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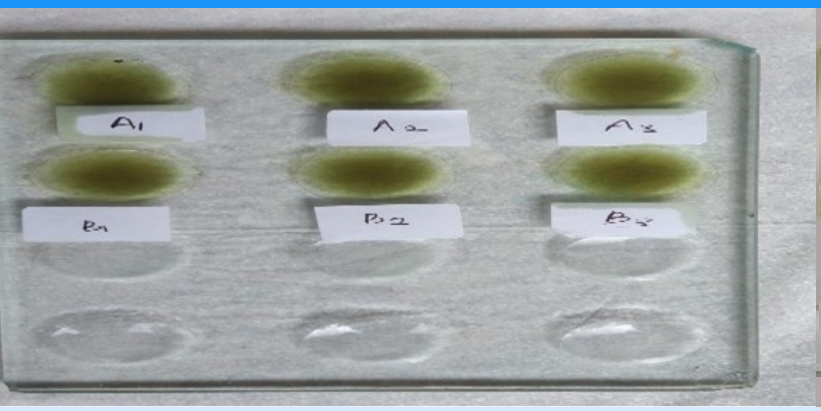

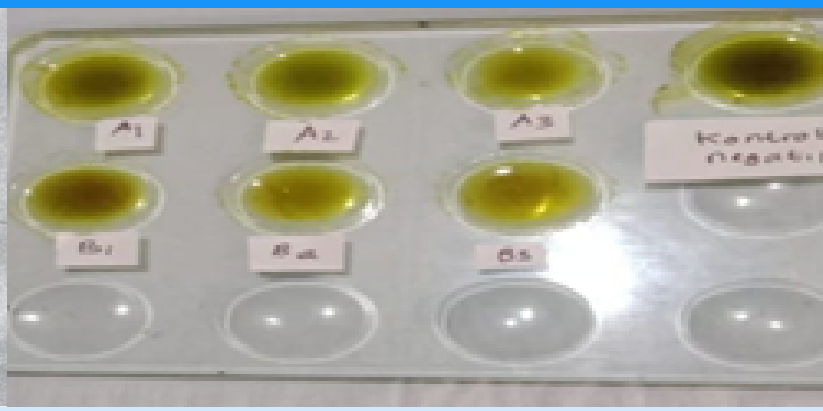

Qualitative analysis of the active flavonoid content of Sungkai leaves
(Jaya and Hasnah, p.23)

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Karakterisasi Senyawa Non-Polar dari Makroalga *Padina minor* Asal Perairan Singkawang dan Aktivitas Antibakteri terhadap *Staphylococcus aureus* dan *Escherichia coli*

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ABSTRACT

Characterization of nonpolar organic compounds of macroalga Padina minor from Singkawang waters and activity against Staphylococcus aureus and Escherichia coli bacteria has been carried out. The stages of this research consisted of maceration, fractionation, phytochemical tests, characterization using FTIR and GC-MS, as well as testing the antibacterial activity with the well-diffusion method. The fractionation process produces n-hexane, dichloromethane, and methanol fractions. The n-hexane fraction was selected for packaging using gradient n-hexane: dichloromethane eluent and yielded 11 combined fractions (F_1RW_1-F_1RW_{11}). The combined fraction F_1RW_2 was separated again using n-hexane: dichloromethane eluent in a gradient manner and 18 combined fractions (F_2RW_1-F_2RW_{18}) were obtained. Then the F_2RW_4 isolate was characterized for GC-MS characterization and the F_2RW_3 isolate was characterized using FTIR. The FTIR interpretation results show the absorption of hydroxyl groups O-H (345.65 cm^{-1}), C=O (1743.65 cm^{-1}), C=C bonds (1633.71 cm^{-1}), C-H (2926.01; 2854.01, 1438.9 and 723.31 cm^{-1}), and C-O-C bonds (1170.79 cm^{-1}). The results of the GC-MS analysis showed that the F_2RW_4 fraction had 2 main compounds, namely methyl decanoate and 11-methyl octadecenoate. The results of the antibacterial activity test showed that the methanol extract and n-hexane fraction of Padina minor were inactive against the inhibiting bacteria Staphylococcus aureus and Escherichia coli.

Keywords: *Padina minor, FTIR, GC-MS, Antibacterial*

ABSTRAK

Telah dilakukan karakterisasi senyawa organik non-polar dari makroalga Padina minor asal kota Singkawang dan aktivitas terhadap bakteri Staphylococcus aureus dan Escherichia coli. Tahap penelitian ini terdiri atas maserasi, fraksinasi, uji fitokimia, karakterisasi menggunakan FTIR dan GC-MS, dan uji aktivitas antibakteri dengan metode difusi sumuran. Proses fraksinasi menghasilkan fraksi n-heksana, diklorometana, dan metanol. Fraksi n-heksana dipilih untuk dilakukan pemisahan menggunakan eluen n-heksana: diklorometana secara bergradien dan menghasilkan 11 fraksi gabungan (F_1RW_1-F_1RW_{11}). Fraksi gabungan F_1RW_2 dipisahkan kembali menggunakan eluen n-heksana: diklorometana secara bergradien dan diperoleh 18 fraksi gabungan (F_2RW_1-F_2RW_{18}). Isolat F_2RW_4 dilanjutkan untuk karakterisasi GC-MS sedangkan Isolat

F_2RW_3 dikarakterisasi menggunakan FTIR. Hasil interpretasi FTIR menunjukkan adanya serapan gugus hidroksil O-H (345.65 cm^{-1}), C=O (1743.65 cm^{-1}), ikatan C=C (1633.71 cm^{-1}), C-H (2926.01; 2854.01, 1438.9 dan 723.31 cm^{-1}), dan C-O-C (1170.79 cm^{-1}). Hasil Analisis GC-MS menunjukkan bahwa fraksi F_2RW_4 memiliki 2 senyawa utama yaitu metil dekanoat dan 11-metil oktadekenoat. Hasil uji aktivitas antibakteri menunjukkan bahwa ekstrak metanol dan fraksi n-heksana Padina minor bersifat tidak aktif dalam menghambat bakteri Sthapylococcus aureus dan Escherichia coli.

***Keyword**: Padina minor, FTIR, GC-MS, Antibakteri*

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1. INTRODUCTION

Macroalgae is a marine biota that contains nutrients such as protein, fat, carbohydrates, and fiber. In addition, seaweed contains secondary metabolites in the form of compounds from alkaloids, flavonoids, phenols, hydroquinones, and tannins (Safia, *et al*, 2020). Macroalgae or seaweed are generally used as food for people living on the coast (Suparmi and Sahri, 2009). Along with the development of technology, seaweed is widely used as a raw material for food, cosmetics and medicines, and others (Alam, 2015).

Seaweed is classified according to the pigments it contains with attractive colors. Seaweed can be grouped into three, namely green seaweed (*Chlorophyta*), red seaweed (*Rhodophyta*), and brown seaweed (*Phaeophyta*). One type of seaweed that is used is brown seaweed (*Phaeophyta)*. Brown seaweed (*Phaeophyta*) is a type of seaweed that can be found in areas with minimal sunlight (Firdaus, 2019).

Padina minor is a type of brown seaweed (*Phaeophyta*). Padina minor has secondary metabolite compounds in the form of alkaloids, saponins, steroids, and triterpenoids (Diachanty, *et al*, 2017). Research conducted by Triastinurmiatiningsih dan Haryani (2008) stated that methanol extract from Padina minor did not show any antibacterial activity against *Escherichia coli*. The methanol extract from *Padina minor* showed antibacterial activity against *Aeromonas hydrophila* and *Vibrio harveyi* (Natrah, *et al*, 2015). The *Padina australis* and *Padina L. nidifica* showed that there was antibacterial activity from Padina australis and *Padina L. nidifica* extracts which could inhibit the growth of *Escherichia coli* and *Staphylococcus aureus* (Yulheriwarni, *et al*, 2016).

The potential antibacterial activity possessed by brown seaweed (*Phaeophyta*) can be developed as a source of antibacterial raw materials. According to Menurut Radji (2011), many Indonesian people suffer from infectious diseases caused by bacteria, including *Escherichia coli* and *Staphylococcus aureus* bacteria. *Escherichia coli* bacteria are present in the digestive tract which can cause diarrhea and urinary tract infections (Rahayu, *et al*, 2018). *Staphylococcus aureus* bacteria can cause skin problems in children and adults, for example, infections of hair follicles, boils, and infections of wounds (Arfani, 2021). Both of these bacteria are normal flora that can be found in the human body, but if the presence of bacteria outside of their original habitat develops excessively can cause disease in humans (Jawetz, *et al*, 2001).

P. minor macroalgae can be found in Singkawang City. In addition, Padina minor is underused by the community and is mostly found on the beach, where some of the water is carried by the waves. In addition, research on the components of organic compounds and antibacterial activity of macroalgae Padina

minor from Singkawang City has not been found.

2. MATERIALS AND METHODS

2.1. Materials

The materials used in this study were *Padina minor* macroalgae, agar, aquades (H_2O), hydrochloric acid (HCl), *S. aureus* and *E. coli* bacteria, barium (II) chloride ($BaCl_2$), technical dichloromethane, technical methanol, technical *n*-hexane, sodium chloride (NaCl), peptone, ferrous (III) chloride reagent, Dragendorff reagent, Liberman-Burchard reagent, Mayer reagent, cerium sulfate reagent, Wagner reagent, magnesium powder, tetracycline, and yeast extract. The tools used in this study were aluminum foil, aerator, autoclave, stir bar, spray bottle, vial, petri dish, glass funnel, separating funnel, Erlenmeyer, measuring cup, beaker glass, hot plate, Fourier Transform Infra-red instrument. Shimadzu, Gas Chromatography and Mass Spectroscopy instrument GCMS-QP2010S Shimadzu, caliper, loop needle, cotton, column, laminar flow, micropipette, drop pipette, volume pipette, TLC plate, rotary evaporator DLAB RE100-pro, silica gel 60 (70 -230 mesh), spatula, test tube, maceration container, and wrapping.

2.2. Methods

Sample Preparation and Extraction

Samples of *P. minor* seaweed were washed clean and dried, then the samples were mashed. Samples that have been mashed extracted by maceration method of as much as 800 grams. Then immersed in methanol solvent for 6 x 24 hours. The macerate obtained is filtered using filter paper and evaporated. Then the methanol extract was weighed and the yield was determined (Marzuki, *et al* ,2020).

$$^0/_0\, Yield = \frac{Extract\ Weight}{Dry\ Sampel\ Weight} \times 100\%$$

Fractionation

Fractionation was carried out by partitioning using methanol, dichloromethane, and *n*-hexane. The methanol extract was dissolved and put into a separatory funnel, and *n*-hexane solvent was added, then shaken. The mixture was allowed to stand until two layers were formed, namely the methanol fraction and the n-hexane fraction. Furthermore, the *n*-hexane fraction which is located at the top is separated and accommodated into the container. Then the fractionation was continued with dichloromethane solvent into a separatory funnel and shaken. The obtained methanol, *n*-hexane, and dichloromethane fractions were concentrated using a rotary evaporator. The yield of the fraction obtained is calculated using the following equation (Rudiansyah, *et al*,2018).

$$^0/_0\,\text{Yield} = \frac{Fraction\ Weight}{Methanol\ Extract\ Weight} \times 100\%$$

Phytochemical Test

Phytochemical tests were carried out to identify groups of alkaloids, flavonoids, phenolic compounds, saponins, terpenoids, and steroids in samples with changes in color and precipitate. Identification of secondary metabolite content was carried out on the following compounds (Harbone,1987).

Flavonoids

As many as 3-5 drops of the extract and fraction solution were dripped onto the drip plate and a little magnesium powder was added, then 3-5 drops of HCl solution were added. The presence of flavonoids was indicated by the formation of orange to red color.

Steroids/Terpenoids

As many as 3-5 drops of extract and fraction solution were dripped into the drop plate and added with Liberman-Buchard reagent qualitatively. The presence of steroids is indicated by the formation of a green or blue color in the solution. While the presence of terpenoids is indicated by the formation of orange-red color.

Alkaloids

As many as 3-5 drops of extract and fraction solution were put into the drip plate, then added Dragendroff reagent, Mayer reagent, and Wagner react. The presence of alkaloids using the Dragendroff reagent is indicated by the formation of an orange to browns color, the Mayer reagent is indicated by a color change with a white precipitate, whereas using the Wagner reagent is characterized by a brown-to-yellow color change.

Saponins

The sample solution and fractions were put into a test tube then added with distilled water and shaken. The presence of saponin compounds was indicated by the formation of stable 1-10 cm high foam in no less than 10 minutes.

Phenolic

A total of 3-5 drops of sample solution and fractions were put into the drip plate and added qualitatively 1% $FeCl_3$ solution. The presence of phenolic compounds was indicated by the formation of dark blue, green to black colors.

Thin Layer Chromatography (TLC)

Extracts and fractions resulting from maceration and partitioning were followed by the identification of secondary metabolite spot patterns using the TLC method. The extract and each fraction were spotted on the TLC plate at a distance of 1 cm from the bottom using a capillary tube. Then eluted using the mobile phase in the form of 100% n-hexane and n-hexane: dichloromethane (9:1, 8:2, and 6:4). The stains formed were then observed under UV light with a wavelength of 254 and 366 nm, then sprayed using cerium sulfate and heated on a hotplate (Forestryana dan Arnida, 2020).

Flash Column Chromatography (FCC)

The packing of the chromatography column uses the wet method, in which silica gel (70-230 mesh) is soaked and occasionally stirred with *n*-hexane for 1 hour, then packed in the column. A total of 1.501 grams of the n-hexane fraction was dissolved and impregnated onto silica gel 60 (70-230 mesh). Then the impreg silica was added to the packed column and eluted using the eluent composition n-hexane: dichloromethane in a gradient manner. The elution results were collected as much as 5 mL in a vial, and TLC was carried out and sprayed with cerium sulfate as a stain remover and heated. Stains that have the same pattern were combined and TLC was repeated. KKT has carried out 2 repetitions (Sayekti, *et al*, 2013).

Activity Test Against Bacteria

Activity test against bacteria was carried out on methanol extract and *n*-hexane fraction. This research refers to research conducted by (Barrow dan Feltham, 1993; Kurniawati, *et al*, 2017) with the following stages.

Concentration Variation Manufacturing

P. minor macroalgae methanol extract and *n*-hexane fraction sample solutions were made with several concentration variations, namely 50 µg/µL, 25 µg/µL, 12.5 µg/µL, and 6.25 µg/µL. The positive controls used were tetracycline 1 µg/µL, methanol, and *n*-hexane as negative controls.

Tool Sterilization

Tool sterilization can be done by washing the equipment that will be used to test the antibacterial activity of the *P. minor* macroalgae extract, then drying it and wrapping it in the paper. Furthermore, it was put into the autoclave at 121˚C.

Rejuvenation of Pure Bacterial Cultures

Rejuvenation of pure bacterial cultures was carried out with nutrient agar (NA) (0.5 g peptone, 0.5 g NaCl, 0.2 g yeast, and 2 g agar) dissolved in 100 mL distilled water and heated. Then sterilized in an autoclave at 121˚C. Pure cultures of *S. aureus* and *E. coli* bacteria were taken as much as 1 ose and streaked on the agar medium. Then incubated at 37˚C for 18-24 hours.

Preparation of Bacterial Test Suspension

Bacterial inoculum using nutrient broth (NB) (0.5 gr peptone, 0.5 gr NaCl, 0.2 gr yeast) then dissolved in 100 mL of distilled water and heated. Then sterilized in an autoclave at 121˚C. Bacterial cultures of *S. aureus* and *E. coli* were taken as much as 1 ose and spread in nutrient broth. Then in the shaker for 24 hours.

Activity Testing Testing

The activity of methanol extract and n-hexane fraction of *P. minor* on the growth of *S. aureus* and *E. coli* bacteria was carried out using the well diffusion method. Nutrient agar (NA) (1 gram of peptone, 1 gram of NaCl, 0.4 grams of yeast, and 4 grams of agar) is dissolved in 200 mL and heated on a hotplate while stirring. Then it was sterilized in an autoclave at 121˚C and poured into a cup of as much as 20 mL and allowed to solidify. Then 200 µL of bacterial suspension was added to NA and wells were made. As much as 20 µL of test solution, positive and negative controls were put into the well. Then incubated at room temperature for 24 hours. The diameter of the inhibition zone formed was measured using a caliper.

3. RESULTS AND DISCUSSION

3.1. Maceration

Maceration was carried out by soaking for 6 x 24 hours with methanol solvent at room temperature. Methanol viscous extract in the form of a green-black paste with a mass of 15.104 grams with a yield of 1.88%. The resulting yield value indicates that the secondary metabolite content contained is small.

3.2. Fractionation

The methanol extract 9,8088 gr was then partitioned in stages starting from a nonpolar solvent, namely n-hexane, to a semipolar solvent, namely dichloromethane. The results of the brown seaweed fractionation P. minor can be seen in (Table 1).

3.3. Phytochemical Test

The extracts and fractions obtained from P. minor species were subjected to phytochemical tests aimed at identifying the contents of terpenoids, steroids, alkaloids, flavonoids, phenolics, and saponins. The test results for the methanol extract, n-hexane fraction, dichloromethane fraction, and methanol fraction from P. minor can be seen in Table 2 and Figure 1.

Table 1. Fractionation Results of Macroalgae *P. minor*

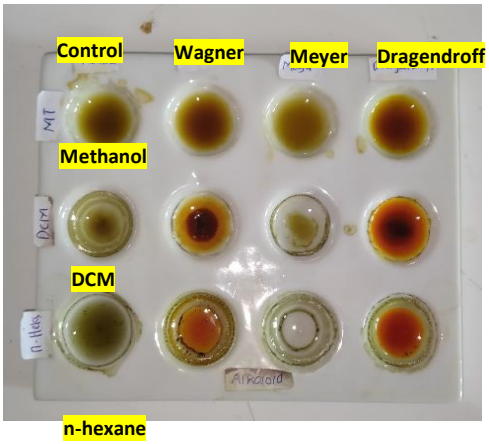
Fraction	Fraction Weight (grams)	Yield (%)
n-hexane	2,209	22,53
Dichloromethane	0,918	9,36

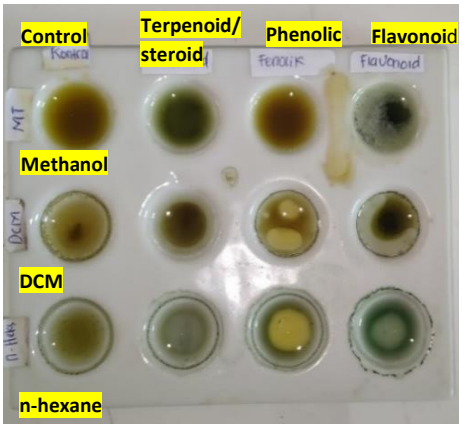
Methanol	3,072	31,32

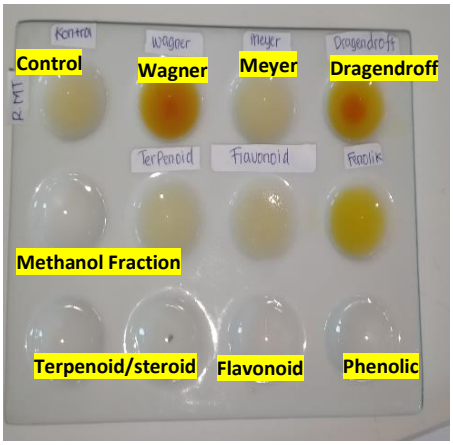
Table 2. Phytochemical Test Result of Macroalgae *P. minor*

Compound	Reagent	Test Result			
		Extract of methanol	Fractions of *n*-hexane	Fractions of dichloromethane	Fractions of methanol
Alkaloids	Dragendroff	++	+++	++	++
	Mayer	-	-	-	-
	Wagner	-	++	++	++
Phenolic	$FeCl_3$	-	-	-	-
Terpenoids/ Steroids	Lieberman-Burchard	+++	++	+++	+
Flavonoids	Mg powder + HCl	+	-	++	-
Saponins	Aquades	+++	+	+++	++

Information: +++ : Strong ++ : Moderate + : Weak - : None







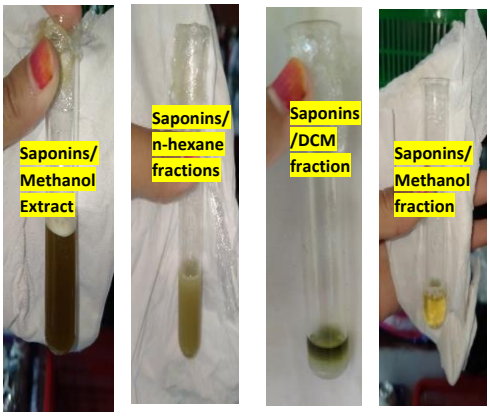


Figure 1. Results of Phytochemical Test

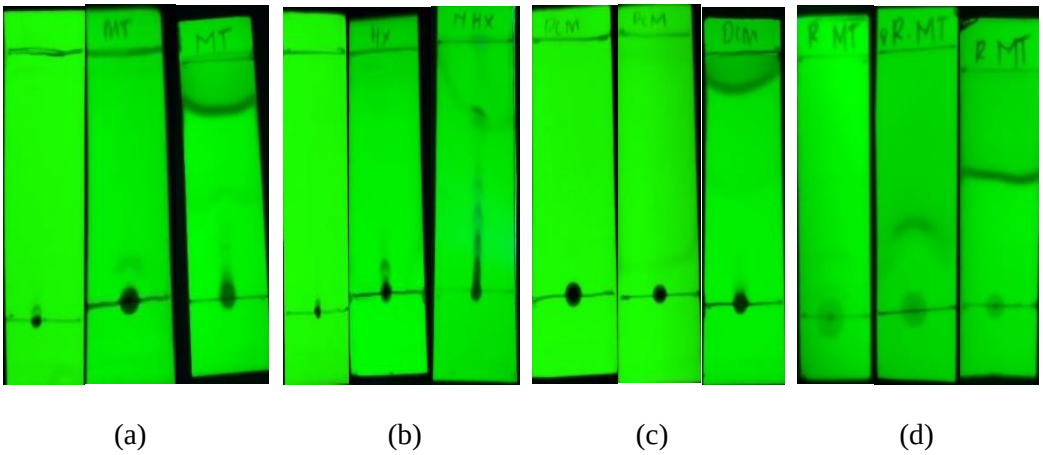
The terpenoid test is orange and the steroid test produces a blue or green color when tested using the Lieberman-Burchard reagent. This is because terpenoid and steroid compounds have the ability to form colors by sulfuric acid in acetic anhydrous solvents, while the color differences produced between terpenoids and steroids are due to differences in groups on C-4 (Marlina and Saleh, 2011). The alkaloid test was identified using Dragendroff reagent to form an orange-to-brown color, tested with Wagner's reagent to form a brown-to-yellow color change, and used Mayer to form a white precipitate. In the alkaloid test, there was a change of ligand, nitrogen which has a lone electron pair (PEB) in the alkaloid formed a covalent coordination bond with K^+ ions originating from potassium tetraiodobismutate to produce a potassium-alkaloid complex which could precipitate (Haryati, *et al*, 2015).

The flavonoid test forms an orange-to-red color when tested using mg powder and concentrated HCl. The mg metal functions to attract the carbonyl groups to bond with each other, while the use of hydrochloric acid aims to reduce the αα-benzopyrene nucleus present in the flavonoid structure and form orange or red flavillum salts (Afriani, *et al*, 2016). The phenolic test will form a color change to dark blue, and green to black when tested using the $FeCl_3$ reagent, this is because FeCl3 reacts with aromatic -OH groups and forms complex compounds with Fe^{3+} ions (Haryati,*et al*,2015). Saponin uses distilled water and is shaken with the marked formation of constant foam because saponins have glycosyl as a polar group and a steroid or triterpenoid group as a nonpolar group so that they are surface active and form micelles when reacting with water (Sangi, *et al*, 2008).

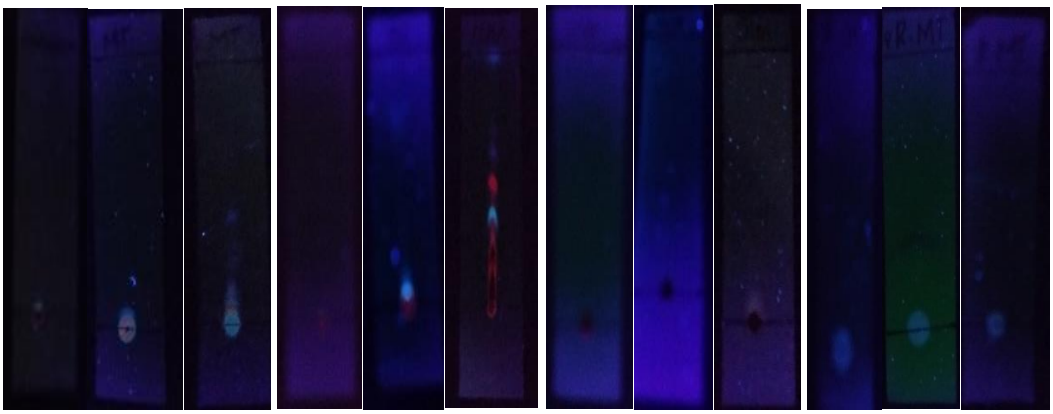
(Table 2.) shows that *P. minor* contains secondary metabolites in the form of alkaloids, terpenoids, steroids, flavonoids, and saponins. This is following the results of research by Diachanty *et al* (2017) that *P. minor* has secondary metabolite compounds in the form of alkaloids, saponins, steroids, and triterpenoids originating from the Seribu Islands Waters. These compounds are dispersed in the extract and its fractions. Methanol extract, *n*-hexane fraction, dichloromethane fraction, and positive methanol fraction contained alkaloids, terpenoids, steroids, flavonoids, and saponins. However, the n-hexane and methanol fractions did not contain flavonoids.

3.4. Thin Layer Chromatography (TLC)

Extracts and fractions that have been obtained are then carried out by TLC using eluent as the mobile phase and polar silica as the stationary phase. The methanol extract, n-hexane fraction, dichloromethane fraction, and methanol fraction were analyzed by TLC using various eluents namely n-hexane 100% and n-hexane: dichloromethane (9:1, 8:2, and 6:4). The results obtained at the TLC orientation that had the best separation were eluents with a ratio of n-hexane: dichloromethane (6:4) in the n-hexane fraction (Figure 2).



(a) (b) (c) (d)

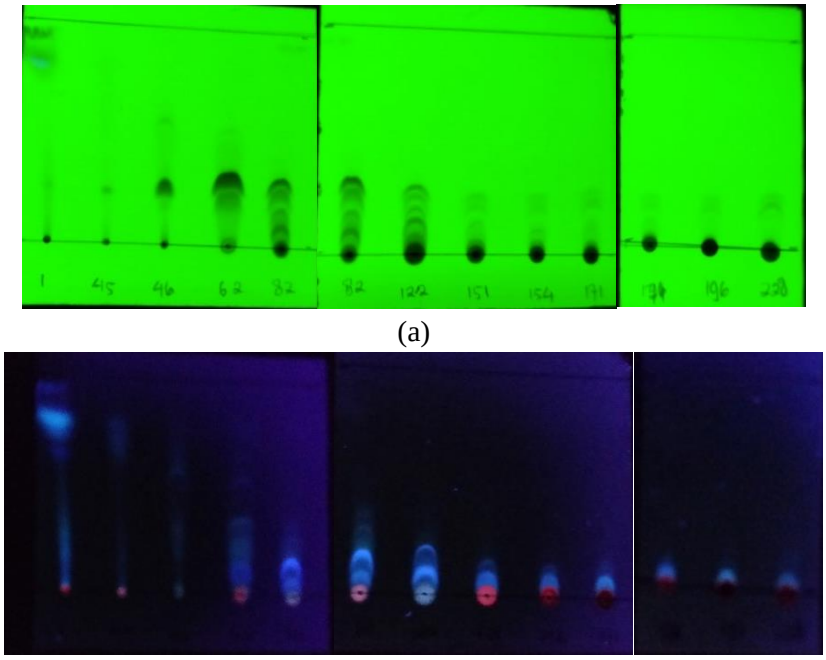


(a) (b) (c) (d)

Figure 2. Eluent Search Chromatogram Profile at UV 254 and 366 nm
(a) Eluent methanol Extract (*n*-hexane 100%, *n*-hexane: dichloromethane (8:2 and 6:4))
(b) Eluent *n*-hexane fraction (*n*-hexane 100%, *n*-hexane: dichloromethane (8:2 and 6:4))
(c) Eluent dichloromethane fraction (*n*-hexane 100%, *n*-hexane: dichloromethane (8:2 and 6:4))
(d) Eluent methanol fraction (*n*-hexane 100%, *n*-hexane: dichloromethane (8:2 and 6:4))

3.5. Flash Column Chromatography (FCC)

The n-hexane fraction was separated on a column using a silica gel 60 stationary phase (70-230 mesh). A total of 1.709 grams of the n-hexane fraction was impregnated, then put into an FCC column which had been packed wetly, where the silica was soaked using n-hexane for 1 hour. Summit was conducted to see the complexity of the compounds in each fraction. This aims to determine the fraction that will proceed to the purification stage. Fractions having the same stain pattern were combined to obtain 11 combined fractions (F_1RW_1-F_1RW_{11}) (Figure 3).

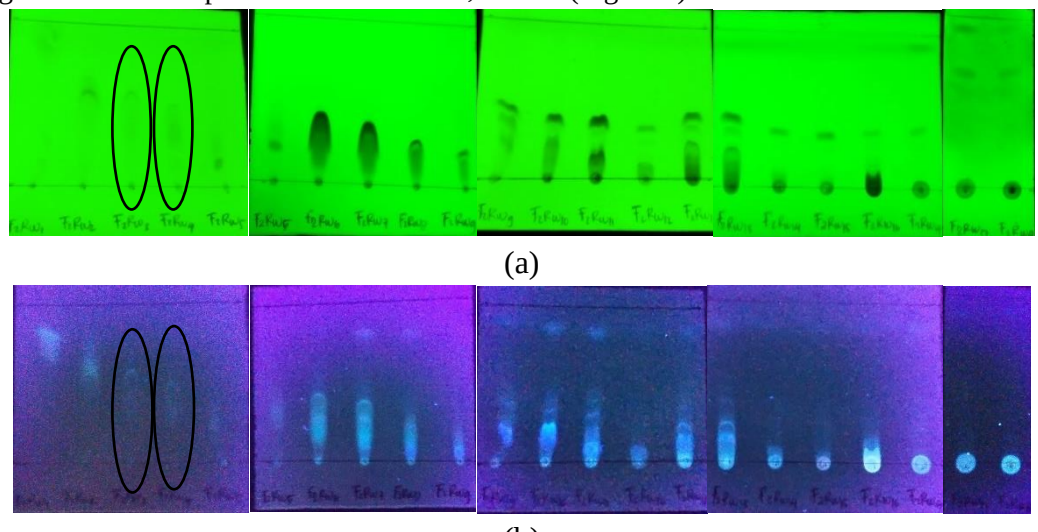


(a)

(b)

Figure 3. First FCC Combined Chromatogram Profile at UV (a) 254 nm (b) 366 nm

Based on the results of the combined TLC, the F_1RW_2-F_1RW_5 were recombined and again separated using a second column of pressure chromatography (FCC). The F_1RW_2-F_1RW_5 fraction has physical characteristics that tend to be the same, namely a yellow color like oil in the fraction. The second FCC fraction having the same stain pattern was combined, resulting in 18 combined fractions (F_2RW_1-F_2RW_{18}), and the combined TLC was performed using n-hexane: dichloromethane as eluent. The combined TLC profile shows a single spot for F_2RW_4 as the target isolate. F_2RW_4 isolates were analyzed by GC-MS and F_2RW_3 isolates were analyzed by FTIR (Figure 4).



(a)

(b)

Figure 4. Second FCC Combined Chromatogram Profile at UV (a) 254 nm (b) 366 nm

3.6. Isolate Analysis

FTIR analysis of Isolate F_2RW_3

Interpretation of the FTIR spectrum (Figure 5) of isolate F_2RW_3 shows the absorption of the hydroxyl functional group -OH at 3450.65 cm^{-1}, carbonyl group C=O at 1743.65 cm^{-1}), C=C bond (1633.71 cm^{-1}), alkane bond C-H (2926.01 cm^{-1}, 2854.01 cm-and 723.31 cm^{-1}), C-OH bonds (1438.9 cm^{-1}), C-O bonds (1026.49 cm^{-1}), C-O-C bonds (1170.79 cm^{-1}). The absorption by functional groups from the F_2RW_3 isolate can be seen in Table 3 and compared with references. Based on the results of this interpretation it indicates the presence of ester compounds in isolate F_2RW_3.

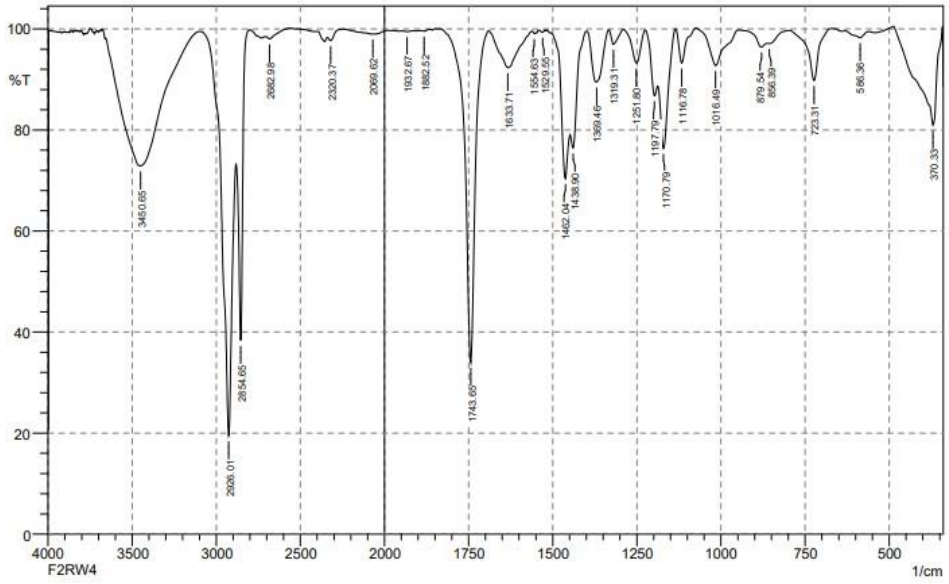


Figure 5. FTIR Spectrum of F_2RW_3 Isolate

Table 3. Interpretation of the FTIR Spectrum Result from $F_2RW_{3\ Isolate}$

Fraction Absorption F_2RW_3	Reference Lutfia, *et al*, 2020	Functional Groups
3450.65	3421	Hydroxyl (-OH)
2926.01; 2854.01	2935	Alkyl (C-H)
1743.65		Carbonyl (C=O)
1633.71	1630	Bond (C=C)
1438.9	1420	Alkyl (C-H)
1170.79		Bond (C-O-C)
1026.49	1036	Bond (C-O)
723.31		Alkyl (C-H)

GC-MS analysis of F_2RW_4 Isolate

The results of the GC-MS analysis (Figure 6) on isolate F_2RW_4 showed that there were 6 components of organic compounds (Table 4) and two of them were the main components of the ester group with a similarity index above 90%. Ester compounds obtained on GC-MS have a correlation with the functional groups produced on the FTIR instrument, namely the presence of ester groups.

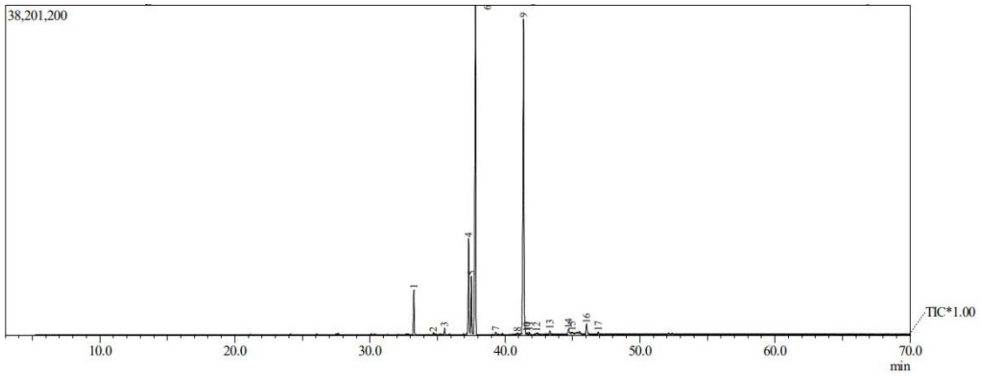


Figure 6. GC Chromatogram of F_2RW_4 Isolate

Table 4. Interpretation of GC-MS Spectrum Result of F_2RW_4 Isolate

No.	Retention Time	Compounds	Molecular Formula	Area (%)
1.	33.256	Metyl 14-pentadecanoate	$C_{17}H_{34}O_2$	4.06
2.	37.317	Methyl 2-undecyl cyclopropane pentanoate	$C_{20}H_{38}O_2$	8.94
3.	37.820	Metyl decanoate	$C_{11}H_{22}O_2$	37.57
4.	41.383	Metyl 11-Oktadecenoate	$C_{19}H_{36}O_2$	38.90
5.	43.330	Decylacetate	$C_{12}H_{24}O_2$	0.37
6.	46.910	1-Hexadecanol acetat	$C_{18}H_{36}O_2$	0.24

The GC-MS results in the table show that there are chemical compounds that make up F_2RW_4 isolate with a similarity level with an index above 90. The main compound that will produce the highest peak is methyl 11-octadecenoate/methyl oleate (38.90%) with an SI of 93%, methyl decanoate/ methyl caprate (37.57%) with an SI of 91%. The brown macroalgae *P. australis* has a high concentration of polyunsaturated fatty acids, namely oleic acid (21.62%) (Kumari, *et al*, 2013). Whereas a study conducted by Caf, *et al* (2015) stated that *P. pavonica* has a high monounsaturate content in the form of palmitic acid of (42.89%), eicosapentaenoic acid with a PUFA/SPA ratio of 0.68%. Based on this, Padina macroalgae can be used as a food supplement.

Figure 7 is the mass spectra of the 11-octadecenoate methyl compound ·+$C_{19}H_{36}O_2$ with m/z = 296. This compound can be fragmented to form +$C_{18}H_{33}O$ (265 m/z) by releasing CO (31 m/z), then the +$C_{18}H_{33}O$ fragment (265 m/z) is fragmented by releasing C_2H_2O (42 m/z) to form +$C_{16}H_{31}$ (223 m/z). Fragment +$C_{16}H_{31}$ (223 m/z) forms +C_9H_{17} (125 m/z) by releasing C_7H_{17} (98 m/z), then fragment C_9H_{17} +(125 m/z) releases C_4H_6 (54 m/z) to form +C_5H_{11} (71 m/z). The +C_5H_{11} fragment (71 m/z) was fragmented by releasing CH_2 (14 m/z) to form +C_4H_9 (57 m/z), +C_3H_7 (43 m/z), and +C_2H_5 (29 m/z). Another fragment of the 11-octadecenoate methyl compound ·+$C_{19}H_{36}O_2$ by releasing $C_{12}H_{25}$ (169 m/z) forms +$C_7H_{13}O_2$ (129 m/z). Fragment +$C_7H_{13}O_2$ (129 m/z) fragmented to form +$C_4H_7O_2$ (87 m/z) by releasing C_3H_6 (42 m/z), then fragment +$C_4H_7O_2$ (87 m/z) was fragmented by releasing CH_2 (14 m/z) to form +$C_3H_5O_2$ (73 m/z) and +$C_2H_3O_2$ (59 m/z).

Figure 8 is the mass spectra of the compound methyl decanoate ·+$C_{11}H_{22}O_2$ (186 m/z). The methyl decanoate compound is fragmented to form +$C_9H_{17}O_2$ (157 m/z) by releasing C_2H_5 (29 m/z). The $C_9H_{17}O_2$ +(157 m/z) fragment was fragmented by releasing CH_2 (14 m/z) to form +$C_8H_{15}O_2$ (143 m/z) and +$C_7H_{13}O_2$ (129 m/z). Fragment +$C_7H_{13}O_2$ (129 m/z) fragmented into +$C_5H_9O_2$ (101 m/z) by releasing C_2H_4 (28 m/z), then fragment +$C_5H_9O_2$ (101 m/z) fragmented into +$C_4H_7O_2$ (87 m/z) by releasing CH_2 (14 m/z). The +$C_4H_7O_2$ (87 m/z) fragment fragmented into +$C_2H_3O_2$ (59 m/z) by releasing C_2H_4 (28 m/z). Another fragment of the methyl decanoate compound ·+$C_{11}H_{22}O_2$ (186 m/z) can form +$C_{10}H_{22}O$ by removing CO (31 m/z), then forming +C_7H_{15} (155 m/z) by releasing C_3H_4O (56 m/z). Fragment +C_7H_{15} (155 m/z) forms +C_5H_{11} (71 m/z) by releasing C_2H_4 (28 m/z). +C_5H_{11} (71 m/z) fragmented releasing CH_2 (14 m/z) to form C_4H_9 +(57 m/z) and C_3H_7 +(43 m/z).

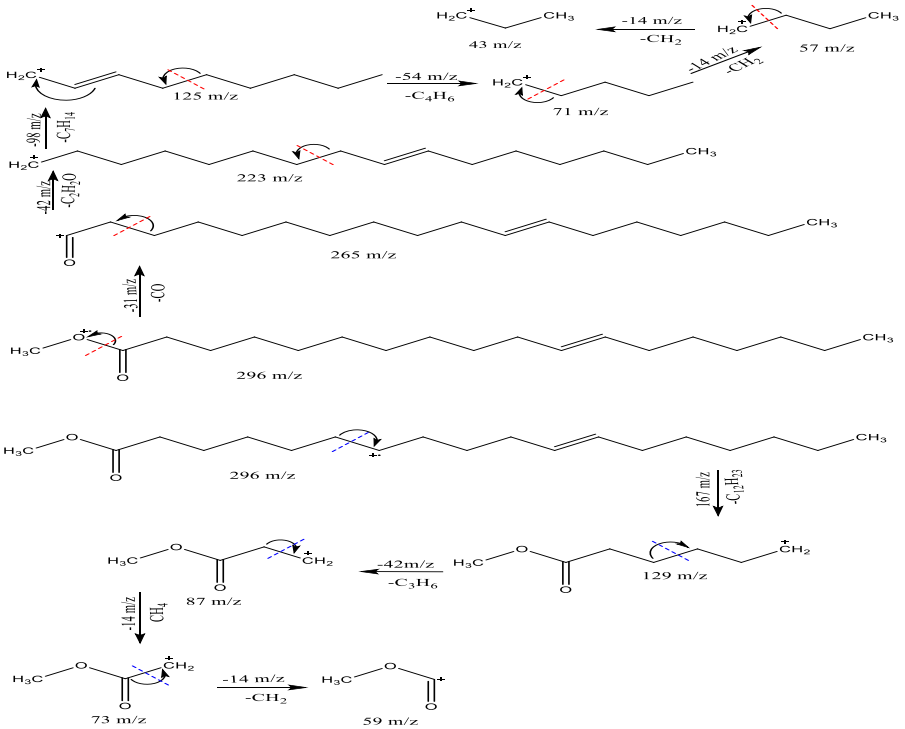


Figure 7. Schematic Diagram on The Fragmentation of Methyl 11- Octadecenoate Compounds

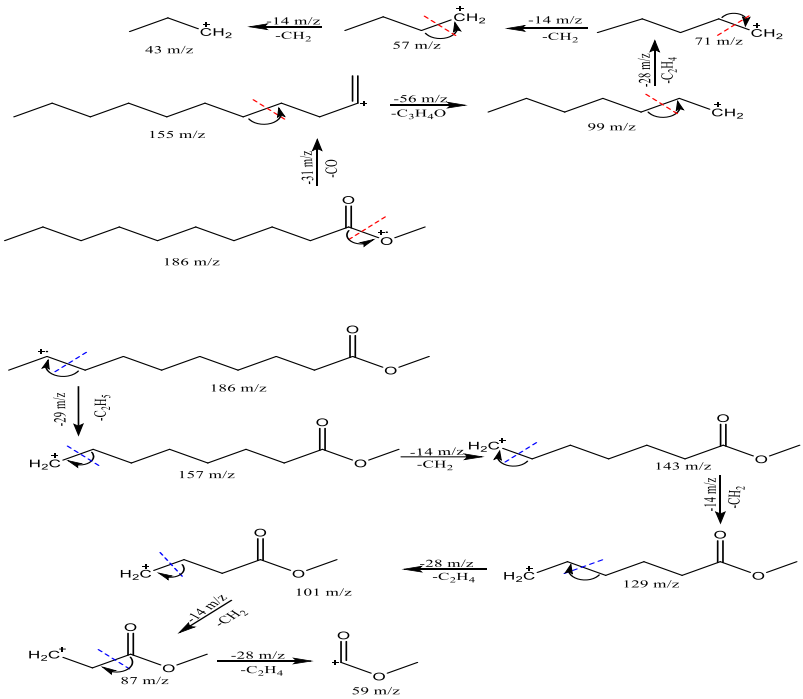
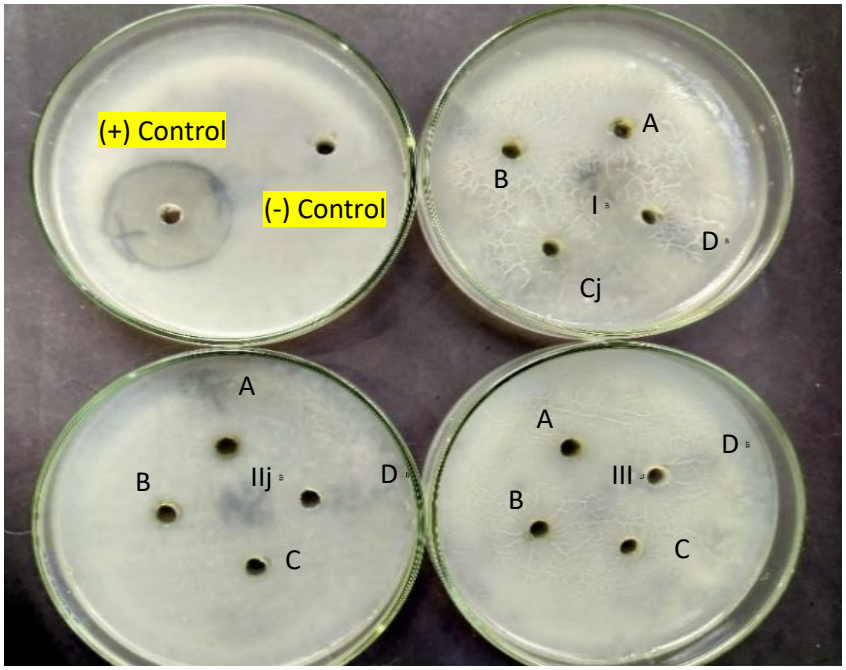


Figure 8. Schematic Diagram on the Fragmentation of Methyl Decanoate Compounds

3.7. Bacterial Activity Test

The antibacterial activity test of brown seaweed *P. minor* from Singkawang waters was carried out using the well-diffusion method. Activity test in this study used concentrations of 50 µg/µL, 25 µg/µL, 12.5 µg/µL, and 12.5 µg/µL. The test results showed no inhibitory activity in the methanol extract and *n*-hexane fraction against *S. aureus* and *E. coli* bacteria with no clear zones around the wells (Figure 9 and Figure 10). Whereas the positive control that used tetracycline had inhibition against *S. aureus and E. coli* bacteria. Tetracycline is an antibiotic that has the ability to inhibit cell wall synthesis (Nasronudin, 2011).

Four factors that affect antibacterial activity, namely the concentration of the extract, the content of metabolites, the type of bacteria that is inhibited, and the diffusion power of the extract (Jawetz, *et al*, 1996). Based on the test results, the antibacterial activity can be affected by the diffusion power of the extract. The methanol extract and the n-hexane fraction of *P. minor* in the well (Well) will inhibit the growth of bacteria by diffusing into the media containing the test bacteria. The type of bacteria and concentration can affect diffusion. In addition, the thickness of the media and the diameter of the wells (Well) can affect the rate of diffusion of metabolites.



(a)

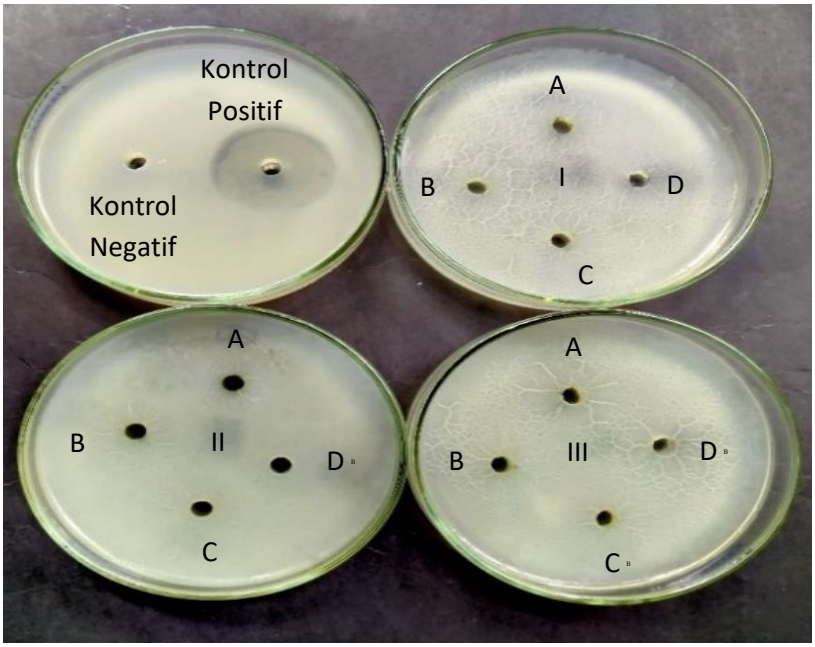
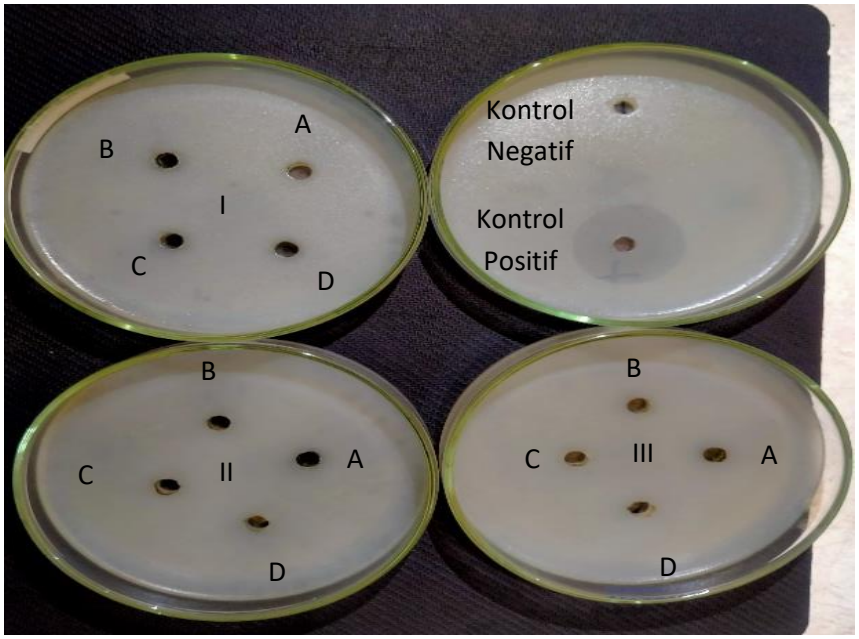
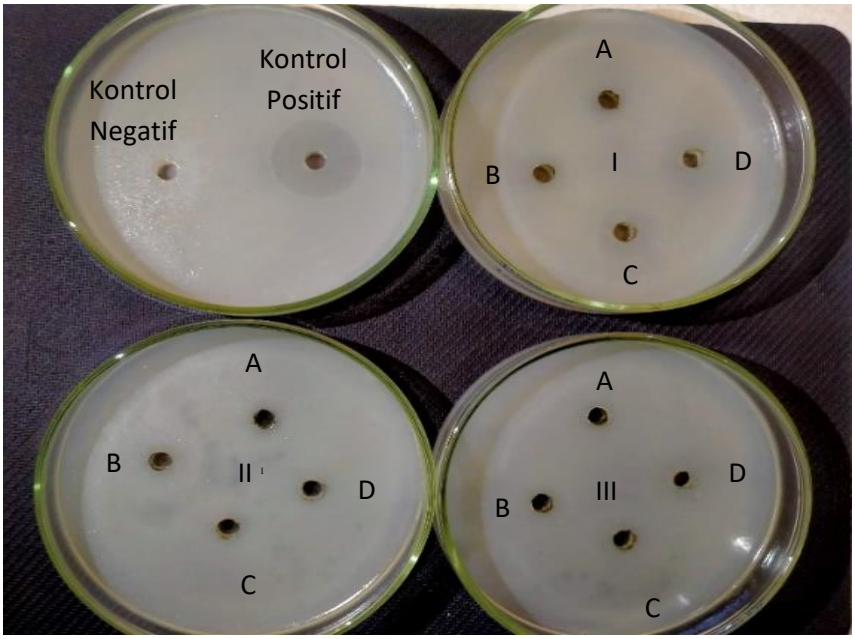


Figure 9. Results of antibacterial activity test of *Padina minor* methanol extract against bacteria (a) *Stahphylococcus aureus* and (b) *Escherichia coli* (A) 50 µg/ µL (B) 25 µg/ µL (C) 12.5 µg/ µL (D 6.25 µg/ µL



(a)



(b)

Figure 10. Results of the antibacterial activity test of the Padina minor n-hexane fraction against bacteria (a) *Stahphylococcus aureus* and (b) *Escherichia coli* (A) 50 µg/ µL (B) 25 µg/ µL (C) 12.5 µg/ µL (D) 6.25 µg/ µL

5. CONCLUSION

Based on the research that has been done, it can be concluded that: a. The results of characterization using the FTIR spectrum on the F_2RW_3 fraction identified the absorption of O-H hydroxyl groups (345.65 cm^{-1}), C=O (1743.65 cm^{-1}), C=C bonds (1633.71 cm^{-1}), C-H (2926.01; 2854.0, 1438.9 cm^{-1} and 723.31 cm-1), and C-O-C bonds (1170.79 cm^{-1}). While the results of the GC-MS analysis showed that the F_2RW_4 fraction had 2 main compounds, namely Methyl 11-Octadecenoate and Methyl Decanoate. The activity test results on the methanol extract and *n*-hexane fraction against *Staphylococcus aureus* and *Escherichia coli* were inactive.

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Comparative Analysis of Maceration and Soxhlation Extraction for The Total Flavonoid Content of Sungkai Leaves (*Peronema canescens* Jack.)

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Abstract

*The sungkai leaf (*P. canescens *Jack.) is an indigenous plant of Indonesia that has been utilized as a mouthwash and for treating minor wounds in traditional medicine. This study aimed to determine the total flavonoid content of* P. canescens *Jack leaf extract using maceration and soxhlation extraction methods. The total flavonoid content in the leaf extract of* P. canescens *Jack was determined using a UV-Vis spectrophotometer and a colorimetric technique ($AlCl_3$) at a wavelength of 412 nm. The results were reported as the total flavonoid content in quercetin equivalent (EQ). The maceration step yielded 7.30%, whereas the soxhlation process yielded 15.34%. The maceration method yielded a total flavonoid content of 81.19 mgEQ/gram extract, whereas the soxhlation process yielded a flavonoid content of 69.068 mgEQ/gram extract.*

Keywords: *Sungkai leaf, flavonoids, extraction, maceration, soxhlation*

Abstrak

Daun sungkai (P.canescens Jack.) merupakan tanaman asli Indoensia yang dikenal dengan nama daun sungkai telah lama digunakan dalam pengobatan tradisional sebagai obat kumur dan luka ringan. Penelitian ini bertujuan untuk mengetahui kadar flavonoid total dari ekstrak daun P. canescens Jack dengan menggunakan metode ekstraksi secara maserasi dan sokhletasi. Penentuan kadar flavonoid total pada ekstrak daun P. canescens Jack dilakukan secara spektrofotometer UV-Vis dengan metode kolorimetri ($AlCl_3$) pada λ 412 nm dan dinyatakan sebagai flavonoid total dalam ekuivalen kuersetin (EQ). Rendemen yang didapat dari proses maserasi sebesar 7,30% dan sokhletasi sebesar 15,34%. Kadar flavonoid total yang didapatkan dari proses maserasi sebesar 81,19 mgEQ/gram ekstrak dan sokhletasi sebesar 69,068 mgEQ/gram ekstrak..

Keywords: Daun sungkai (P.canescens Jack.), flavonoid, ekstraksi, maserasi, sokhletasi.

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1. INTRODUCTION

Sungkai (Peronema canescens Jack.) is a plant commonly found in Kalimantan. Sungkai has long been employed to treat minor wounds and as a mouthwash. The Dayak and Banjar tribes utilize the sungkai plant as a medicinal remedy to enhance the body's immune system, treat bruises, alleviate fever, and provide a bath for postpartum mothers (Sari et al., 2023). Multiple research findings also indicate that

sungkai plants have interesting activities. Yani et al. (2014) found that administering sungkai extract increased the number of leukocytes by 36% at a dose of 0.56 mg/kgBB in mice. Dillasamola et al. (2021) discovered that administering sungkai leaf extract enhanced the activity and capacity of macrophage cells and increased the number of leukocyte cells and overall leukocyte count in mice. These two studies demonstrate that the sungkai plant possesses immune-boosting properties. The antibacterial properties of Sungkai leaves have been scientifically studied and confirmed by Fransisca et al. in 2020. The preclinical evaluation of sungkai leaves has shown promise as an anti-hyperuricemic agent since it effectively lowers blood uric acid levels in rats (Latief et al., 2021).

Sungkai leaves have strong antioxidant activity with an IC50 value of 50,838 ppm for young leaves and 52,835 ppm for old leaves (Okfrianti et al., 2022). Kasumawati and Hasnah (2021) investigated the impact of the sungkai leaf simplicia drying method on the antioxidant activity of the ethanol extract. The results showed that the antioxidant activity of sungkai leaf ethanol extract was very strong in all samples with different drying methods. The antioxidant activity of samples with oven drying at 50°C had an IC50 value of 13,340 ppm, oven drying at 70°C was 17,034 ppm, wind drying at 14,610 ppm, and sun drying at 16,799 ppm. Sungkai leaves are rich in bioactive substances such as triterpenoids, alkaloids, flavonoids, phenolics, steroids, and saponins (Pindan et al., 2021). Ramadhani et al. (2022) stated that the total phenol content of sungkai bark extract was 14.97 ± 1.28 mgGAE/g extract and a total flavonoid content of 29.41 ± 0.64 mgEQ/gram extract. This study aimed to compare the overall flavonoid content of sungkai leaves using two distinct extraction techniques: maceration and soxhlation. These two extraction procedures are frequently employed in diverse research investigations. The total flavonoid content in sungkai leaf extract was quantified using a UV-Vis spectrophotometer using the colorimetric method. The results were reported as the total amount of flavonoids in quercetin equivalent (mgEQ/gram)..

2. MATERIALS AND METHODS

2.1. Materials and Tools

The materials used were sungkai leaf (Peronema canescens Jack.) obtained from the Banua Botanical Gardens in Banjarbaru, filter paper, ethanol p.a, methanol p.a, distilled water, acetic acid, sodium hydroxide, quercetin, and $AlCl_3$.

The tools used were analytical scales, ovens, porcelain dishes, glass beakers, test tubes, macerators, soxhlet extraction apparatus, water baths, Thermo Scientific Genesis 30 UV-VIS spectrophotometers, vortex mixer, and rotary evaporators.

2.2. Preparation of sungkai leaf simplicia powder

The preparation process commenced with wet sorting, separating dirt or foreign materials from sungkai leaves. The material was washed with clean water to remove soil and other impurities. The drying process was carried out in an oven at 50 ± 2°C. Dry sorting was then carried out to separate foreign organic materials and simplisia damaged by the previous process. The dry-sorted samples were pulverized into a coarse powder using a blender. Simplicia powder was stored in a clean and dry container (Praditya, 2016; Puspitasari & Proyogo, 2017).

2.3. Qualitative analysis of sungkai leaf flavonoid using chemical reaction methods

A total of 100 mg of sungkai leaf powder for the maceration and soxhlation methods was weighed and diluted with methanol p.a. Then, 2 mL of each was taken and put into a test tube. A negative control solution was also made as a

comparison. Then, a few drops of 10% sodium hydroxide were added to each test tube. If a yellow hue is produced, a positive test indicates the presence of flavonoids (Mailuhu et al., 2017).

2.4. Preparation of ethanol extract from sungkai leaves (maceration method)

A total of 25 g of sungkai leaf simplicia powder was put into a 1,000 mL beaker, added with 250 mL of ethanol until the entire sample was submerged, and stirred every 8 hours at room temperature. Extraction was carried out for three days (re-maceration) with solvent replacement every 1 x 24 hours. The liquid extract underwent filtration using filter paper to separate the sediment and extract. The liquid extract of sungkai leaves was evaporated using a rotary evaporator until a thick extract was obtained. The extract was concentrated using a water bath at 50°C until a constant weight was obtained (Leksono et al., 2018). The percent yield was calculated using Equation (1).

$$\%\ Yield = \frac{weight\ of\ extract\ obtained}{weight\ of\ simplicia}\ x\ 100\% \qquad (1)$$

2.5. Preparation of ethanol extract from sungkai leaves (Soxhlation Method)

A total of 25 grams of sungkai leaf simplicia powder was wrapped in filter paper, tied, and put into a sokhlet extractor. 250 mL of ethanol solvent was put into a round bottom flask. Soxhlation was carried out for approximately 18 hours at 64ºC until the solvent was clear. The extract was evaporated using a rotary evaporator at a 65ºC until a thick extract was obtained, then concentrated using a water bath until a concentrated extract was obtained. The extract replication process was repeated thrice (Mokoginta et al., 2013; Puspitasari & Proyogo, 2017; Riyani & Adawiah, 2015; Verawati et al., 2017).

2.6. Determination of total flavonoid levels using a UV-VIS spectrophotometer

2.6.1. Determination of maximum wavelength

A total of 1 mL of 40 ppm quercetin standard solution was put into a test tube and added with 1 mL of 2% $AlCl_3$ and 8 mL of 5% acetic acid. The solution was then homogenized with a vortex mixer and incubated for 30 minutes. Absorption was measured in the wavelength range of 400 – 500 nm (Asmorowati & Lindawati, 2019).

2.6.2. Determination of standard curve

A total of 0.4 mL, 0.6 mL, 0.8 mL, 1 mL, and 1.2 mL of 1000 ppm quercetin standard were put into a 10 mL volumetric flask and then diluted using methanol p.a. to have a final concentration of 40, 60, 80, 100, and 120 ppm. From each solution concentration, 1 mL was taken, put into a test tube, and added with 1 mL of 2% $AlCl_3$ and 8 mL of 5% acetic acid. The solution was homogenized with a vortex mixer and then incubated for 30 minutes at room temperature. The absorption at the maximum wavelength was then measured (Asmorowati & Lindawati, 2019).

2.6.3. Determination of total flavonoid levels

A total of 10 mg of sungkai leaf extract from the maceration method was diluted with methanol in a 10 mL volumetric flask. A total of 1 mL of the extract solution was put into a test tube, then added with 1 mL of 2% $AlCl_3$ reagent, and 8 mL of 5% acetic. The solution was homogenized with a vortex mixer then incubated for 30 minutes. After incubation, the sample absorption was measured using a spectrophotometer at the maximum wavelength previously obtained. Soxhlation results were extracted in the same way to obtain sample absorbance data.

2.7. Determination of Moisture Content

A total of 2 grams of sungkai leaf simplicia powder was put into a silica crucible that had been heated to a temperature of 105°C and measured. The simplicia was flattened by shaking the silica crucible. The silica crucible was placed in an open oven and then dried at 105°C until a constant weight was obtained. The silica crucible cup was cooled and weighed (MOH RI, 2008). Water content was calculated following Equation (2).

$$Moisture\ Content = \frac{initial\ weight\ -\ final\ weight}{initial\ weight\ of\ sample}\ x\ 100\% \quad (2)$$

3. RESULTS AND DISCUSSION

3.1. Plant Sampling

The samples consisted of leaves from the sungkai plant, which were collected in May 2023 at the Banua Botanical Gardens in Banjarbaru, South Kalimantan. The sungkai plant is a tall, woody plant that typically reaches a height of approximately 20-30 meters. Figure 1 displays the visual appearance of the plant. The leaves taken were mature leaves with green leaves and are still fresh because they contain more secondary metabolites than young leaves (Wijaya et al., 2013). Young leaves have a low photosynthetic capacity, while the photosynthesis process influences the content of secondary metabolites, such as flavonoids, in the leaves. Consequently, young leaves have a relatively low flavonoid content (Sjahid, 2008).

3.2. Preparation of Sungkai Leaf Simplicia Powder

The preparation of sungkai leaf simplicia powder started with wet sorting. Wet sorting aims to separate sungkai leaves that are dirty, damaged, and foreign objects. Afterward, it was washed with clean water to remove soil and other impurities attached to the leaves. Drying was carried out in an oven at 50 ± 2ºC. The drying aims to reduce the water content, thereby inhibiting microbial growth (Histifarina et al., 2004). This will prevent the growth of mold and mildew so that simplicia is produced, which is durable and can be stored for a long period (Djumaati et al., 2018). Manoi (2006) states that a water content above 10% will result in enzymatic reactions and microbial degradation.

Afterward, a dry sorting procedure was conducted to segregate samples that have incurred damage due to the preceding step. The sample grinding process was carried out using a blender so that a fine powder was obtained and the extraction process could run well (BPOM RI, 2013).

Figure 1. Plants (a) and sungkai leaves (b) (Personal Documentation)

Figure 2. Sungkai leaf powder

As stated by Kiswandono (2011), using a blender to refine simplicia enhances the interaction between the solvent and the sample during the extraction process.
This facilitates a more effective extraction of secondary metabolites from the sample. The organoleptic test results of sungkai leaf simplicia powder indicated that it was finely powdered, possessed a distinct odor, had a bitter taste, and exhibited a green hue (Figure 2).

3.3. Qualitative Analysis of the Active Flavonoid Content of Sungkai Leaves

Qualitative analysis of flavonoid testing using the chemical reaction method was a preliminary stage in a study that aims to provide an overview of the flavonoid compounds in sungkai leaves. The chemical reaction method was carried out by looking at the color testing reaction using a color reagent (Simaremare, 2014). The ethanol extract of sungkai leaves, obtained using maceration and soxhlation procedures, yielded identical findings in the flavonoid test when treated with NaOH reagent. The findings of this qualitative test indicate that the variation in extraction procedures has no impact on the flavonoid content. Both techniques are capable of extracting flavonoid chemicals. This is because flavonoids are phenolic compounds that can change color when alkali or ammonia are added, so they are easily detected in solution (Rais, 2015). Figure 3 presents the analysis of flavonoids in sungkai leaves using the chemical reaction method with sodium hydroxide reagent.

The presence of flavonoids in sungkai leaves was confirmed through sodium hydroxide tests; positive results were observed, as evidenced by the extract's transformation from dark green to yellow. The color shift is a result of the reaction between the sample and sodium hydroxide, which leads to the formation of a quinoid structure in the ring. This structure has longer and planar conjugated double bonds, allowing it to exhibit fluorescence (Mulyani & Laksana, 2011) (Figure 4).

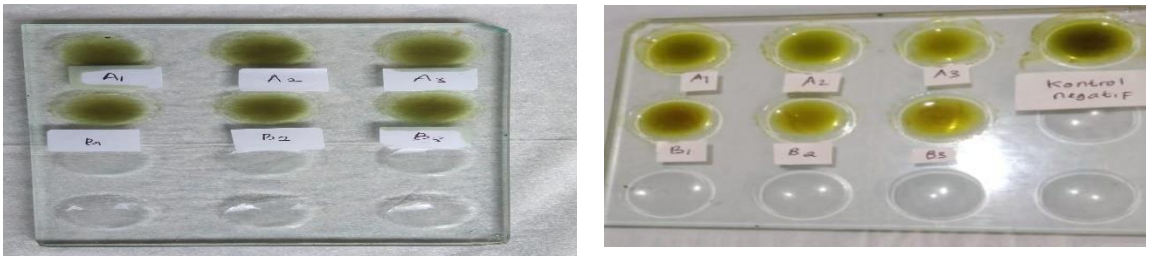

Figure 3. Qualitative analysis of the active flavonoid content of sungkai leaves before (left); and after right the addition of NaOH

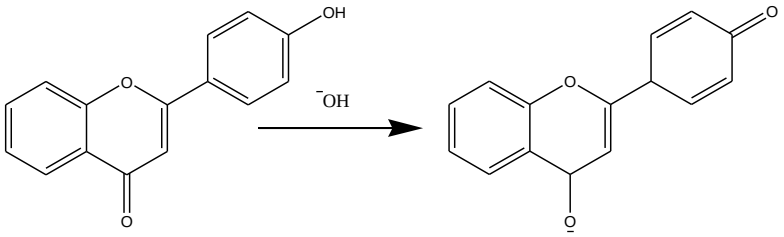

Figure 4. Reaction of flavonoids with NaOH (Mulyani & Laksana, 2011)

3.4. Sungkai Leaf Extraction

a. Maceration Method

The residue was re-macerated with ethanol solvent for two repetitions. The filtrate obtained from each repetition was collected and concentrated using a vacuum rotary evaporator and then heated using a water bath. The yield of ethanol extract of sungkai leaves by maceration method with three repetitions is presented in Table 1.

Table 1. The yield of sungkai leaf extract using the maceration method

Sungkai leaf extract sample	Yield (%)
Repetition-1 (A1)	6.08
Repetition-2 (A2)	7.60
Repetition-3 (A3)	8.24

b. Soxhlation Method

xtraction was carried out until an extract was obtained with a clear solvent. The extraction procedure was conducted from 8 am to 4 pm for three days. Soxhlet extraction was stopped when the solvent in the siphon tube containing the sample was visually clear. The extract solution obtained from the soxhlation process was then concentrated using a vacuum rotary evaporator and heated using a water bath. The yield of ethanol extract of sungkai leaves using the soxhlation method with three repetitions is presented in Table 2.

Table 2. Results of sungkai leaf extract yield using the soxhlation method

Sungkai leaf extract sample	Yield (%)
Repetition-1 (B1)	16.80
Repetition-2 (B2)	13.72
Repetition-3 (B3)	15.52

This study found that the yield of sungkai leaves was higher when using the soxhlation method (Table 2) than the maceration method (Table 1). These findings align with the studies conducted by Rahman (2017) and Firdaus (2019), which demonstrated that the soxhlation method yielded the highest yield of ethanol extract compared to the maceration method. The capabilities of the soxhlation method influence the difference in yield results. The heating process will bind more active compound components in sungkai leaves. Mukhriani (2014) states that the soxhlation extraction method offers the advantage of continuous extraction, where the material is extracted using pure solvent obtained from condensation. This leads to a higher yield compared to maceration extraction. Furthermore, applying heat in the soxhlation method enhances the solvent's capacity to extract insoluble compounds at ambient temperature, resulting in a more efficient chemical withdrawal process (Harborne, 1987). Rosidi et al. (2014) also affirmed that the large number of components extracted during the soxhlation process can cause the high yield value obtained.

3.5. Determination of Total Flavonoid Content Using UV-VIS Spectrophotometry

The colorimetric approach can be employed to quantify the total flavonoid content in the samples. The principle of determining total flavonoid levels is the reaction between flavonoids and $AlCl_3$, which forms a yellow complex whose absorbance is measured at the maximum wavelength. The reaction for determining flavonoid levels using the $AlCl_3$ colorimetric method is presented in Figure 5. The $AlCl_3$ method offers several advantages, including its simplicity, efficiency, and applicability for quantifying flavonoid levels by comparing them to quercetin (Lukman, 2015).

3.5.1. Determination of Maximum Wavelength

The objective of determining the maximum wavelength is to identify the specific wavelength at which the complex formed by AlCl3 and quercetin exhibits the most absorption, resulting in optimal absorbance (Suharyanto & Prima, 2020). The maximum wavelength was determined by measuring a standard solution of 40 ppm quercetin in the 400 – 500 nm range. The maximum wavelength obtained in this study was 412 nm. This maximum wavelength was used to measure the absorbance of the sungkai leaf extract sample.

3.5.2. Preparation of a Standard Curve

The quercetin standard curve was prepared by varying the standard series, which was read at an absorbance wavelength of 412 nm with an incubation time of 30 minutes. A calibration curve was constructed using different concentrations of quercetin, specifically 40 ppm, 60 ppm, 80 ppm, 100 ppm, 120 ppm, and 140 ppm, obtained from a 1000 ppm stock standard solution.

The regression equation was derived from the graph as follows: $y = 0.002x + 0.146$. The linearity value (r) obtained was 0.9995. The accuracy obtained was 99.95%. Linearity states a linear relationship between the concentration and absorbance of the concentration series solution obtained. The closer it is to 1, the more linear the results obtained. This statement is in accordance with the literature, which states that linearity is good if the correlation coefficient (r) value is close to 1 (Nafisa et al., 2015).

3.5.3. Determination of Total Flavonoid Levels

The total flavonoid content in sungkai leaf extract was determined using maceration and soxhlation extraction techniques. The absorbance value of each extract was entered into the quercetin standard curve equation obtained previously, namely $y = 0.002 x + 0.146$. The compound used as a standard in determining flavonoid levels was quercetin because quercetin is a flavonoid from the flavonol group, which has a keto group on the C-4 atom and also a hydroxyl group on the neighboring C-3 and C-5 atoms (Sari & Triyasmono, 2017). The total flavonoid content of sungkai leaves is presented in Table 3.

Table 3. Total flavonoid content of sungkai leaves

Extraction Method	Sample absorbance	Mean absorbance ± SD	Total flavonoid content (mgEQ/gr extract)	Average total flavonoid content (mg EQ/gr extract) ± SD
Maceration	0.312 0.305 0.313	0.31± 0.0044	80.582 80.303 82.673	81.19± 1.295
Soxhlation	0.280 0.290 0.292	0.29± 0.0064	65.048 70.588 71.568	69.068± 3.52

The Shapiro-Wilk test was conducted on the ethanol extract obtained using maceration and soxhlation procedures. The test yielded a significance value greater than 0.05, suggesting the data follows a normal distribution. The test of homogeneity of variances yielded a significance level greater than 0.05, indicating that the data was homogeneous. Subsequently, a One-way ANOVA analysis was carried out. A one-way ANOVA was conducted due to the normal distribution and homogeneity of the data. The One-way ANOVA analysis showed that the maceration and soxhlation methods differed significantly (sig < 0.05) on total flavonoid levels. Due to the notable disparities, the post hoc Test was conducted. The Post Hoc Test analysis findings indicated significant differences in total flavonoid levels between the maceration and soxhlation techniques, as evidenced by a value (sig < 0.05).

The maceration process resulted in a higher total flavonoid content of sungkai leaves compared to the soxhlation method, as shown in Table 3. These findings align with Rahman's (2017) research, which shows that the maceration approach yields a higher total flavonoid content compared to the soxhlation method. Based on the calculation results, the total flavonoid content of the maceration method averaged 81.19 ± 1.295 mg EQ/g extract, while the soxhlation method obtained an average of 69.068 ± 3.52 mg EQ/g extract. Therefore, it can be inferred that the sungkai leaves in this study are sensitive to heat, making them unstable when exposed to high temperatures. As a result, the maceration approach yields a higher flavonoid concentration compared to the soxhlation method.

Precision is expressed by the relative standard deviation (RSD) of a series of data (Alwi, 2017). The % RSD value in sungkai leaf extract using the maceration and soxhlation methods is in accordance with the percent RSD requirement, namely ≤ 4%. This demonstrates that the acquired results possess satisfactory repeatability, hence meeting the validation criteria (Gonzales et al., 2010).

3.6. Moisture Content

Determination of moisture content aims to provide a threshold or range for the amount of water and volatile compounds eliminated in the drying process. The remaining substance was measured by drying at a temperature of 105°C for 1 hour until the weight was constant. Table 4 presents the determination of the water content of sungkai leaves.

Table 4. Moisture content of sungkai leaves

Sungkai leaf extract sample	Moisture Content (%)
Repetition -1	6.71
Repetition -2	6.77
Repetition -3	6.72

According to the Indonesian Ministry of Health (1995), the drying shrinkage values range from no more than 10%. Therefore, the water content obtained from the sungkai leaves meets the specified requirements. A drying process is considered superior quality when the shrinkage value is lower. The water content in traditional medicine should not exceed 10% (Ministry of Health of the Republic of Indonesia, 1995). The material will easily grow mold if the water content exceeds 10%. Materials with low water content are less susceptible to mold contamination. This will affect the purity and contamination of a material (Ratnani et al., 2015).

4. CONCLUSION

The average yield of sungkai leaf extract using the maceration method was 7.30%, while the soxhlation method was 15.34%. The total flavonoid content of sungkai leaf extract from the maceration method was 81.19 mgEQ/gram of extract and the soxhlation method was 69.068 mgEQ/gram of extract.

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The Effect of ZnO Mass Variation on Chitosan/ZnO/Cellulose Acetate Composites from Citronella Waste As A Mask Filter Material

Pengaruh Variasi Massa ZnO Pada Komposit Kitosan/Zno/Selulosa Asetat Dari Limbah Serai Wangi Sebagai Material Filter Masker

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ABSTRACT

Air is an important component that affects human survival, but air quality in Indonesia has greatly decreased due to air pollution. This study used chitosan / ZnO / cellulose acetate composite membranes made from citronella waste as mask filters with ZnO variations of 1%, 2%, and 3%. Composite membranes are made by the phase inversion method and characterized by FTIR, tensile, SEM, and antibacterial tests. Optimum conditions based on the formation of pores measuring 0.17 µm are found in chitosan/ZnO/Cellulose Acetate composite membranes with a variation of 3% ZnO. In addition, this variation also has good mechanical properties, with an elongation value of 2.1177% and an elastic modulus of 6.5560 N/m². Based on antibacterial tests, the composite membrane of the 3% ZnO variation also showed the ability to increase antibacterial activity with moderate antibacterial inhibitory strength.

Keywords: *Composite, Filter Mask, Cellulose Acetate, Chitosan, ZnO*

ABSTRAK

Udara merupakan komponen penting yang mempengaruhi keberlangsungan hidup manusia, namun kualitas udara di Indonesia mengalami banyak penurunan karena pencemaran udara. Pada penelitian ini dilakukan pembuatan membran komposit kitosan/ZnO/selulosa asetat dari limbah serai wangi sebagai filter masker dengan variasi ZnO sebesar 1%, 2%, dan 3%. Membran komposit dibuat dengan metode inversi fasa dan dikarakterisasi FTIR, uji tarik, SEM, dan uji antibakteri. Kondisi optimum yang didasarkan pada terbentuknya pori berukuran 0,17 µm terdapat pada membran komposit kitosan/ZnO/Selulosa Asetat dengan variasi ZnO 3%. Selain itu pada variasi ini juga memiliki sifat mekanik baik, dengan nilai elongasi 2,1177% dan modulus young 6,5560 N/m² Berdasarkan uji antibakteri, membran komposit variasi ZnO 3% juga menunjukkan kemampuan dalam meningkatkan aktivitas antibakteri dengan kekuatan daya hambat antibakteri tergolong sedang.

Keywords: Komposit, Filter Masker, Selulosa Asetat, Kitosan, ZnO

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1. INTRODUCTION

Air pollution in Indonesia has reached alarming levels, ranking fifth in the world for worst air quality. Dust particles, released by human activities and natural sources such as volcanoes, contribute to this pollution and can cause respiratory problems and even chronic diseases if inhaled. To reduce the effects of air pollution, masks are recommended. However, disposable masks made from non-biodegradable materials can cause waste buildup. However, more environmentally friendly cloth masks have lower filtering effectiveness due to their single-layer construction.

The alternative solution in question is a mask filter made of cellulose acetate. Cellulose acetate is an organic ester compound that is odorless, tasteless, non-toxic, and biodegradable. It is commonly used in textiles, plastics, paper coatings, and membrane fibers. The cellulose acetate used in this study comes from citronella waste, a by-product of citronella essential oil production. According to Ververis, *et al*. (2004) citronella waste contains 35,0% cellulose, making it suitable as a raw material for cellulose acetate production.

To improve the mechanical properties of cellulose acetate, the addition of chitosan is needed because according to Afidin (2021), cellulose acetate is easy to absorb water, brittle, and stiff. Chitosan increases the water absorption capacity, brittleness, and stiffness of cellulose acetate. Another additive used in mask filter composite membranes is ZnO (zinc oxide), which provides antibacterial properties. ZnO is known for damaging bacterial cell walls, making it an effective bactericide. Compared to other substances such as CuO and Fe2O3, ZnO has shown the highest antibacterial activity against gram-positive and gram-negative bacteria (Azzam, *et al*., 2012). ZnO can attach to the cell membrane bacteria and has an oxidizing ability that can inhibit the growth of bacteria (Tantini, 2020). The manufacture of mask filters from cellulose acetate with the addition of chitosan has previously been carried out by Zomi (2022) and obtained mask filters that have a good filtering system.

This research aims to develop a chitosan/ZnO/Cellulose Acetate composite from citronella waste as a mask filter with better filtration activity. By utilizing the abundant citronella waste and combining environmentally friendly materials such as cellulose acetate and chitosan, the researchers hope to create a sustainable solution to reduce exposure to air pollution through effective mask filtration.

2. MATERIALS AND METHODS

2.1. Materials

The materials used were citronella waste (leaf part), NaOH, NaOCl, distilled water, acetic anhydride, glacial acetic acid, H_2SO_4, ZnO, acetone, ethanol, pp indicator.

The tools used were beakers, measuring cups, measuring flasks, stirring rods, petri dishes, dropper pipettes, funnels, erlenmeyers, spatulas, pH paper, filter paper, magnetic stirrers, blenders, and scissors. In addition, this study also used analytical scales DENVER Instrument Company, glass plates, THERMOLYNE hotplate stirrer, Alpha II FT-IR Infrared Spectroscopy Spectrometer, and Hitachi Flexsem 1000 Scanning Electron Microscope (SEM).

2.2. Methods

The process of cellulose isolation involves delignification and bleaching. To extract cellulose from dried citronella waste, it is first cut into pieces, blended, and filtered through a

sieve. The resulting waste is then delignified using a sodium hydroxide (NaOH) solution and heated for three hours. Next, citronella waste is washed until the pH is neutral, and then it is bleached with a sodium hypochlorite (NaOCl) solution until it turns white. The bleached waste is neutralized with distilled water and dried in an oven. The cellulose obtained from this process is then characterized using Fourier Transform Infrared Spectroscopy (FTIR).

In the cellulose acetate synthesis, the delignified cellulose obtained from citronella waste is mixed with sulfuric acid and glacial acetic acid. Acetylation is carried out by adding acetic anhydride and glacial acetic acid, and the mixture is heated for an hour. Afterwards, glacial acetic acid and sulfuric acid are added to the solution, which is heated for an additional hour. The solution is then transferred to a beaker containing distilled water, filtered, and rinsed with water until the pH is neutral. The cellulose acetate is then dried in an oven. FTIR is used to characterize the cellulose acetate.

To produce the chitosan/ZnO/cellulose acetate composite, cellulose acetate is dissolved in acetone and cast onto a glass plate. The plate is then placed in a coagulation bath filled with water. Chitosan is prepared by dissolving it in acetic acid and adding ZnO solids. This mixture is applied to the dried cellulose acetate, and the same process is repeated for variations with different concentrations of ZnO. The composite membrane is characterized using FTIR, tensile testing, scanning electron microscopy (SEM), and antibacterial testing.

For the antibacterial test of the composite membrane, the inhibition zone method is employed against gram-negative bacteria such as E. coli. The membrane is cut into small circles, placed on the surface of an E. coli culture medium, and incubated. The formation of a clear zone around the membrane indicates antibacterial activity.

3. RESULTS AND DISCUSSION

Composite synthesis was done by mixing cellulose acetate, chitosan, and ZnO. The mass variation of ZnO was 1%, 2%, and 3%. The making of this composite is based on the phase inversion method. Phase inversion changes a polymer from a liquid to a solid phase through a certain controlling mechanism. Cellulose acetate dissolved in acetone is cast on a glass plate and then dipped into a coagulation bath filled with water. This aims to dissolve the acetone because cellulose acetate cannot dissolve in water. The cellulose acetate membrane that has been dried is then coated with chitosan that has been dissolved in 2% acetic acid and mixed with ZnO variations of 1%, 2%, and 3%. The dried composite is a thin white membrane. The composite membrane obtained was then analyzed for FTIR, mechanical properties, membrane surface, and antibacterial properties.

3.1. FTIR analysis of composite membrane chitosan/ZnO/cellulose acetate citronella waste

Chitosan/ZnO/Sellulose acetate waste citronella composite membrane was characterized by FTIR to determine the functional groups contained in the composite membrane. **Figure 1** shows the FTIR spectrum of chitosan/ZnO/cellulose acetate waste citronella composite membrane.

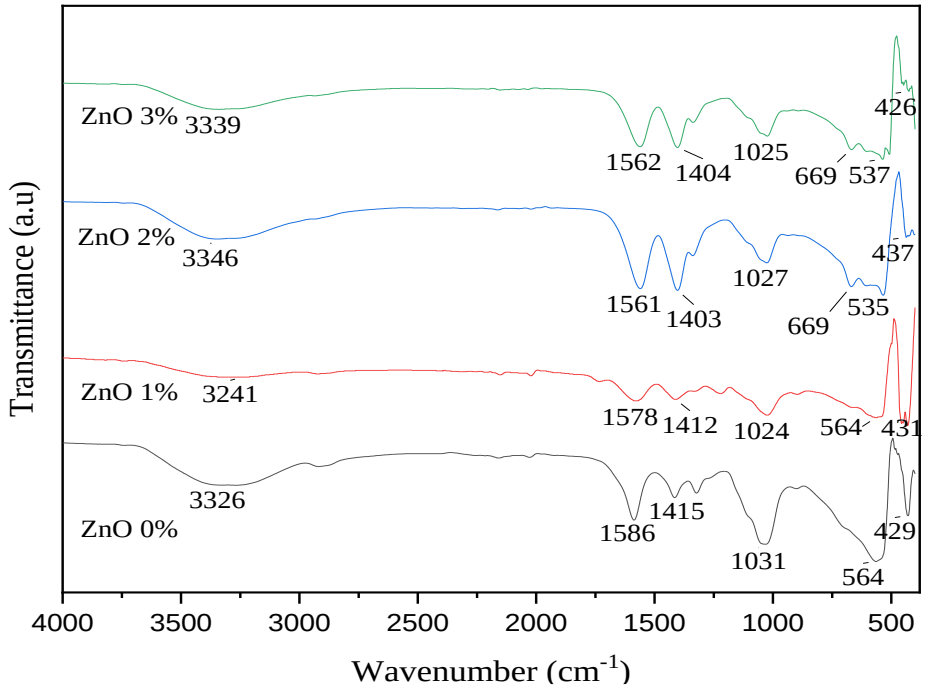

Figure 1. FTIR spectrum of chitosan/ZnO/cellulose acetate composite of citronella waste

The appearance of new absorption in the FTIR spectrum of the composite with the addition of ZnO in the 700-400 cm-1 wavenumber range indicates a ZnO functional group resulting from adding ZnO to the composite (Tantini, 2020). However, similar absorption is found in composites without the addition of ZnO. This can be influenced by chitosan because similar absorption is found in the FTIR of commercial chitosan from the research of Sartika et al. (2016). The absorption indicates that there is still metal content in chitosan. The typical functional group of cellulose acetate, namely C=O, is characterized by absorption in the wavenumber range of 1561-1586 cm-1. The absorption in the wavenumber range of 1024-1031 cm-1 indicates the C-O functional group. The typical chitosan functional group, C-N, is characterized by absorption in the wavenumber range of 1403 - 1415 cm-1. The absorption indicating the -OH functional group at wavenumbers 3750-3000 cm-1 became wider due to the interaction between -OH from cellulose acetate and -NH from chitosan (Rojtica, 2021).

3.2. Analysis of mechanical properties of composite membrane

Chitosan/ZnO/Cellulose acetate waste citronella composite membrane was tested for Tensile strength and then analyzed on the elongation and elastic modulus values to determine the mechanical properties of the composite membrane. Elongation is the maximum strain value of the material when given a force. Meanwhile, elastic modulus (elasticity) is the result of dividing the tensile strength value by the percent elongation (Tantini, 2020). Based on ASTM D838, the elongation value for cellulose acetate-based plastics is 3.10 - 3.50. The results of the composite membrane mechanical properties test can be seen in Table 1.

Table 1 Mechanical properties test results of composite membranes

ZnO variations (%)	Elongations (%)	Elastic modulus (N/m^2)
0	3.1762	6.3029
1	3.1567	7.7188
2	2.4457	5.8997
3	2.1177	6.5560

Adding ZnO to a composite membrane decreases its elongation value, making it more rigid. This is due to the interaction between the polymer matrix and hydroxyl groups on the ZnO particles, which reduces the mobility of the polymer chains. The bond between molecules in the plastic film weakens, causing the pores to open and the composite to become rigid. However, the composite membrane with 1% ZnO variation meets the elongation standard and has potential as raw material for mask filters. The elastic modulus value, which is directly proportional to elongation, is highest in the composite membrane with 1% ZnO mass variation. Adding ZnO reduces the bond density, and agglomeration of the ZnO material can cause a decrease in the elastic modulus value. The 2% ZnO composite has a smaller value than the 3% ZnO composite, possibly due to insufficient bonding between constituent particles.

3.3. Surface analysis of composite membranes

Analysis of the composite membrane surface was carried out using Scanning Electron Spetroscopy (SEM). **Figure 2** shows the SEM results of the composite membrane.

Based on the SEM results, it can be seen that no pores are formed in the chitosan/cellulose acetate composite membrane while in the chitosan/ZnO/cellulose acetate composite with 3% ZnO variation, pores are formed. After analysis, it is known that the pore size formed in the composite membrane is 0.17 µm. This pore size is quite good because it can withstand particles that are harmful to humans which have sizes ranging from ≤ 10 µm and ≤ 2.5 µm and microorganisms measuring 0.1 - 100 µm found in dust. This composite membrane almost meets the ASTM-F2100 mask standard, which can withstand droplets that have a size of 3 µm but is less optimal in holding particles that have a size of 0.1 µm.

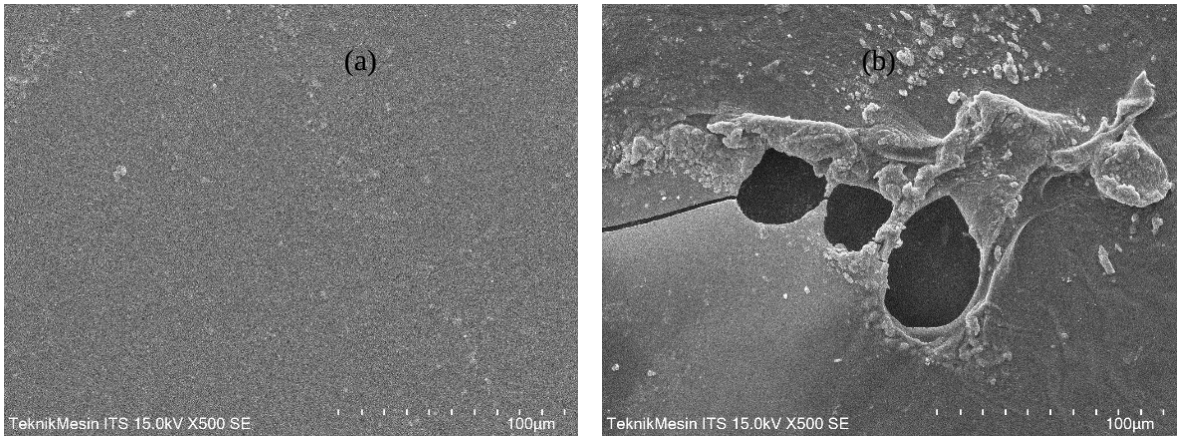

Figure 2. Morphology of composite membrane (a) Chitosan/Cellulose Acetate (b) Chitosan/ZnO/Cellulose Acetate

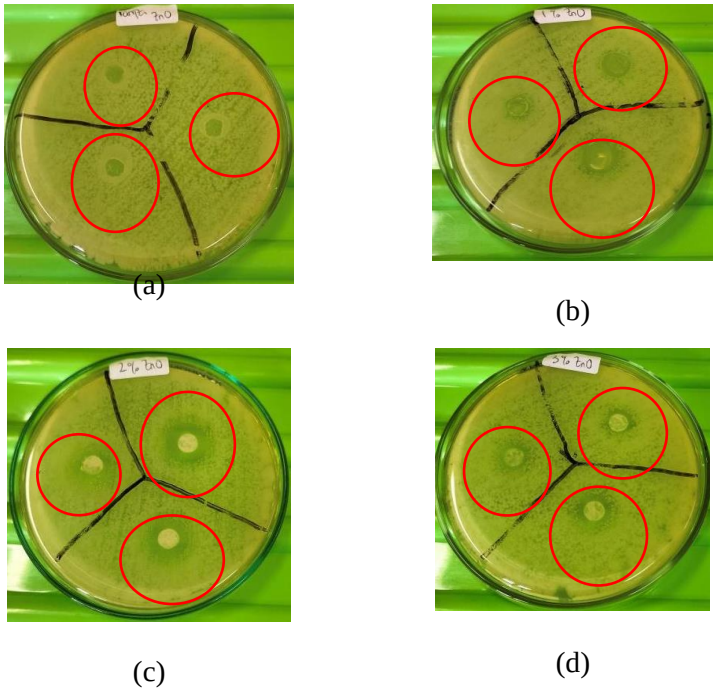

Figure 3. Antibacterial test results of composite membrane (a) 0% ZnO (b) 1% ZnO (c) 2% ZnO (d) 3% ZnO

3.4. Antibacterial properties analysis

The bacteria chosen in this test are E.coli bacteria because they can be carried in the air (Trisno, *et al*., 2019). The zone of inhibition formed on the test sample indicates the presence of antibacterial activity. The positive control used is Erythromycin because it has the ability to kill and inhibit bacterial growth. **Figure 3** shows the zone of inhibition formed in the antibacterial test.

In the picture above, it can be seen that no clear zone is formed on the composite membrane without the addition of ZnO, while the composite membrane with the addition of ZnO forms a clear zone. The clear zone formed is then measured and calculated to determine the strength of antibacterial inhibition. The strength of antibacterial inhibition of the composite membrane can be seen in **Table 2**.

Table 2 Strength of Antibacterial Inhibition

Sample	ZnO variations	Zone of Inhibition Diameter (mm)	Strength of Antibacterial Inhibition
Cellulose Acetate/Chitosan Composite Membrane	ZnO 0%		Weak
Cellulose Acetate/Chitosan/ZnO Composite Membrane	ZnO 1%	5	Medium
	ZnO 2%	9	Medium
	ZnO 3%	6	Medium
Positive Control		30	Very strong

Based on **Table 2**, it is known that the composite membrane without the addition of ZnO has weak antibacterial properties. Liu et al. (2010) said that membranes with the addition of chitosan have less effective antibacterial properties. The addition of ZnO to the composite membrane shows the ability of the membrane to inhibit bacterial growth with moderate inhibition strength. ZnO inhibits bacterial growth by attaching to the surface of the bacterial cell membrane, causing protein denaturation and membrane permeability changes. The bacterial cell wall has a negative charge due to peptidoglycan, composed of long carbohydrate chains intersecting with short amino chains. Zn^{2+} ions from ZnO will bind to the negative charge on the bacterial cell wall, causing the outer membrane of the cell to be damaged. The diameter of the inhibition zone showed an increase along with the addition of ZnO but decreased in the composite membrane with the addition of 3% ZnO. This decrease can be influenced by agglomeration, which causes oxide compounds that inhibit bacterial growth to decrease.

4. CONCLUSIONS

Based on the results of the research that has been done, it can be concluded that based on the pores formed, the optimum condition is found in the chitosan/ZnO/Cellulose Acetate composite membrane with 3% ZnO variation. This is characterized by the presence of pores measuring 0.17 µm. This pore size has met the ASTM-F2100 standard. In addition, this variation also has good mechanical properties, with an elongation value of 2.1177% and a young modulus of 6.5560 N/m2. Based on the antibacterial test, the 3% ZnO variation composite membrane also shows the ability to increase antibacterial activity with an inhibition zone diameter of 6 mm and a moderate antibacterial inhibition strength. This makes the chitosan/ZnO/cellulose acetate composite membrane potentially applied as a mask filter material.

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Phytochemical Screening and Cytotoxicity Test of *Sidaguri* Leaf (*Sida rhombifolia* Linn.) Fractionated Extract with Brine Shrimp Lethality Test (BSLT) Method

Skrining Fitokimia Dan Uji Sitotoksisitas Ekstrak Fraksinasi Daun Sidaguri *(Sida rhombifolia Linn.)* dengan Metode *Brine Shrimp Lethality Test*

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ABSTRACT

Traditional medicine can be an alternative in the treatment of cancer. One of the traditional medicines used as herbal ingredients that have the potential to be anticancer is sidaguri (Sida rhombifolia Linn.). The leaves of S. rhombifolia are plants belonging to the family Malvaceae. S. rhombifolia plants contain chemical compounds. This study aims to determine the chemical content of S. rhombifolia extract and potential cytotoxicity based on LC_{50} (Lethal Concentration 50) values in the death of nauplii Artemia salina Leach after administration of ethanol extract and *n*-hexane fraction, ethyl acetate and water. The method used S. rhombifolia leaves that have been in the form of simplisia were extracted with 70% ethanol solvent using the ultrasonication method. Then tested for the content of chemical compounds. Phytochemical screening results show that S. rhombifolia ethanol extract contains alkaloid compounds, flavonoids, saponins, tannins, and triterpenoids. The ethanol extract was further fractionated using solvents *n*-hexane, ethyl acetate and water. The cytotoxicity of S. rhombifolia extract was tested using the Brine Shrimp Lethality Test (BSLT) method using ethanol extract, *n*-hexane fraction, ethyl acetate fraction and toxic water fraction against Artemia salina or less than 1000 ppm, of the four test extracts that had the smallest LC_{50} value were ethanol extract which was 320.15 µg / ml and alkaloid compounds fractionated from ethanol extract of S. rhombifolia leaves *n*-hexane fraction 500.10 µg / ml, ethyl acetate 575.06 µg/ml and water 873.21 µg/ml. It was concluded that the results obtained that ethanol extract is more cytotoxicity compared to fractionation
Keywords : BSLT; Sidaguri; Cytotoxicity

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1. INTRODUCTION

Cancer is a disease that occurs due to uncontrolled and indefinite cell division (Ma'at, 2003). Cancer cells can spread to other parts of the body so that it can cause death (Anderson *et al*, 1991); Medical cancer treatment is usually handled with surgery, radiotherapy, chemotherapy and drugs that cause many side effects. External factors that can cause cancer, namely radiation, free radicals, ultraviolet light, viruses, infections, cigarettes and chemicals from food. While internal factors that cause cancer, namely genetic or congenital factors, hormonal factors, psychiatric factors, and immune (Utari *et al*, 2013). Only some types of cancer can be treated, especially if treated at an early stage, cancer is also synonymous with expensive treatment costs. Through several studies it has been known that there are various types of anticancer medicinal plants that are around us and easier to obtain (Mangan, 2009).

Bionatural materials derived from plants, animals, and microorganisms have been widely used for human needs including medicines. Indonesian people still use plants for traditional medicine commonly referred to as jamu (Lenny, Barus & Sitopu, 2010) Indonesia is the second largest center of biodiversity or biodiversity in the world, where about 80% of tropical forest plant sources are found in Indonesia (Handa, Rakesh and Vasisht, 2006). One plant that has quite promising potential is Sidaguri. This plant is in the form of a shrub that is often found on the roadside. Sidaguri belongs to the family *Malvaceae*, with the Latin name *Sida rhombifolia* this plant can grow in areas with tropical climates, both in the highlands and in the lowlands. The height *S. rhombifolia* can reach 2 meters. Research conducted by Mu'im & Hanani (2011) mentioned S. *rhombifolia* plants contain chemical compounds alkaloids, calcium oxalate, saponins, tannins, phenols, amino acids, steroids, essential oils. The content contained in S. *rhombifolia* plants is used for health, such as scabies, curing ulcers, preventing rheumatism, overcoming helminthiasis, overcoming diarrhea, kidney stones, gout. [8] Pharmacological research that has been conducted on this plant has proven its potential as an anti-inflammatory (Atsana, 2017), antibacterial (Dewi, 2012), antioxidant (Muammar, 2014).

The antioxidant power of ethanol extracts from the roots, stems, leaves, and all parts of the *S. rhombifolia* plant has also been evaluated through research conducted by (Rahman *et al*, 2011) Anti-inflammatory and antihyperuricemia effects were observed by looking at the inhibitory activity of Sidaguri on xanthinoxidase. In this study, simplisia powder was extracted successively by stratified maceration using four types of solvents based on polarity levels, namely petroleum ether, ethyl acetate, n-butanol and 96% ethanol. All extracts can inhibit xanthin oxidase activity with IC_{50} value of 1.71 µg/ml for n-butanol extract, ethyl acetate extract with IC_{50} 2.38 µg/ml, ethanol extract with IC_{50} 4.64 µg/ml, and in petroleum ether extract with IC_{50} 9.52 µg/ml (Lestari, 2012). The selection of the concentration is desirable in order to obtain linear results. This cytotoxicity examination uses the Brine shrimp lethality test (BSLT) method and uses *Artemia salina Leach nauplii*. This method is the beginning of the search for anti-cancer compounds because the results of toxicity tests have a positive correlation with anti-cancer cytotoxicity activity (Meyer *et al*, 1982). The use of the Brine shrimp lethality test (BSLT) method has several advantages compared to other methods, namely easy to do, cheap, fast, quite accurate, does not require aseptic conditions and is reliable (Dachrinus, Oktima & Stanias, 2005; Fajarningsih *et al*, 2006). The data will be analyzed with the SPSS 20.0 probit analysis

program to look for the relationship between the concentration of the test solution and the nauplii mortality response of Artemia salina Leach (Silva *et* al, 2007) and determine the IC_{50} value (Lethal Concentration 50). The tested extract can be said to have a toxic effect if the IC_{50} value is less than 1000 µg/ml [13].

2. MATERIALS AND METHODS

2.1. Determination S. rhombifolia

S. *rhombifolia* samples were determined at the Center for Tropical Biopharmaceutical Studies LPPM IPB, to determine the identity of the sample species.

2.2. Preparation sample

Preparation of S. rhombifolia based on modified method (Ardianti and Kusnadi, 2014). The leaves of S. rhombifolia are washed using running water, dried in an oven at 40-45°C and made a 40 mesh powder using a blender.

2.3. Determination of water content

Water content is determined based on the SNI 01-2891-1992 method. Empty porcelain dishes are dried in an oven at 105°C for 1 hour then cooled in a desiccator for 15 minutes and weighed with an analytical balance. A total of 1 gram of sample was put into a saucer and heated at 105°C for 3 hours and then cooled in a desiccator for 15 minutes and then weighed. The cup containing the sample is weighed and recorded in mass. The drying of the cup containing the sample is repeated until it reaches a constant mass (mass change of not more than 0.003 grams). The moisture content of the sample is calculated based on the following equation:

$$Water\ content\ (\%) = \frac{a-b}{a} \times 100\%$$

Information:
a = mass of cup and sample before drying (g)
b = mass of cup and sample after drying (g)

2.4. Sample Extraction

Extraction of S. *rhombifolia* was performed based on a modified method (Sasongko *et al*, 2017). First, S. *rhombifolia* is dried in the oven at 40°C for 2-3 days. The dry sample is then mashed using a blender. S. *Rhombifolia* simplisia of 50 grams each was put into a beaker and then added 70% ethanol as much as 500 mL of extraction using the ultrasonication method at 25°C with an ultrasonic frequency of 20 kHz for 25 min. The sonicated extract is filtered to separate the filtrate and its residue. The filtrate that still contains solvent is concentrated with an evaporator at 40°C. The yield of the extract obtained is calculated using the formula:

$$Yield = \frac{\text{mass of extract produced}}{\text{mass of simplicia extracted}} \times 100\%$$

2.5. Phytochemical screening

Determination of phytochemical content of samples is carried out based on the modified method (Setiyono & Bermawie, 2014).

Test alkaloids as much as 10 mg extract dissolved with 2 mL chloroform and 2 mL ammonia, filtrate filtered and accommodated. The filtrate obtained is then added 5 drops of concentrated H_2SO_4. The solution is whipped to form 2 layers. The top layers are then taken and tested with Meyer, Wagner and Dragendorf reagents respectively. The presence of alkaloids in the sample is indicated by the formation of white or yellow deposits in Meyer's reagents, brown to black deposits in Wagner reagents and orange deposits in Dragendorf reagents. Flavonoid test as much as 10 mg sample reacted with 10 mL of water then heated. The mixture is separated and the filtrate is fed with a powder of 0.1 Mg, 1 mL of 5 concentrated HCl and 1 mL of amyl alcohol. A positive test is characterized by the appearance of a red or yellow color on the amyl alcohol layer. Test saponins as much as 10 mg of extract is put into a test tube, then added 10 mL of hot water and then cooled. The

test solution is shaken vertically for 10 seconds, then observed for 10 minutes. The formation of foam 1.10 cm high indicates the presence of saponins in the sample. On the addition of 1 drop of HCl 2N foam does not disappear. The tannin test solution is prepared by reacting 10 mg of the sample with 5 mL of aquades, then heated to boiling for 5 minutes and the filtrate is filtered. A total of 5 mL of test solution is inserted into the test tube, then a few drops of $FeCl_3$ are added. The formation of dark blue or greenish-black indicates the presence of tannins. Test terpenoids and steroids as much as 10 mg extract added 0.5 mL chloroform, 0.5 mL acetic anhydride, and dripped with concentrated sulfuric acid through the tube wall. The result at the second boundary of the solution is formed brownish-red or purple cicin indicates the presence of terpenoids and the result of the solution at the top becomes green or purple indicating the presence of steroids or triterpenoids.

2.6. Fractionation of extracts

Fractionation is carried out on the basis of a modified method (Salni, Marisa and Muti, 2011). Fractionation is carried out with 3 solvents namely *n*-hexane, ethyl acetate, and water with a separate funnel. as much as 1 gram of ethanol extract is dissolved in 100 mL of water, then put into a separate funnel. then into the split funnel added *n*-hexane as much as 100 mL of mixture shaken slowly every 10 minutes for three times and allowed to stand for 30 minutes until two layers of *n*-hexane fraction (top) and water phase (bottom) are formed. Furthermore, the nonpolar phase (*n*-hexane) is separated from the polar phase (water) and accommodated as the *n*-hexane fraction. The polar phase is then inserted into the split funnel and fractionated again using ethyl acetate. The polar phase (water) is further separated from the semipolar phase (ethyl acetate) and then accommodated in a container. The three fractions are evaporated solvent using a vacuum evaporator at 40°C.

2.7. Brine Shrimp Lethality Test (BSLT) Method

Cytotoxicity activity testing was performed on the basis of a modified method (McLaughluin, Rigers & Anderson, 1998). The larvae of A. *salina* shrimp were used as test animals. A. *salina* larvae were placed in Erlenmeyer filled with seawater and hatched for 2×24 hours with a constant oxygen supply and under a 25 Watt TL lamp at room temperature of 25°C. Ten milligrams of extract were added to a test tube containing 50 µL of Tween80 in 10 mL of seawater to obtain a stock solution (2000 µg/mL). This study used concentration variations of 10, 100, 500 and 1000 µg/mL. Each concentration of the extract was tested three times. Incubation is carried out at room temperature under the light of a 25-watt TL lamp for 24 hours, then the number of live larvae is calculated. Negative control is carried out by the same procedure without the addition of extracts. Larvae of A. salina are considered dead if they do not show any movement during 10 seconds of observation. The extract is considered toxic to A. *salina* larvae if it has an LC_{50} (Letha1 Concentration) value of less than 1000 ppm (Meyer *et al*, 1982).

2.8. Data analysis

The research data will be processed and presented in table form. Data from the toxicity test will be analyzed using linear regression analysis using SPSS 23 to determine the LC_{50} value .

3. RESULTS

3.1. Determination results of Sida Rhombifolia samples

Plant determination is carried out to establish the identity of a plant. Determination in this study is important to determine and ensure the type (species) and the purpose of

determination is to ensure the identity of a plant so as to avoid errors in sample selection. The determination of S. rhombifolia in this study was carried out by sending leaf samples to the Center for Tropical Biopharmaceutical Studies LPPM IPB. The results of the determination showed that the sample was S. *rhombifolia* with the scientific name S. *rhombifolia* Linn.

3.2. Water content and yield extract Sida rhombifolia

Determination of water content aims to provide a limit or maximum range of water content in simplisia which is useful for minimizing the growth of microorganisms, so that simplisia can be stored for a long time. Determination of water content is carried out gravimetrically through heating at a temperature of 105°C. Based on the results of water content measurement, the water content of S. *rhombifolia* simplisia was 2.75% with the percentage yield of S. *rhombifolia* ethanol extract from sonicated extraction was 3.78% of the total weight of dry simplisia of 500 grams. The extract that has been obtained is then phytochemical screening to identify the presence of secondary metabolite compounds in the extract.

3.3. Phytochemistry of ethanol extract of Sida rhombifolia

Based on the results of phytochemical tests conducted to identify the compound content in the ethanol extract of S. rhombifolia leaves. The basic principle is to see the change in reaction formed by adding a reagent specific to a particular content (Oktari, Fitmawati & Nery, 2014). From the test results it was obtained that simplisia and leaf extract of S. rhombifolia contain compounds as shown in Table 1.

Table 1 Screening test results phytochemicals *Sida r hombifolia*

Phytochemicals Compound	Extract ethanol *Sida r hombifolia*
Alkaloids	
Dragendrof	+++
Meyer	++
Wagner	++
Flavonoids	+++
Saponin	+++
Tannin	++
Triterpenoids	++

Description : (+++) : Contains more compounds / concentrated colors
(++) : Contained light compounds/colors

3.4. Fractionation extract ethanol Sida rhombifolia

Based on the results of fractionation using 3 solvents of different polarity, namely n-hexane (nonpolar), ethyl acetate (semipolar) and water (polar). Data on the fractionation of ethanol extract with the three types of solvents are presented in Table 2.

Table 2 Percent yield fraction extract ethanol S. *rhombifolia*

Sample	Yield (%)
n- hexane fraction	12.70
Fraction ethyl acetate	14.30
Water	60.46

3.5. Cytotoxicity extract Sida rhombifolia

The method of testing extracts on A. *salina* shrimp larvae known as BSLT is a pre-screening method for anticancer activity. BSLT test was performed on ethanol extract, *n*-hexane fraction, ethyl acetate and S. *rhombifolia* water. The results obtained are then calculated as the LC50 value of the extract, where the concentration of the extract that can cause the death of A. salina is as much as 50% of the number of shrimp larvae. The death of low-level organisms such as A. *salina* can be used as a guiding tool for screening and searching for new bioactive ingredients. The cytotoxic activity of S. rhombifolia leaf extract and fraction against shrimp larvae can be seen in Table 3.

Table 3 Activities cytotoxic extracts and fractions leaf S. *rhombifolia* against *Artemia salina*

Sample	LC_{50}
Extract ethanol	320.15
n-hexane fraction	500.10
Fraction ethyl acetate	575.06
Water	873.21

4. DISCUSSION

4.1. Water Content and Yield Extract Ethanol Sida rhombifolia

Determination of water content of simlipsia is very important to provide a maximum limit of water content contained in S. *rhombifolia* simplisia. This is because a high amount of water can be a growing medium for microorganisms that can damage and change the content of chemical compounds in simplisia. The moisture content requirement of simplisia is based on the applicable standard parameters according to Food and Drug Monitoring Agency (2014) the moisture content of a good simplisia is not more than 10% to prolong storage. Based on the results of measuring the moisture content of S. rhombifolia simplisia, a simplisia moisture content of 2.80% was obtained, this was due to the drying process that was less than optimal or due to lack of heating. This shows that the moisture content of S. *rhombifolia* simplisia has met the requirements for good water content of simplisia and is suitable for use as a herabal material that is eligible for further testing.

Dried S. *rhombifolia* simplisia is powdered by blending so that the sample size becomes smaller. A smaller sample size can expand the contact and increase its interaction power with the solvent. Samples with powder size will make it easier for solvents to enter and bind to compounds in the sample. The simplisia powder was further extracted using 70% ethanol solvent. The choice of ethanol as a solvent is based on its relatively non-toxic nature and is able to dissolve metabolite compounds optimally (Azis, Febrizky, & Mario, 2014). The extraction method used in this study was sonication extraction. Sonication extraction is an extraction method by utilizing the help of ultrasonic waves generated by the sonicator. This method has advantages over other extraction methods because it can extract metabolites in a material faster and produce a yield that is not much different from other conventional extractions (Jos, Pramudono & Aprianto, 2011). During the extraction process, the sonicator will emit ultrasonic waves. The resulting ultrasonic vibrations will cause the solvent to easily diffuse into the cell, so that the cell undergoes lysis. As a result of cell lysis, the content of metabolite compounds contained in the sample tissue can be deposited into the solvent.

Based on the extraction results using ultrasonication method, the yield percentage of S. *rhombifolia* ethanol extract was 3.78%. The low percentage of yield is thought to come from the comparison between the amount of solvent and simplisia that is not right. According to Meiyanto *et al* (2008), the amount of solvent in the extraction process is one of the factors that greatly determines the percentage of yield, this is because the more the amount of solvent used in extraction, the greater the contact between the material and the solvent so that it has the potential to increase the percentage of yield. Another factor that affects the low yield percentage is the fast length of extraction time. The extraction process that takes place too quickly can allow extraction is not optimal so that it can indirectly have an impact on the percentage of yield produced.

4.2. Phytochemistry of Ethanol Extract of Sida rhombifolia

Phytochemical screening is the initial stage to qualitatively identify the content of plant secondary metabolites. The basic principle is to see the changes in reactions formed by adding a reagent that is specific to a particular content. In addition, this test is also intended to prove that the difference in the location of the region and rainfall from the origin of this plant will show a difference in the composition of secondary metabolites contained in it. Estimation of the class of compounds that play a role in inhibiting cell proliferation can be done with this test. Secondary metabolite compounds commonly found in plants are flavonoids, alkaloids, tannins, saponins, steroids, and terpenoids. Based on previous studies conducted Okoli *et al* (2007), the results of phytochemical screening of S. *rhombifolia* ethanol extract as a whole showed positive results against all tests (table 1). This is in accordance with the statement Okoli *et al* (2007) that the content of secondary metabolites of a plant is strongly influenced by geographical location, altitude and rainfall from which the plant originates. According to Dulay and Decastro (2016), the content of secondary metabolite compounds present in plants is a very important component in their biological activities related to treatment. Some secondary metabolite compounds such as alkaloids, flavonoids, terpenoids, tannins and saponins are said to have biological activities that can act as antimicrobial, antioxidant, anticancer, antiallergic and anti-inflammatory. This is supported by the results from Fartyal and Kumar (2016) which proves that some metabolite compounds such as alkaloids, flavonoids and steroids also have a very important role in the world of medicine, which can be used as natural anticancer agents.

4.3. Fractionation of S. rhombifolia Ethanol Extract

Fractionation is carried out with the aim of separating secondary metabolite compounds in S. *rhombifolia* ethanol extract by using solvents based on polarity properties, namely *n*-hexane solvents, ethyl acetate and water, Polar solvents will dissolve compounds that are polar, while nonpolar solvents will dissolve compounds that are nonpolar, as well as semipolar solvents (Arifianti, Oktarina & Kusumawati, 2014). The selection of these three types of solvents is based on their ability to dissolve compounds secondary metabolites with optimal. Before fractionation, ethanol extract is first dissolved in water until it blends to facilitate fractionation. The dissolved ethanol extract is then put into the split funnel and added *n*-hexane to it for fractionation. When *n*-hexane is inserted into the split funnel, both mixtures of solution form 2 layers. The same thing also happens when the fraction of water fractionated with *n*-hexane is fractionated again using ethyl acetate. The formation of both layers in the solvent mixture is caused by differences in density and polarity between solvents. The fractionation process is carried out by shaking the solvent mixture so that the metabolite compounds from the ethanol extract of S. *rhombifolia* will be partitioned into each solvent according to polarity. The fractionation results that still contain solvent are then evaporated so that a pure fraction is obtained which is expressed in % yield. Based on the results of fractionation that has been done, the water fraction of S. *rhombifolia* has a higher yield percentage than the ethyl acetate fraction and the *n*-hexane fraction (Table 2). The high percentage of yield in the water fraction compared to other fractions is suspected that the secondary metabolites contained in the ethanol extract of S. *rhombifolia* tend to be polar, so that almost all chemical components are more dissolved in water solvents than ethyl acetate and *n*-hexane.

4.4. Cytotoxicity of S. rhombifolia Extract

The Brine Shrimp Lethality Test (BSLT) method is widely used to bioascertain the bioactivity of an extract. Cytotoxicity test of secondary metabolite compounds in S. *rhombifolia* extract using BSLT method using *Artemia salina* shrimp larvae. This method is an inexpensive screening method and does not require a certain specialization in its implementation and this method is a 95% confidence method to observe the toxicity of a compound in plant extracts and does not require a code of ethics in testing.

This test aims to determine an extract has the ability to inhibit the growth of cancer cells and undergo further procedures in the process of finding anticancer drugs (Kumar *et al*, 2013; McLaughlin, Rogers & Anderson, 1998) stating that the method of testing the extract on A. *salina* shrimp larvae known as BSLT is a pre-screening method for anticancer activity. A. *salina* larvae are thought to represent zoological organisms for in vivo mortality testing. The test results conducted to compare the mortality test for A. *salina* and the proliferative inhibition test for carcinoma showed a positive correlation of the toxicity of the test compound to both types of tests. The BSLT test was carried out by observing the mortality rate caused after being given an extract to shrimp larvae after incubating for 1 x 24 hours. The results obtained are then calculated as the LC_{50} value of the extract, where the concentration of the extract that can cause *Artemia salina* death as much as 50%. Based on the LC_{50} value in Table 3, the highest level of toxicity was found in ethanol extract, *n*-hexane fraction, ethyl acetate fraction and lowest toxicity level in water fraction. The smaller the LC_{50} value indicates the higher the content of bioactive compounds from a natural material.

An extract is categorized as active and has a toxic effect if it can cause 50% death of A. salina at a concentration of less than 1000 ppm and is non-toxic if it has a concentration of more than 1000 ppm and the death of Artemia SalinaL each shrimp larvae due to the presence of secondary metabolite compounds that are toxic, toxic compounds present in the extract can enter through the mouth of Artemia SalinaLeach shrimp larvae and be absorbed into the The digestive tract occurs absorption process through the cell membrane. After the absorption process continued with the distribution of toxic compounds into the body of Artemia SalinaLeach shrimp larvae, and a process of metabolic reaction damage occurred (Meyer *et al*, 1982). The anatomical structure of the body of *Artemia salina* L. shrimp larvae at the naupli stage is still very simple, consisting of layers of skin, mouth, anthena, digestive tract or digestion that is still simple, and prospective thoracopods. Drastic changes in the concentration gradient between inside and outside the cell cause toxic compounds to spread well to the body of Artemia SalinaLeach shrimp larvae. The effects of metabolic damage caused by rapid occurrence can be detected within 24 hours, causing 50% death of *Artemia salina L*. shrimp larvae (Dachrinus, Oktima & Stanias, 2005).

5. CONCLUSIONS

Based on the results of the research that has been carried out can be concluded as follows:

1. Secondary metabolites contained in ethanol extract in the leaves of S. rhombifolia by phytochemical screening method are alkaloids, flavonoids, saponins, tannins, and triterpenoids.
2. Ethanol extract of S.rhombifolia leaves has a potential toxicity with an LC_{50} value of 320.15 µg/mL. Alkaloid compounds fractionated from ethanol extract of S.rhombifolia leaves Fraction *n*-hexane 500.10 µg / mL, ethyl acetate 575.06 µg / mL and water 873.21 µg / mL results obtained that ethanol extract is more cytotoxic than fractionation.

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Superhydrophobic Silica Ultrasonic Coating Based on Sinabung Volcanic Ash as Anti-Corrosion of Ferrous Metal Materials

Pelapisan Ultrasonik Superhidrofobik Silika Berbasis Abu Vulkanik Sinabung Sebagai Anti Korosi Material Logam Besi

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ABSTRACT

Superhydrophobic materials are substances with a water contact angle exceeding 150°. They can be employed as coatings to prevent corrosion on ferrous metals. This occurs due to the ability of superhydrophobic materials to hinder water adhesion to the metal surface, resulting in less interaction between the metal, water, and oxygen. Ultrasonication is a technique to blend, standardize, and enhance the dispersion of particles. This research uses ultrasonic waves to disperse superhydrophobic silica into a homogeneous mixture. It is then applied as a protective coating material on the surface of ferrous metal to increase its ability to inhibit ferrous metal corrosion. The main material used in this research was Sinabung volcanic ash-based silica, which was synthesized using the sol-gel method. Superhydrophobic silica material was added to the paint by dispersing (0; 0.6; 1.2; 1.8) g with 10 mL of paint using an ultrasonic homogenizer for 15 minutes. The corrosion test was carried out by immersing the sample in a corrosive solution for 96 hours. The results show the effect of sonification and variations in the composition of superhydrophobic silica on the performance of the superhydrophobic silica layer on the surface of iron against corrosive solutions of 15% HCl (v/v) and 3.5% NaCl (w/v). The optimal conditions for varying compositions of superhydrophobic silica were determined to achieve the lowest corrosion rate in different corrosive solutions. Specifically, the corrosion rate was 64.51 mpy at 15% HCl (SS3) and 1.31 mpy at 3.5% NaCl (SS6). These corrosion rates were inversely proportional to the inhibitor efficiency, measured at 83.40% in 15% HCl (SS3) and 93.75% in 3.5% NaCl (SS6). Therefore, the superhydrophobic silica material is highly suitable as an additional component in iron coatings to protect against corrosion effectively.

Keywords: superhydrophobic silica, ultrasonically dip coating, corrosion rate, corrosion inhibitor.

ABSTRAK

Bahan superhidrofobik adalah bahan yang memiliki sudut kontak air lebih besar dari 150° yang dapat digunakan sebagai bahan pelapisan untuk penghambat korosi pada logam besi. Hal ini karena bahan superhidrofobik dapat mencegah air menempel pada permukaan logam, sehingga dapat mengurangi kontak antara logam dengan air dan oksigen. Ultrasonikasi dapat digunakan untuk mencampurkan, menghomogenkan, dan memperluas penyebaran suatu partikel. Penelitian ini memanfaatkan gelombang

ultrasonik untuk mendispersikan superhidrofobik silika dan cat menjadi suatu campuran yang homogen dan mengaplikasikannya menjadi bahan lapisan pelindung dipermukaan logam besi dengan tujuan untuk meningkatkan kemampuanya dalam penghambatan korosi logam besi. Bahan utama yang digunakan dalam penelitian ini adalah silika berbasis abu vulkanik Sinabung yang disintesis dengan metode sol-gel. Penambahan bahan superhidrofobik silika pada cat dilakukan dengan mendispersikan (0; 0,6; 1,2; 1,8) g dengan 10 mL cat menggunakan alat ultrasonic homogenizer selama 15 menit. Uji korosi dilakukan dengan merendam sampel dalam larutan korosif selama 96 jam. Hasilnya terdapat pengaruh sonifikasi dan variasi komposisi superhidrofobik silika pada kinerja lapisan superhidrofobik silika di permukaan besi terhadap larutan korosif HCl 15% (v/v) dan NaCl 3,5% (b/v). Kondisi optimum pada variasi komposisi superhidrofobik silika diperoleh dengan laju korosi terendah dimasing-masing larutan korosif sebesar 64.51 mpy pada HCl 15% (SS3), dan 1.31 mpy pada NaCl 3,5% (SS6), yang berbanding terbalik dengan efisiensi inhibitor yang tertinggi dimasing-masing larutan korosif sebesar 83,40% pada HCl 15% (SS3), dan 93,75% pada NaCl 3,5% (SS6), sehingga bahan superhidrofobik silika cukup layak dipakai sebagai bahan tambahan pada pelapisan besi untuk melindunginya dari korosi.

Kata Kunci: superhidrofobik silika, pelapisan celup ultrasonik, laju korosi, penghambat korosi

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1. INTRODUCTION

Metal possesses numerous benefits but is also susceptible to corrosion (Affandi, 2020). The cause of this phenomenon can be attributed to the elevated levels of humidity and acidic air in Indonesia (Priyotomo, 2020). Applying a protective layer to metal surfaces is a technique to inhibit corrosion (Amri, 2020). However, previous research on metal coating methods has not used practical, efficient, and cost-effective technology (Miranda, 2020). Therefore, further research is needed to develop metal coating methods that are more practical, efficient, and cost-effective, enabling their widespread use to mitigate metal corrosion.

Research on developing hydrophobic coatings employing fluorocarbon compounds like fluoroalkylsilane (FOK) is being discontinued due to its adverse effects on health and environmental contamination (Ilham, 2019). Furthermore, hydrophobic coating studies shifted to using more environmentally friendly compounds such as trimethylchlorosilane (TMCS) and xylene. An inherent drawback of this chemical is its relatively low hydrophobicity, measuring less than 110. Consequently, water can still permeate the sample's surface (Setyawan D. H., 2014; Setyawan Y. & ., 2015). The next step involves utilizing a blend of hydrophobic and coupling agents to enhance the hydrophobic properties of the material surface by employing silicates. An identified weakness of the superhydrophobic coating method is its lower efficiency, as highlighted by Qiao (2018). Satish (2013) researched superhydrophobic properties in silica-based coatings, specifically focusing on the durability and restoration of these properties. The study utilized sol-gel dip coatings, as described by Mahadik (2013). The resulting superhydrophobic layer had a contact angle of 148.24°. Theoretically, a surface is categorized as superhydrophobic if the contact angle is >150°. The silica coating on iron metal is suboptimal due to the weak adhesion between silica and iron. It is believed to be due to the less efficient coating technique being used. To address this, it is recommended that the technique be updated by employing the ultrasonic dip coating method. This technique

offers the advantage of enhancing the uniformity and stability of the mixture and facilitating the efficient and rapid dissolution of the sample. Adopting this technique allows the coating process to be carried out effectively and smoothly (Haser, 2018).

Research related to hydrophobic materials has attracted researchers' attention because of its lotus effect. The lotus effect is a phenomenon when water droplets that fall on the surface of lotus leaves form dots and roll, carrying pollutants or dust that stick to the surface of the leaves. This phenomenon is often known as the "Lotus effect" (Barthlott, 1997). This is caused by the presence of microscopic structures on the surface of lotus leaves, which are capable of producing high water contact angles (>150°) (Putri, 2018). Based on these findings, researchers tried to apply the lotus effect mechanism to coating systems in various ways. The hydrophobic coating offers the benefit of being water-repellent since it can effectively prevent water from adhering to the metal surface. This can minimize corrosion problems because water is a corrosion medium. The hydrophobic coating on a metal surface ensures that water droplets roll off without leaving any marks, thereby maintaining a clean appearance. This makes the metal surface always look clean. Furthermore, hydrophobic coating can reduce friction between the fluid and the metal surface. This can increase work efficiency in equipment that uses fluids. When hydrophobic coatings are used on metal coatings, they can effectively reduce corrosion issues, enhance job productivity, and lower maintenance expenses (Hasanah, 2018).

Silica is an oxide material with potential high-tech applications, including metal coating. Silica particles with small to nanoscale sizes offer several benefits, including chemical resistance, low density, heat resistance, robust atomic bonding, and high adhesion (Sinaga, 2019). Silica is a material that is strong, durable, and environmentally friendly. Silica can serve as a protective layer for metal, shielding it from corrosion, abrasion, and elevated temperatures. The ash from Mount Sinabung has a high concentration of silicon (Si) and can be easily obtained, making it a promising supply of silica for metal coatings (Simatupang & D., 2019; Manurung, 2019). This research aimed to determine the results of mixing the composition of silica and TMCS-xylene on the coating of the coated material to determine the performance of superhydrophobic silica solutions with various composition variations to inhibit the rate of iron corrosion in 15% HCl and 3.5% NaCl solutions and to analyze the effect of sonification (ultrasonically dip coating) on the effectiveness of coating on iron metal in 15% HCl and 3.5% NaCl solutions.

2. MATERIALS AND METHODS

2.1. Materials

The research material was volcanic ash from Mount Sinabung, Kabanjahe Regency, Beras Tepu Village. Test samples included metal plates (Strip Plate), Iron paint (ABC Brand), velvet cloth (Aura Velvet Brand), metal polish (Autosol Brand), and sandpaper. Other supporting materials were NaOH (E-Merck), 37% HCl (E-Merck), NaCl (E-Merck), xylene (E-Merck), trimethylchlorosilane (TMCS), and aqua DM. The equipment was an oven, furnace, 200-mesh sieve, laser cutting, analytical scales, and grinding equipment (mortar). The analytical instruments used were X-ray diffraction (XRD) (D8 Advance Eco (Bruker), Bragg-Bentano Diffraction), and Scanning Electron Microscopy (SEM) (Zeiss type EPOMH 10 Zss).

2.2. Extraction of silica from the volcanic ash of Mount Sinabung

Silica (SiO_2) extraction from Sinabung volcanic ash was carried out using the sol-gel method. First, the volcanic ash was sieved with 200 mesh and weighed 20 grams. The ash was soaked in 12 M HCl for 24 hours to remove metal ions. After that, the ash was neutralized with distilled water and dried in an oven at 120°C for 6 hours. Second, dry volcanic ash was destroyed by dissolving it in 8 M NaOH. The solution was boiled while stirring until it thickened. After almost drying, the solution was put into the furnace at 750°C for 3 hours. After cooling, the solution was added with 200 mL of distilled water and left for 12 hours. Third, the mixture was filtered, and 20 mL of the filtered solution was taken. The solution was dripped with 3 M HCl while stirring with a magnetic stirrer until the pH approached 7. A white silica precipitate was formed. The precipitate was filtered and washed with distilled water. The precipitate was dried in an oven at 120°C for ± 3 hours (Bariyah, 2020).

2.3. Preparation of superhydrophobic silica materials

Silica with various concentrations (0.6 g, 1.2 g, and 1.8 g) was dispersed in 10 mL of 4M NaOH while heated at 50ºC (1 hour) until a completely dissolved Na_2SiO_3 solution was obtained. Next, TMCS 13% (v/v) was added, then xylene solvent (v/v) slowly into the mixture and heated at 50ºC (2 hours) until a homogeneous solution was obtained (Silviana, 2021). The next stage was to apply a layer of material on the iron surface. Each superhydrophobic silica solution of various concentrations was added to the paint with a paint volume of 10 mL for each composition variation and dispersed for 15 minutes using Ultrasonic Homogenizers (OMNI SONIC Ruptor 400 with a wavelength of 40 kHz, power 400 watts, amplitude 40%) until a homogeneous mixture was obtained.

2.4. Coating 'Ultrasonically Dip Coating'

The sample used was an iron metal plate measuring 2×2 cm and 3 mm (0.3 cm) thick, which was prepared with 1500 grit to flatten and remove scratches on the sample surface. The coating process was carried out using a simple dip coating method (Ultrasonically Dip Coating) for 15 minutes with a frequency of 40 kHz, power 40% in an Ultrasonic Water Bath — an ultrasonic bath (BANDELIN BactoSonic Type BS 14.2, 800 W, 40 kHz), and drying for 24 hours at room temperature (Haser, 2018).

2.5. Corrosion testing

Iron corrosion testing was carried out by immersing an iron plate that had been coated with superhydrophobic silica material in the test solution for 96 hours. The test solution was a corrosive solution, namely a 15% HCl solution and a 3.5% NaCl solution. The 15% HCl acid solution represents an acidic environment, while the 3.5% NaCl solution represents a salty environment which causes corrosion.

3. RESULTS AND DISCUSSION

3.1. Silica (sio_2) extraction results from mount Sinabung volcanic ash

The silica extraction process from Sinabung mountain ash was carried out in three stages. First, sodium silicate (Na_2SiO_3) from Sinabung mountain ash was prepared using NaOH. The dehydration process will occur in this condition, and the second OH^- will bond with hydrogen to form water. The two Na^+ ions will balance the negative charge formed and interact with the SiO_3^{2-} ion to form Na_2SiO_3 in Equation 3.1. Second was the preparation of silicic acid and $Si(OH)_4$. At this stage, the sodium silicate solution was reacted with a strong acid (HCl) until a precipitate (silica gel) was formed, which was still mixed with NaCl. In this reaction, the hydroxyl group

(OH^-) in sodium silicate was replaced by a hydrogen group (H^+). The released hydroxyl groups then react with water to form silicic acid. The silicic acid formed was then hydrated to form silica gel in the chemical reaction Equations 3.2 and 3.3. Third was the preparation of SiO_2 using the $Si(OH)_4$ silica gel drying process (Bariyah, 2020). The final stage was to evaporate the water with an oven. The final result of this drying process was white silica powder, as in Figure 1, with the following formation reaction:

$$SiO_{2\,(s)} + 2NaOH_{\,(aq)} + H_2O_{\,(l)} \rightarrow Na_2SiO_{3\,(aq)} + 2H_{2\,(g)} \quad (3.1)$$

$$Na_2SiO_{3\,(aq)} + 2H^+_{\,(aq)} \rightarrow 2Na^+_{\,(aq)} + O{=}Si(OH)_{2\,(aq)} \quad (3.2)$$

$$O{=}Si(OH)_{2\,(aq)} + H_2O_{\,(l)} \rightarrow Si(OH)_{4\,(s)} \quad (3.3)$$

Figure 1. Silica powder extracted from volcanic ash from Mount Sinabung.

Silica gel is a material that has a unique texture and diverse properties. The texture can be hydrogel, xerogel, or airgel, depending on the manufacturing process. The result of this silica extraction is silica xerogel, which has a hard and brittle texture. Silica gel has inert, hydrophobic, and transparent properties (Silviana, 2021). Its inert nature means it does not easily react with other chemicals. Its hydrophobic nature means it does not absorb water easily. Its transparent nature makes it usable for applications that require transparent materials. Silica gel also has other beneficial properties, such as high mechanical strength and thermal stability, and does not expand in organic solvents (Dirna, 2020). Silica gel is also stable towards hydrogen except fluorine and inert towards all acids except HF. The quality of silica gel is determined by various factors, namely internal structure, particle size, porosity, surface area, resistance, and polarity. Silica gel has several weaknesses: the active site is only siloxane and silanol. This silanol group has low acidity, in addition to having a weak donor atom. However, siloxane and silanol groups are also advantageous because they allow modification (Suchithra, 2012). Silica gel can be modified to improve its properties and be used for wider applications.

3.2. Ultrasonically dip coating of iron plates

Using the Ultrasonically Dip Coating technology, the iron plate's surface was coated with a superhydrophobic silica material. The process involved different versions, including a blank coating without inhibitors labeled as C1/C2 and a coating with superhydrophobic silica material (SS). The variations were determined by the amount of silica used as a superhydrophobic substance, specifically 0.6 g, 1.2 g, and 1.8 g for every 10 mL of paint. Variations in material composition were labeled: SS1/SS4 (0.6:10) g/mL, SS2/SS5 (1.2:10) g/mL, SS3/SS6 (1.8:10) g/mL, each of which represents the test in a corrosive solution of 15% HCl/3.5% NaCl. The documentation for the iron plate that has been coated after drying for 24 hours is presented in Figure 2. The iron plate's surface, solely covered with paint, has greater shine. Conversely, the surface of the iron plate, which is covered with a superhydrophobic silica substance, appears rougher. This is because of the presence of silica particles. In addition, the ultrasonically superhydrophobic silica coating (SS1) findings demonstrate that the superhydrophobic silica material forms a thin layer that tightly and densely adheres to

the iron plate sample's surface, effectively filling any gaps. This coating technique allows the superhydrophobic silica material to conform to the shape of the substrate surface, resulting in a strong bond between the substrate and the coating material through mechanical interlocking (Farid, 2019).

3.3. XRD and EM analysis of superhydrophobic silica coating on iron plates

a. XRD (X-Ray Diffraction) characterization

X-ray diffraction (XRD) was employed to characterize the crystal structure that developed on the iron plate. X-rays with a wavelength (λ) were directed at the crystal surface at an angle of 2θ. This XRD characterization used a type D8 Advance Eco diffractometer (Bruker), Bragg-Bentano Diffraction, equipped with Automatic Powder Diffraction software, and used a Cu anod tube with a wavelength of 1.54060 A. The data obtained from XRD analysis was a diffractogram, a graph of the relationship between intensity (I) spectrum peaks and diffraction angles (2θ). The diffractogram shows the spectrum peaks that appear in the sample. Figure 3 presents the XRD test results of iron plate samples before and after superhydrophobic silica coating in the origin program view.

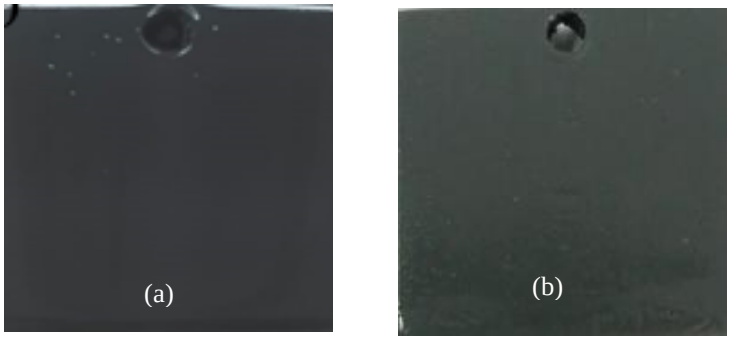


Figure 2. (a) Iron surface with a blank layer (C), (b) Iron surface with a superhydrophobic silica layer (SS1).

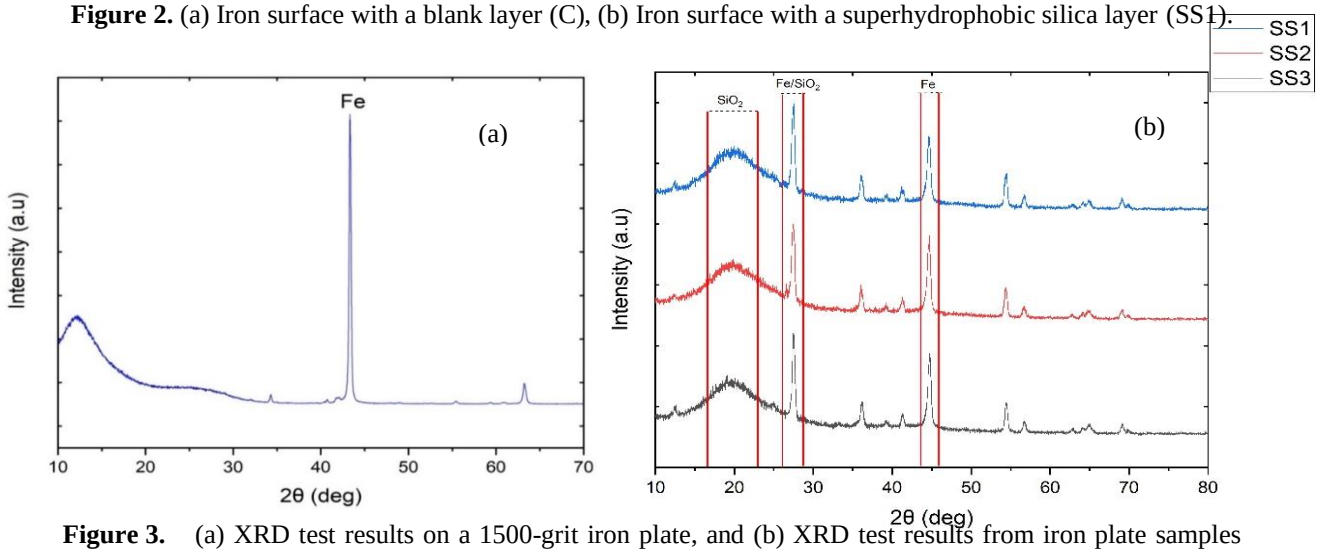


Figure 3. (a) XRD test results on a 1500-grit iron plate, and (b) XRD test results from iron plate samples after superhydrophobic silica coating.

The peak intensity was plotted on the y-axis, and the measured diffraction angle was plotted on the x-axis, where the diffraction angle was (2θ), which was between 10° - 70°.

Based on the XRD test results, the highest peak was obtained at the angle 2θ = 44.081° (JCPDS 06-0696), which was the Fe element with an intensity of 10233.5 cps. Specimens that have been coated with superhydrophobic silica material using Ultrasonically Dip Coating are presented in Figure 3(b). Each was labeled according to variations in the addition of silica additives with a combination of paint/silica nanoparticles, namely SS1 (0.6: 10) g/mL, SS2 (1.2:10) g/mL, and SS3 (1.8:10) g/mL. Based on the diffractogram graph where the diffraction angle (2θ) was between 10° - 80°, the XRD pattern for the SiO_2 compound widened at an angle of 2θ = 20.8° (JCPDS 0.36-1451) which showed an amorphous nature where the pattern was in the shape of a hump. The amorphous nature of the resulting silica gel is beneficial when this material is further modified because it is very reactive. Then, a sharp peak appeared at 2θ = 26.38° (JCPDS 05-0490), the peak of the Fe crystalline phase coated with SiO_2 quarts. The silica phase in the silica paint mixture becomes crystalline due to the influence of ultrasonication treatment, which, during the cavitation process, will result in bubble collapse, namely the breaking of small waves caused by sound. As a result, a hotspot event will occur, namely very intense local heating, which will cause the crystalline level of silica to increase, resulting in the silica particles becoming more crystallized (Guo, 2016). Then, the appearance of a diffraction peak at 2θ = 40° - 50° indicates that the crystal phase is Fe (JCPDS 06-0696). Based on the description above, variations in the composition of superhydrophobic silica applied to each iron plate do not significantly change its structure. Scientific evidence has demonstrated that sound waves used in the coating process do not alter the chemical characteristics or harm the chemical bonds in iron plates with varying compositions.

Figure 4 presents diffractograms of XRD test results on iron plate samples immersed in 15% HCl and 3.5% NaCl solutions, respectively.

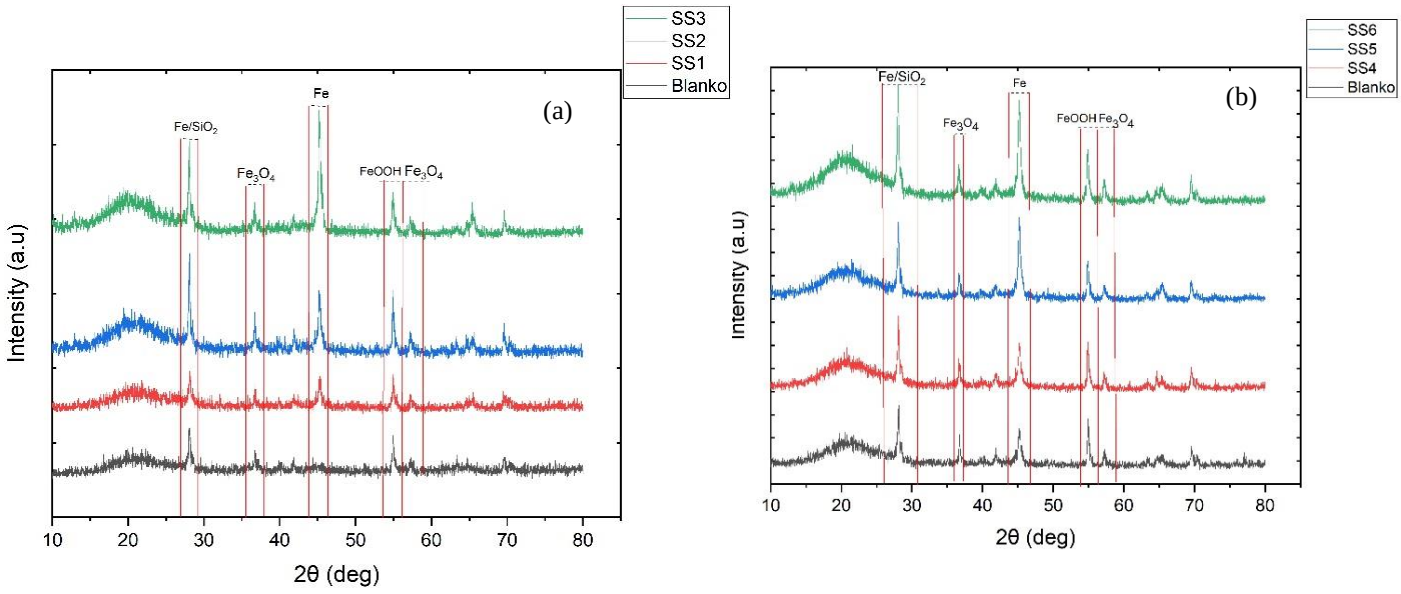


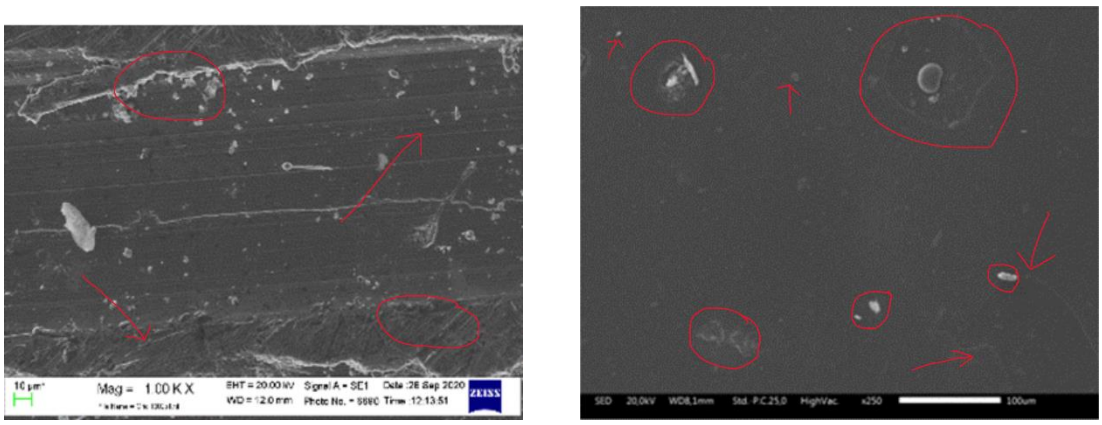
Figure 4. XRD diffractogram of an iron plate in solution, (a) HCl 15% (v/v) and (b) NaCl 3.5% (w/v).

Based on the diffractogram graph in Figure 4, the diffraction angle (2θ) is between 10°-80°. The XRD pattern for the amorphous SiO2 phase compound broadens at an angle of 2θ =

20.8° (JCPDS 0.36-1451) with intensity increasing as the composition of the superhydrophobic material is added. In the image above, the diffraction pattern of an iron plate coated with paint (blank) shows the peak of Fe content at 2θ = 40° - 50°, the smallest compared to iron plates coated with paint and superhydrophobic silica. This is due to the formation of deposits, which are corrosion products resulting from the interaction between the iron plate and the corrosion environment, namely 15% HCl and 3.5% NaCl solutions. The deposits could be Fe_2O_3, Fe_3O_4, and FeOOH, which are corrosion products in the form of a reddish-brown substance that is brittle and porous (Koch, 2017). These deposits would cover the Fe so that the Fe content becomes less. From the composition, the addition of superhydrophobic silica of 0.6 grams, 1.2 grams, and 1.8 grams shows that the Fe peak at 2θ = 40°- 50° is increasing (sharp). This shows that adding superhydrophobic silica mass to the paint will increase the Fe content, as indicated by the higher Fe peak at 2θ = 40°-50°. This indicates that adding silica and surface smoothness greatly influences the corrosion rate. This is in accordance with the theory put forward by Janariah (2022) that the tendency for corrosion is smaller in smooth specimens because the surface is flat. On the iron plate, you can also see the appearance of a new peak that is not too sharp when immersed in a corrosive solution. It can be seen at 2θ = 60°- 70°, which is a Fe_2O_3 corrosion product. The appearance of Fe_2O_3 indicates that during the immersion process with a corrosive solution, the oxidation process of Fe_2O_3 by oxygen occurs (Abdeen, 2020).

b. SEM (Scanning Electron Microscopy) characterization

Scanning Electron Microscopy (SEM) test was carried out to determine the morphology and distribution of sample composition. SEM is utilized to verify the morphological composition of a substance, allowing for the examination of its grain size, surface topography, and structural imperfections (Setyawan D. H., 2014). The iron plate samples included in this study were arranged in a grid pattern of 1500 to homogenize and eliminate any scratches present on the surface of the samples. The results of the 1500 Grid Iron Plate characterization test using SEM referred to Simatupang et al. (2023). Figure 5 (a) shows the presence of lines on the surface of the sample due to the blasting process, which makes the surface of the iron plate sample look smooth and even with a uniform level of roughness so that the sample can be coated with superhydrophobic silica (Simatupang L. S., 2023). SEM testing was conducted on iron plate samples coated with a superhydrophobic silica layer of composition 10:0.6 (SS1). Figure 5(b) shows ultrasonically coating superhydrophobic silica (SS1). The SEM images depict the morphology of several iron plate samples. The coating surface of the iron plate samples appears smooth and devoid of any scratches following the ultrasonic coating process, as opposed to the SEM morphology shown in Figure 5 (a).

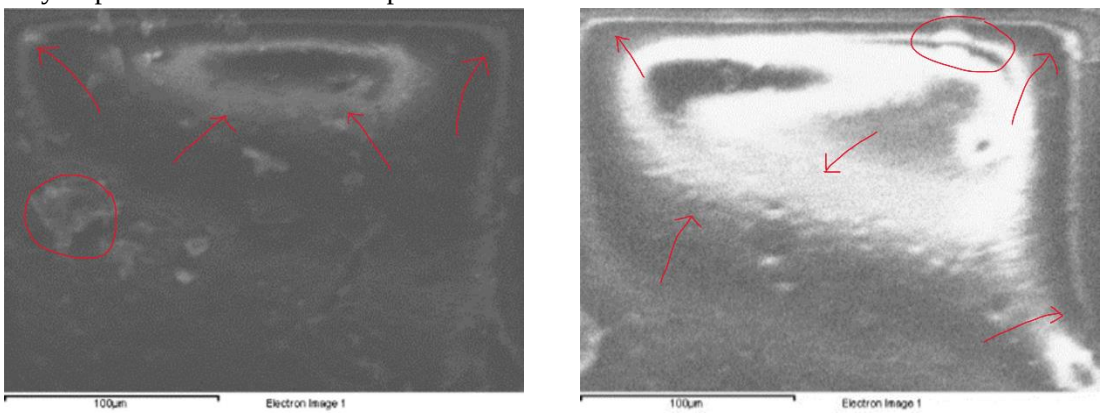


(a) (b)

Figure 5. SEM test results of iron plates before corrosion testing (a) Surface of 1500 grit iron plate (Simatupang et al., 2023), and (b) Surface of superhydrophobic silica layer (SS1).

Superhydrophobic silica coating carried out using a simple ultrasonically dip coating method shows that superhydrophobic silica is able to penetrate well into the blasting holes. The ultrasonic coating method enables superhydrophobic silica to conform to the contours of the substrate surface (Marhamah, 2022). The SEM test findings indicate that the interface between the substrate and the superhydrophobic silica layer exhibits a strong connection, allowing for mechanical interlocking between the substrate and the paint/silica nanoparticles.

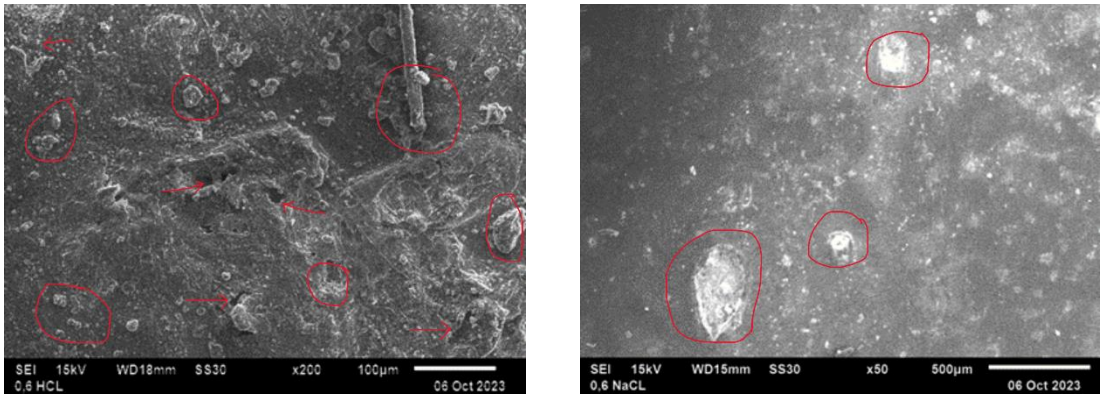
The process of creating a superhydrophobic silica material protective layer was achieved by subjecting it to ultrasonic treatment for 15 minutes. The cavitation effect of sound waves will push superhydrophobic silica particles to fill the empty space on the iron plate. SEM characterization shows the morphological appearance of the protective layer of superhydrophobic silica material on the test sample after corrosion testing. The results show that the superhydrophobic silica layer will inhibit the contact of the iron plate with a corrosive environment (Korb, 1992). The results of the SEM characterization test on the surface of the iron plate after coating 10 mL of paint (blank) are presented in **Figure 6**.



(a) (b)

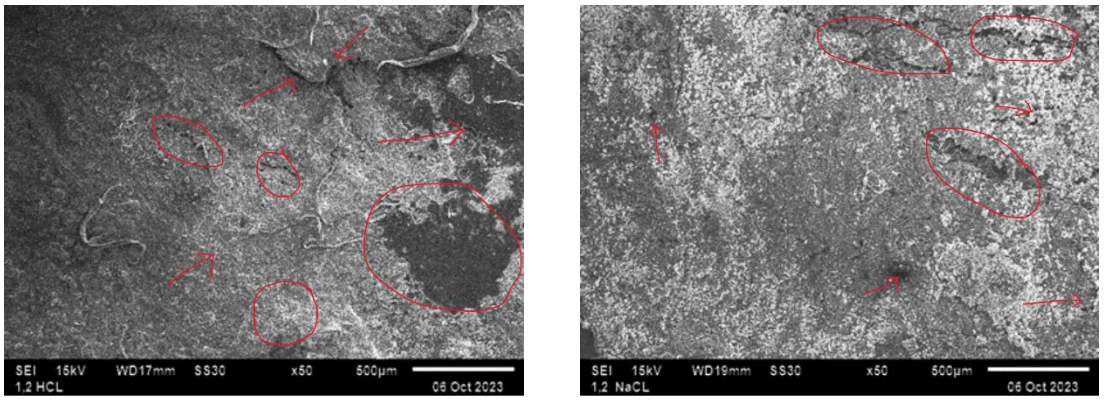
Figure 6. SEM test results of the surface of a blank iron plate after corrosion testing (a) immersed in 15% HCl and (b) immersed in 3.5% NaCl.

Figure 7 shows the results of the SEM characterization test on the surface of the iron plate after it was coated with 0.6 g superhydrophobic silica and 10 mL paint. The results of the SEM characterization test on the surface of the iron plate after being coated with 1.2 g superhydrophobic silica and 10 ml paint are shown in **Figure 8**. **Figure 9** shows the SEM characterization test results on the surface of the iron plate after it was coated with 1.8 g superhydrophobic silica and 10 mL paint.



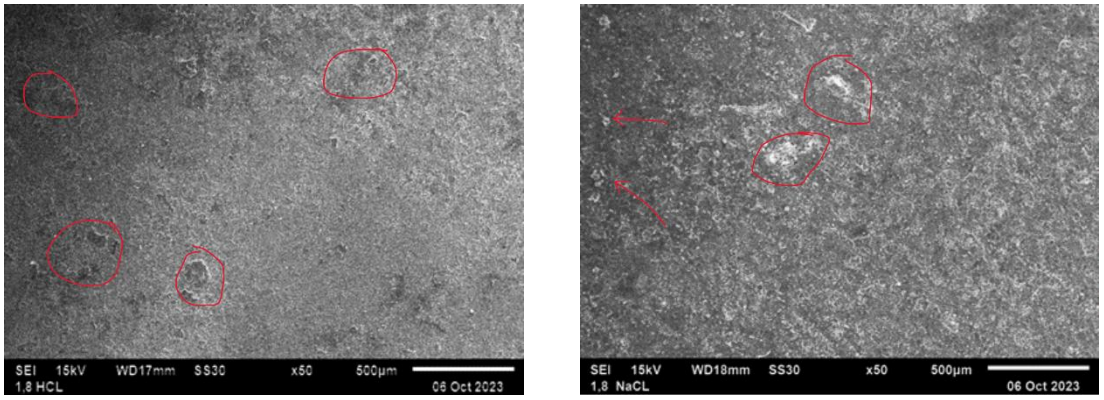
(a) (b)

Figure 7. SEM test results of the surface of an iron plate after corrosion testing (a) sample SS1 in 15% HCl and (b) sample SS4 in NaCl 3.5%.



(a) (b)

Figure 8. SEM test results of the iron plate surface after corrosion testing (a) SS2 sample in 15% HCl and (b) SS5 sample in 3.5% NaCl.



(a) (b)

Figure 9. SEM test results of the surface of an iron plate after corrosion testing (a) SS3 sample in 15% HCl and (b) SS6 sample in 3.5% NaCl.

Based on the SEM test results, it can be seen that the layer on the surface of the iron plate only experienced slight cracks, and some small holes appeared, which became a way for Cl^- ions to damage the surface of the iron plate. The corrosion caused by the HCl solution is greater than that caused by the NaCl solution due to its highly corrosive nature. The reaction between the iron plate and the environment involves the transfer of electrons, and the metal is oxidized into ions (A. F. 'Adzimaa, 2013). When immersed in a corrosive solution, the difference between an iron plate coated with superhydrophobic silica and paint is clearly visible. Superhydrophobic silica materials can reduce the rate of metal corrosion in a chloride environment by donating silica ions to the iron so that a thin membrane or deposit forms that covers the iron surface. Silica remains stable on the surface; it is proven that silica has stability in acidic media. In other words, to improve the corrosion protection performance of the coating, corrosion inhibitors must be added to the coating system. Superhydrophobic silica mixed with paint can provide maximum protection to iron plates from corrosion attacks by acidic media (Qiao, 2018). The SEM test photo in **Figure 9** shows the distribution of the white Si element, which is more abundant and evenly distributed as the variation in the composition of the superhydrophobic silica material in the surface layer increases. The wide distribution of the Si element shows the influence of ultrasonic waves in the superhydrophobic silica coating process on the surface of iron plates by filling voids and gaps on the substrate surface, thus creating a strong passive layer between the silica and the substrate.

3.4. Corrosion rate and efficiency of superhydrophobic silica materials

The corrosion rate test assesses the specimen's resistance to rust or corrosion. The corrosion rate was determined by conducting corrosion experiments over a specific duration, during which the alteration in the weight of a material caused by corrosion was measured. Then, the corrosion rate value was calculated. The corrosion rate will provide insight into the effectiveness of applying superhydrophobic silica materials. The corrosion rate (LK) calculation was based on the gravimetric method, specifically the weight loss method. The average corrosion rate using this method was obtained through Equation 3.4 (MF, 2012; A. F. 'Adzimaa, 2013).

$$\boldsymbol{Corossion\ rate\ (mpy)} = \frac{\boldsymbol{W \times K}}{\boldsymbol{DA_s T}} = \frac{\boldsymbol{(W_0 - W_1) \times K}}{\boldsymbol{DA_s T}} \quad (3.4)$$

Note:

mpy = Corrosion rate in mils per year (mpy)

W = Weught loss (g)

w_0 = Initial sample weight (g)

w_1 = Sample weight after corossion (g)

K = Corrosion rate factor constant 3.45×10^6 (mpy)

D = Specimen density (g/cm^3) for iron metal 7.874 g/cm^3

A_s = Sample surface area (cm^2) of iron plate (p = 2 cm; l = 2 cm; t = 3 mm = 0.3 cm)

$A_s = 2 \cdot (p \times l + p \times t + l \times t)$

$A_s = 2 \cdot (2 \times 2 + 2 \times 0.3 + 2 \times 0.3)$ cm

$A_s = 10.4\ cm^2$

T = Length of testing time (hours) for 96 hours

Calculating the corrosion rate can be obtained by taking several data such as weight loss, submerged surface area, immersion time, and the density of the metal being tested. By calculating the corrosion rate, the efficiency of the superhydrophobic silica material from each corrosion test solution can be determined. The superhydrophobic efficiency of silica was calculated using Equation 3.5 (Irianty, 2012; A. F. Adzimaa, 2013).

The corrosion activity of the test samples that occurred in an acid solution (HCl 15%) was higher than immersion in a salt solution (NaCl 3.5%). Acidic solutions are very corrosive. Metals in an acidic medium will easily undergo oxidation because it is an anode reaction. The corrosion rate is influenced by variations in the application of superhydrophobic silica material. The activity of silica as a precursor to additives in paint at micro sizes influences the performance of superhydrophobic silica to inhibit corrosion. The ability of superhydrophobic silica to coat metal surfaces increases due to reduced particle size conditions. This gives the product different properties that can improve its quality and activity.

$$\boldsymbol{Efficiency}\ (\%) = \frac{\boldsymbol{LK_0 - LK_1}}{\boldsymbol{LK_0}} \times \mathbf{100}\% \quad (3.5)$$

Note:

LK_0 = Non-additive corrosion rate (mpy)

LK_1 = Additive corrosion rate (mpy)

Table 1. Corrosion Rate and Efficiency of superhydrophobic silica materials

No	Material	Corrosion Rate (mpy)		Efficiency (%)	
		HCl 15%	NaCl 3,5%	HCl 15%	NaCl 3,5%
1	Paint	388.82	21.06	-	-
2	SS1 (0.6:10)	186.51	3.94	52.03	81.25
3	SS2 (1.2:10)	96.54	2.63	75.16	87.50
4	SS3 (1.8:10)	64.51	1.31	83.40	93.75

* Description: C: Paint 10 mL (Blank); SS: Superhydrophobic Silica

The iron plate coated alone with paint (C1-C2) exhibited a higher corrosion rate in comparison to the iron plate coated with superhydrophobic silica (SS1-SS3). Thus, there is an influence of variations in silica composition on the performance of paint coated on an iron surface in a corrosive solution of 15% (v/v) HCl (A) and 3.5% (w/v) NaCl (B), as shown in **Figures 10** and **Figure 11**. As the amount of silica additive increases, the corrosion rate on the iron plate decreases. This relationship is inversely related to the inhibitor efficiency, as stated by Koch (2017) and Janariah (2022), and is directly proportional to the corrosion rate value. The SEM test findings confirm that the superhydrophobic silica layer, applied to the iron plate samples by ultrasonically dip coating, effectively penetrates the substrate layer. This coating technique employs the cavitation phenomenon generated by ultrasonic waves in a liquid medium. The waves propel the paint/silica nanoparticles into the cavities formed by blasting, conforming to the shape of the substrate surface and forming a protective layer. Ultrasonic wave irradiation improves the efficiency of filling hard-to-reach cavities on the substrate with superhydrophobic silica. This increases the total surface area and creates a strong bond between the substrate and the superhydrophobic silica, enhancing the adhesion strength of the layer during electrochemical reactions.

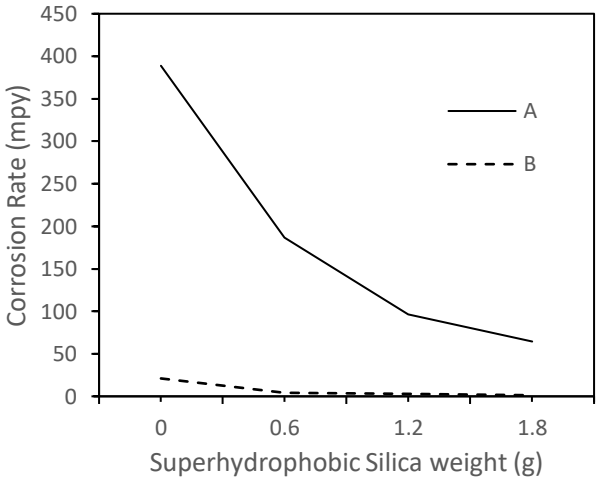


Figure 10. Corrosion Rate of the iron plate for various superhydrophobic silica compositions.

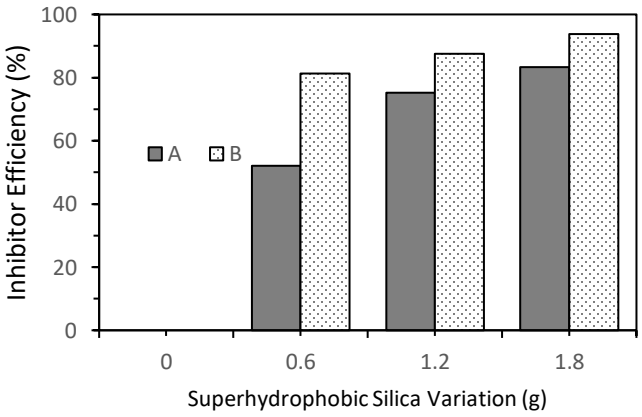


Figure 11. Inhibitor efficiency for various superhydrophobic silica compositions.

4. CONCLUSIONS

There is an influence of sonification (ultrasonically dip coating) and variations in the composition of superhydrophobic silica on the performance of ultrasonic superhydrophobic silica coating on the iron surface against corrosive solutions of 15% HCl and 3.5% NaCl. The most optimum conditions resulting in the lowest corrosion rate for each solution were seen for sample SS3, with a corrosion rate of 64.51 mpy in a 15% HCl (v/v) solution, and for sample SS6, with a corrosion rate of 1.31 mpy in a 3.5% NaCl (w/v) solution. The maximum level of inhibitor effectiveness was observed in the SS3 sample, with an efficiency of 83.40%, when exposed to a 15% HCl (v/v) solution. Similarly, the SS6 sample exhibited the highest inhibitor efficiency of 93.75% when exposed to a 3.5% NaCl (w/v) solution. Ultrasonically coated superhydrophobic silica material could protect against iron corrosion in HCl and NaCl acid solutions. Adding superhydrophobic silica to paint could improve the coating's performance in protecting iron from corrosion. Therefore, superhydrophobic silica material is quite suitable as an additive for iron coatings to provide protection against corrosion. Based on the SEM test results, increasing the concentration of superhydrophobic silica in the paint mixture will form a thicker and more uniform layer. Additionally, this leads to a reduction in the corrosion rate.

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Comparing the Effectiveness of Methods and Solvents on the Yield and Phytochemicals of Gerga Citrus Peel Essential Oil (*Citrus nobilis L.* Var RGL) from Kerinci Regency

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Abstract

Gerga orange was one of the leading commodities of Bengkulu province and also widely cultivated in Kerinci, Jambi. Nearly 75% of gerga orange peel was wasted without any processing. Orange peel was a plant that can be produced in to essential oil. However, the extraction effectiveness can be affected by the solvent and method. The purpose of this study was to determine the best solvent and method for extracting gerga orange peel essential oil. In addition, this research also aims to analyze the phytochemicals and yield. In this study, maceration and soxhletation methods will be compared to extract gerga orange peel essential oil with a variety of solvents with different polarities. The simplicia was macerated with ethanol, ethyl acetate and n-hexane solvents respectively for 3×24 hours. Then it was distilled to evaporate the ethanol. While the soxhletation method the simplicia are percolated using ethanol, ethyl acetate, and n-hexane solvents respectively for 4 hours. The results obtained yield of gerga orange peel essential oil by maceration method with ethanol, ethyl acetatee, n-hexane solvents, respectively, 23.04%; 16.05%; 11.80%, whereas with the soxhletation method 10.36%; 3.02%; and 2.04%. The phytochemical screening results of extractions using maceration and soxhlet methods indicate that all essential oils contain flavonoids and phenolics. The highest content of flavonoids and phenolics was obtained from extraction using ethanol solvent with the maceration method. The use of these three solvents in the maceration method, especially ethanol, was found to be more effective in extracting essential oils from bitter orange peel. Based on the phytochemical screening results using specific reagents with color changes indicating the presence of certain metabolites in the sample, it is revealed that the dominant secondary metabolites in the essential oil of bitter orange peel belong to the phenolic group. Furthermore, the essential oil of bitter orange peel also contains flavonoids and does not detect the presence of saponin compounds..

Keywords: maceration, soxhletation, Citrus nobilis L. Var RGL, phytochemicals.

Abstrak

Jeruk gerga merupakan salah satu komoditas unggulan provinsi Bengkulu dan juga banyak dibudidayakan di Kerinci, Jambi. Hampir 75% kulit jeruk gerga terbuang begitu saja tanpa ada pengolahan. Kulit buah jeruk salah satu tanaman yang dapat menghasilkan minyak atsiri. Akan tetapi, efektivitas ekstraksinya dapat dipengaruhi oleh pelarut dan metode. Tujuan penelitian ini untuk mengetahui pelarut dan metode terbaik untuk mengekstraksi minyak atsiri kulit jeruk gerga. Selain itu, penelitian ini juga bertujuan untuk analisis fitokimia dan rendeman hasil ekstraksi. Pada penelitian ini akan dibandingkan metode maserasi dan sokletasi untuk mengekstraksi minyak atsiri kuit jeruk gerga dengan variasi pelarut dengan kepolaran. Pada metode maserasi

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simplisia dimaserasi dengan masing-masing pelarut etanol, etil asetat dan n-heksana selama 3x24 jam. Kemudian maserat didistilasi untuk menguapkan etanol. Pada metode sokletasi, simplisia disokletasi menggunakan masing-masing pelarut etanol, etil asetat dan n-heksana selama 4 jam. Hasil penelitian menunjukan bahwa rendeman minyak atsiri kulit jeruk gerga dengan metode maserasi dengan pelarut etanol, etil asetat, n-heksana masing-masing yaitu 23,04%; 16,05%; 11,80%, sedangkan dengan metode sokletasi 10,36%; 3,02%; dan 2,04%. Hasil skrining fitokimia dari ekstraksi menggunakan metode maserasi dan sokletasi menunjukkan bahwa semua minyak atsiri mengandung flavonoid dan fenolik. Kandungan flavonoid dan fenolik tertingi diperoleh dari ekstraksi menggunakan pelarut etanol dengan metode maserasi. Penggunaan ketiga pelarut Metode maserasi dengan pelarut etanol lebih efektif untuk mengekstrak minyak atsiri kulit jeruk gerga. Berdasarkan hasil skrining fitokimia sesuai dengan indikator pada masing-masing reagen spesifik ditandai dengan perubahan warna pada sampel, menunjukkan bahwa kandungan metabolit sekunder pada minyak atsiri kulit jeruk gerga paling dominan adalah golongan fenolik. Selain itu, minyak atsiri jeruk gerga juga mengandung flavonoid dan tidak terdeteksi golongan senyawa saponin
.

Kata Kunci: maserasi, sokletasi, Citrus nobilis L. Var RGL, fitokimia

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1. INTRODUCTION

Gerga Lebong Rimau Orange was one of the leading commodities of Bengkulu province which was the main ingredient for making fruit syrup. Utilization of this citrus fruit certainly produces waste in the form of fruit peels that cannot be handled properly. Wastes, if not handled well can be one of the causes of global warming even though fruit peels contain higher antioxidant compounds than other parts of the fruit (R.E.Abdelazem et al., 2021). Orange peel also contained phenolic compounds consisting of phenolic acids, falvonones, and polymethoxylated flavones, as well as carotenoids and ascorbic acid (N.A. Indrastuti et al., 2019).

Essential oils were important compounds that can be isolated from plants, from woods, seeds and leaves. Essential oil was colorless but has a distinctive aroma. Consumption of essential oils in the world increases every year because the use of essential oils were very wide in scope, namely in the manufacture of detergents, soaps, cosmetics, medicines, perfumes, soft drinks, and insecticides (E.A. Hassan et al., 2009; M.D. Asfaw et al., 2022). Indonesia produced 40 types of essential oils out of a total of 150 types worldwide (Salsabila et al., 2022). The essential oil components found in orange peel include limonene (95%), mircene (2%), noctanal (1%), pinene (0.4%), linanool (0.3%), deanal (0.3%), sabiena (0.2%), geranial (0.1%), dodecanal (0.1%), and other compounds (0.5%) (A. Kurniawan et al., 2008; A.S. Ananda et al., 2022).

The process of extracting essential oils from orange peels can be done by solid-liquid extraction methods, namely maceration, percolation, reflux, and soxhletation. Maceration and soxhletation methods were widely used because they were simple, economical and fast (R. Hasibuan et al., 2021). According to Febrina et al. (2017) by using distillation method, the yield of essential oil from Siamese orange peel was very small (about 0.5-1.7%). The same method was used by Wibaldus et al. (2016), the eyield of essential oil from lime peel was 0.23%. Compared with the study of Kawiji et al. (2015) essential oil from kaffir lime peel was 9.638%. Furthermore, Ananda et al. (2022) who used the maceration method for extraction, produced a higher yield reached 0.8496%. On the other hand

Salsabila et al. (2022) was also carried out the essential oil from lime by soxhletation method and the result was 3.25%.

The use of solvents in the orange peel extraction process will affect the effectiveness of the extraction. An ideal solvent can dissolve specifically, has a low boiling point, insoluble in water, inert, and cheap and easy to obtain. However, no solvent was ideal. Based on previous research, n-hexane was able to dissolve essential oils from lime because its polarity was close to that of essential oils (Salsabila et al., 2022). While, a study was reported by Adiyasa et al. (2015) found that the most effective solvent for producing mandarin orange peel extract was ethanol 96%. According to Rafsanjani and Putri (2015) ethanol, ethyl acetatee, and water can extract essential oils from mandarin orange peels. This study will compare the ability of n-hexane, ethyl acetatee, and ethanol to gain the maximum yield of essential oil from gerga orange peel. Gerga orange was widely consumed in Bengkulu and Kerinci. Lack information utilization of gerga orange peel. Therefore, further research needs to be carried out to determine the best method and solvent to utilize wasted peel of gerga to essential oil. This research will provide valuable information about the best method, solvent and also phytochemical of gerga orange essential oil.

2. MATERIALS AND METHODS

2.1. Materials

The tools used were erlenmeyer, analytical balance, a set of soxhleting tools, test tubes, sieve 60 mesh, beaker, stirring rod, spatula, a set of distillation apparatus, whatman filter paper no 1, dropper pipette and drip plate. The materials used were gerga orange peel obtained from Lolo Gedang, Kerinci Regency, ethyl acetate p.a (Supelco), ethanol p.a (Supelco), n-hexane p.a, methanol p.a (supelco), Mg powder (Merck), concentrated HCl (Merck), Mayer's reagent, Dragendorff's reagent, Wagner's reagent, Liebermann Burchard's reagent (acetic acid anhydride-H_2SO_4), chloroform, and 1% $FeCl_3$.

2.2. Simplicia Preparation

Gerga oranges were obtained from Lolo Gedang, Kerinci Regency. As much as 8 kg of yellow gerga oranges separated by skin and fruit. Gerga orange peels were cleaned under running water and cut into ± 8 cm pieces. Gerga orange peels were dried for 3x24 hours, then crushed and sieved using sieve 60 mesh to obtain simplicia powder.

2.3. Maceration Method

Maceration method according to study conducted by Handayani (2014) and Damayanti et.al (2021). 20 grams of each simplicia was macerated with ethanol, ethyl acetate and n-hexane (1:10) in a closed container to be stored for 3 days (in a closed room at 28ºC and protected from sunlight). During the maceration process, stirring is also carried out every day. After 3 days of maceration, filtering was carried out using filter paper. The resulting filtrate was then distilled to separate the oil and solvent (distillation temperature; ethanol 78°C, n-hexane 70°C and ethyl acetate 77°C) for 3 hours. The distilled extract oil was evaporated again with the oven based on solvent temperature (ethanol 78°C, n-hexane 70°C and ethyl acetate 77°C) to remove the remaining solvent and weighed (Ramli et al., 2019).

2.4. Soxhletation Method

Each of 20 grams of gerga orange peel simplicia was put into filter paper that has been formed into a cylinder and tied. Then it was inserted into the socket with 300 mL of solvent (respectively ethanol, ethyl acetate and n-hexane). Then heated with heating mantle depend on solvent temperature (ethanol 78°C, n-hexane 70°C and ethyl acetate 77°C) extraction for 4 hours. The socket that has been assembled with cooling back is closed on top with grease-free cotton (Adam et al., 2019; Dewi et al.,

2022). Essential oil yields determination according to Chen et al. (2015).

2.5. Phytochemical Screening Test

Phytochemical tests such as alkaloids, flavonoids, phenolics, saponins, terpenoids and steroids refer to Harborne (2013). The standard used refers to Noviarni et al. (2020).

Alkaloid Test

Test for alkaloid compounds using dragendorf, wagner, and mayer reagents which were dripped into the extract. The positive result for the dragendorf reagent produced a red precipitate, the Wagner reagent produced a brown precipitate, and the Mayer reagent produced a white precipitate.

Flavonoid Test

A small quantity of sample was put on the drip plate then added Mg powder and 2 drops of concentrated hydrochloric acid into the same plate, observed the color change. Formation of orange to red color indicated the presence of flavonoids.

Phenolic Test

A small quantity of the sample put on the drip plate, then added 1-2 drops of 1% FeCl3 reagent into the plate, observed the color change. The presence of phenolic was indicated by a color change to green or blue-black.

Saponin Test

Took a few mL of the water layer then put it in a test tube and shaked it, and observed the changes that occur. The presence of saponins was indicated by the formation of foam which remained for 5 minutes.

Terpenoids and Steroids Test

The sample was dissolved in chloroform then added Lieberman Buchard's reagent (acetic acid anhydrous-H_2SO_4), observed the changes that occur. A positive result for the presence of steroids is indicated by a change in color to green-blue and red-purple for terpenoids.

3. RESULTS AND DISCUSSION

3.1. Determination of Yield Essential Oil

Extraction of gerga orange peel essential oil was carried out using various solvents (ethanol, ethyl acetate, n-hexane) and methods (maceration and soxhletation). The essential oils had different colors. The essential oil extracted using ethanol and ethyl acetate was yellow-orange in color. The color of essential oils on extraction with ethanol was more concentrated than ethyl acetate. While the extraction using n-hexane essential oil was yellow. Based on the study that conducted, the highest yield was in the maceration method with ethanol solvent, as shown in (Table 1.) and the lowest was in n-hexane. This is in line with the research reported by Hossain et al. (2011), which suggests that essential oils produced from more polar solvents (such as ethanol) are superior to those extracted using ethyl acetate and n-hexane (methanol extract > ethyl acetate extract >chloroform> butanol > hexane extract)

Table 1. Yield (%) of Essential Oil

Solvents	Ethanol	Ethyl acetate	*n*-hexane
Maceration yield (%)	23,04	16,05	11,80
Soxhletation yield (%)	10,36	3,02	2,04

The differences in extraction methods affect to efficiency and percentage of yield. In line with the research conducted by Ramli et al. (2017), maceration was more effective in producing a higher yield than the soxhletation method. It was because the soxhletation method involved the use of heat which resulted in the possibility of heat-resistant compounds to be degraded. While the maceration method had not involved heat therefor it was suitable for the extraction of easily degraded compounds. Adam et al. (2019) also reported that essential oils from extracts of Sudan's medicinal plants using the maceration

method produced a higher yield than the soxhletation method. The solvents used were methanol, chloroform, and n-hexane. The highest yield was obtained from extraction using methanol solvent with the percentage of yield of the maceration method 7.123% while the soxhletation method was 6.137%.

The highest yield produced from both methods was by using ethanol solvent. According to Adiyasa et al. reaserch in Dewi et.al (2022), ethanol was the most effective solvent among n-hexane, ethyl acetate, and ethanol. The yield of essential oil was 47.69% from mandarin orange peel. The difference in yield was due to the polarity of ethanol which was higher than other solvents since the secondary metabolites in the sample were more extracted to ethanol solvents (R.E Abdelazem et.al., 2021).

3.2. Phytochemical Screening

The results of the phytochemical tests showed that each essential oil extracted using ethanol, ethyl acetate and n-hexane contained phenolic. Tables 2 and 3 showed the results of the phytochemical tests using the maceration and soxhletation methods. The highest phenolic detected in essential oils extract with ethanol and the lowest in ethyl acetate. It was indicated by a change in color intensity to blackish green which was more concentrated in the essential oil extracted with ethanol.

Essential oil extracts had not contained saponins and there had no color change when Dragendorf reagent had added as shown in Tables 2 and Table 3. Steroid groups in Gerga orange essential oil was not detected in extraction using ethanol and ethyl acetat solvents. However, in ethanol solvents, triterpenoid groups were detected. It was indicated by a red-purple color change when Lieberman Buchard's reagent was added.

Steroids typically have higher solubility in polar solvents, such as ethanol, compared to nonpolar solvents like ethyl acetate and n-hexane. Therefore, in extractions using ethyl acetate and n-hexane, steroid compounds are less likely to be extracted or may not be extracted at all. More polar solvents have a better affinity for polar compounds like steroids.

Polar ethanol will dissolve steroids in the first stage of extraction, whereas with n-hexane solvent, steroids are not detected. Abdelazem et al. (2021) reported a similar finding, stating that based on phytochemical screening results on orange peel, saponins were not detected in samples extracted using ethyl acetate and other nonpolar solvents.

Table 2. Phytochemical Test Results of Essential Oil Produce by Maceration Method

Phyto-chemical	Reagent	Ethanol	Ethyl acetate	*n*-hexane
Alkaloid	Mayer	-	+	++
	Wagner	+++	++	+++
	Dragendroff	-	-	-
Flavonoid	Mg + HCl	+++	++	+
Phenolic	$FeCl_3$	+++	+	++
Saponin	Distillated water	-	-	-
Triterpenoids and Steroids	Lieberman Buchard			
	Triterpenoids	++ -		-
	Steroids	- -		++

Explanation: (-): not detected. (+) detected. Amount of (+) show the color intensity

Table 3. Phytochemical Test Results of essential oil produce by Soxhlet Method

Phyto-chemical	Reagent	Ethanol	Ethyl acetate	*n*-hexane
Alkaloid	Mayer	-	++	++
	Wagner	++	+	+++
	Dragendroff	-	-	-
Flavonoid	Mg + HCl	++	+	+
Phenolic	$FeCl_3$	+++	+	++
Saponin	Distillated water	-	-	-
Triterpeno-ids and Steroids	Lieberman Buchard			
	Triterpenoids	+	+	-
	Steroids	-	-	+

Explanation: (-): not detected. (+) detected. Amount of (+) show the color intensity

The differences of secondary metabolites detected depend on the type of solvent and method used. In addition, the more concentrated the color intensity change when added with the reagent, showed the higher concentration of the compound group. The more intense the color changed that appeared, illustrated the higher content of phtyochemical in essential oil (Table 3).

Besides the phenolic group, the essential oil from gerga orange peel which has been extracted by the maceration method contains flavonoids. The highest flavonoids in essential oils extracted with ethanol solvent were characterized by a more intense orange color intensity. Based on research by Oikeh et al. (2020) the generally in orange peels were flavonoids and phenolics. In addition, Hasibuan et.al reported that lemon peel contains a high content of limonene. Syamsuhidayat and Hutapea (1991) found that the essential oil content present in lime peel was siral, limonene, feladren, and hedperidin glycosides.

4. CONCLUSIONS

Based on research, the maceration method was more effective for extracting essential oil from gerga orange peel than the soxhletation method. Among ethanol, ethyl acetate, and n-hexane, the effective solvent was ethanol with yield value was 23.04%. The phytochemical screening of essential oil from gerga orange peel showed the most dominant group were phenolics and flavonoids, which were the higest phytochemical contained on extract that produced by maceration method and solvent was ethanol.

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