

Web evaluation of maceration length period on antioxidant potency of *Sonneratia caseolaris* leaf

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Abstract. Diabetic and cancer, as an examples of degenerative diseases in human may arise as consequences of free radicals, i.e. highly reactive molecules. The reactivity of free radicals can be constrained by a substance, known as antioxidant. Mangroves that inhabitant of highly dynamic waters of estuary is likely have defend activity by produce some substances those known as bioactive compounds that may have potency of antioxidant. The quality and quantity of bioactive compounds may affected by extraction method. This study aims to evaluate antioxidant potency of *Sonneratia caseolaris* leaf with three different length time (24, 48 and 72 hours) of maceration by applying of DPPD (1,1-diphenyl- β -picryl hydrazyl) method. Result based on 50% of inhibition concentration (IC₅₀) value suggest that the best maceration time is 24 hours (IC₅₀ was 6.35 ppm), following by 72 and 48 hours (IC₅₀ were 11.5 and 17.4 ppm, respectively). In addition, overall of different maceration time show IC value lower than 50 ppm, indicate that *Sonneratia caseolaris* leaf have very strong potential of antioxidant activity.

1 Introduction

Degenerative diseases, such as cancer, stroke, rheumatism, heart disease, kidney failure and hypertension were triggered by several causes, one of them is free radicals. In living organisms, free radicals are produced as metabolism residual of protein, carbohydrates and fat. Antioxidants are compounds used to reduce free radicals that can be originated naturally from plant fluids or from factory production [4]. One of the compounds contained in plants is bioactive compounds. Bioactive compounds are one of the secondary metabolites produced by plants through a series of secondary metabolic reactions. This secondary metabolite compound in plants is usually used as a defense and protection mechanism to fight predators [3]. In addition, the higher the environmental pressure given to plants, the more secondary metabolites are produced [13]. Bioactive compounds in plants also have advantages for other living organisms, especially for humans. These compounds have various benefits, including as source of antioxidants, antibacterial, anti-inflammatory and can also be used as a

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prevention of cancer [1]. The content of bioactive compounds can also function as immunomodulators, metabolic blocking prevention for dyslipidemia, diabetes and obesity [2].

Plants that have the potential as medicinal plants contain bioactive compounds such as alkaloids, terpenoids, phenolics, steroids, and flavonoids with varying amounts. Mangroves are believed to contain bioactive compounds that have the potential as natural antioxidants. Mangrove *S. caseolaris* has a potential as a natural antioxidant due to several bioactive compounds such as terpenoids, flavonoids, tannins and phenol hydroquinones had been identified from this plant [10]. Parts of fruit, bark, and leaves of this mangrove species have been reported being used to make of traditional medicine [12]. Withdrawing an active substance in plant can be accomplished with extraction process using solvents. In this study the extraction process was performed by maceration.

The length of maceration time as part of extraction process may affect the quantity and quality of the bioactive compounds that can be extracted from a substance, thus influencing their bioactivity such as antioxidants. Shorter maceration time will lead to insoluble bioactive compounds, whereas longer maceration time which will lead to damage the bioactive compounds of the sample [14]. Therefore, to find the optimal antioxidant activity of *S. caseolaris* leaf, the length of maceration time should be evaluated. The study objective is to evaluate different length of maceration of extraction (24, 48 and 72 hours) towards antioxidant activities of *S. caseolaris* using 1,1-diphenyl- β -picryl hydrazyl) (DPPH) method.

2 Materials and methods

Research was conducted from January to April 2018. Sample of *S. caseolaris* leaf was collected from the coastal area of Serang, Blitar Regency, East Java and laboratory analysis was conducted at the Fisheries and Marine Resources Exploration Laboratory of the Faculty of Fisheries and Marine Sciences, Universitas Brawijaya. The research was experimental research performed with two-way ANOVA design, with first factor is the maceration length time consisting of A (24 hours), B (48 hours) and C (72 hours) whereas the second factor is the extract concentration consisting of 5 ppm, 10 ppm, 20 ppm and 40 ppm. All treatments were repeated 3 times.

2.1 Samples preparation

Samples of mangrove leaves of *S. caseolaris* taken were leaf that were dark green and the shape is still intact, then weighed overall. The collected *S. caseolaris* mangrove leaf samples were washed using clean running water to remove the impurities in the leaf samples. Leaf samples that have been washed and then wiped with tissue. Drying the sample at the next stage is carried out for 16 days in the green house to reduce the water content in the sample.

2.2 Samples extraction

Sample extraction method used maceration by soaking 200 grams of dried leaf sample in 600 ml of methanol solvent for 24 hours (treatment A), 48 hours (treatment B) and 72 hours (treatment C). When the maceration process is complete, the solvent was filtrated using Whatman no. 42, then the filtrate was evaporated at 44°C for 60 minute and the extract resulted will be used for antioxidant testing.

2.3 Antioxidant testing

The antioxidant activity of *S. caseolaris* leaf extract was carried out with DPPH (1,1-diphenyl-2-picrylhydrazyl) method follows [6], with the concentration of each treatment which is 5 ppm, 10 ppm, 20 ppm and 40 ppm. The antioxidant activity was evaluated base on qualitative result (sample color changes) and quantitative result (50% inhibition concentration or IC₅₀) applying absorbance value at 571 nm. Absorbance of blank solution in the antioxidant test was carried out by mixing 0.5 mM DPPH (1,1-diphenyl-2-pikrillhidrazil) solution and methanol with a ratio of 1: 3. This solution is then homogenized and incubated for 30 minutes at dark room temperature and then the absorbance is measured by a UV-Vis spectrophotometer with a wave length of 517 nm. Positive control on the antioxidant test used ascorbic acid with concentrations of 2, 4, 6 and 8 ppm.

3 Result and discussion

Qualitative test of antioxidant shows that color change of sample from purple to yellow was occurred at sample concentration of 20 ppm and 40 ppm, while at sample concentration of 5 ppm and 10 ppm the color changed was purple to light purple. This indicates that higher sample concentration has higher antioxidant activity that can be seen when the sample color change from purple to yellow (Figure 1). This is supported by [8] that the presence of antioxidant activity in the samples tested was marked by a change in purple to yellow. This is because the antioxidant compounds from *Sonneratia caseolaris* mangrove leaf extract donate protons to DPPH free radicals (1,1-diphenyl-2-picrylhydrazyl). According to [9], antioxidant compounds will react with DPPH radicals through the mechanism of donating hydrogen atoms and causing changes in color from purple to yellow. The purple, DPPH color intensity will decrease according to the concentration and ability of the compound to inhibit free radicals. From the qualitative test it can be inferred that the higher the concentration of the sample used during testing, the greater the color change that occurs from purple to yellow. The greater concentration of samples used, the higher the antioxidant content that will have an impact on the level of inhibition of free radicals carried out by antioxidants. This is in accordance with [5] suggestion that the higher the concentration used, the higher the percentage inhibition value.

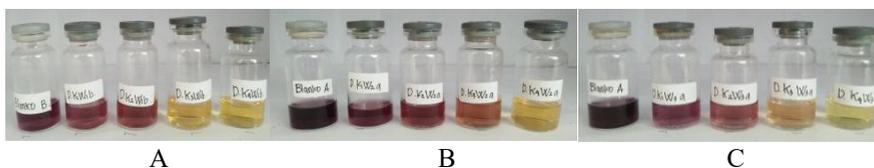


Fig. 1. Result of DPPH test of *S.caseolaris* leaf extract for different maceration time; (A) 25 hours; (B) 48 hours and (C) 72 hours; from left to right were different concentrations of 0, 5, 10, 20 and 40 ppm, respectively

Quantitative antioxidant test results were obtained from measurements of absorbance values using a UV/VIS spectrophotometer at a wavelength of 517 nm. From the absorbance value can be calculated the percentage of inhibition value with the formula:

$$\frac{(\text{Blank absorbance} - \text{sample absorbance})}{\text{Blank absorbance}} \times 100\% \quad (1)$$

IC₅₀ values show the amount of sample concentration that can inhibit 50% of free radicals. IC₅₀ values were obtained by applying linear regression equations which illustrate the relationship between the sample concentrations (x axis) and percent of inhibition (y axis) and the IC₅₀ value for each treatment can be seen in Figure 2.

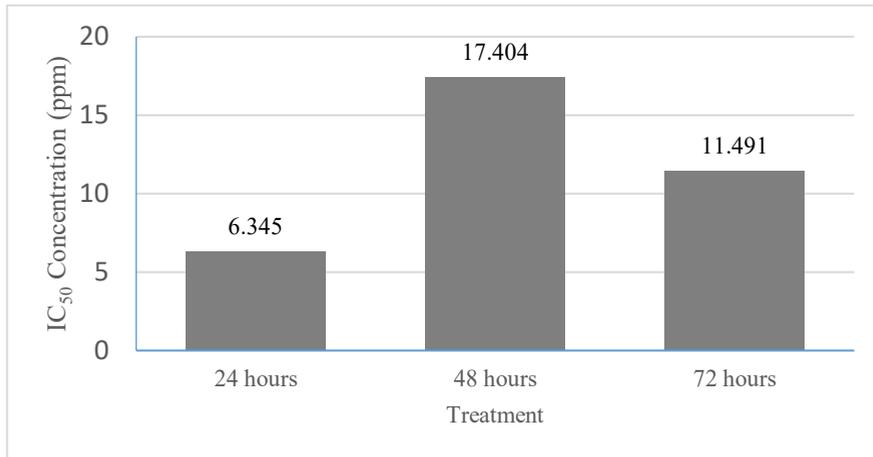


Fig. 2. IC₅₀ value of *S. caseolaris* leaf

Figure 2 shows that IC₅₀ values of all the maceration treatment that have been observed were lower than 50 ppm. This indicates that all methods resulted in very strong antioxidant activity. The two-way ANOVA results showed that the treatment of 24-hour maceration of *S. caseolaris* extract leaf had higher antioxidant activity compared to the treatment of 48 hours and 72 hours maceration (Sig.0,000 <0,05). The lowest IC₅₀ values was obtained from maceration 24 hour, indicates that this length of maceration time is the best method that yielded highest antioxidant activity. According to [11], the lower the IC₅₀ value, the higher the ability to inhibit free radicals. Generally, extract of a compound is considered to be; a very strong antioxidant if the IC₅₀ value <50 ppm, strong antioxidant for IC₅₀ is ranged at 50-100 ppm, moderate antioxidant if it is ranged 100-150 ppm, and weak antioxidant if the IC₅₀ value is higher than 151 ppm.

Based on the statistical analysis results it can be seen that the extract concentration has an effect on the antioxidant activity (Sig. 0,000 <0,05) while the interaction between the length of maceration and the concentration of *S. caseolaris* leaf extract shows no interaction between them (Sig. 0,05). The further results of the antioxidant activity showed that there was no significant difference of antioxidant activity at extract concentration of 5 ppm with a concentration of 10 ppm, but it had a significant difference with concentrations of 20 ppm and 40 ppm. Based on the mean difference value between concentrations, the concentration of leaf extract *S. casolaris* which showed the highest antioxidant activity was a concentration of 40 ppm.

4 Conclusion

Based on the value of IC₅₀ and the length of time use for maceration, the 24 hours of maceration was the best extraction method that has the highest antioxidant activities whereas the best extract of *S. caseolaris* leaf with the highest antioxidant activities is a concentration of 40 ppm.

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