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Toxicity and potential bioactive compounds of *Rhizophora apiculata* and *Bruguiera sexangula* leaf extracts from two small islands on the coast of South Sumatra

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ABSTRACT

Mangroves grow on the coast of large to small islands with unique ecology, known as a source of potential bioactive compounds. This study aims to evaluate the toxicity and potential bioactive compounds from leaf extracts of *Rhizophora apiculata* and *Bruguiera sexangula* growing on two small islands with different habitats, namely Payung Island and Maspari Island on the coast of South Sumatra. A total of 1,000 grams of leaf samples of *R. apiculata* and *B. sexangula* were taken from both islands and then dried in the sun using the indirect sunlight method covered with black cloth. A total of 100 grams of fine powdered leaf samples were macerated in 96% ethanol with a ratio of 1:10 (b/v) for 24 hours. Then the maceration solution was filtered and evaporated to a concentrated extract using a rotary evaporator at 60 °C. Toxicity testing of extracts using the Brine Shrimp Lethality Test (BSLT) method, and identification of toxic bioactive compounds in extracts, is done through phytochemical tests, total phenols, and GC-MS analysis. The test results showed that leaf extracts of *R. apiculata* and *B. sexangula* from Payung Island showed higher toxicity than those from Maspari Island, with LC_{50} values of 407 µg/mL and 337 µg/mL, respectively (medium toxic category), while those from Maspari Island were 654 µg/mL and 868 µg/mL (weak toxic category). Total phenolics in leaf extracts from Payung Island were recorded at 168.06 gGA/g for *R. apiculata* and 529.46 gGA/g for *B. sexangula*. Phytochemical tests identified the presence of alkaloids, saponins and terpenoids in both types of extracts from Payung Island, while GC-MS analysis revealed 20 bioactive compounds in *R. apiculata* extracts and 3 compounds in *B. sexangula* that have the potential as source of bioactive compounds for health and pharmacology.

Keywords: *Bruguiera sexangula*, Maspari Island, Payung Island, *Rhizophora apiculata*, toxicity.

INTRODUCTION

Mangrove ecosystems generally grow in coastal areas of large islands in the world, especially in sheltered areas such as bays, lagoons, deltas, estuaries, and river banks that are influenced by tidal dynamics. Mangroves provide a variety of ecosystem services that are very important in coastal areas. These services include habitat for a variety of marine life and fishery products, water quality improvement, nutrient cycling, eco-tourism, and blue carbon sequestration (Anu et al., 2024). In addition, mangroves are also known as a potential source of bioactive compounds in the field of pharmacology. The content of plant bioactive compounds is influenced by environmental limiting factors and geographical conditions (Gololo et al., 2018). Mangroves adapt to changes in temperature, salinity, waves, tides, and sedimentation (Ellison, 2021).

This adaptation process forces mangroves to perform chemo-physiological processes to produce toxic bioactive compounds (Lakshmanrao et al., 2018; Mitra et al., 2021).

Small island areas on the coast of South Sumatra such as Payung Island (Hermialingga et al., 2020) and Maspari Island (Rozirwan et al., 2019) Payung Island is a unique habitat for a variety of mangrove species, including *R. apiculata* and *B. sexangula*. These two mangrove species grow in different environmental conditions, Payung Island is located at the mouth of the Musi River, with brackish water conditions and muddy substrates (Ulqodry et al., 2019). In addition, the Musi River Estuary has become a polluted area due to anthropogenic activities that produce waste, such as industrial activities, plantations, and ship transportation along the Musi River (Rozirwan et al., 2021). In contrast, Maspari Island is located in the open sea with sandy beach conditions, high salinity, large waves and extreme tides. These conditions make the mangrove species *R. apiculata* and *B. sexangula* able to produce toxic and diverse bioactive compounds.

Previous studies have revealed that various parts of mangrove plants, including leaves, contain toxic bioactive compounds that have important biological activities. Phytochemical analysis of *R. apiculata* leaf extracts identified compounds such as flavonoids, saponins, tannins, steroids, and terpenoids (Syawal et al., 2020). Bioactive compounds from *R. apiculata* leaf extracts have been shown to have anticancer activity (Maulana and Sasmito, 2021), anti-inflammatory, antibacterial, and antioxidant (Ganesh et al., 2024). Meanwhile, *B. sexangula* leaf extract contains a variety of bioactive compounds such as phenolics, proteins, steroids, alkaloids, tannins, anthocyanins, carbohydrates, fatty acids, hydrocarbons, and lipids (Mitra et al., 2021). Bioactive compounds from *B. sexangula* leaf extracts have been reported to have activities as antioxidants, antityrosinase, and antielastase (Machana et al., 2017). The potential of these bioactive compounds opens up opportunities for the development of mangrove resources in the pharmaceutical industry. Although there have been several studies exploring the bioactive activities of mangroves, information on the toxicity and phytochemical profile of mangrove leaf extracts of *R. apiculata* and *B. sexangula* from unique habitats and different characteristics on small islands such as Payung Island and Maspari Island in coastal South Sumatra has not been conducted.

Initial screening of the toxic potential of mangrove leaf extracts can be done through in-vitro testing before further testing in-vivo. Brine Shrimp Lethality Test (BSLT) is one of the simple and accurate methods to measure initial toxicity before further testing in-vivo (Osamudiamen et al., 2020; Rozirwan et al., 2022). Identification of phytochemical components in the most toxic extracts of *R. apiculata* and *B. sexangula* leaves can be done using a qualitative phytochemical test observing color changes (Eswaraiah et al., 2020), total phenol content analysis (Vittaya et al., 2022) and gas chromatography-mass spectrometry analysis (GC-MS) to determine the components of bioactive compounds (Lalitha et al., 2021). This study was conducted to evaluate the level of toxicity and potential bioactive compounds in leaf extracts of *R. apiculata* and *B. sexangula* growing from two small islands with unique and different ecological habitats, namely Payung Island on the coast of Banyuasin Regency and Maspari Island on the coast of Ogan Komering Ilir Regency, South Sumatra Province.

MATERIAL AND METHOD

Study area

Leaf samples of *R. apiculata* and *B. sexangula* were taken from two different islands in June 2024, namely Payung Island on the coast of Banyuasin Regency and Maspari Island on the coast of Ogan Komering Ilir Regency, South Sumatra Province, Indonesia (Figure 1). Sample preparation and toxicity testing of *Artemia salina* larvae were conducted at the Marine Bioecology Laboratory, Department of Marine Science, Faculty of Mathematics and Natural Sciences, Sriwijaya University. Analysis of phytochemical compounds and total phenolics was conducted at the Chemistry Laboratory, Faculty of Teacher Training and Education, Sriwijaya University, while GC-MS analysis was conducted at the Analytical Chemistry and Instrumentation Testing Laboratory, Faculty of Mathematics and Natural Sciences, Sriwijaya University.

Preparation and extraction of *R. apiculata* and *B. sexangula* leaf samples

A total of 1000 grams of mangrove leaves of *R. apiculata* and *B. sexangula* were taken from Payung Island on the coast of Banyuasin Regency

Figure 1. Sampling location map

and Maspari Island on the coast of Ogan Komering Ilir Regency. The leaves were put into plastic clips and then brought to the laboratory for the preparation process. The preparation process includes washing the leaves until they are clean from dirt in the form of attached sediments, then the leaves are cut into small pieces to speed up the drying process, and the leaves are dried in the sun using the indirect sunlight method which is covered using a black cloth (Eswaraiah et al., 2020; Robbiyan et al., 2021). The dried leaves were pulverized with a blender and stored in plastic clip bags to maintain sample quality. A total of 100 grams of *R. apiculata* and *B. sexangula* mangrove leaf powder was extracted using maceration method with 96% ethanol at a ratio of 1:10 (b/v). This mixture was stirred until homogeneous and allowed to stand for 24 hours. After that, the macerated solution was filtered, and the filtrate was evaporated using a rotary evaporator at 40°C (Mitra et al., 2023).

Toxicity test brine shrimp lethality test of extracts

The test refers to the method Sarah et al., (2017), 2 g of *A. salina* eggs were hatched in artificial seawater with a salinity of 30 psu for 48 hours until the larvae were ready for use. Stock solution with a concentration of 10.000 mg/mL was prepared from 1 gram of mangrove leaf extract of *R. apiculata* and *B. sexangula* mixed with distilled water to 100 μL, then diluted into five test concentrations of 1000, 500, 250, 100, and 50 μg/mL as much as 50 ml volume. Each test concentration was taken as much as 5 mL and then put into a vial bottle and given *to A. salina* larvae as many as ten individuals (Rozirwan et al., 2022). The test was conducted in three replicates of each concentration, and negative and larval mortality was observed for 24 hours.

Calculation of the percentage of mortality of *A. salina* larvae using Abbott's formula (1925) equation 1 (Kalpana et al., 2024). Mortality categories refer to De Alencar et al., (2014), highly toxic (75–100%), moderately toxic (50–75%), and non-toxic (< 50%). LC_{50} is used to determine the dose of extract that can cause 50% mortality in test larvae using linear regression Harlan, (2018). Equation 2 (y): is the independent variable; a is the constant; (b): is the regression coefficient; and (x): is the independent variable. The leaf extracts of *R. apiculata* and *B. sexangula* were classified based on the categories of Clarkson et al. (2004). These are highly toxic (LC_{50} 0–100 μg/mL), moderately toxic (LC_{50} 100–500 μg/mL), weakly toxic (LC_{50} 500–1000 μg/mL), and non-toxic (LC_{50} > 1000 μg/mL).

$$Mortality = \frac{Number\ of\ dead\ larvae}{Number\ of\ test\ larvae} \cdot 100\% \quad (1)$$

$$y = a + b \quad (2)$$

Bioactive compound analysis (phytochemical test, total phenol test and GC-MS)

Leaf extracts of *R. apiculata* and *B. sexangula* leaf extracts were subjected to the phytochemical test, total phenol test, and GC-MS analysis to determine bioactive compounds that have toxic biological activity against larvae. Phytochemical tests were carried out qualitatively by looking at changes in color and physical appearance (Rani and Vijayanchali, 2021), with reference to the methods in the research Khan et al., (2024) include test parameters for alkaloid compounds, flavonoids, steroids, tannins, saponins, and triterpenoids. To determine the amount of phenol compounds contained in the extract, testing was carried out using the Folin-Ciocalteau reagent. (Shaikh and Patil, 2020). Furthermore, to determine the group of bioactive compounds that are volatile and toxic in both extracts tested using Gas Chromatography-Mass Spectrometry Analysis (Jasna and Khaleel, 2020; Pathak et al., 2023).

RESULTS AND DISCUSSION

Mortality test values of *R. apiculata* and *B. sexangula* leaf extracts against *A. salina*

R. apiculata leaf extract from Payung Island and Maspari Island

The results of toxicity testing against *A. salina* larvae showed that the mortality rate of *R. apiculata* leaf extracts from Payung Island was higher than that of leaf extracts from Maspari Island (Table 1). *R. apiculata* leaf extract from Payung Island caused mortality of 50% at a concentration of 500 µg/mL and 63% at a concentration of 1000 µg/mL which is categorized as moderately toxic. In contrast, the leaf extract of *R. apiculata* from Maspari Island only showed a mortality of 57% at a concentration of 1000 µg/mL, categorized as moderately toxic.

B. sexangula leaf extract from Payung Island and Maspari Island

The results of the toxicity test against *A. salina* larvae showed that the percentage of mortality of *B. sexangula* leaf extract from Payung Island was also higher than that of *B. sexangula* leaf extract from Maspari Island (Table 2). *B. sexangula* leaf extract from Payung Island caused mortality of 57% at concentrations of 250 µg/mL and 500 µg/mL and 60% at a concentration of 1000 µg/mL which is categorized as moderately toxic. Meanwhile, *B. sexangula* leaf extract from Maspari Island only showed a mortality rate of 50% at a concentration of 1000 µg/mL, with a moderate toxic category.

Table 1. Mortality test value of *A. salina*

Concentration (µg/mL)	% Mortality			
	RA (P)	* Toxicity category	RA (M)	* Toxicity category
1000	63	Moderately toxic	57	Moderately toxic
500	50	Moderately toxic	43	Non-toxic
250	47	Non-toxic	37	Non-toxic
100	33	Non-toxic	23	Non-toxic
50	17	Non-toxic	13	Non-toxic
Control (-)	0	-	0	-

Note: RA (P): *R. apiculata* leaf extract from Payung Island; RA (M): *R. apiculata* leaf extract from Maspari Island; *: Toxicity categories refer to De Alencar et al., (2014).

Table 2. Mortality test values against *A. salina*

Concentration (µg/mL)	% Mortality			
	BS (P)	* Toxicity category	BS (M)	* Toxicity category
1000	60	Moderately toxic	50	Moderately toxic
500	57	Moderately toxic	43	Non-toxic
250	57	Moderately toxic	30	Non-toxic
100	30	Non-toxic	17	Non-toxic
50	20	Non-toxic	10	Non-toxic
Control (-)	0	-	0	-

Note: BS (P): Leaf extract of *B. sexangula* from Payung Island; BS (M): *B. sexangula* leaf extract from Maspari Island; *: Toxicity categories refer to De Alencar et al., (2014).

LC_{50} test value of *R. apiculata* and *B. sexangula* leaf extracts against *A. salina*

LC_{50} (lethal concentration 50%) analysis was used to determine the concentration of *R. apiculata* and *B. sexangula* leaf extracts that can cause death in 50% of *A. salina* larvae. The results of the analysis (Table 3) showed that the LC_{50} values of *R. apiculata* and *B. sexangula* leaf extracts from Payung Island were 407 µg/mL and 337 µg/mL, respectively, while those from Maspari Island were 654 µg/mL and 868 µg/mL. Based on the toxicity classification Clarkson et al. (2004), *R. apiculata* and *B. sexangula* leaf extracts from Payung Island fall into the moderate toxicity category, while extracts from Maspari Island fall into the weak toxicity category.

Mangroves from the genus Rhizophora and Bruguiera are known to have toxic biological activities. Various studies have shown the potential toxicity of leaf extracts from both genera to test organisms. *R. apiculata* leaf extract is known to be highly toxic to *A. salina* shrimp larvae with varying LC_{50} values, namely LC_{50} 81 µg/mL (Laith, 2021), LC_{50} 99.07 µg/ml (Fadilah et al., 2023), and LC_{50} 49.45 µg/mL (Maulana and Sasmito, 2021). In addition, *R. apiculata* leaf extract is also toxic to *Aedes aegypti* mosquito larvae with an LC_{50} of 0.085 µg/L (Shinde et al., 2018). Another species in the same genus, namely *R. mucronata*, showed toxicity to *A. salina* with an LC_{50} value of 402.45 µg/mL (Zulfahmi et al., 2024). On the other hand, research on the toxicity of *B. sexangula* leaf extract is still very limited, although research on other species in the genera *Bruguiera* has indicated the presence of toxic biological activity. For example, *B. gymnorrhiza* leaf extract is toxic to *A. salina* with a value of LC_{50} 241.4 µg/ml (Karim et al., 2020). while *B. cylindrica* leaf extract showed an LC_{50} value of 16.628 µg/mL against the same organism (Islam et al., 2017). Genetic variability of populations within the genera Rhizophora and Bruguiera is also thought to influence the toxic biological activity of these species.

Table 3 shows that leaf extracts of *R. apiculata* and *B. sexangula* from Payung Island are more toxic than those from Maspari Island. The toxicity of plant extracts is strongly influenced by the content of bioactive compounds, and the content of bioactive compounds depends on habitat conditions and environmental limiting factors in the vicinity (Gololo et al., 2018). Mangrove habitats on Payung Island face higher ecological pressure than Maspari Island. In addition to the natural limiting factors of mangrove growth, such as temperature, pH, DO and salinity, there are anthropogenic activities along the Musi River to the estuary that produce heavy metal waste. (Purwiyanto et al., 2020). Heavy metals will accumulate in the sediments of the mangrove ecosystem on Payung Island and will suppress its growth. Study Khotimah et al., (2024), found that the concentration of heavy metal Pb in *E. agallocha* roots reached 2.89 ± 0.033 mg/kg, while heavy metal Cu in *A. alba* reached 10.57 ± 0.38 mg/kg in the Payung Island area. Then study Rozirwan et al., (2022), also reported that mangrove leaf extracts taken from areas affected by port activities were positively correlated with the content of toxic bioactive compounds.

Bioactive compounds and biological potential of *R. apiculata* and *B. sexangula* leaf extracts

Analysis of bioactive compounds was conducted on extracts that had the highest LC_{50} values in the toxicity category. Based on the LC_{50} values shown in Table 3, leaf extracts of *R. apiculata* and *B. sexangula* from Payung Island showed moderate toxicity, compared to leaf extracts of *R. apiculata* and *B. sexangula* from Maspari Island which were classified as weak toxicity. *R. apiculata* and *B. sexangula* leaf extracts from Payung Island were then further

Table 3. LC_{50} value against *A. Salina*

Sample	Linear regression			LC_{50} (µg/mL)	* Toxicity category
	a	b	R2		
RA (P)	2.626377834	0.909479481	0.949743972	407	Moderately toxic
RA (M)	2.285648875	0.964044701	0.985582986	654	Weakly toxic
BS (P)	2.756016246	0.887591155	0.868681021	337	Moderately toxic
BS (M)	2.027910299	1.011408092	0.991875686	868	Weakly toxic

Note: RA (P): *R. apiculata* from Payung Island; RA (M): *R. apiculata* from Maspari Island; BS (P): *B. sexangula* from Payung Island; BS (M): *B. sexangula* from Maspari Island (*): Toxicity categories refer to Clarkson et al. (2004).

analyzed using phytochemical tests, total phenol tests, and GC-MS analysis to identify toxic compounds. The results of qualitative phytochemical analysis showed that the leaves of *R. apiculata* and *B. sexangula* contained alkaloid, saponin, and terpenoid compounds (Table 4).

Alkoloid, saponin, and terpenoid compounds found in mangrove leaf extracts of *R. apiculata* and *B. sexangula* have toxic biological activities that cause mortality in *A. salina* larvae test. The results of the phytochemical test of the mangrove leaf extract of *R. apiculata* only found 3 compounds, this result is different from the previous study which found 6 compounds. The mangrove leaf extract of *R. apiculata* contains alkaloids, saponins, terpenoids, tannins, flavonoids and steroids (Laith 2021; Karga 2023). The results of the phytochemical test of *B. sexangula* mangrove leaf extract are also different from previous studies. *B. sexangula* mangrove leaf extract contains flavonoid and tannin compounds (Machana et al., 2017). The difference in these compounds can be caused by the habitat and geographical location of their growth. Alkaloid, saponin, and terpenoid compounds have toxic effects that are deadly through various mechanisms of exposure to test animals. Alkaloid compounds can damage cell membranes by entering cells and disrupting the nervous system of larvae (Purba and Muliarta, 2024). Saponin compounds function by irritating the mucosa in the digestive tract and have a bitter taste, which reduces the appetite of the larvae and ultimately causes death (Moniharapon et al., 2020). Terpenoid compounds can act as stomach poisons; when they enter the body of larvae, these compounds will disrupt their digestive system and cause death (Annashr et al., 2024).

The compounds found in the mangrove leaf extracts of *R. apiculata* and *B. sexangula* also have potential biological activity in various fields, especially health and pharmacology. Alkoloid compounds have various pharmacological activities that provide benefits to human health, including antimicrobial, anticancer, antidiabetic, anti-inflammatory, antimalarial, antihypertensive, and antioxidant properties (Rajput et al., 2022). Terpenoid compounds function as antibacterials that are effective in inhibiting the growth of bacteria, fungi, viruses, and protozoa (Syawal et al., 2020). Saponin compounds are known to have a variety of biological activities, including antimicrobials, antivirals, anticancer, fungicides, anti-inflammatories, and sources of antioxidants (Juang and Liang, 2020).

The higher total phenol content in the extracts of *R. apiculata* and *B. sexangula* suggests it is a potential source of antioxidants. Antioxidants play an important role in protecting cells from damage caused by free radicals (Mitra et al., 2021). The total phenol content of *R. apiculata* and *B. sexangula* leaf extracts from Payung Island was 168.06 gGA/g and 529.46 gGA/g (Table 5). The total phenol content in *R. apiculata* leaf extract is higher than the results of previous studies, which is 66.79 mg gGA/g in the n-hexane extract, but lower than the ethyl acetate extract of 222.97 GAE/g and the ethanol extract which reaches 929.04 mg GAE/g (Maulana and Sasmito, 2021). The total phenolic content of *B. sexangula* leaf extract was higher than the n-hexane, ethyl acetate, and methanol leaf extracts of *B. gymnorrhiza*, namely 12.41, 97.57, and 30.07 mg GAE/g (Nurjanah et al., 2016), and 13.82 mg GAE/g (Rozirwan et al., 2023a). The total phenol content in *R. apiculata* and *B. sexangula* leaf extracts was much higher than the total phenol content of other mangrove leaf extracts, such as *A. marina* 9.0258 mg GAE/g, and *S. alba* 9.4969 mg GAE/g (Rozirwan et al., 2023a); *A. officinalis* 100.64 mg GAE/g (Bui et al., 2021). In addition, phenols also exhibit other biological activities, such as antimicrobial, antioxidant, anti-inflammatory, and antiproliferative (Albuquerque et

Table 4. Phytochemical compounds of *R. apiculata* and *B. sexangula* leaf extracts

Phytochemical test parameters	RA (P)	BS (P)
Alkoloids	+	+
Tannins	-	-
Flavonoids	-	-
Saponins	+	+
Terpenoids	+	+
Steroids	-	-

Note: RA (P): *R. apiculata* from Payung Island; BS (P): *B. sexangula* from Payung Island.

Table 5. Total phenol compounds of *R. apiculata* and *B. sexangula* leaf extracts

Sample	Total flavonoid content (g GA / g)
RA (P)	168.06
BS (P)	529.46

Note: RA (P): *R. apiculata* from Payung Island; BS (P): *B. sexangula* from Payung Island.

al., 2021; Zahra et al., 2021). This ability makes phenol a compound that plays an important role in the health sector, including in natural remedies and pharmaceutical products.

Based on the results of GC-MS analysis, *R. apiculata* leaf extract from Payung Island on the chromatogram graph detected 20 peak points at ret. time 4.32 to 36.66 (Figure 2). The chromatogram graph informed as many as 20 compounds contained in *R. apiculata* leaves (Table 6). The compound names are 4-Chloro-3-methylbut-2-en-1-ol (2.29%), Nonanoic acid, 9-oxo, methyl ester (0.66%), 2-Methyl-d-glucose (1.29%), 3-O-Methyl-d-glucose (6. 54%), Hexadecanoic acid, methyl ester (36.15%), 9-Octadecenoic acid (Z)-, methyl ester (16.91%), 10-Octadecenoic acid, methyl ester (0.60%), Phytol d (2.46%), Methyl stearate (3. 22%), Oxiraneundecanoic acid, 3-pentyl-, methyl ester, cis (0.63%), cis-13-Eicosenoic acid (0.63%), Squalene (0.74%), Vitamin E (2.31%), Ethyl iso-allocholate (0.71%), Stigmasterol (1. 15 %), ç-Sitosterol (4.10%), 6a,14a-Methanopicene, perhydro-1,2,4a,6b,9,9,12a-heptamethyl-10-hydroxy (2.23), á-Amyrin (1.76%), Lup-20(29)-en-3-one

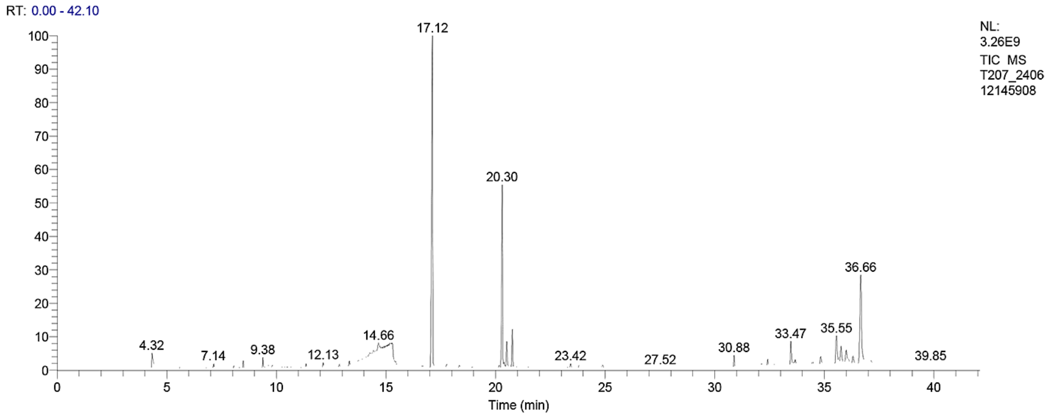

Figure 2. GC-MS chromatogram of *R. apiculata* leaf extract from Payung Island

Table 6. Bioactive compounds in *R. apiculata* leaf extracts from Payung Island

Ret. time	Peak area%	Name of compounds	Formula
4.32	2.29	4-Chloro-3-methylbut-2-en-1-ol	$C_5H_9C_lO$
9.38	0.66	Nonanoic acid, 9-oxo-, methyl ester	$C_{10}H_{18}O_3$
14.66	1.29	2-Methyl-d-glucose	$C_7H_{14}O_6$
15.28	6.54	3-O-Methyl-d-glucose	$C_7H_{14}O_6$
17.12	36.15	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$
20.30	16.91	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$
20.38	0.60	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$
20.51	2.46	Phytol	$C_{20}H_{40}O$
20.76	3.22	Methyl stearate	$C_{19}H_{38}O_2$
23.82	0.63	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, cis	$C_{19}H_{36}O_3$
24.88	0.63	cis-13-Eicosenoic acid	$C_{20}H_{38}O_2$
30.88	0.74	Squalene	$C_{30}H_{50}$
33.47	2.31	Vitamin E	$C_{29}H_{50}O_2$
33.67	0.71	Ethyl iso-allocholate	$C_{26}H_{44}O_5$
34.83	1.15	Stigmasterol	$C_{29}H_{48}O$
35.55	4.10	ç-Sitosterol	$C_{29}H_{50}O$
35.76	2.23	6a,14a-Methanopicene, perhydro-1,2,4a,6b,9,9,12a-heptamethyl-10-hydroxy	$C_{30}H_{50}O$
36.00	1.76	á-Amyrin	$C_{30}H_{50}O$
36.31	1.01	Lup-20(29)-en-3-one	$C_{30}H_{48}O$
36.66	14.60	Lupeol	$C_{30}H_{50}O$

(1.01%) and Lupeol (14.60%). Previous studies have shown that *R. apiculata* leaf extract contains bioactive compounds such as Phytol, Squalene, and Vitamin E found in Pichavaram, Tamil Nadu, India. These compounds are known to have antibacterial properties that can reduce diseases in rice plants and shrimp farming (Kannappan et al., 2018; Lakshmanan et al., 2019). In addition, the compounds 3-O-Methyl-d-glucose and Lupeol found in Guntur District, Andhra Pradesh, India, have antidepressant and nootropic activities (Mande et al., 2023). Hexadecanoic acid methyl ester found in Pichavaram, India, also exhibits antibacterial activity (Paranjothi and Murali, 2018). Stigmasterol and β-Sitosterol compounds found in *R. annamalayana* leaf extract in India have antifungal activity (Mahalakshmi et al., 2020).

In addition, the compounds Amyrin, Lup-20(29)-en-3-one, and Ethyl iso-allocholate were found in the leaf extract of *R. stylosa* in Alue Naga Coast, Banda Aceh, Indonesia. (Saidi et al., 2024). Octadecenoic acid methyl ester compound was found in *R. mucronata* leaf extract in Pichavaram, India (Chitra et al., 2019). Several other compounds such as 4-Chloro-3-methylbut-2-en-1-ol, Nonanoic acid 9-oxo-, methyl ester, 2-Methyl-d-glucose Methyl stearate, Oxiraneundecanoic acid, 3-pentyl-, methyl ester, cis, cis-13-Eicosenoic acid, and 6a,14a-Methanopicene, perhydro-1,2,4a,6b,9,9,12a-heptamethyl-10-hydroxy have not been reported in previous studies focusing on the *R. apiculata* species or the Rhizophora genera.

Another study also revealed that the compounds contained in *R. apiculata* leaf extract have similarities with other plant extracts and show potential biological activity. Nonanoic acid, 9-oxo-, methyl ester exhibits antifungal, antimicrobial, antioxidant, and larvicidal properties (Sen et al., 2017); 2-Methyl-d-glucose is antimicrobial (Belmessieri et al., 2017); 3-O-Methyl-d-glucose functions as antitumor and anti-inflammatory (Hussein et al., 2016; Mickymaray et al., 2016). 9-Octadecenoic acid (Z)-, methyl ester acts as anticancer and hypotensive (Chenniappan et al., 2020), while 10-Octadecenoic acid, methyl ester is antibacterial and antidiabetic (Rosa and Jesam, 2024). Phytol functions as an anticancer, antimicrobial, anti-inflammatory, and diuretic (Chenniappan et al., 2020), while Methyl stearate has antidiarrheal and antiproliferative properties (Saha et al., 2020). Oxiraneundecanoic acid, 3-pentyl-, methyl ester, cis has antioxidant properties (Najah and Alshawish, 2023), and cis-13-Eicosenoic acid act as antifungal, anti-inflammatory, antibacterial, and anticancer (Osman et al., 2024; Naeim et al. 2020; Kadhim et al., 2016).

In addition, Squalene is known to have antioxidant, antitumor, antibacterial, detoxification, and immunity activities (Cheng et al., 2024), while Vitamin E functions as an effective antioxidant to inhibit aging and glycation (Song et al., 2021; Rizvi et al., 2014). Ethyl iso-allocholate has antimicrobial, anti-inflammatory, anti-arthritic, and antioxidant activities (Kumar et al., 2022; Gopu et al., 2021). Stigmasterol is antimicrobial, antifungal, anti-inflammatory, antioxidant, antiviral, and anticancer (Mukherjee et al., 2022; Lestari et al., 2024). Ç-Sitosterol is known to be a source of antioxidants (Gyesi et al., 2019), and 6a,14a-Methanopicene have antimicrobial activity (Mohamed et al., 2019). Furthermore, α-Amyrin is antifungal and antibacterial in nature (Ekalu et al., 2019). Lup-20(29)-en-3-one, as the oxidized form of lupeol, has sugar-regulating, lipid-lowering, anti-inflammatory, antimutagenic, and antioxidant properties (Tsai et al., 2016), while Lupeol exhibits antimicrobial, antiprotozoan, antiinvasive, antiangiogenic, antiproliferative, and

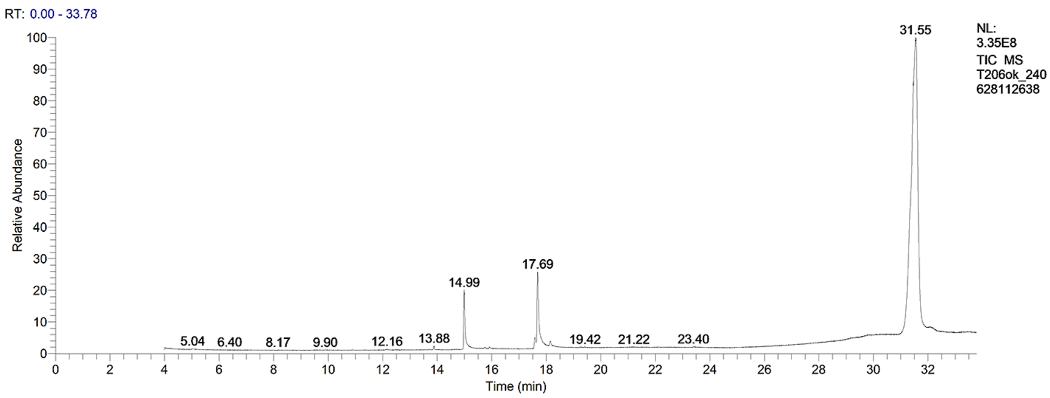

Figure 3. GC-MS chromatogram of *B. sexangula* leaf extract from Payung Island

Table 7. Bioactive compounds in leaf extracts of *B. sexangula* from Payung Island

Ret. time	Peak area%	Name of compounds	Formula
14.99	4.71	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$
17.68	8.05	trans-13-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$
31.55	87.24	Benzoic acid, 3,5-dicyclohexyl-4-hydroxy-, methyl ester	$C_{20}H_{28}O_3$

anti-inflammatory activities (Tiwari et al., 2021). Then for the leaf extract of *B. sexangula* from Payung Island, the chromatogram graph detected 3 peak points at ret. time 14.99, 17.68 and 31.55 (Figure 3). The chromatogram graph informs as many as 3 compounds contained in the leaves of *B. sexangula* (Table 7). The compound names are Hexadecanoic acid, methyl ester (4.71%), trans-13-Octadecenoic acid, methyl ester (8.05%), and Benzoic acid, 3,5-dicyclohexyl-4-hydroxy-, methyl ester (87.24%).

Previous studies have revealed the compounds Hexadecanoic acid, methyl ester and trans-13-Octadecenoic acid, methyl ester found in *A. marina* leaf extract from the protected area of Sembilang National Park, South Sumatra, Indonesia (Rozirwan et al., 2023b). Other research reveals that the compound hexadecanoic acid, methyl ester is an antioxidant compound (Anyasor et al., 2015), antimicrobials, and anti-inflammatories (Abubakar and Majinda, 2016; Ojekale et al., 2016). Trans-13-Octadecenoic acid, methyl ester, functions as an anticancer, anti-inflammatory, and insecticide (Abdullah et al., 2020). while Benzoic acid, 3,5-dicyclohexyl-4-hydroxy-, methyl ester has antibacterial and antifungal properties (Ojinnaka et al., 2015). The biological activity of 4-Chloro-3-methylbut-2-en-1-ol is not yet known for certain.

CONCLUSIONS

In conclusion, leaf extracts of *R. apiculata* and *B. sexangula* from Payung Island showed higher toxic activity than extracts from Maspari Island. The LC_{50} values of *R. apiculata* and *B. sexangula* extracts from Payung Island were 407 μg/mL and 337 μg/mL (medium toxic category), while those from Maspari Island were 654 μg/mL and 868 μg/mL, respectively (weak toxic category). The total phenol content of the extracts from Payung Island was recorded at 168.06 gGA/g for *R. apiculata* and 529.46 gGA/g for *B. sexangula*. Phytochemical tests of *R. apiculata* and *B. sexangula* leaf extracts from Payung Island identified the presence of alkaloids, saponins, and terpenoids, while GC-MS analysis showed the presence of 20 bioactive compounds in *R. apiculata* extracts and 3 compounds in *B. sexangula* that have important biological activities and can be further developed in the fields of health and pharmacology.

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