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FULL PAPER

Drying method comparison of black mangrove leaves (*Rhizophora mucronata*) for an antioxidant activity assay

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Abstract

Ethanolic extracts of black mangrove leaf (*Rhizophora mucronata*) can be a natural source of antioxidants. The phenolic content can affect the activity of antioxidants in the sample. This study aimed to determine black mangrove leaf (*Rhizophora mucronata*) chemical content primarily focused on total phenolic content and antioxidant activity during sun and wind drying using DPPH, ABTS and CUPRAC analysis methods. Ethanolic extracts were obtained from the maceration method with 1:5 sample and solvent ratio. The total phenolic content value was 1691.2 mg GAE/g with an extract yield of 12.57%. The antioxidant activity of DPPH, ABTS, and CUPRAC method values were 16.61 ppm, 11.07 ppm and 103.52 mg ascorbic acid/g extract and classified as a very strong antioxidant. The best result showed that the highest content of phenolic content and antioxidant activity could be found in the wind-dried extract. The result indicated that ethanolic extract of *Rhizophora mucronata* leaves can be utilized as a strong antioxidant agent. Drying methods can be used as a good way to preserve mangrove leaves while maintaining their antioxidant activity.

1. Introduction

Mangroves grow in almost all coastal environments in Indonesia. Mangroves thrive in tropical climate conditions (Cruz *et al.,* 2015). *Rhizophora mucronata* is a mangrove plant that grows a lot in Indonesia. This plant is commonly found in many brackish waters that tend to be close to the sea. *Rhizophora mucronata* is known to inhibit the process of coastal abrasion due to the shape of its roots. Residents have widely used this plant species as food and medicine from fruit and leaves. *Rhizophora mucranata* has a relatively high active component in the leaves, so it can be developed as a natural antioxidant to ward off free radicals (Tarman *et al.,* 2013).

Antioxidants are compounds that can inhibit and delay the oxidation process due to free radicals. Antioxidants work by providing electron/hydrogen donors to free radicals to stop the chain reaction of free radicals (Neha *et al.,* 2015). Synthetic antioxidants were initially applied to inhibit free radicals, but synthetic antioxidants have carcinogenic properties, so their use is increasingly being abandoned (Firdayani *et al.,* 2015). Antioxidant compounds, such as vitamin C, vitamin E, carotene, polyphenols, and flavonoids, can be found in various leaves and vegetables. Various plants generally produce antioxidants as secondary metabolites. Black mangrove leaves are an example of a natural source of antioxidants.

Black mangrove plant species have been known to have the potential as natural antioxidants. Purwaningsih *et al.* (2013) investigated the antioxidant content of old black mangrove fruit with an IC50 value of 10.26 ppm. The results of research conducted by Ernawati and Hasmila (2015) show that the methanol extract of black mangrove leaves contains flavonoids and steroids, which can then be applied as an anti-bacterial compound. Research conducted by Wahyuni *et al.* (2015) showed that the results of the DPPH method from black mangrove leaf extract *Rhizophora mucronata* were categorized as powerful antioxidants (5 g/mL). The results of this study can prove that the plant *Rhizophora mucronata* has the potential as a natural antioxidant.

Leaves have a high enough water content, so they are easy to rot. The solution that can be done to prevent the decay process is to use drying. Materials with low

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water content tend to have a longer shelf life due to decreased microbial activity due to drying processes (Alp and Bulantekin, 2021). Mild and short thermal processing enhances the antioxidant activity by increasing the bio-accessible phenolic content and total antioxidant activity while extreme heating may result on degradation of leaf phenolic content (Nwozo *et al.,* 2016). The advantage of using leaf drying at low temperatures is that the active components are still in good condition, but the material is feared to be overgrown with mold because the process takes a long time (Susiani *et al.,* 2017). The drying method commonly used by the community is sun drying and wind drying. Black mangrove leaves that are dried by this method are thought to still have antioxidant activity. The leaves of *Rhizophora mucronata* have been known to have the potential as a vital source of antioxidants. However, until now, there has been no research on changes in the value of the antioxidant activity content due to the sun-drying process and wind-drying. This information needs to be strengthened by different antioxidant analysis methods such as (1,1-diphenyl-2-picrylhydrazyl), ABTS (2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid), and CUPRAC (Cupric Reducing Antioxidant Capacity). This study aimed to determine black mangrove leaf (*Rhizophora mucronata*) chemical content primarily focused on total phenolic content and antioxidant activity during sun and wind drying using DPPH, ABTS and CUPRAC analysis methods.

2. Materials and methods

2.1 Sample preparations

The primary materials used in this study were samples of black mangrove leaves (*Rhizophora mucronata*) obtained from Indah Kapuk Beach, North Jakarta. The materials used for extraction were ethanol solvents. The materials used for testing antioxidant activity with the DPPH method are DPPH solution and ascorbic acid (Vitamin C). The materials used for antioxidant testing with the ABTS method are ABTS powder and potassium persulfate. The materials used for testing the antioxidant activity of the CUPRAC method were 96% ethanol, $CuCl_2.2H_2O$, ethanolic neocuproine, ammonium acetate buffer, and Trolox solution. The tools used are a knife, spatula, glassware (Pyrex), micropipette (Gilson), vortex (VM-300), digital scale, aluminum foil, desiccator, ashing furnace oven, rotary evaporator (RV 10 digital V), orbital shaker, and UV-VIS spectrophotometer (UV-2500).

2.2 Extraction of leaf

Fresh and dried leaves are each mashed with a blender until they become leaf powder. Proximate analysis of fresh leaf powder included moisture, fat, protein, ash, and fiber content. Samples of fresh, sun-dried, and wind-dried black mangrove leaves were each extracted by single maceration based on the method carried out by Rahman *et al.* (2011) with modifications. Maceration extraction was carried out by soaking the sample powder in 70% ethanol in a ratio of 1:5 (w:v). Maceration was carried out for 48 hours at room temperature using an orbital shaker. The filtrate was filtered using Whatman 42 filter paper and evaporated with a rotary vacuum evaporator at 40°C to obtain a crude extract of black mangrove leaves.

2.3 Proximate analysis

The primary parameter for the analysis is water, ash, protein, fat and carbohydrate content. Proximate analysis was carried out on fresh black mangrove leaves, referring to the AOAC (2005) standard for proximate test.

2.4 Total phenolic content

Analysis of total phenol content (Apostolidis and Lee, 2010) was carried out using the Folin-Ciocalteu method by mixing 1 mL of sample extract, 1 mL of ethanol pro analysis, 5 mL of distilled water, and 0.5 mL of 50% Folin-Ciocalteau reagent. The mixture was allowed to stand for 5 mins, then added with 1 mL of 5% Na_2CO_3 and allowed to stand in the dark for ±60 mins. The absorbance was measured using a spectrophotometer at a wavelength of 765 nm. The standard used in the analysis of total phenol content is gallic acid. The absorbance value was then converted into total phenol, expressed in mg GAE/g sample weight.

2.5 DPPH

The antioxidant activity of black mangrove leaf extract using the DPPH method (Boeing *et al.,* 2014) was done by mixing 4.5 mL of sample extract and 0.5 mL of 1 mM DPPH, then vortexed for 30 seconds. Measurement of the blank was done using a mixture of 4.5 mL of ethanol pro analysis and 0.5 mL of DPPH solution. The mixture was incubated at room temperature in the dark for 30 mins, and then the absorbance was measured at a wavelength of 517 nm using a spectrophotometer. The sample concentrations were 10, 20, 30, 40 and 50 ppm. The positive control used ascorbic acid with concentrations of 1, 2, 3, 4 and 5 ppm. The calculated percentage of inhibition was converted into IC50 by inserting 50 as Y value in the regression equation. The inhibition percentage is described as the following formula;

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$$\text{Inhibition (\%)} = \frac{\text{blank absorption-sample absorption}}{\text{blank absorption}} \times 100\%$$

2.6 ABTS

ABTS stock solution (2,2-Azinobis 3-ethyl benzathiazoline-sulfuric acid) was prepared by dissolving ABTS in deionized water to obtain a concentration of 7.4 mM. A stock solution of potassium persulfate was prepared with a concentration of 2.6 mM. The solution to be reacted was made by mixing ABTS stock solution with potassium persulfate stock solution in a ratio of 1:1 (v/v). The oxidation reaction lasted 16-18 hours in the dark. The solution was then diluted with deionized water to obtain an absorbance of 1.1±0.02 units at a wavelength of 750 nm. A total of 100 L of the sample was mixed with 200 L of ABTS radical (2,2-Azinobis 3-ethyl benzathiazoline-sulfuric acid) in a microplate reader and placed at room temperature for 10 mins. Absorbance measurements were carried out at a wavelength of 750 nm (Kusumaningtyas *et al.,* 2015). The calculated percentage of inhibition was converted into IC50 by inserting 50 as Y value in the regression equation. The inhibition percentage is described as the following formula:

$$\text{Inhibition (\%)} = \frac{\text{blank absorption-sample absorption}}{\text{blank absorption}} \times 100\%$$

2.7 CUPRAC

The antioxidant activity of black mangrove leaf extract using the CUPRAC method (Apak *et al.,* 2008) was done by mixing 0.3 mL of sample extract with 1 mL of 0.01 M $CuCl_2.2H_2O$; 1 mL 0.0075 M ethanolic neocuproine; 1 mL ammonium acetate buffer pH 7 1M; and 0.8 mL of distilled water. The sample and reagent mixture were vortexed, then incubated at room temperature in the dark for 30 mins. The absorbance was measured at a wavelength of 450 nm. Calibration curves were made using Trolox solutions with various concentrations.

3. Results and discussion

3.1 Extraction yield and water content

Black mangrove leaves (*Rhizophora mucronata*) have an elliptical leaf shape that extends to elongate. In addition, the shape of the leaf's tip is tapered. The leaves tend to have dark green along with the presence of black dot underneath the leaf surface (Shamin-Shazwan *et al.,* 2021). Mangrove growth can be different for each place where it grows because each environment where the mangrove grows can have a variety of values of salinity, temperature, humidity, light intensity and water nutrients (Kasitowati *et al.,* 2017). The fresh and dried black mangrove leaves (*Rhizophora mucronata*) can be seen on Figure 1.

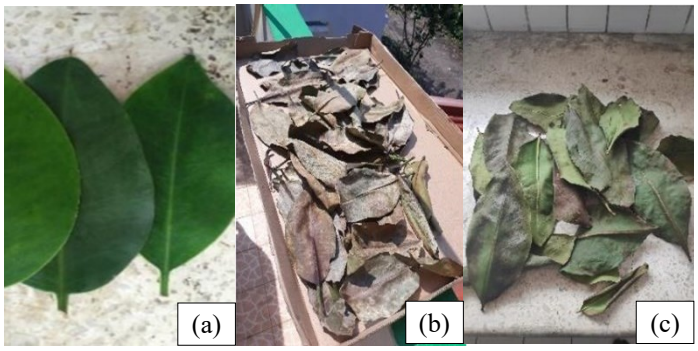

Figure 1. Fresh leaves (A), Sun-dried leaves (B) and Wind-dried leaves (C) of black mangrove (*Rhizophora mucronata*).

The leaves obtained from the area are then prepared to be extracted. Material is extracted to separate a desired active compound from the material. The selection of the extraction method and the solvent used is based on the solubility of the desired compound and the nature of the material used. The result of the extraction is then evaporated, and the yield is calculated, which is the ratio between the weight of the resulting extract and the initial weight of the sample used and expressed in percent (%). The yield of black mangrove leaf extract with various drying methods is presented in Table 1.

The chemical composition of fresh black mangrove leaves (*Rhizophora mucronata*) presented in Table 1 shows that black mangrove leaves have the highest water content of 76.2%. This value has similarities with the research of Dia *et al.* (2015) with a sample of lindur leaves (*Bruguiera gymnorhiza*). High water content in leaves accelerates leaf rot due to microbial activity, such as bacteria and fungi that proliferate in water media. Materials with high moisture content tend to have a shelf life that is not too long (Mohos, 2017).

Table 1 shows the yield of mangrove leaf extract with different drying methods using a dry basis calculation. The yield obtained is still higher when

Table 1. Black mangrove (*Rhizophora mucronata*) leaves extract yield and water content.

Leaves	Drying duration	Water content (%)	Yield (%)
Fresh	-	76.2±1.05^{c}	11.02±0.5^{a}
Sun-dried	30 hours	6.7±0.34^{a}	14.99±0.6^{c}
Wind-dried	288 hours	11.13±0.8^{b}	12.57±0.4^{b}

Values are presented as mean±SD of three replications. Values with different superscripts within the same column are statistically significantly different (P<0.05).

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compared to the research results by Ridlo *et al.* (2017), with the same sample using 3.27% methanol as a fresh sample (4.07% on a wet basis). The difference is caused by using solvent comparisons with smaller samples in the literature to reduce the extract results. The results of maceration extraction are green black to brown, but after the solvent is evaporated the extract obtained is dark brown. The green color in the extract is a chlorophyll compound which is also extracted during the maceration extraction process. Chlorophyll tends to be easily attracted to polar compounds such as water and ethanol as well as methanol (Kwartiningsih *et al.* 2020).

The yield of black mangrove leaf extract has different results in each treatment of the drying method. The yield obtained tends to increase as the water content decreases. The yield obtained concurred with the research of Susiani *et al.* (2017), which also tends to yield more along with the increase in temperature of the cat whiskers leaf extract. However, there is a decrease in the number of simplicia obtained at extreme temperatures due to damage to the extract by excessive heat. The treatment of fresh black mangrove leaves had the lowest yield due to the thick cell walls and high-water content. Fresh leaves inhibit the extraction process due to the solvent's difficulty in binding the material in the cell wall of fresh leaves. However, the drying process can reduce the water content in the material so that it can also damage the cell wall structure in plants, and the solvent can bind the material in the cell wall (Saifullah *et al.,* 2019). Mild drying without the use of extreme heat will provide better retention of chemical properties such as antioxidant activity of leaves (Raja *et al.,* 2018).

3.2 Proximate analysis

Chemical composition analysis was carried out to determine the information contained in black mangrove leaves. This analysis is used to assess the quality of feed or food ingredients, especially on food substance standards such as water, protein and fat. The results of the chemical composition of black mangrove leaves (*Rhizophora mucronata*) are presented in Table 2.

Ash is an inorganic content in a material. Ash can represent the content of various minerals in the material. Ash is obtained by the combustion reaction of organic components at high temperatures. The results of analysis showed that the ash content of black mangrove leaves were 3.11%. The ash content or inorganic material from leaves is very dependent on the environmental conditions in which the plant lives, such as temperature, salinity, humidity and light intensity (Momin and Kadam 2011). The fat content of black mangrove leaves is 0.52%. This value tends to be smaller than the study of Dia *et al.* (2015), which obtained lindur leaf fat content of 1.12%. The fat content in the leaves is influenced by the temperature of the environment in which it grows. Fat acts as an energy source and helps transport fat-soluble vitamin compounds. Fats can play a role in protecting tissues in an organism (Isitua *et al.,* 2015)

The proximate test results showed that black mangrove leaves had a protein content of 2.78%. The protein content obtained was more significant than the study of Dia *et al.* (2015), who obtained a protein content of lindur leaves of 2.16%. Plant protein content tends to be low compared to animal protein. However, plant protein sources are healthier than animal proteins, because they have less total fat, saturated fat, and cholesterol but have more fiber than equivalent protein from animal sources. Plant protein is also good to use as a dietary supplement (Tripathi *et al.,* 2014). Carbohydrates are the products of plant photosynthesis that come from the reaction between CO_2 and H_2O. Carbohydrates can be from monosaccharides such as glucose to higher molecular weight polysaccharides such as cellulose. Carbohydrates act as plant food reserves by accumulating starch in the leaves (Braun *et al.,* 2016). The carbohydrate content in black mangrove leaves is 12.21% which tends to be lower than the study of Dia *et al.* (2015), with a carbohydrate content of 19.45%. The difference can be caused by differences in the rate of photosynthesis of the leaves. According to Kothari and Seshadri (2010), methanol and water extracts have high antioxidant activity because the extracted phenolic content is more and has a high ability to scavenge free radicals.

3.3 Phenolic content

The phenolic content of the material is expressed in terms of gallic acid equivalent (GAE). Tests for total phenol content were carried out on fresh, sun-dried and wind-dried leaves. Total phenol testing was carried out to determine the phenol content of the material that could represent its antioxidant activity. The results of the total phenol test of black mangrove leaf extract are presented in Table 3.

Table 3 shows the results of the total phenol test of black mangrove leaves with different drying methods. The drying treatment showed an increasing trend in the total phenol content of black mangrove leaves. The lowest total phenol was obtained in the treatment of fresh leaves of 1331.06 mgGAE/g extract. The total phenol content of the sun-dried mangrove leaves was 1559.43 mgGAE/g extracts. The highest total phenol obtained by wind-dried was 1691.2 mgGAE/g. The total phenol content obtained significantly differs from the study of Banerjee *et al.* (2008), who obtained the total phenol content of *Rhizophora mucronata* leaves of

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23.81mgGAE/g extract. Differences can influence this difference in the value of total phenol content in solvents, living habitats, and extraction time. Heat treatment can stimulate enzyme activation in the leaves. Activation of these enzymes can significantly impact increasing the total phenol content and antioxidant activity of the leaves (Leng *et al.,* 2017). The increase in the total phenol value from the leaves is not always directly proportional to the drying temperature used. Research Hihat *et al.* (2017) showed that drying coriander leaves at temperatures above 60°C tended to decrease. The decrease can be caused by the extreme temperatures experienced by the leaves so that the total phenol content and existing antioxidant compounds are damaged and do not increase.

Phenolic compounds can be widely found in almost all plants, from roots, leaves, stems, and flowers. Plants need phenolic compounds for pigment, reproduction, growth, and resistance to pathogenic bacteria. Plants produce phenol as a secondary metabolite (Oksana, 2012). Phenol compounds are soluble in water and play an essential role in maintaining the health of the human body. The content of phenols that are often consumed in daily life comes from tea, coffee and chocolate. Phenol can inhibit lipid oxidation, act as a radical scavenger, and prevent other free radical chain reactions (Salas, 2010). Black mangrove leaves contain phenols such as flavonoids. Almost all mangrove species can produce flavonoid compounds because the environment is quite extreme. These flavonoid compounds can protect mangroves from stress in the environment in which they live (Mierziak *et al.,* 2014).

3.4 Antioxidant activity

The antioxidant activity value of black mangrove leaf extract (*Rhizophora mucronata*) was tested using DPPH (1,1-diphenyl-2-picrylhydrazyl), ABTS (2,2-azino -bis-3-ethylbenzothiazoline-6-sulfonic acid) and CUPRAC (Cupric Reducing Antioxidant Capacity). Testing antioxidant activity is not enough just to use one method because each method has different principles and produces different values (Buyuktuncel *et al.,* 2014). These three methods have characteristics and have been commonly used in testing antioxidant activity. The DPPH (1,1-diphenyl-2-picrylhydrazyl) method is a method that is widely used in testing antioxidant activity. DPPH compounds act as stable free radicals that can react with compounds capable of donating hydrogen atoms and help test an extract's antioxidant activity. The principle of the DPPH antioxidant test method is based on the reaction of hydrogen atom capture by DPPH contributed by antioxidant compounds. The advantages of this method are that it is fast, simple, and easy to do. The antioxidant activity of the sample was indicated by a color change in the DPPH solution from purple to pale yellow. The absorbance of DPPH is usually measured from a wavelength of 515-520 nm (Molyneux, 2004).

The ABTS method (2,2-Azinobis 3-ethyl benzathiazoline-sulfuric acid) can be used to determine antioxidant activity. The ABTS compound (2,2-Azinobis 3-ethyl benzathiazoline-sulfuric acid) can only be dissolved in water or organic solvents. Its antioxidant activity was measured based on the compounds in the test sample's hydrophilic and hydrophobic properties. The principle of ABTS testing is the loss of blue color due to the reduction of ABTS by antioxidants. The ABTS method (2,2-Azinobis 3-ethyl benzatiazoline-sulfuric acid) reacts energetically with molecules able to donate hydrogen atoms or electrons, such as phenolic compounds (Cerretani and Bendini, 2010). The antioxidant activity of black mangrove leaf extract analyzed by DPPH and ABTS methods can be seen in Figure 2.

The CUPRAC (Cupric Ion Reducing Antioxidant Capacity) method is one of the methods used to determine the antioxidants of a material. The principle of the CUPRAC test is to utilize copper (II)-neocuproine reagent as an oxidizing agent, which gives color to the solution. The preparation of the test solution included mixing the antioxidant solution with $CuCl_2$, neocuproine, and ammonium acetate solutions at pH 7. The absorbance of the Cu(I) chelate formation was the result of redox by reducing polyphenols measured at a wavelength of 450 nm. Cu(I)Ne spectra were obtained by reacting various concentrations of ascorbic acid with a CUPRAC reagent. The advantages of this method are that the CUPRAC reagent can react quickly, is more stable, and is easy to obtain and apply (Apak *et al*., 2007). The antioxidant activity value of black mangrove leaf extract (*Rhizophora mucronata*) analyzed by CUPRAC method is presented in Figure 3.

Figure 2 shows each value of the antioxidant activity of DPPH and ABTS of black mangrove leaves from each treatment. The results of antioxidant analysis using the DPPH method showed that IC50 tended to decrease with the drying treatment. The highest IC50 value can be found in fresh black mangrove leaves, which are $29.28^{c}\pm2.41$ ppm. Meanwhile, the IC50 value of the sun-dried treatment was $24.91^{c}\pm0.82$ ppm. The lowest IC50 concentration obtained by wind-dried leaves with value of $16.61^{b}\pm044$ ppm while ascorbic acid as a standard has an IC50 value of $4.34^{a}\pm0.50$ ppm. IC50 is the concentration of substances that can cause free radical inhibition by 50%. The higher the IC50 number, the lower the antioxidant activity. Fresh, sun-dried and wind

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-dried extracts of black mangrove leaves are classified as potent antioxidants. The studies showed similarities with Rumengan *et al.* (2021), who reported that ethanolic leaf extract of *Rhizophora mucronata* obtained from North Sulawesi have a DPPH IC50 value of 20.99 ppm (very strong). However, the value obtained was different from the research of Ridlo *et al.* (2017), who got the IC50 value from black mangrove leaf extract obtained from Central Java using methanol of 113.41 ppm (medium). It is also reported by Vigneswaran *et al.* (2018) that dried *Rhizophora mucronata* leaf extract exhibits an excellent DPPH and ABTS assay. Factors that can influence this difference include the place of the environment, the type of solvent, the size of the leaves and the length of the extraction time.

The results of antioxidant analysis using the ABTS method showed that the drying treatment of black mangrove leaves caused a decrease in the IC50 value. Fresh black mangrove leaves have an immense IC50 value of $20.08^{d}\pm0.37$ ppm. The IC50 value of sun-dried and wind dried black mangrove leaves had a smaller value of $17.69^{c}\pm0.58$ ppm and $11.07^{b}\pm0.47$ ppm, respectively. Ascorbic acid as a standard has an IC50 value of $3.92^{a}\pm0.04$ ppm. The result shows that every treatment has a significance different in IC50 value, indicating that ABTS analysis has more precision on testing black mangrove leaves antioxidant activity. It also classifies all extracted black mangrove leaves into potent antioxidants because they have an IC50 value of less than 50 ppm. The research done by Kumar *et al.* (2016) obtained the IC50 value of maja leaf extract (*Aegle marmelos*) of 282.42 ppm, which is classified as weak. ABTS is proven as an effective radical scavenger compared to DPPH specifically using a polar extract such as ethanol (Noreen *et al.,* 2017). The antioxidant found was often caused by the presence of secondary metabolites in plant part such as leaves. The presence of flavonoids, phenolics, saponins and tannins may contribute to the antioxidant value obtained (Nengsih *et al.,* 2021).

Antioxidant analysis of black mangrove leaves using the CUPRAC method got different results for each treatment shown in Figure 3. The results of the CUPRAC antioxidant analysis showed that the drying treatment of black mangrove leaves resulted in a more excellent value. Fresh black mangrove leaves had the lowest value, $77.02^{a}\pm0.98$ mg ascorbic acid/g. The treatment of sun-dried and wind dried mangrove leaves had a higher value of $90.18^{ab}\pm2.2$ mg ascorbic acid/g and $103.52^{b}\pm3.4$ mg ascorbic acid/g. The higher the number obtained, the better the ability of a material to ward off free radicals. Additionally, utilizing the CUPRAC assays, the antioxidant ability of the examined extracts was assessed in terms of reducing power. The ionization potentials of the antioxidants, the spin distribution of the radical cations, and the bond dissociation energy of the phenolic O-H bond were the main determinants of the reducing potential of antioxidants (Sadeer *et al.,* 2019). With the aid of the CUPRAC reagent, the reactive hydroxyl groups of polyphenolics and oligomeric flavonoids are converted to the corresponding quinines. The extract is said to be a possible antioxidant because it contains phenolic compounds. In using reducing power tests, antioxidants can decrease iron from its oxidized form (Fe^{3+}) to its reduced form. By contributing an electron, (Fe^{2+}) is created (Mondal *et al.,* 2019). The results obtained are still smaller than those of Diachanty *et al.* (2017) who obtained the antioxidant activity values of brown seaweed *S. polycystum* and *P. minor* using ethanol as a solvent201 mol trolox/g extract and 163,429 mol trolox/g extract, respectively. The difference is caused by the environmental conditions of an organism, the extraction temperature, and the extraction time.

The antioxidant activity of black mangrove leaf extract with drying treatment has a better value than fresh black mangrove leaves. The difference is caused by damage to the cell wall due to the shrinkage process during drying. In the condition of fresh leaves, the cell walls are still intact and solid, so the solvent is difficult to bind the content in the leaf cell walls (Saifullah *et al.,* 2019). Hihat *et al.* (2017) obtained the highest DPPH value of coriander leaves at a drying temperature of 60°C with an inhibition value of 80% and decreased significantly at higher temperatures. The findings indicate that each leaf has an optimal temperature that can increase its antioxidant activity. Differences that can influence differences in the antioxidant activity of a material are the type of solvent, extraction method, and method of testing the antioxidant activity carried out (Shalaby and Sanaa, 2012).

Total phenol content is closely related to the antioxidant activity of black mangrove leaves. Most of the antioxidant components come from the phenol group, such as flavonoids. The higher the total phenol content obtained, the better the antioxidant activity obtained (Meenakshi *et al.,* 2009). The test results of antioxidant activity on dried black mangrove leaves get a higher antioxidant activity value and increased total phenol levels compared to the fresh one. Phuyal *et al.* (2020) reported that the total phenol content of the sample is directly proportional to the increasing value of the antioxidant activity. The increase in a specific temperature experienced by the leaves is thought to be able to stimulate the work of enzymes in the leaves, thereby increasing the phenol concentration. The use of extreme heat will destroy the phenol content in the

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leaves, considering the nature of phenols that are susceptible to high heat temperatures (Leng *et al.,* 2017).

Conclusion

The drying method (sun-dried and wind-dried) that applied on fresh black mangrove leaves has been proven to affect the extract yield, total phenol, and antioxidant activity of black mangrove leaf extract (*Rhizophora mucronata*). Based on the experiment conducted, the wind-dried black mangrove leaves have been proven to be the best treatment out of sun-dried and fresh leaves as it has higher value of phenolic content and antioxidant activity. The drying method can increase the antioxidant activity value of black mangrove leaves by damaging the leaf cell walls and stimulating the work of enzymes that can increase the total phenol content in the leaves and increasing the extraction efficiency**.** Mild drying methods can be utilized as an efficient way to preserve mangrove leaves while maintaining their antioxidant activity.

Conflict of interest

The authors declare no conflict of interest.

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