methylthio) benzothiazole	13.786	181.0007	181.00199	-7.1
	4-Hydroxybenzaldehyde	16.76	122.03615	122.03678	-5.2
Fr. 6	Trigonelline	1.179	137.04672	137.04768	-7.0
	Nicotinamide	1.257	122.04733	122.04801	-5.6
	Nicotinic acid	1.258	123.03132	123.03203	-5.8
	Biopterin	1.448	237.08675	237.08619	2.4
	3-Indoleacrylic acid	3.850	187.06232	187.06333	-5.4
	4-Indolecarbaldehyde	7.655	145.05182	145.05276	-6.5
	Apigenin	10.618	270.05338	270.05282	2.0
	2-(Methylthio) benzothiazole	13.761	181.00066	181.00199	-7.4
	Shogaol	18.030	276.17528	276.17254	9.9
	4-Methoxycinnamic acid	20.506	178.06173	178.06299	-7.1

Part per million (ppm) is the common unit used to report the mass error (Brenton & Godfrey 2010). However, Chernonosov et al. (2021) stated that high-resolution mass spectrometry (HRMS) detects the samples using accurate mass with an error of 10 ppm or less. Therefore, this study chose compounds with mass error < 10 ppm (Table 1). Several studies have applied LC-HRMS to characterize bioactive compounds from plants extract (Adriani et al. 2022; Christina et al. 2021; Safitri et al. 2020). Moreover, 19 compounds were successfully identified using LC-HRMS from Fr. 5 and Fr. 6 based on predetermined criteria. Several of these compounds have been reported from various mangrove species with incredible biological activities such as antidiabetic, antiinflammatory, antihypertensive, antiobesity, and antihypercholesterolemic (Sachithanandam et al. 2019). Then, these compounds were continued for *in silico* analysis.

Compound drug-like molecule activity

Druglikeness analysis aims to screen drug candidate compounds with specific parameters in the early stages of new drug discovery (Luqman et al. 2020). Parameters consisting of molecular mass, LogP, hydrogen bond acceptors, donors, and molar refractivity refer to the Lipinski Rule of Five, and bioavailability predictions were used in this study (Nugraha et al. 2021). The drug candidate compound must have a molar refractivity of 40-130, >10 hydrogen bond acceptors, >5 hydrogen bond donors, >5 LogP, and a molecular mass of>500 Daltons, a positive prediction as a drug-like molecule is that the candidate compound must meet at least 2 rules (Wijaya et al. 2021). All chemical compounds from *R. apiculata* act as drug-like molecules because they follow a minimum of two Lipinski Rule of Five and bioavailability scores >0.17 (Table 2). Hence, all metabolites are predicted to enter the body's circulation.

Antioxidant and antidiabetic probability

This study uses the PASSOnline server for antioxidant and antidiabetic prediction. PASSOnline server predicts 4,000 bioactivities of a chemical compound consisting of the mechanism of action, side effects, toxicity, enzyme interactions, and gene expression (Prahasanti et al. 2021). The ability of bioactivity refers to the value of the probability of activity (Pa) and the probability of inhibition (Pi); this study uses Pa>0.3 or medium confidence or theoretically proven predictions (Riyadi et al. 2021; Susanto et al. 2018). Table 3 shows the result of antioxidant and antidiabetic prediction.

Among 19 compounds from *R. apiculata* leaves, 17 were predicted to have antioxidant activity, and 15 had the potential to have antidiabetic activity. Furthermore, these compounds were expected to be the reason for the strong antioxidant and (-glucosidase inhibitory activity of Fr. 5 and Fr. 6 based on the in vitro assay (Figures 2 and 3). In addition, several of these compounds have been reported as antioxidant and antidiabetic agents (Aldakinah et al. 2017; Allahghadri et al. 2010; Luo et al. 2004; Peng et al. 2012).

The molecular mechanism of anticancer

Compounds with anticancer abilities play a role in inhibiting the activity of oncoproteins so that cancer cells cannot develop (Lichota and Gwozdzinski 2018). This study used oncoprotein tyrosine kinase (c-Kit) in P388 Murine Leukemia Cell as a target binding of compounds from the prospective fractions to predict anticancer mechanisms. Tyrosine kinase protein (c-Kit) as a target for anticancer development is a common strategy; inhibition in such proteins can trigger proliferation and survival failure in cancer cells (Yamaoka et al. 2018). Simulation of the

mechanism of interaction of ligands with targets and prediction of molecular mechanisms are performed through molecular docking; cytarabine as the control drug for the treatment of Leukaemia is also used in docking simulations.

Compounds with a binding affinity value are more negative than the control, with a similarity of the position of interaction with the control predicted to trigger activities such as inhibition on the target. Binding affinity is negative energy produced by the interaction of molecules according to the ligand-protein complex (Ansori et al. 2022). In addition, weak bonds are produced in the ligand-protein complex that aims for stability and triggers a specific protein response (Dibha et al. 2022). Several weak bonds are van der Waals, hydrogen, hydrophobic, pi, alkyl, and electrostatic formed in the ligand-protein complex (Kharisma et al. 2020).

Several compounds from the prospective fractions, namely 3-hydroxyphenylacetic acid, 2-(methylthio)benzothiazole, 3',4'-dimethoxyacetophenone, 4-indole-carbaldehyde, carvone, apocynin, apigenin, cumin aldehyde, decanamide, jasmone, n-butylbenzene-sulfonamide, biopterin, 3-indoleacrylic acid, 4-methoxycinnamic acid, and shogaol had a more negative affinity binding than cytarabine (Table 4).

The strongest bond activity of fifteen compounds with binding affinity was more negative than the controls produced by apigenin. Apigenin also had similar binding sites with cytarabine via Asp677, Asn680, Phe811, and Leu595. In addition, apigenin can form weak bond interactions consisting of van der Waals, hydrogen, and Pi, which play a role in triggering the inhibitory activity of the tyrosine kinase protein (c-Kit) (Figure 6).

Table 2. The results of drug-likeness and bioavailability prediction

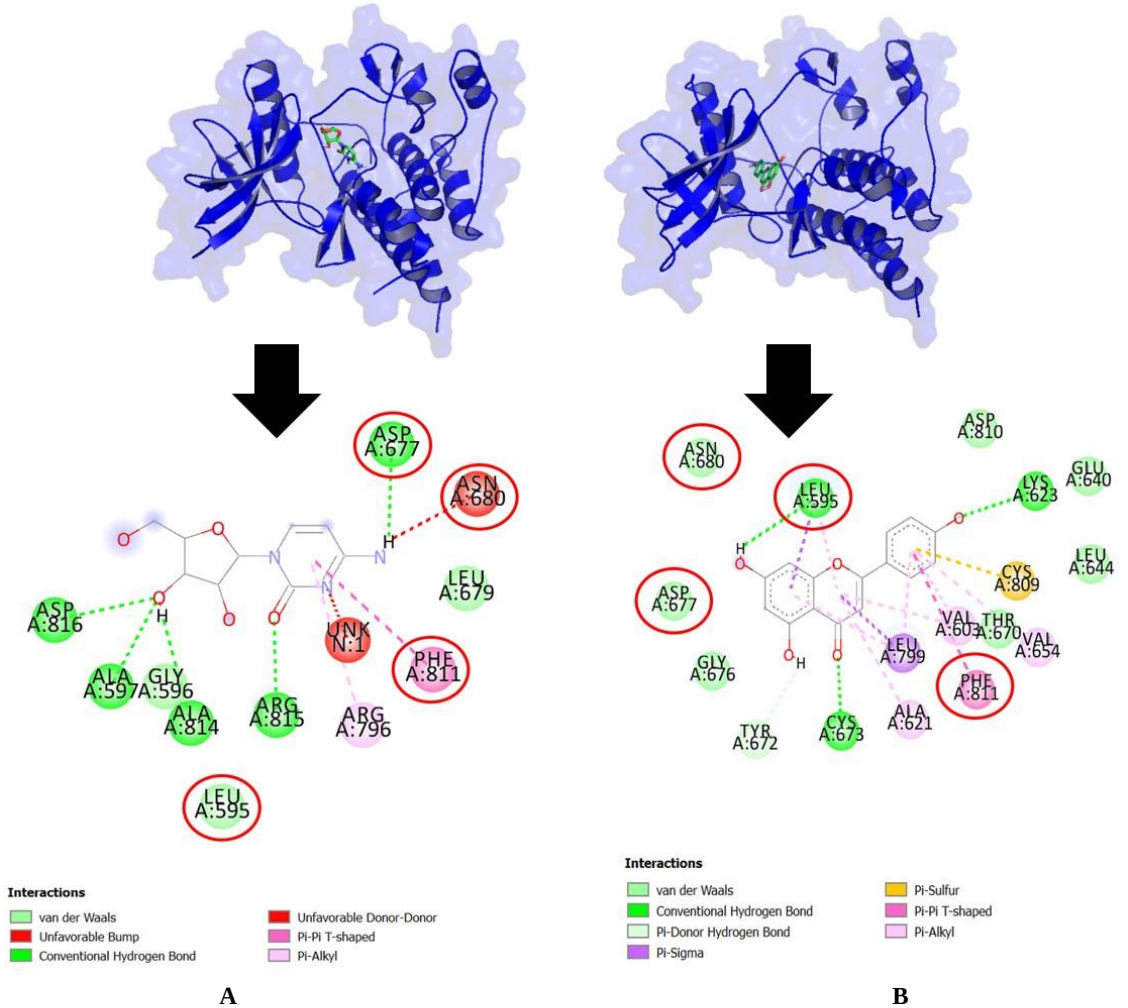
Compounds	MS (Dalton)	HBD	HBA	LOGP	MR	BA	Probable
3-Hydroxy phenylacetic acid	152.000	2	3	1.019	39.446	0.85	Drug-like Molecule
2-(Methylthio) benzothiazole	181.000	0	1	3.018	51.401	0.55	Drug-like Molecule
3',4'-Dimethoxy acetophenone	180.000	0	3	1.906	49.550	0.55	Drug-like Molecule
4-Indolecarbaldehyde	145.000	1	1	1.980	43.686	0.55	Drug-like Molecule
4-Hydroxy benzaldehyde	122.000	1	2	1.204	33.494	0.55	Drug-like Molecule
Carvone	150.000	0	1	2.487	46.301	0.55	Drug-like Molecule
Apocynin	166.000	1	3	1.603	44.663	0.55	Drug-like Molecule
Apigenin	270.000	3	5	2.419	70.813	0.55	Drug-like Molecule
Cuminaldehyde	148.000	0	1	2.622	45.918	0.55	Drug-like Molecule
Decanamide	171.000	2	2	2.612	51.804	0.55	Drug-like Molecule
Jasmone	164.000	0	1	3.022	50.988	0.55	Drug-like Molecule
N-Butylbenzene sulfonamide	213.000	1	3	2.845	56.582	0.55	Drug-like Molecule
Biopterin	237.000	5	8	-1.419	57.421	0.55	Drug-like Molecule
3-Indoleacrylic acid	187.000	2	2	2.265	54.968	0.85	Drug-like Molecule
4-Methoxycinnamic acid	178.000	1	3	1.793	49.663	0.85	Drug-like Molecule
Nicotinic acid	123.000	1	3	0.779	31.196	0.85	Drug-like Molecule
Shogaol	276.000	1	3	4.038	81.268	0.55	Drug-like Molecule
Trigonelline	137.000	0	2	-0.330	34.150	0.55	Drug-like Molecule
Nicotinamide	122.000	2	3	0.180	32.754	0.55	Drug-like Molecule

Table 3. Antioxidant and antidiabetic compound bioactivity value

Compounds	Antioxidant			Antidiabetic		
	Pa	Pi	Prediction result (Pa > 0.3)	Pa	Pi	Prediction result (Pa > 0.3)
3-Hydroxyphenylacetic acid	0.629	0.017	+	0.485	0.025	+
2-(Methylthio) benzothiazole	0.288	0.195	-	0.377	0.016	+
3',4'-Dimethoxy acetophenone	0.615	0.020	+	0.373	0.073	+
4-Indolecarbaldehyde	0.405	0.092	+	0.159	0.044	-
4-Hydroxybenzaldehyde	0.611	0.020	+	0.192	0.138	-
Carvone	0.387	0.103	+	0.317	0.031	+
Apocynin	0.616	0.019	+	0.340	0.129	+
Apigenin	0.732	0.004	+	0.320	0.029	+
Cuminaldehyde	0.485	0.056	+	0.222	0.048	-
Decanamide	0.750	0.004	+	0.454	0.012	+
Jasmone	0.482	0.057	+	0.392	0.049	+
N-Butylbenzene sulfonamide	0.458	0.066	+	0.312	0,005	+
Biopterin	0.274	0.214	-	0.419	0.039	+
3-Indoleacrylic acid	0.490	0.054	+	0.224	0.137	-
4-Methoxycinnamic acid	0.664	0.011	+	0.376	0.050	+
Nicotinic acid	0.749	0.004	+	0.398	0.013	+
Shogaol	0.743	0.003	+	0.330	0.149	+
Trigonelline	0.678	0.009	+	0.491	0.005	+
Nicotinamide	0.796	0.003	+	0.509	0.005	+

Table 4. Binding affinity from docking simulation

Compounds	CID	Target	Minimize energy (kcal/mol)	Binding affinity (kcal/mol)
3-Hydroxyphenylacetic acid	12122	Receptor Tyrosine Kinase (c-Kit)	+89.47	-6.3
2-(Methylthio)benzothiazole	11989	Receptor Tyrosine Kinase (c-Kit)	+237.16	-6.3
3',4'-Dimethoxyacetophenone	14328	Receptor Tyrosine Kinase (c-Kit)	+238.37	-6.8
4-Indolecarbaldehyde	333703	Receptor Tyrosine Kinase (c-Kit)	+214.02	-6.4
4-Hydroxybenzaldehyde	126	Receptor Tyrosine Kinase (c-Kit)	+57.74	-5.7
Carvone	7439	Receptor Tyrosine Kinase (c-Kit)	+108.72	-7.2
Apocynin	2214	Receptor Tyrosine Kinase (c-Kit)	+155.32	-6.6
Apigenin	5280443	Receptor Tyrosine Kinase (c-Kit)	+230.49	-10.1
Cuminaldehyde	326	Receptor Tyrosine Kinase (c-Kit)	+71.07	-6.9
Decanamide	75347	Receptor Tyrosine Kinase (c-Kit)	+40.01	-6.1
Jasmone	1549018	Receptor Tyrosine Kinase (c-Kit)	+219.45	-7.1
N-Butylbenzenesulfonamide	19241	Receptor Tyrosine Kinase (c-Kit)	+473.28	-7.1
Biopterin	135403659	Receptor Tyrosine Kinase (c-Kit)	+152.76	-6.2
3-Indoleacrylic acid	5375048	Receptor Tyrosine Kinase (c-Kit)	+229.39	-7.9
4-Methoxycinnamic acid	699414	Receptor Tyrosine Kinase (c-Kit)	+104.34	-7.2
Nicotinic acid	938	Receptor Tyrosine Kinase (c-Kit)	+58.97	-5.6
Shogaol	5281794	Receptor Tyrosine Kinase (c-Kit)	+156.73	-7.4
Trigonelline	5570	Receptor Tyrosine Kinase (c-Kit)	+69.00	-5.5
Nicotinamide	936	Receptor Tyrosine Kinase (c-Kit)	+73.71	-5.2
Cytarabine (Control Drug)	6253	Receptor Tyrosine Kinase (c-Kit)	+313.29	-5.9



A **B**

Figure 6. Molecular visualization of 3D and 2D interaction from the docking results. A. Cytarabine_Tyrosine Kinase (c-Kit). B. Apigenin_Tyrosine Kinase (c-Kit). The red round shape on amino acid residues is a binding site similarity between cytarabine and apigenin

Rhizophora apiculata leaves are predicted to be anticancer for leukemia disease through the interaction of apigenin with the tyrosine kinase protein (c-Kit) with stronger inhibitory activity than the control drug (Cytarabine) and has similar binding sites with controls (Cytarabine).

In conclusion, Fractions 5 and 6 of *R. apicualata* leaves showed potential biological properties as antioxidants, α-glucosidase inhibitors, and cytotoxic agents against P388 Murine Leukemia Cells. The predicted results of drug-likeness and bioavailability showed that all selected compounds acted as drug-like molecules. In addition, 17 compounds were predicted to have antioxidant activity, and 15 had antidiabetic activity. Molecular docking analysis predicts the interaction of apigenin with protein tyrosine kinase (c-Kit) as a key cytotoxicity mechanism against P388 Murine Leukemia Cells. Moreover, it is suggested to conduct *in vivo* assay to confirm the pharmacological effect of the prospective fractions.

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