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Total Phenolic And Antioxidant Avtivity Of *Pnematophores* Root Extract Of Sonneratia Caseolaries

Netti Herawati[1], Muharram[2]
Makassar State University
Email: urfatami.unm74@gmail.com

Abstract. Total phenolic content and antioxidant activity of various *Sonneratia caseolaries* pneumatophores root extracts were evaluated. The total phenolic content of the extract was determined based on the Folin-Ciocalteau method which showed that methanol contained the greatest total phenolic ($119{,}44 \pm 3{,}99\ \mathrm{mg\,GAE/g}$). Antioxidant activity was determined through measurement of DPPH free radical scavenging activity (IC_{50}) and reduction capacity. The IC_{50} values of extracts were 19.50, 49.36, 96.16, and 119 µg / mL respectively for methanol, ethyl acetate, chloroform and n-hexane extract. Reduction capacity can be seen from the ability to transform Fe^{3+} - Fe^{+2} ions that methanol extract shows the highest capacity. Solvent polarity affects antioxidant activity and total phenolic content. Similarly, total phenolic content is positively and significantly correlated with antioxidant activity. It can be concluded that the roots of *S.caseolaries* have potential as a source of pharmaceutical raw materials.

Keywords: Total phenolic, antioxidant activity, *Sonneratia caseolaries,* pneumatophores root

INTRODUCTION

Sonneratia caseolaries like halophyte plants generally grow well in extreme areas such as high salinity and ultraviolet radiation which can cause oxidative damage to plant cells (Jithest et al., 2006; Xiong & Zhu, 2002). Plants that have the ability to adapt to extreme areas like this, certanly have a unique defense system to protect them from damage. One of these defense systems is in the form of secondary metabolites found in halophyte plants (mangroves) which include alkaloids, phenolics, steroids and terpenoids. These compounds have important toxic, pharmacologic and ecological effects (Bandaranayake, 2002; Kokpol, 1990). Phenolic compounds are known to protect plants from herbivores, and the main function of most phenolic compounds is to protect plants from damage due to excessive light by acting as antioxidants, and their levels vary according to environmental conditions, and there is a tendency to increase the production of phenolic compounds in mangroves when grow and survive under stressful conditions (Banerjee et al. 2008; Agati et al. 2007; Close & McArthur, 2002). Various studies on the phytochemicals of *S. Caseolaries* plants have been carried out. Tian et al. (2009)

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reported a variety of compounds including phenolics from S stem bark, caseolaries. Bunyapraphatsara et al. (2003) studied 32 species of mangrove plants and found that extracts of various parts of S. caseolaris and *S. alba* plants had antioxidant activity. Sadhu et al. (2006) isolated two flavonoids and determined their antioxidant activity from leaves of S. caseolaris. Latif et.al (2018) reported that the ethyl acetate extract of S.alba root contains β-sitosterol and stigmasterol.

Crude extracts from fruits, vegetables, grains and other plant materials that are rich in phenolic compounds have attracted attention in the food industry, this is due to the ability of these compounds to prevent lipid degradation so that food nutrition is maintained (Kahkonen et al., 1999 ; Rice Evans et al., 1995). The extraction, characterization and utilization of natural antioxidants that can act as potential candidates against carcinogenesis and aging are being promoted (Namiki, 1990; Mathew and Abraham, 2006). Thus the isolation, identification and quantification of active compounds (phytochemicals) contained in plants and evaluation of their potential uses in health are very important.

The active components of plants are distributed in various edible parts of the plant, although they are also often found in the inedible parts. Mangrove habitat in the form of sandy mud in river estuaries, is often found in areas that jut into the sea with relatively high salinity (Anonymous, 2008). Thus, the roots of mangrove plants face high environmental stress. The root part is the object of this research due to the extreme environmental conditions, so the roots are likely to contain unique compounds and contribute highly to the protection and functioning of the roots properly.

In this study, the antioxidant activity of various S. caseolaries root extracts was evaluated based on DPPH free radical scavenging activity and reduction capacity. Total phenolic content was also measured according to the Folin-Ciocalteau method. The results of this study can help to understand about this plant and its potential in the pharmaceutical industry

METHODE

Tools and Materials

Tools: the extraction process uses a variety of glassware and a rotary evavorator. To measure the antioxidant activity and total phenolic content, the Cary 100 Conc.UV-Vis variant spectrometer was used.

Materials: 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteau reagent, n-hexane, chloroform, ethyl acetate, methanol, ascorbic acid, Na2CO3, phosphate buffer, potassium fericinide [K3Fe (CN) 6], acid trichloroacetate, FeCl3. All chemicals used are pro analysis grade.

Sample Preparation

The roots of the mangrove plant, *S. caseolaries*, were obtained from the mangrove forests around the mouth of the Waetuo River, Bone Reagency, South Sulawesi Province, Indonesia. For methanol extraction, 500 g of dried root is ground

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into a fine powder and macerated with 500 mL of methanol solvent. The extraction continues until the extraction solvent becomes colorless. The extract obtained was filtered with Whatman No.1 paper and the filtrate was collected, then methanol was removed by a rotary evaporator at 50 ° C. The same treatment was used to obtain the extract, n-hexane, chloroform and ethyl acetate.

Determination of Total Phenolic

A total of 0.1 g of S.caseolaries mangrove root extract was dissolved in 10 mL of distilled water. The sample solution was pipette 0.2 mL and then added with 1 mL of Folin-Ciocalteu reagent, 15.8 mL of distilled water then shaken and let stand 10 minutes. Then 3 mL of 20% Na2CO3 were added and then shaken until homogeneous. Let stand at room temperature for 2 hours. The sample solution was then measured for its absorbance using a UV-Vis (Ultraviolet-visible) spectrophotometer at a predetermined maximum wavelength. This absorbance value will be used to calculate the total phenol content using the equation y = ax + b that was previously obtained using gallic acid.

Determination of DPPH Radical Scavenging Activity

The free radical scavenging activity of the extract was evaluated with DPPH • radical following the methodology described by Blois (1958).), where the rate of free radical scavenging DPPH • is monitored at a characteristic wavelength in the presence of the sample. In its radical form, DPPH absorbs at 517 nm, but after being reduced by antioxidants or radical species its absorption decreases (Elmastas et al., 2006). Briefly, 0.1 mM solution of DPPH • in ethanol was prepared and 1 mL of this solution was added to 3 mL of aqueous solution of the compound at different concentrations. After 30 minutes later, the absorbance was measured at 517 nm. The lower absorbance of the reaction mixture indicates a higher free radical scavenging activity.

$$\%\ \text{DPPH free radical scavenging} = \frac{([\![\text{Akontrol}]\!]\_(517) - [\![\text{Sample}]\!]\_517)}{[\![\text{Akontrol}]\!]\_517} \times 100\%$$

Determination of Reduction Capacity

The reduction capacity of the compound was determined using the Oyaizu (1986) method. Various concentrations of extract (25-200µg / ml) in 1 ml of distilled water mixed with phosphate buffer (2.5 ml; 0.2 M; pH 6.6) and potassium fericinide [K3Fe (CN) 6] (2.5 mL , 1%). The mixture was incubated at 50 ° C for 20 minutes. 2.5 ml trichloroacetic acid 10% was added to the mixture, then centrifuged for 10 minutes at 1000 rpm. The top layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl3 (0.5 mL, 0.1%), then the absorbance was measured at 700 nm using a UV-Vis spectrophotometer. An increase in the absorbance of the mixture indicates an increase in reduction capacity.

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RESULTS AND DISCUSSION

Total phenolic content of S. caseolaries root extract

Gallic acid was used as the standard for determining phenolic content in the Folin-Ciocalteau method. The phenolic content is expressed as the gallic acid equivalent per gram of sample. The phenolic content level in the extract determined by this method does not measure absolutely the amount of phenolic present, but is based on the fact that the chemical reduction capacity of phenolic is relative to the equivalent of the reduction capacity of gallic acid. Based on table 1, it can be seen that n-hexane contains the lowest total phenolic, then chloroform, ethyl acetate and methanol extracts. the polarity of the solvent used in the extraction affected the total phenolic content of the S. caseolaries root extract. The more polar the solvent, the higher the phenolic content, so it can be said that the phenolic compounds in the root extract of S. caseolaries are semipolar to polar.

Table 1. Total Phenolics and Antioxidant Activity of S. caseolaries root extracts

Extract	Total Phenolics (mg GAE/g)	IC_{50} (µg/ml)
n-Hexane	19,50 $\pm$ 0,48	140.70 $\pm$ 2.15
Cloroform	49,36 $\pm$ 4,69	124$\pm$ 4.60
Ethyl acetat	96,16 $\pm$ 5,44	27.98 $\pm$ 2.06
Methanol	119,44 $\pm$ 3,99	18.55$\pm$ 1.83
Ascorbic Acid	-	12.52 $\pm$ 1.05

Data is average $\pm SD$ (n = 3)

DPPH Free Radical Scavenging Activity

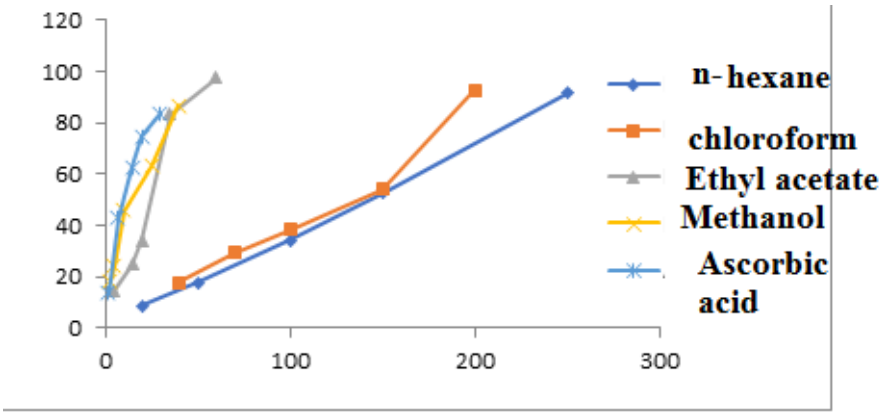

Figure 1. Percentage of DPPH free radical scavenging from S. caseolaries root extracts

Methanol extract showed the greatest DPPH radical scavenging activity followed by ethyl acetate, chloroform and n-hexane extracts (Table 1 and Figure 1). Although the activity of methanol extract was still smaller than ascorbic acid which was used as a positive control, this activity value was high according to the criteria; IC50 <50 µg / ml is classified as a strong antioxidant, 50 <IC50 <100 µg / ml is

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considered moderate, while IC50> 100 µg / ml is classified as a weak antioxidant (Makhmoor, 2005; Shetty & Whalqvist, 2004). Methanol extract most likely has a compound with high radical scavenging activity, although the activity shown by the extract may be due to the synergistic effect of the compounds present in the extract.
The polarity of the S. caseolaries root extracting solvent affected the activity of free radical scavenging. The more polar the solvent the greater the activity. This is supported by several previous studies which stated that the extraction solvent used in isolating antioxidant compounds has an effect on the amount and activity of antioxidants due to differences in polarity of these compounds (Falleh, 2008; Marinova & Yanishlieva, 1997). The increase in polarity of the solvent used for fractionation resulted in an increase in activity, indicating that semi-polar to polar compounds such as phenol group compounds contributed to the activity.

Reducing Capacity

Table 2. Absorbance of S. caseolaries root extract at various concentrations in the ion transformation Fe3 + - Fe2 +.

Extract	**Consentration of Extract (µg/mL)**					
	25	50	75	100	150	200
n-Hexane	0.054	0.09625	0.188	0.278	0.31175	0.2945
Chloroform	0.161	0.18375	0.266	0.336	0.46725	0.63525
Ethyl acetate	0.27497	0.267905	0.41354	0.75838	1.216955	1.59478
Methanol	0.2	0.32625	0.48125	0.9725	1.64	2.1125

Note: Data are average of absorbance, measure at 700 nm (n=3)

Reducing capacity has been used as an important parameter of antioxidant ability in traditional medicine (Duh et al., 1999: Duh and Yen, 1997). The antioxidant effect of various compounds has been shown to be related to the presence of reductors that show antioxidant activity through breaking free radical chain reactions through the donation of hydrogen atoms (Qi et al., 2005). The reduction capacity of a compound can be a significant indicator of potential antioxidant activity. In this study, all the root extracts of S. caseolaries had activity in reducing Fe3 + ions to Fe2 + ions in the order of the highest namely methanol, ethyl acetate, chloroform and n-hexane extracts (Table 2 and Figure 2). The order of this ability is the same as the DPPH radical scavenging activity. The dependence of the reduction capacity on the concentration shows that all extracts contain compounds that can donate electrons, can react with free radicals to turn them into stable products, so that they can stop radical chain reactions (Liu, et al., 2008).

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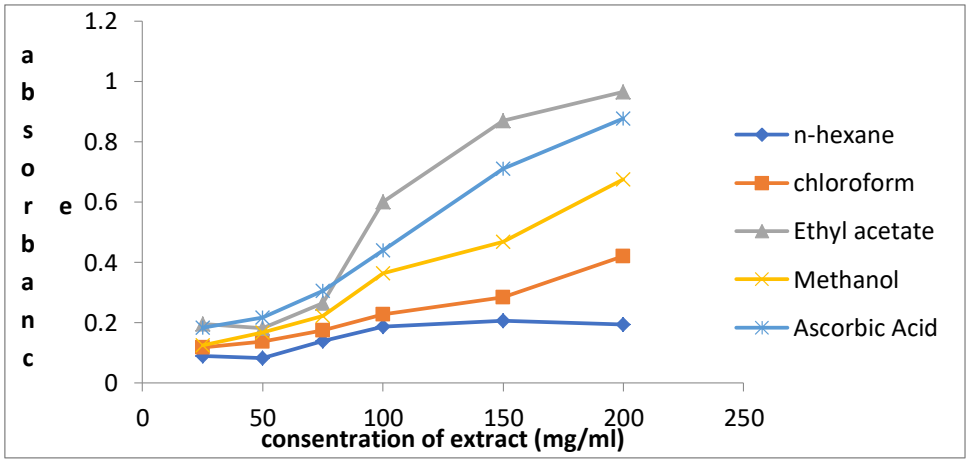

Figure 2. Reduction capacity of S. caseolaries root extract as measured by transforms FE3 + to Fe2 +

The relationship between antioxidant activity and the total extract phenolic content

Extracts with high radical scavenging activity have a high total phenolic content with good correlation. In this study, regression analysis showed a strong correlation between IC50 for DPPH radical scavenging activity and total phenolic content with a correlation coefficient value, r2 of 0.995 (Figure 3). Strong correlations between these two variables have been reported from various studies (Borneo, 2008; Katalinic et al., 2006; Parejo et al., 2003; Silva et al., 2007). This fact is supported by several researchers who state that the antioxidant properties of plant extracts are generally caused by phenolic compounds, such as flavonoids, phenolic acids, and tannins (Tian et al., 2008; Sighn et al., 2007; Sighn & Jayaprakasha, 2002; Pietta , 2000; Revilla & Ryan, 2000). However, Ou (2003) reported the opposite, namely that there was no strong correlation between total phenolic content and antioxidant activity. This difference is likely due to different researchers using different methods of determining antioxidant activity. Different methods have different research conditions and principles (Borneo, et al., 2003).

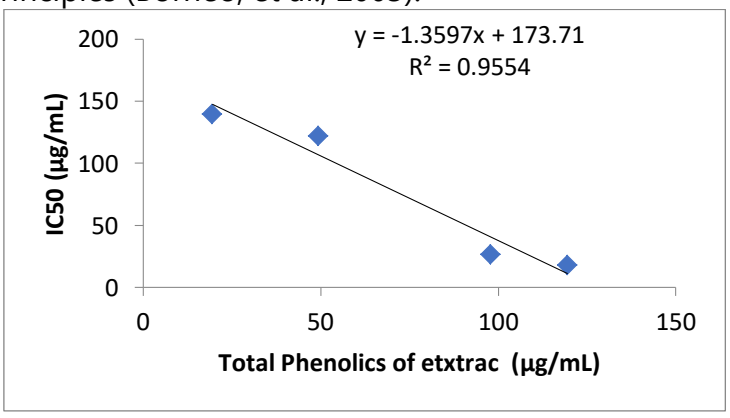

Figure 3. Correlation Between Total Phenolics and an Antioxidant Activity of Extract of *S. caseolaries* Root

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CONCLUSION

The root of S. caseolaries has high antioxidant activity related to DPPH free radical scavenging activity and reduction capacity. Its antioxidant activity is related to the total phenolic content in the extract. The more polar the extracting solution the greater the phenolic content and the higher the antioxidant activity. Thus, phenolic compounds have a major contribution in the high antioxidant activity of S. caseolaries root extracts. To better understand the potential of this plant, it is necessary to carry out various bioactivity tests and isolate the compounds responsible for their activity.

REFERRENCE

Agati, G., Matteini, P., Goti, A., and Tattini, M. 2007. Chloroplast located flavo noids can scavenge singlet oxygen. New Phytologist. 174: 77-8

Bandarnayake W.M 2002. Bioactivities, bioactive compounds and chemical constituents of *mangrove* plants. Wetlands Ecol. Manage. 10: 421-452.

Banerjee.D., Chakrabarti. S., Hazra.A.K., Banerjee.S., Ray.J., Mukherjee.B, 2008., Antioxidant activity and total phenolics of some *mangrove*s in Sundarbans. African Journal of Biotechnology Vol. 7 (6), pp. 805-810

Blois, M.S. 1958. Antioksidan determination by Use of a Stable Free Radikal. Nture, 181, 119-200

Borneo, R, A.E. León A.E, Aguirre A, Ribotta P, Cantero J. 2008.Antioxidant capacity of medicinal plants from the Province of Córdoba (Argentina) and their in vitro testing in a model food system. Food Chemistry 112 (2009) 664–670

Chanwitheesuk, A. Teerawutgulrag, N. Rakariyatham, 2005. Screening of antioxidant activity and antioxidant compounds of some edible plants of Thailand, Food Chem. 92; 491–497.

Close, D.C. and McArthur, C. 2002. Rethinking the role of many plant phenolics – protection from photodamage not herbivores? Oikos. 99: 166-172. Cowan, M.M., 1999. Plant products as antimicrobial agents. Clinic. Microbiol. Rev. 12, 564–582.

Duh, P.D., Yen, G.C., 1997. Antioxidant activity of three herbal water extracts. Food Chemistry 60, 639–645.

Duh, P.D., Tu, Y.Y., Yen, G.C., 1999. Antioxidant activity of waterextract of Harng Jyur (Chrysanthemum morifolium Ramat). Lebensmittel-Wissenschaft und-Technologie 32, 269–277.

Elmastas M, Demirtas I, Isildak O, Aboul-Enein HY. 2006a. Antioxidant activity of S-carvone isolated from spearmint (Mentha Spicata L. Fam Lamiaceae). J Liquid Chromatogr Relat Technol 29: 1564–1475.

Falleh.H., Riadh.K., Kamel.C., Najoua.K-B., Najla.T. Mondher.B., Chedly.A. 2008. Phenolic compotition of *Cynara cardunculus* L. organs, and their biological activities. C.R.Biologies 331 372-379.

ISBN: 978-623-7496-62-5

Jithesh M, Prasanth SR, Sivaprakash KR, Parida A .2006. Monitoring expression profiles of antioxidant genes to salinity, iron, oxidative light and hyperosmotic stresses in the highly salt tolerant gray *mangrove*, *Avicennia marina* (Frosk.)Vierh. by mRNA analysis. Plant Cell Rep., 25: 865-876.

Liu.X., Zhao.M., Wang.J., Yang.B., Jiang.Y., 2008. Antioxidant activity of methanolic extract of emblica fruit (*Phyllanthus emblica L*) from six region in China. Journal of Food Composition and Analysis 21 219-228.

Makhmoor.T. 2005. Antioksidan from medicinal plants and synthetic origin. P.hD Dissertation. H.E.J. Research Institute of Chemistry International Centre for Chemical Science University of Karachi, Pakistan.

Marinova, E. M., & Yanishlieva, N.VI. 1997. Antioxidative activity of extracts from selected species of the family Lamiaceae in sun- ¯ower oil. Food Chemistry, 58, 245-248.

Mathew M, Abraham TE. 2006. In vitro antioxidant activity and scavenging effects of Cinnamomum verum leaf extract assayed by different methodologies. Food Chem Toxicol 44: 198–206.

Namiki M. 1990. Antioxidants/antimutagens in food. Crit Rev Food Sci Nutr 29: 273–300.

Kahkonen MP, Hopia AI, Vuorela HJ et al. 1999. Antioxidant activity of plant extracts containing phenolic compounds. J Agric Food Chem 47: 3954–3962.

Katalinic, V., Milos, M., Kulisic, T., & Jukic, M. 2006. Screening of 70 medicinal plants extracts for antioxidant capacity and total phenolics. Food Chemistry, 94, 550–557.

Kokpol, U., D. H. Miles, A. M.. Payne, and V. Chittawong, 1990. Chemical Constituents and Bioactive Compounds from *Mangrove* Plants – in Atta-ur-Rahman, , Studies in Natural Products Chemistry, (Ed), Vol.7, Elsevier Science Publishers B. V., Amsterdam.

Ou, B., Huang, D., Hampsch-Woodili, M., & Flanagan, J. A. 2003. When the east meets west: The relation yin–yang and antioxidation–oxidation. The FASEB Journal, 17, 127–129.

Oyaizu, M., 1988. Antioxidantive activities of browning products ofglucosamine fractionated by organic solvent and thin-layer chromatography.Nippon Shokuhin Kogyo Gakkaishi 35, 771–775.

Parejo, I., Viladomat, F., Bastida, J., Rosas-Romero, A., Saavedra, G., Murcia, M. A., Jiménez, A. M., & Codina, C.2003. Investigation of Bolivian plant extracts for their radical scavenging activity and antioxidant activity. Life Sciences, 72, 1667–1681.

Qi, H., Zhang, Q., Zhao, T., Chen, R., Zhang, H., Niu, X., et al. 2005. Antioxidant activity of different sulfate content derivatives of polysaccharide extracted from Ulva pertusa (Chlorophyta) in vitro. International Journal of Biological Macromolecules, 37(4), 195–199.

ISBN: 978-623-7496-62-5

Rice Evans C, Miller NJ, Bolwell GP, Bramley PM, Pridham JB. 1995. The relative antioxidant activities of plant derived polyphenolic flavonoids. Free Radic Res 22: 375–383.

Shetty.K.,and Wahqvist.M. 2004. A model for role of proline-linked pentose phosphate pathway in phenolic phytochemical biosynthesis and mechanism of action for human health and environmental application. Asia Pacific.J.Clinic.Nutrition 13: 1-24

Silva, E. M., Souza, J. N. S., Rogez, H., Rees, J. F., & Larondelle, Y. 200). Antioxidant activities and polyphenolic contents of fifteen selected plant species from the Amazonian region. Food Chemistry, 101, 1012–1018.

Sighn,R.P, Murthy K.N.C, Jayaprakasha,G.K. 2002. Studies on the antioxidant activity of pomegranate *Punica granatum*) peel and seed extracts using in vitro

Tian , F., Bo., L , Baoping J, Jinhua Y, Guizhi Z, Yang C, Yangchao L.2009. .Antioxidant and antimicrobial activities of consecutive extracts from Galla chinensis: The polarity affects the bioactivities. Food Chemistry 113: 173–179.

Xiong . L & Zhu. J.K. 2002. Molecular and genetic aspects of plant responses to osmotic stress. Plant cell Environ. 25: 131-139.