

Extraction of bioactive compounds fruit from Rhizophora mucronata using sonication method

by Ernawati Ernawati

Submission date: 15-Nov-2021 06:56PM (UTC+0700)

Submission ID: 1703342779

File name: 15_2019_IOP_extraction_bioactive_compound_rhizophora.pdf (126.77K)

Word count: 2507

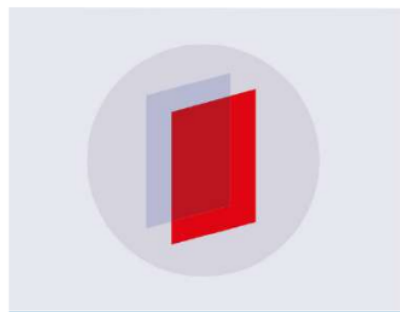
Character count: 14112

PAPER · OPEN ACCESS

8
Extraction of bioactive compounds fruit from *Rhizophora mucronata* using sonication method

4
To cite this article: Ernawati *et al* 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **236** 012122

View the [article online](#) for updates and enhancements.



IOP ebooks™

Bringing you innovative digital publishing with leading voices to create your essential collection of books in STEM research.

Start exploring the collection - download the first chapter of every title for free.

Extraction of bioactive compounds fruit from *Rhizophora mucronata* using sonication method

Ernawati^{1*}, E Suprayitno², Hardoko² and U Yanuhar²

¹Doctoral Program Faculty of Fisheries and Marine Science Brawijaya University Malang, Indonesia

¹Fishery Products Technology Study Program Faculty of Agriculture Yudharta University Pasuruan, Indonesia

²Faculty of Fisheries and Marine Science Brawijaya University Malang, Indonesia

*Corresponding author: ernawati_thpi@yudharta.ac.id

Abstract. Mangrove species *Rhizophora mucronata* fruit is currently widely used as a processed food ingredient. This aims of this research to determine the characteristics of fruit, phytochemical screening and rendement of extract in various solvents. Phytochemical research is qualitative descriptive using several reagents which include sample preparation, extraction, and phytochemical screening. From result of research got calculation of successive yield to extract methanol, ethyl acetate and n-hexane is 5,38%; 3.34% and 1.26%, The results of phytochemical screening of *Rhizophora mucronata* fruit extract on methanol solvent contain compounds: alkaloids, flavonoids, saponins, triterpenoids and tannins. In the ethyl acetate solvent contains compounds: alkaloids, triterpenoids, steroids and tannins. In the n-hexane solvent contain triterpenoid.

1. Introduction

Indonesia is a tropical country with abundant biodiversity. The mangrove forest is one of the tropical forests that store immense ecological benefits, biological, and economical. Mangrove contain bioactive compounds, such as alkaloids, carbohydrates, glycosides, tannins, proteins and amino acids, flavonoids, saponins, sterols, acidic compounds, resins, peroxid and polyuronoid [1]. Some of these compounds could potentially have anti-cancer activity, anti bacterial [2][3], anti-inflammatory [4], antidiabetes [5], antioxidant [6] and others. The bioactive compound is a component derived from natural ingredients and scientifically proven to have positive effects on health [7].

Particulars of bioactive components in food that affect physiologically to promote health, prevent and treat various diseases, known as phytochemicals. Foodstuffs that contain phytochemicals, in addition to its function as a source of nutrients, also called functional food.

Exploration of the active compound of marine biological materials still require a lot of research. Before the phytochemical screening is done, the materials must be extracted using a solvent extraction method and certain to get a phytochemical compounds from the cell wall material. Various studies on the extraction method of the *Rhizophora mucronata* have been conducted including extraction using maceration with methanol, ethanol, acetone [7]. Extraction methods of microwave assisted extraction with chloroform and acetone [8]. Sonication extraction method using ultrasonic waves with methanol, ethanol and acetone [9]. Results from several studies above found that the method of sonication is the best extraction method with the highest yield using methanol. Based on the description above, in the



mangrove exploration development business, is necessary to extract the phytochemical screening *Rhizophora mucronata*. Phytochemical screening was conducted to determine the content of bioactive compounds in *Rhizophora mucronata* that health benefits.

Sonication method is a method of maceration modified with the help of ultrasound (high-frequency signals, 20 – 50 kHz). Container containing the powder sample was placed in a container of ultrasonic and ultrasound. This is done to provide the mechanical stress on the cell to produce a cavity in the sample. Cell damage can lead to increased solubility of the compound in a solvent and increasing the extraction yield.

This study aims to determine the characteristics of fruit, phytochemical screening and rendement of extract in various solvents using sonication method.

2. Methodology

2.1. Materials and chemical reagents

Mangrove *Rhizophora mucronata* was collected from Kalianyar village, Bangil, Pasuruan, East Java, Indonesia. While the chemicals used were 96% methanol, n-hexane, ethyl acetate, ethanol, HCl, magnesium, H₂SO₄ and distilled water were purchased from CV. Makmur Sejati and all other chemical used in this study were of reagent grade.

2.2. Sample preparation

The experiment begins with a process of flouring *Rhizophora mucronata*. Process flouring this sample include: sorting fruit; stripping the skin of the fruit, soaking in fresh water and replaced the water until the water no longer change color; boiling for 15 minutes; drying using a low temperature in the range of between 50-70 °C to reach moisture content 10-11%; Willey flouring mill using milling tool with a sieve size of 60 mesh; When finished flour dried fruit back for 10 minutes at 70 °C, in order to completely dry and prevent the flour into sour. The flour fruit *Rhizophora mucronata* 50 g was then extracted with various solvent methanol 70% of 250 ml, ethyl acetate, n-hexane. Biomass mixture and the solvent was extracted by ultrasonic waves at a frequency of 50 kHz for 30 minutes. The extraction results filtered with filter paper to get rid of the waste in order to obtain filtrate and residue. Furthermore, the residue sonicated back with the same treatment up to 3x more repetitions in order to obtain the filtrate. Concentrated using a vacuum rotary evaporator temperature of 40 °C, 60 rpm, and a pressure of 200 mBar until no solvent drips.

2.3. Phytochemical assay

Active phytochemical test compound with a reagent test of the extract of methanol, ethyl acetate and n-hexane of *Rhizophora mucronata* test conducted on alkaloids, flavonoids, terpenoids, steroids, saponins and tannins. In order to screen the presence of secondary metabolites content from each extracts, the analysis of phytochemicals was conducted according to the previous study by Harborne [10] with slight modification.

The crude extract (0.5 mg) was dissolved in 5 mL distilled water then heated. Two mL of the extract then was added with 0.4 mL amyl alcohol containing 37 % HCl and 96 % ethanol, 0.5 mg magnesium, and 70 % alcohol. The presence of red or orange color indicated the flavonoid content. Subsequently, in order to identify the tannin content, 2 drops of 1 % FeCl₃ and H₂SO₄ was added in 2 mL extract. The presence of blackish green precipitate indicated the tannin content. Then, 2 mL extract was added with 0.5 mL of HCl 2% and the solution was divided into two tubes. First tube add 2-3 drops of reagent Dragendorff, second tube added 2-3 drops reagent Mayer. The presence orange color in the dragendorff reagent tube and the second tube yellowish precipitates showed alkaloids. Extract then added water (1: 1) while shaken for 1 minute, if the cause of foam is added HCl 1 N, the foam formed can last for 10 minutes with a height of 1-3 cm, then the positive extract contains saponins. Extract 2 mL was dissolved in 0.5 mL of chloroform, and then supplemented with 0.5 ml of acetic acid anhydride. This mixture is added with 1-2 mL of concentrated H₂SO₄ through the tube wall. If the results obtained in the form of brownish or violet ring on the border of two solvents showed

triterpenoids, whereas if it formed a bluish green color indicate the presence of steroids. Each phytochemical assay done 3 times repetition.

2.4. Ultraviolet visible spectroscopy (UV-Vis)

A spectrophotometer is the tool used to analyze both quantitative and qualitative compounds, by measuring transmittance or absorbance of a sample as a function of concentration. Determination qualitatively based on the peaks produced on the spectrum a certain element at a certain wavelength, while determining quantitatively based on the absorbance value generated from the spectrum [11]. Absorption spectrum in the areas of ultraviolet and visible light is composed of one or several of the absorption band. For spectrophotometry system, UV-Vis most widely available and most popular use. Ease of this method is it can be used both for color samples also for colorless samples such as organic compounds based on transition or and therefore require a chromophore in the molecule. This transition occurs in a region of the spectrum from 200-800 nm.

3. Results and discussion

3.1. The yield of extract

The results of the extract of *Rhizophora* fruit flour were obtained from the percentage of weight concentrated extract divided by the weight of the *Rhizophora* sample used. The resulting yield is different for each solvent extraction. This is influenced by many factors such as type of solvent, solvent concentration, time, temperature, pH, number of steps, liquid-to-solid ratio and particle size of the plant material [12].

In our study, we used sonication extraction methods with methanol, ethyl acetate, and n-hexane solvents. The purpose of using sonication extraction method to produce maximum extraction, due to cavitation process is the formation of micro bubbles that increased pressure due to ultrasonic waves. These bubbles are not stable so they break easily. The rupture of these bubbles involves a large amount of energy and helps the contact between the solvent and the ingredients in the extraction so that the extraction results are maximized. The mechanical effects of the sonication method can increase solvent penetration into the material cell and increase mass transfer so that the time needed for cell breakdown is only a few minutes [13]. From the results of the research, it was obtained that the yield of methanol, ethyl acetate and hexane extract was: 5.38%, 3.34%, 1.26%. The highest yield in methanol extract was 5.38%.

3.2. Screening of phytochemical contents from *Rhizophora mucronata*

Phytochemical qualitative test of *Rhizophora mucronata* methanol extract showed a positive response to alkaloids, flavonoids, saponins, triterpenoids and tannins. *Rhizophora mucronata* ethyl acetate extract showed a positive response to alkaloids, triterpenoids, steroids and tannins. n-hexane extract of *Rhizophora mucronata* showed a positive response to triterpenoids (Table 1).

Table 1. Results of analysis extract phytochemical screening flour *Rhizophora mucronata*.

Group Active Compounds	Extract		
	methanol	ethyl Acetate	n-hexane
Alkaloids			
- Mayer	+	+	-
- Dragendorff	+	+	-
Flavonoids	+	-	-
Saponin	+	-	-
Triterpenoids	+++	++	+
steroids	-	+	-
Tanin			
- FeCl3	++	+	-

- gelatin + - -

Information:
 (+) = Positive containing compound
 (-) = do not contain compounds

Chemically, saponins have extensive structural diversification, and certain saponin compounds with their surfactant properties can cause cell wall lysis. Tannins are generally defined as polyphenol compounds which have a fairly high molecular weight (more than 1000) and can form complexes with proteins. Tannins have biological activity as antioxidants, so tannins will have an effect on antioxidant activity. The secondary metabolite group owned by *Rhizophora mucronata* has the potential to have a variety of biological activities, such as anticancer, antiproliferation, antioxidant or antibacterial.

3.3. UV-Vis result

UV-VIS spectroscopy is a very useful technique for extract analysis. UV-Vis analysis in Figure 1 shows the UV-VIS spectrum obtained in *Rhizophora mucronata* extract using methanol solvent (figure 1A), ethyl acetate (figure 1B), and n hexane (figure 1C). Figure 1A identifies the maximum wavelength at 204 nm with an absorbance of 3,371. the results shown in the figure 1B identifies the maximum wavelength in the range 237-288 nm with absorbance of 10 and figure 1C showed in a maximum wavelength of 228 nm with an absorbance of 3.73.

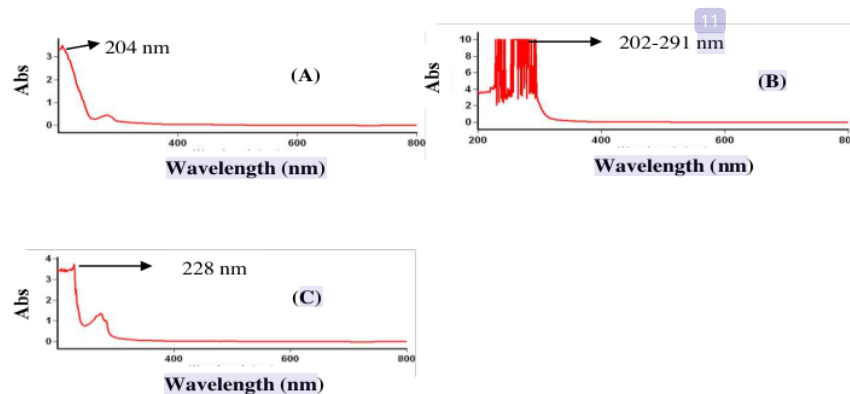


Figure 1. UV-Vis spectra of the methanol (A), ethyl acetate (B) and n-hexane (C) extract of *Rhizophora mucronata*.

In the UV-VIS spectrum, the appearance of one or more peaks in this region from 200 to 400 nm is an indication of the presence of unsaturated groups and heteroatoms such as S, N, O. The peak of the hydroalkoolic extract is characterized by absorption in the UV region of 250-450 nm, according to phenolic acids and their derivatives (flavones, flavonols, phenylpropene, quinon). At 280 nm it was identified specific wavelengths for phenolic acids and between 300-350 found flavonoids, quinines, coumarin [14].

The use of UV-visible spectrophotometry in complex media analysis is limited by the inherent difficulties in assigning absorption peaks to certain constituents in the system. So, UV-VIS findings must be equipped with a number of other analytical techniques such as GC/MS, LC/MS etc., to enable proper extraction of constituent characterization and identification.

4. Conclusion

The results showed that the highest yield was at 5.38% methanol extracts using high-frequency ultrasound with 20 kHz. Phytochemical screening of *Rhizophora mucronata* methanol extract contains

compounds: alkaloids, flavonoids, saponins, triterpenoids and tannins. Phytochemical screening of ethyl acetate extract contains compounds: alkaloids, triterpenoids, steroids and tannins. Phytochemical screening of n-hexane extract contains triterpenoids. Extract compounds were characterized using UV-VIS showed the existence of phenolic acids and their derivatives (flavones, flavonols, phenylpropene, quinon at 250-450 nm.

5. References

- [1] Kumar S, Abhay K P 2013 *The Scient. World J.* 1-16
- [2] Behbahani B A, Farideh T Y, Fakhri S, Hamid N, Alireza V, Ali A 2018 *J. Microb. Pathogen.* **114** 225-232
- [3] Saad S, Muhammad T, Deny S, Haitham Q, Anis F I 2012 *Asian Pac. J. Tropic. Biomedic.* 427-429
- [4] Chakraborty K, Vamshi K R 2016 *J. Phytochem.* **XXX** 1-9
- [5] Hardoko, Eddy S, Puspitasari, Amalia 2016 *Int. Food Res. J.*, **22(3)** 953-959
- [6] Ravikumar S, Gnanadesigan M 2012 *J. Exp. Clinic. Medic.* **4(1)** 66-72
- [7] Joel E L, Valentin B 2010 *Asian Pac. J. Tropic. Medic.* 602-604
- [8] Cahyani, Maulidya D, Novidayasa 2016 *Ekstraksi Zat Warna Alami dari Kayu Bakau Rhizophora mucronata Dengan Metode Microwave Assisted Extraction* Undergraduate Theses (Surabaya: Institut Teknologi Sepuluh Nopember)
- [9] Giacometti J, Gordana Z, Marta Z 2018 *Food J.* MDPI.
- [10] Harborne 1984 *Phytochemical Methods, A Guide to Modern Techniques of Plant Analysis* 2nd edition (USA: Chapman and Hall)
- [11] Perkampus H H 1992 *UV-VIS Spectroscopy and Its Applications* Springer Laboratory Translated by Grinter H C and Threlfall T L
- [12] Tan M C, Tan C P, Ho C W 2013 *Int. Food Res. J.* **20(6)** 3117-3123
- [13] Ashley K, Andrews R N, Cavazos L, Demange M 2001 *J. Analytic. Atomic Spectro.* **16** 1147-1153
- [14] Zhu H, Wang Y, Liu Y, Xia T, Tang Y 2010 *Food Anal. Method.* **3** 90-97

Acknowledgment

The authors would like to thank those who have assisted in the research especially Materia Medika laboratory Batu, Fishery and Marine Science Faculty of Brawijaya University, has provided excellent facilities and services during this research.

Extraction of bioactive compounds fruit from *Rhizophora mucronata* using sonication method

ORIGINALITY REPORT

14%

SIMILARITY INDEX

7%

INTERNET SOURCES

12%

PUBLICATIONS

%

STUDENT PAPERS

PRIMARY SOURCES

1	eprints.itn.ac.id Internet Source	2%
2	Abdullah, Angelina, Mauhibah Yumna, Rita Arbianti, Tania Surya Utami, Heri Hermansyah, Sri Ningsih. " Flavonoid isolation and identification of mother-in-law's tongue leaves () and the inhibitory activities to xanthine oxidase enzyme ", E3S Web of Conferences, 2018 Publication	2%
3	ifrj.upm.edu.my Internet Source	2%
4	repository.petra.ac.id Internet Source	2%
5	M F Zikri, A Tibrizi, Y Marsino, T S Utami, R Arbianti, H Hermansyah. "The Effect of NADES Ratio Solvent and Sonication Time in Extraction of Papaya Sap for Bio-insecticide Production", E3S Web of Conferences, 2018 Publication	1%

6	Solomon Durodola, Abolanle Adekunle, Lukman Olasunkanmi, John Oyekunle. "Inhibition of Mild Steel Corrosion in Acidic Medium by Extract of Spilanthes uliginosa Leaves", <i>Electroanalysis</i> , 2020 Publication	1 %
7	I M Widiastuti, A M S Hertika, M Musa, D Arfiati. "Acute toxicity test and LC50 value of mercury on tubifex tubifex", <i>Journal of Physics: Conference Series</i> , 2019 Publication	1 %
8	sinta.ristekbrin.go.id Internet Source	1 %
9	Iqbal, Z.. "Anthelmintic activity of <i>Calotropis procera</i> (Ait.) Ait. F. flowers in sheep", <i>Journal of Ethnopharmacology</i> , 20051114 Publication	1 %
10	hal.archives-ouvertes.fr Internet Source	<1 %
11	Jonathan R. Tischler, M. Scott Bradley, Vladimir Bulović, Jung Hoon Song, Arto Nurmikko. "Strong Coupling in a Microcavity LED", <i>Physical Review Letters</i> , 2005 Publication	<1 %

Exclude bibliography On

Extraction of bioactive compounds fruit from *Rhizophora mucronata* using sonication method

GRADEMARK REPORT

FINAL GRADE

/0

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6
