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Characterization of Bioactive Compounds and Stability of Mangrove Extract Rhizopora Sp.

Dirk Y. P. Runtuboi[1*], Ervina Indrayani[2], Imam Misbach[2] , Gabriela Olivia Karisoh[2]

[1] Department of Biology, Faculty of Mathematics and Natural Science, Cenderawasih University, Jayapura, Papua, Indonesia.
[2] Department of Marine Science and Fisheries, Faculty of Mathematics and Natural Science, Cenderawasih University, Jayapura, Papua, Indonesia.

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Corresponding Author:
Dirk Y. P. Runtuboi
dirk.runtuboi@fmipa.uncen.ac.id

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Abstract: In recent decades, bioactive compounds contained in plants have increasingly attracted the attention of researchers. This is because these bioactive compounds have the potential to be used as raw materials in the manufacture of health products, medicines, and cosmetics. However, in developing products based on bioactive compounds in Rhizopora sp. mangroves, sufficient knowledge about these compounds is needed. The main objective of this study was to identify bioactive compounds and evaluate the stability of Rhizopora sp. mangrove extract obtained from Youtefa Bay area, Jayapura City. The methods used include mangrove leaf sampling, maceration extraction using methanol, followed by fractionation and phytochemical tests to detect the content of active compounds. Data analysis was performed using UV and IR spectroscopy. The results showed that the isolated Rhizophora sp. mangrove leaf extract obtained from Youtefa Bay waters contained several bioactive compound activities such as alkaloid, flavonoid, phenolic, and saponin bioactive compound groups in methanol solvents. In conclusion, mangrove Rhizophora sp. from Youtefa Bay has great potential as a source of bioactive compounds that can be further developed in various commercial products.

Keywords: Bioactive compounds; Characterization; Mangrove extracts of rhizopora Sp.; Stability

Introduction

The urgency of finding new bioactive compounds that are useful for health in this era is increasing. One source of bioactive compounds that continues to be developed is plants (Bergonzi et al., 2022). The utilization of plants for the treatment of various diseases is increasingly being carried out to reduce the level of chemical drug consumption (Chaachouay & Zidane, 2024; Nasim et al., 2022). One type of plant that has potential as a source of medicines is mangrove (Rahayu et al., 2019). Mangrove plants are known to contain various compounds that have the potential as a source of natural ingredients to treat diseases (Cerri et al., 2022; Sanjaya et al., 2023). Rhizopora sp. is one type of mangrove that is widespread in coastal areas of Indonesia, including in the Youtefa Bay Nature Park Jayapura City. In Rhizopora sp. plants there are bioactive compounds such as flavonoids, tannins, saponins and polyphenols that have been shown to have antioxidant and anti-inflammatory activities (Ridlo et al., 2019).

In the last few decades, bioactive compounds contained in plants have increasingly attracted the attention of researchers. This is because these bioactive compounds have the potential to be used as raw materials in making health products, medicines and cosmetics (Martinez-Burgos et al., 2024; Mutik et al., 2022; Nieto et al., 2023). Bioactive compounds in the mangrove Rhizopora sp. It is also known to have

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antimicrobial, anticancer, antioxidant and anti-inflammatory properties (Kumar & Pola, 2023). However, in developing products based on bioactive compounds in the mangrove Rhizopora sp. sufficient knowledge regarding these compounds is required (Rosulva et al., 2022). Although the bioactive potential of these plants is known, more in-depth research on the characteristics and stability of these compounds, especially in Rhizopora sp. from Youtefa Bay Nature Park, Jayapura City, is still very limited. The geographical and ecosystem conditions in this area may affect the content of bioactive compounds, making this research relevant to identify unique compounds that may not be found in other locations. In addition, the stability of bioactive compounds is very important in determining their effectiveness in pharmaceutical and cosmetic products. Unstable compounds tend to degrade, reducing their therapeutic potential (ElGamal et al., 2023).

Previous research by Mutik et al. (2022) showed the results of phytochemical tests on Rhizopora apiculata from the waters of Awur Bay, Jepara, by finding various classes of bioactive compounds. In n-hexane solvent, alkaloids and steroids were detected; in ethyl acetate solvent, alkaloids, phenolics, and steroids were found; while in methanol solvent, alkaloids, flavonoids, phenolics, and saponins were detected. Nevertheless, this study concluded that Rhizopora apiculata does not have potential as an antibacterial agent against multi-drug resistant (MDR) bacteria.

Another study by Syawal et al. (2020) revealed that Rhizopora apiculata leaf extract contains saponins, tannins, flavonoids, steroids, and terpenoids. Meanwhile, research by Jha et al. (2022) showed that polysaccharides from Rhizophora mucronata leaves have promising antioxidant and antibiofilm properties, making them potential therapeutic agents in the field of functional foods and medicines**.**

This latest research has novelty by focusing on the characterization of bioactive compounds as well as the stability of Rhizopora sp. mangrove extracts originating from the Youtefa Bay Natural Tourism Park area in Jayapura City. The research findings are expected to provide important information regarding the content of bioactive compounds in Rhizopora sp. mangrove extract from Youtefa Bay Nature Park, Jayapura City, and its stability which is an important factor in the development of pharmaceutical and cosmetic products.

In the context of public health, this research can also contribute to the development of alternative therapies for diseases that are still difficult to cure with conventional therapy. This is important considering that more and more people are turning to alternative medicine to find solutions to the chronic diseases they suffer from.

Thus, this research has great urgency in exploring the potential of Indonesia's natural resources which have not been utilized optimally and contributing to the development of alternative therapies for chronic diseases.

Method

The Youtefa Bay Natural Tourism Park area is located in the city of Jayapura, Papua, with an area of around 10 hectares. This area is a strategic place to take mangrove samples because it has a high diversity of mangrove types and is located in an area that is protected from pollution and environmental damage (Sari et al., 2023). The image (Figure 1) mangrove sampling process is carried out by selecting healthy and mature mangrove trees with a trunk diameter of at least 10 cm and a minimum height of 3 meters. The samples were then picked carefully to ensure that there was no damage to the mangrove trees and the roots were taken. Next, the samples were identified morphologically and environmental parameters such as water temperature, salinity, pH and soil moisture were measured. The following is a map of the research location.

Figure 1. Sampling location

Research on Characterization of Bioactive Compounds and Stability of Mangrove Extracts

Rhizopora sp. carried out in the Youtefa Bay Natural Tourism Park Area, Jayapura City. Mangrove Rhizopora sp. was chosen as a research object because it has potential as a source of bioactive compounds that can be used in the development of medicines and health supplements. To take mangrove samples, this is done using a purposive sampling method, namely by selecting locations that have environmental conditions that support mangrove growth.

Research method: Characterization of Bioactive Compounds and Stability of Mangrove Extract

Rhizopora sp. in the Youtefa Bay Natural Tourism Park Area, Jayapura City can be explained in detail and in stages as follows: The first stage is the extraction of bioactive compounds using ethanol solvent. 100 grams of dried mangrove leaves were extracted with 1 liter of 70% ethanol in an Erlenmeyer flask. The flask was placed in a shaker incubator for 24 hours at 30°C with a stirring speed of 150 rpm. After that, the extract was filtered using filter paper to obtain a crude extract, which was then evaporated with a rotary evaporator until a dry extract was obtained.

The next step is fractionation using column chromatography. The dry extract was fractionated with a 60-200 mesh silica gel column, using n-hexane: ethyl acetate eluent in a ratio of 9:1 to 1:9 gradually. Each fraction produced was tested with thin layer chromatography (TLC) to determine the content of bioactive compounds in each fraction.

Furthermore, bioactive compounds were identified using TLC and spectrophotometer. TLC was performed with n-hexane: ethyl acetate solvent in a ratio of 6:4 as the mobile phase. TLC bands were then tested using p-anisaldehyde-sulfuric acid reagent to identify the type of compound in each fraction. A spectrophotometer was used to measure the absorbance of each fraction at a specific wavelength.

The final stage is the evaluation of the stability of Rhizopora sp. mangrove extract through heat and humidity tests. The heat test was carried out by heating the extract at 40°C, 60°C, and 80°C for 24 hours. The humidity test was conducted by storing the extract at 75% humidity at 30°C for 24 hours. After that, the stability of the extract was evaluated by measuring the content of bioactive compounds before and after the heat and humidity tests.

Meanwhile, the following are the targeted achievement indicators in this study, first, the extraction results reflect the efficiency of the extraction process calculated by comparing the weight of the extract with the weight of the raw material, with the expected results ranging from 5-10%. Second, the level of bioactive compounds contained in the extract is measured using a spectrophotometer, with a target level of bioactive compounds in the range of 10-20 mg/mL. Third, the indicator of the number of fractions resulting from the fractionation process using column chromatography is expected to reach 3-5 fractions.

Furthermore, the successful identification of bioactive compounds contained in the extract using thin layer chromatography and spectrophotometer techniques. The expected results in this study are the success of identifying certain bioactive compounds and determining their relative levels. After that, the focus is on extract stability which shows how stable the Rhizopora sp. mangrove extract is against heat and humidity. Stability is measured by conducting heat and humidity tests and observing changes in bioactive compound levels after testing. Stable extracts are expected to maintain the levels of bioactive compounds well under different storage conditions.

In addition, isolation and purification of active compounds were carried out on the ethyl acetate fraction using vacuum column chromatography with silica gel as the stationary phase and several eluent systems as the mobile phase. The chromatography results were collected in bottles and subjected to KLT test. The bottles that showed spots with the same Rf value were combined and then concentrated using a rotary evaporator to obtain a thick isolate. Pressurized column chromatography was used to obtain sub-fractions or pure compounds. The compounds obtained were purified by crystallization/recrystallization and their boiling points were determined. Identification of compounds was carried out using infrared and UV-Vis spectrophotometry.

By referring to the achievement indicators that have been determined, it is hoped that this research can provide useful information regarding the characterization of bioactive compounds and the stability of Rhizopora sp mangrove extract. in the Youtefa Bay Natural Tourism Park Area, Jayapura City.

The output of this research is that the bioactive compounds contained in the mangrove extract Rhizopora sp. in the Youtefa Bay Natural Tourism Park Area, Jayapura City. Apart from that, this research will also provide information about the stability of the extract, both in heat and humidity tests. The results of this research can contribute to increasing the potential of mangrove extract Rhizopora sp. as a natural ingredient for various applications, especially in the health sector. It is also hoped that the results of this research can become a reference for further research in the same or related fields**.**

Result and Discussion

The Table 1 presents the fractionation results of the Thin Layer Chromatography (TLC) analysis of mangrove Rhizophora sp. extract. The data includes values for four extract samples, three fractionated samples (Fractions 1, 2, and 3), and two sets of residue values (Res 1 and Res 2).

The data in Table 1, shows some important observations. Firstly, the values for Res 1 and Res 2 are consistent across the fractions in each extract sample, indicating a uniform pattern among the residues. Secondly, the residue values (Res 1 and Res 2) showed variation in positive and negative responses, indicating different levels of chemical activity or concentration in the samples. In addition, the fraction values varied

among the different extracts, indicating the presence of different components or compounds in each fraction. This illustrates the complexity of the chemical composition in the extract samples.

Table 1. Results of TLC Fractionation of Mangrove Rhizophora sp. Extract

Extract	Fraction 1	Fraction 2	Fraction 3	Residue 1	Residue 2
25	10	15	28	-.36440	-.36440
28	18	17	30	.83812	.83812
30	25	20	32	-.29152	-.29152
22	21	18	29	-.01822	-.01822
26	23	16	31	-.16398	-.16398

Normality Test

The results of the One-Sample Kolmogorov-Smirnov Test indicate that the unstandardized residuals have a mean of 0.0000000 and a standard deviation of 0.48668321. The most extreme differences are 0.315 (absolute), 0.315 (positive), and -0.227 (negative). The test statistic is 0.315, with an asymptotic significance (2-tailed) value of 0.118. Based on these results, we can conclude that the data follows a normal distribution, as the significance value is greater than 0.05, indicating that there is no significant deviation from normality.

Table 2. Calculation of Normality Test One-Sample Kolmogorov-Smirnov Test

N		Unstandardized Residual
		5
Normal Parameters[a,b]	Mean	0.0000000
	Std. Deviation	0.48668321
Most Extreme Differences	Absolute	0.315
	Positive	0.315
	Negative	-0.227
Test Statistic		0.315
Asymp. Sig. (2-tailed)		0.118[c]

a. Test distribution is Normal.
b. Calculated from data.
c. Lilliefors Significance Correction.

One way ANOVA Test

The one-way ANOVA analysis was conducted to examine the effect of the predictors Fraksi_1, Fraksi_2, and Fraksi_3 on the dependent variable extract. The results revealed that the regression model accounted for a significant portion of the variance in extract, with a sum of squares of 35.853, a mean square of 11.951, an F-value of 12.614, and a significance level of 0.203. The residual sum of squares was 0.947, with 1 degree of freedom. The total sum of squares was 36.800 with 4 degrees of freedom. This suggests that while the model explains a substantial amount of variability, the significance level indicates that the predictors may not be statistically significant in explaining the variance in extract.

Table 3. One-way ANOVA test calculation

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	35.853	3	11.951	12.614	0.203b
	Residual	0.947	1	0.947		
	Total	36.800	4			

a. Dependent Variable: extract
b. Predictors: (Constant), Fraksi_3, Fraksi_2, Fraksi_1

Linear Test

The linear regression analysis aimed to further investigate the relationship between the predictors and the dependent variable Extract. The model demonstrated a strong fit, with an R value of 0.987 and an R-squared value of 0.974, indicating that approximately 97.4% of the variability in Extract can be explained by the predictors. The adjusted R-squared value was 0.897, with a standard error of the estimate at 0.973.

The analysis indicates that the predictors Fraksi_1, Fraksi_2, and Fraksi_3 are important in explaining the variability in the dependent variable extract, with Fraksi_3 showing the strongest positive effect. However, the significance levels suggest that these predictors may not be statistically significant.

Table 4. Result Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	0.987[a]	0.974	0.897	0.973

a. Predictors: (Constant), Fraksi_3, Fraksi_2, Fraksi_1
b. Dependent Variable: Extract

Table 5. Residuals Statistics Result

Parameters	Minimum	Maximum	Mean	Std. Deviation	N
Predicted Value	22.02	30.29	26.20	2.994	5
Residual	-.364	0.838	0.000	0.487	5
Std. Predicted Value	-1.397	1.367	0.000	1.000	5
Std. Residual	-.374	.861	0.000	0.500	5

a. Dependent Variable: Extract

Table 6. Coefficients Analysis Result

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-70.767	16.189		-4.371	0.143
	Fraksi_1	-0.768	0.187	-1.482	-4.112	0.152
	Fraksi_2	0.564	0.380	.358	1.485	0.377
	Fraksi_3	3.405	0.612	1.775	5.567	0.113

a. Dependent Variable: Extract

UV Light Fractionation of Mangrove R. apiculata Extract

Based on the identification results, it was found that the mangrove plants used as samples are of the Rhizophora apiculata type, obtained from the Youtefa Bay Nature Tourism Park Area in Jayapura City. Mangrove leaves were chosen as samples because they have a simple tissue structure and the ability to produce flavonoids, as has been proven in several previous studies (Glasenapp et al., 2019; Muliyana, 2020; Roy & Dutta, 2021; Rozirwan et al., 2023; Sari, 2021). Flavonoids are one of the main compounds targeted in this study.

Extraction of mangrove leaves was carried out by maceration method using 96% ethanol solvent. The maceration method has several advantages, including simplicity of process (Asworo & Widwiastuti, 2023), minimal physical disturbance, and is able to produce high yields (Hasnaeni & Wisdawati, 2019). In addition, maceration also increases extraction efficiency due to the reflux process assisted by heating at 40-60°C (Saifudin, 2014).

The image (Figure 3) shows the results of UV light fractionation of the Mangrove R. apiculata extract, highlighting the presence and separation of different compounds within the extract under UV light.

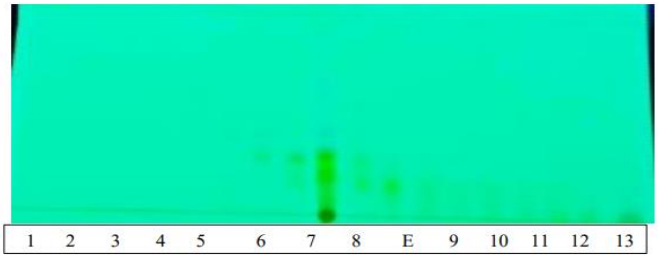

Figure 2. Results of UV light fractionation of Mangrove *R. apiculata* extract

Based on the identification results, it was found that the mangrove plants that will be used as samples are the Rhizopora apiculata type obtained in the Youtefa Bay Nature Tourism Park Area, Jayapura City. Mangrove leaves were chosen as samples because their tissue is simple and can produce flavonoids. The image (picture 4) flavonoids are one of the target compounds in this research. Mangrove nut leaf extraction uses the maceration method with 96% ethanol solvent. The advantages of maceration are that it is simple, not difficult, has little physical disturbance, high yield, and is more efficient in extracting extracts because reflux is assisted by heating 40-60°C (Saifudin, 2014).

Figure 3. Mangrove *R. Apiculata*

The Figure 3 drying stage of the Mangrove R. apiculata samples involves laying out the collected leaves to ensure they are thoroughly dried before extraction. This step is crucial as it reduces the moisture content in the leaves, which can otherwise interfere with the extraction process. Proper drying ensures that the bioactive compounds within the leaves remain stable and concentrated for the subsequent extraction steps (Widarta & Wiadnyani, 2019).

Figure 4. Mangrove *R. apiculata* samples Drying Stage

In the maceration extraction process (Figure 5), 100 grams of dried Mangrove R. apiculata leaves were placed in an Erlenmeyer flask containing 1 liter of 70% ethanol solvent. The flask was then placed in a shaker incubator for 24 hours at 30°C, with a shaking speed of 150 rpm. This process allows the ethanol to effectively penetrate the plant material and dissolve the bioactive

compounds. After 24 hours, the mixture was filtered using filter paper to separate the liquid extract from the solid plant material, resulting in a crude extract.

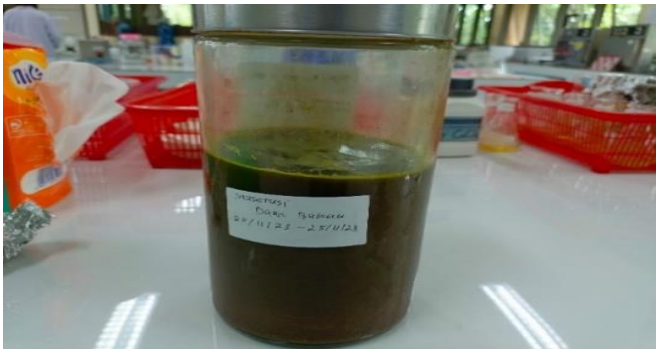

Figure 5. Maceration extraction of *R. apiculata* mangrove samples

The crude extract obtained from the maceration process was further processed using a rotary evaporator, as shown in the Soxhletation stage. This step involves evaporating the solvent under reduced pressure to concentrate the extract and remove any remaining ethanol. The use of a rotary evaporator allows for efficient solvent removal while preserving the integrity of the bioactive compounds. The final product (Figure 6) is a dry extract, which can be used for further analysis and testing.

Figure 6. Soxhletation stage of *R. apiculata* mangrove samples

Discussion

Thin Layer Chromatography is an analytical method to separate compounds based on their polarity (Hamka & Arief, 2022). In KLT there are two phases, namely the stationary phase which is a flat plane and a phase that will move or expand along the stationary phase. This phase is called the mobile phase and occurs due to the influence of capillaries (Pratiwi et al., 2023). The selection of the mobile phase can be done by reading various supporting literature or by using the trial and error method or trying it yourself (Gandjar & Rohman, 2007). In the TLC test, the stationary phase is silica gel with the eluent or mobile phase hexane-ethyl acetate (7:3).

The simplest method for carrying out this analysis is using a TLC plate (La et al., 2020). The advantage of this method compared to phytochemistry is that the data obtained is more reliable because it is able to describe the differences in metabolites precisely from the differences in stain characteristics on the TLC plate. Differences in Rf values and the color of each stain indicate different types of metabolites (Karthika & Paulsamy, 2015). In the results of fractions no. 1-6, no spots appeared on the TLC, possibly because it still used a solvent with a low polarity level, so there were no compounds that were attracted to fractions no. 1-6.

Antioxidant activity is expressed in inhibition concentration (IC50) values. This value means the minimum concentration needed to inhibit free radicals is 50%, so the smaller the IC50 value, the better the activity (Sibero et al., 2019). The antioxidant test results of R. apiculata leaf extract showed that the methanol extract provided the highest antioxidant activity with an IC50 value of 83,299 ± 0.1483 ppm, followed by ethyl acetate with an IC50 value of 116.69 ± 0.307 ppm and the weakest antioxidant activity was shown in the n-hexane extract with IC50 value 468.77 ± 0.117 ppm. Dwijayanti et al. (2023); Souhoka et al. (2019); Zulfajri (2019) stated that an IC50 value of 50-100 ppm is categorized as a strong antioxidant. Methanol extract has the strongest antioxidant capacity compared to ethyl acetate and n-hexane extracts. This is thought to be because the methanol solvent has high polarity so it is able to extract polar and non-polar bioactive compounds.

According to Ridlo et al. (2017), phenolic compounds will break the radical chain reaction and will donate their hydrogen atoms so that more stable free radicals are produced. Based on the results obtained, this study found that the methanol extract of R. apiculata leaves has the potential to be used as an antioxidant. Antioxidant activity is influenced by the presence of secondary metabolites contained in a sample (Wardani et al., 2020). Compounds from phenolic groups such as flavonoids, tannins and quinones are known as excellent antioxidant agents (Mutha et al., 2021; Sun & Shahrajabian, 2023). These results are in accordance with the report by Rumengan et al. (2021) which states that Rhizophora mangrove leaves contain various natural pigments that have the potential to act as antioxidants such as lutein, neoxanthin, violaxanthin, and -carotene. Moreover, carotene pigment is often found in the last stain with the largest Rf value on the TLC plate (Zeb & Murkovic, 2010).

Conclusion

Based on the results of UV and IR spectroscopy data, the isolation results from mangrove leaf extract Rhizophora sp. obtained from the waters of Youtefa Bay

contains several bioactive compound activities such as: alkaloid, flavonoid, phenolic and saponin bioactive compound groups in methanol solvent.

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Author's Contributions
Dirk Y.P. Runtuboi, wrote the introduction, methods, results, discussion and conclusion. Ervina Indrayani supervised and edited.

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Conflict of Interest
There is no conflict of interest in this writing.

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