Pharmacogn J. 2018; 10(5):958-962
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com | www.journalonweb.com/pj | www.phcog.net

Original Article

Optimization of Extraction Condition to Obtain Antioxidant Activity and Total Phenolic Content of Seagrass *Thalassia hemprichii* (Ehrenb.) Asch from Indonesia

Nely Suryani Nopi[1], Effionora Anwar[1]*, Tati Nurhayati[2]

ABSTRACT

Background: *Thalassia hemprichii* (Ehrenb.) Asch is one of the commonly found seagrasses spread over the coast of Indonesia and has not been utilized. Previous studies have reported its potency as an antioxidant, however, studies on optimal extraction conditions using ethanol as a solvent to obtain higher antioxidant activity are still limited. **Objective:** The purpose of this study is to optimize the extraction condition for obtaining the highest antioxidant activity and total phenolic content of the seagrass *T. hemprichii*. In an addition, percentage yield of extract was also evaluated. **Methods:** In this study, factorial design with independent factors of ethanol concentrations (50%, 70%, and 100%), the use of HCl 1 N (Yes/No), and temperature (30^0C, 60^0C) was developed. The analysis of variance (ANOVA) was used to determine the significance of the above factors towards antioxidant activity and total phenolic content. The antioxidant activity was evaluated with 2,2-diphenyl-1-picrylhydrazyl (DPPH) method and the total phenolic content was determined by Folin-Ciocalteu method. **Results:** The highest antioxidant activity (IC_{50}=83.48 µg/mL) was shown in sample R1 (extracted with 50% ethanol, added with HCl 1 N, and 60^0C). The sample was also showed total phenolic content 41.03 mg GAE per gram and 22% yield extract. Among the factors examined, the used of HCl 1 N showed the most significant effect to the antioxidant activity and the total phenolic content, with the p-value of 0.048 and 0.034 respectively (p<0.05). **Conclusion:** This extraction condition can be applied to obtain higher antioxidant activity and total phenolic content from the plant.

Key words: *Thalassia hemprichii*, Factorial design, DPPH, Total phenolic content.

Nely Suryani Nopi[1], Effionora Anwar[1]*, Tati Nurhayati[2]

[1]Faculty of Pharmacy, University of Indonesia, Depok, INDONESIA.
[2]Department of Aquatics Product Technology, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University, Bogor, INDONESIA.

Correspondence

Effionora Anwar

Faculty of Pharmacy, University of Indonesia, Depok, 16424, INDONESIA.

Phone no : +62813-8329-3995

E-mail: effi.nora@gmail.com

History
- Submission Date: 08-04-2018;
- Review completed: 23-05-2018;
- Accepted Date: 03-07-2018

DOI : 10.5530/pj.2018.5.162

Article Available online
http://www.phcogj.com/v10/i5

INTRODUCTION

Seagrasses are flowering plants (Angiospermae) that live inundated in shallow sea water[2] and have been used for the treatment of indigestion, muscle pain, skin diseases, wounds and fever as traditional medicine.[1] One of seagrasses commonly found spread throughout Indonesian coast among 13 species recorded is *Thalassia hemprichii* (Ehrenb.) Asch, belong to the family of Hydrocharitaceae.[2]

T. hemprichii has been reported to contain polyphenolic compounds such as phenolic acids (chicoric acid, ferulic acid, p-coumaric acid, caffeic acid, gallic acid, and p-hydroxybenzoic acid)[3-4] and flavonoids (thalassiolin A-B[4] and thalassiolin D).[5] Its potency as a source of antioxidant has also been reported.[1,3,6] Several studies reported that polyphenolic content in a plant contributes to their antioxidant properties.[1,3,7-8] Phenolic compounds of *T. hemprichii* showed wide range of pharmacological properties as antiviral HCV protease,[5] antibiotic[9] and skin-regenerating activities.[10] The ethanol extract of *T. hemprichii* has also been reported its potency as an anti-wrinkle cosmetics.[11]

Extraction of phytochemicals from plant materials is impacted by many factors such as solvent, solvent concentration, extraction time, temperature and pH.[8,12] Significant effect on the antioxidant activity and phenolic content from different pH and temperature of extraction conditions have been studied by Inggrid *et al.*[13] and Wong *et al.*[14] Moreover, studies on the evaluation of antioxidant activity from *T. hemprichii* generally have been reported from the methanol, ethyl acetate, and n-hexane extract, while the ethanol extract is still limited.[1,3,6] Hence in this study, ethanol will be chosen and the extraction factors from various ethanol concentration, the addition of HCl, and temperature will be optimized using factorial design to obtain higher total phenolic content and antioxidant activity from seagrass *T. hemprichii*.

Cite this article: Nopi NS, Anwar E, Nurhayati T. Optimization of Extraction Condition to Obtain Antioxidant Activity and Total Phenolic Content of Seagrass *Thalassia hemprichii* (Ehrenb.) Asch from Indonesia. Pharmacog J. 2018;10(5):958-62.

MATERIALS AND METHODS

Materials

Ascorbic acid, gallic acid, quercetin, and 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich. Sodium carbonate, sodium acetate, and aluminum chloride were obtained from Merck. Folin-Ciocalteu, methanol, ethanol, and other solvents used were analytical grade purchased from Merck.

Plant material

T. hemprichii seagrass collected in August 2017 from Pasauran Beach, located in Serang, Banten, Indonesia. The identification of sample was determined by Center for Marine Studies, Biology Department, University of Indonesia. After harvested, seagrass directly rinsed to remove salt, sand, and foreign particles attached. All parts of samples were then dried at room temperature, shattered into powder and kept in a tight container.

Methods

Optimization of extraction

The extraction was carried out using factorial design with three factors namely ethanol concentration (50%, 70%, and 100%), the addition of HCl 1 N (Yes-No), temperature (60^0C, 30^0C); and with two responses, the IC_{50} value of antioxidant activity and total phenolic content. The significant factors on responses will be analyzed using ANOVA.

Method of extraction refers to Jeyapragash *et al.*[1] with modifications. About five grams of sample was soaked in 100 mL solvent for 24 h at room temperature under dark condition, followed by acidification of 0.5 mL HCl 1 N and kept for 24 h at a temperature based on the matrix described in Table 1. The mixture solution was then filtered and centrifuged at 3000 rpm for 5 min. The filtrate was then evaporated with vacuum rotary evaporator and dried on water bath at 50^0C.

Table 1: Factorial design experimental

Sample ID	Factors		
	A	B	C
R1	50	Yes	60
R2	50	Yes	30
R3	50	No	60
R4	50	No	30
R5	70	Yes	60
R6	70	Yes	30
R7	70	No	60
R8	70	No	30
R9	100	Yes	60
R10	100	Yes	30
R11	100	No	60
R12	100	No	30

Note: A – Ethanol concentration (%); B – Addition of HCl 1 N; C – Temperature (^{0}C)

% Yield of extract

The percentage yield of all samples was calculated by comparing the weight of dry extracts obtained to the initial weight of the sample.

DPPH Scavenging Activity

The antioxidant activity was evaluated using radical scavenging DPPH method refers to Bobo-Garcia *et al.*[15] with slight modifications. About 20 µL sample solution was added with 180 µL DPPH solution (150 µmol/L) under dark condition. After stored for 40 min, the absorbance was read at wavelength 519 nm using Versamax Microplate Reader (USA). A control solution was applied by adding 20 µL of 80% methanol instead of sample. Ascorbic acid used as positive control and 80% methanol as blank. The scavenging activity was obtained from the equation follows:

$$\%\ \text{inhibition} = \frac{\text{Absorbance of the control} - \text{Absorbance of the extract}}{\text{Absorbance of the control}} \times 100$$

The sample concentration capable of providing DPPH radical inhibition by 50% (IC_{50}) was then calculated using the linear regression $y = a + bx$, where x is the sample concentration (µg/mL) and y is the percentage of the inhibition.

Total Phenolic Content (TPC)

Folin-Ciocalteu method was applied in total phenolic content (TPC) with reference to Bobo-Garcia *et al.*[15] Under dark condition, 20 µL of extract solution was added with 100 µL of 20% diluted Folin - Ciocalteu and homogenized for 1 min. After 4 min stand, then added with 75 µL 10% sodium carbonate and shaken for 1 min. The mixture was stored for 120 min and the absorbance was measured at wavelength 750 nm using microplate reader. TPC of each sample was calculated based on the calibration curves of 20-100 µg/mL gallic acid standard solution and stated in gallic acid equivalent per gram sample (mg GAE per gram).

Statistics analysis

The measurement was conducted in triplicate and results were represented as mean of three determination. The significant extraction factor against total phenolic content and antioxidant activity was analyzed using ANOVA test.

RESULTS

% Yield of extract

Higher percentage yield of extract was generally obtained from the sample of 50% ethanol compared to other concentrations of ethanol. The highest yield was shown in sample R1 (22%), while the lowest was in R11 (4%) as shown in Figure 1.

Antioxidant Activity

The antioxidant activity of seagrass *T. hemprichii* in this experiment is expressed in IC_{50} value. Higher antioxidant properties of a compound in an extract was indicated by the lower IC_{50}. Refers to Molyneux,[16] strong antioxidant activity was shown in R1 and R9 samples with IC_{50} under 100 µg/mL; intermediate activity was shown in R2-R8 with IC_{50} below 150 µg/mL, whereas the R10-R12 had a weak activity with IC_{50} above 200 µg/mL as presented in Figure 2. The highest antioxidant activity was shown in R1 with IC_{50} 83.48 µg/mL and the lowest activity was shown from R12 with IC_{50} 499.24 µg/mL.

Total Phenolic Content (TPC)

TPC of all sample were calculated using the equation of gallic acid calibration curve obtained, y = 0.0688x + 0.005 (r=0.9996). All samples showed total phenolic content in a range of 9.92 – 41.63 mg GAE per gram (Figure 3). Sample R9 had shown the highest total phenolic content (41.63 mg GAE per gram), while the lowest was in sample R12 (9.92 mg GAE per gram).

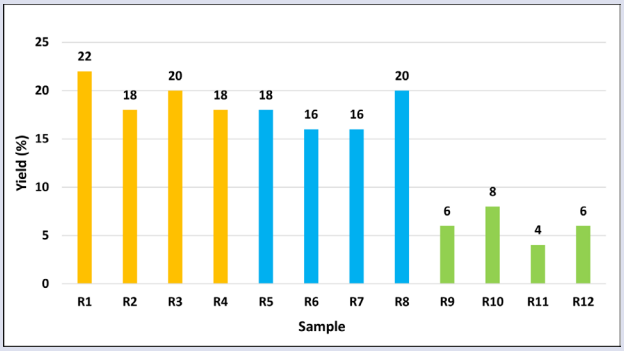


Figure 1: Percentage yield of extract.

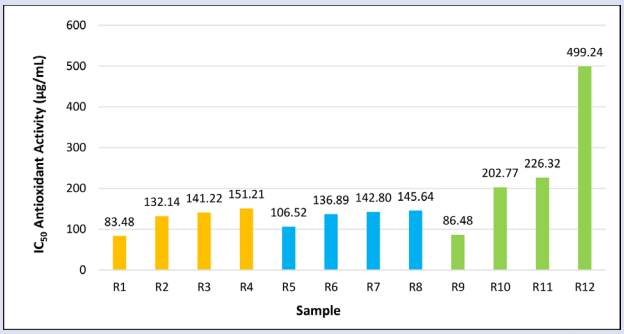


Figure 2: IC_{50} Values of Antioxidant Activity.

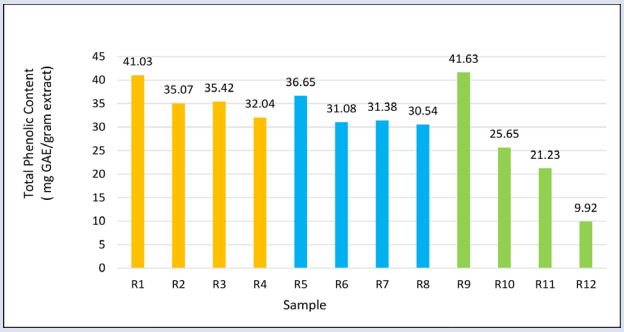


Figure 3: Total Phenolic Content of *T. hemprichii* extract.

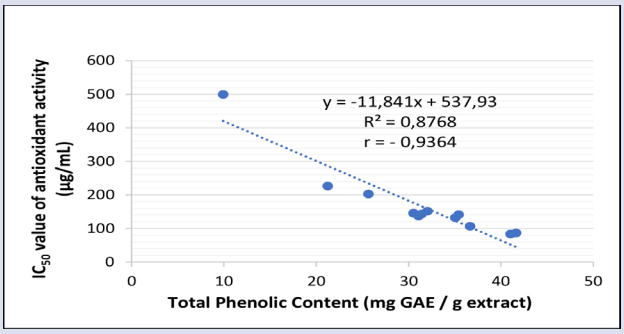


Figure 4: Linear regression between IC50 values of antioxidant activity and total phenolic content.

Pearson correlation test revealed a strong liniear relationship (R^2= 0.8768; r=0.936) between IC_{50} values of antioxidant activity and the total phenolic content as displayed in Figure 4.

Statistical analysis of the results

The experimental design used in this study was factorial design with variable factors of ethanol concentration, the addition of HCl, and temperature. The ethanol concentration was divided in three treatment levels, namely 50%, 70%, and 100%. The HCl factor was divided into two levels, namely added (Yes), and not added (No), while the temperature factor was divided into two levels of 30^{0}C and 60^{0}C. The response variable was antioxidant activity and total phenolic content. From the above treatment factors analyzed by ANOVA, the HCl factor gave the significant influence compared with other factors, in the response of antioxidant activity with a p-value of 0.048, and in the response of total phenolic content with a p-value of 0.034 (p-value <0.05). This suggests that the addition of HCl has the most significant effect on the antioxidant activity and total phenolic content of *T. hemprichii* extract.

DISCUSSION

T. hemprichii is one of the scattered seagrasses on the coast of Indonesia and yet has not been utilized. Several studies have shown their potency as an antioxidant, therefore, the authors are interested to explore it. Phenolic acids and flavonoids have been reported in this seagrass.[3,4,5] The phenolics were reported capable of scavenging free radical chain by donating the hydrogen,[17] and flavonoids also contribute to the antioxidant activity because of their hydroxyl functional group at the site of the structure.[18]

Extraction played important role in isolating phytochemicals from the plant materials. One of the factors that could affect the extraction process is a selection of solvent. The ethanol was chosen in this study because its considered safer, and environmentally friendly compared to other solvents. Moreover, several studies have demonstrated higher antioxidant activity as well as total phenolic content when extracted with ethanol in *Caesalpinia bonduc,*[19] seagrass *T. testidunum,*[10] palm kernel-by product[14] and in black tea and mate tea.[20]

Another significant influence in obtaining the phenolic content and antioxidant activity is the proportion of water in ethanol.[14] According to Markham,[21] the best solvent for extracting phytochemicals compounds is a mixture of alcohol and water, rather than water or alcohol alone. The possible reason is due to the plant swelling by the water through the increase of surface contact area as also reported by Xiao *et al.*[22]

In this study, other than the solvent, an addition of HCl and temperature have also shown significant influence on antioxidant activity and total phenolic content of seagrass *T. hemprichii* as seen in the results (Figure 2 and 3). The used of HCl and high temperature in the extraction will make the plant cell wall becomes more fragile so that the intracellular compounds can be extracted out into the solvent. A similar trend was also reported by Inggrid *et al.*[6] in obtaining the antioxidant activity and phenolic content from strawberry fruits. According to Inggrid *et al,*[6] the stability of some phenolic compounds e.g anthocyanin strongly affected with pH and temperature. Higher temperature from 5^{0}C to 60^{0}C could increase diffusion rate and solubility of phenolic content, moreover, lower pH could also affect the result of phenolic content.[6]

The used of factorial design in this study was to investigate the effect of all experimental factors on responses and finding the most significant factors through ANOVA test. The results of ANOVA showed that the significant influence on antioxidant activity and total phenolic content was from the addition of HCl. The correlation test between the result of antioxidant activity (IC_{50}) and total phenolic content of seagrass showed

a strong linear correlation (R^2 = 0.8768, r= -0,936) and similar with the one reported by Jeyapragash *et al.*[1] (R^2 = 0.864).

The percentage yield of extracts showed in Figure 1 was generally increased by the increasing portion of water in ethanol. Samples extracted in 50% ethanol solvent showed a higher yield of extract compared to samples in 70% and 100% ethanol. This is probably because 50% ethanol has the same ratio of ethanol and water so the phenolic compounds that soluble in organic solvents or in water can be extracted into that solvent.

CONCLUSION

The extraction condition used in this study has been able to obtain higher antioxidant activity and total phenolic content from the seagrass *T. hemprichii*. Higher antioxidant activity (IC_{50}) 83.48 µg/mL was shown in sample R1 (extracted with 50% ethanol, added with HCl 1 N, and 60^{0}C). The sample showed total phenolic content 41.03 mg GAE per gram and 22% yield extract.

ACKNOWLEDGEMENT

Nely Suryani Nopi would thanks to the National Agency of Food and Drug Control (NADFC) Indonesia for giving the scholarship and opportunity for doing this research. The authors would also like to thank to Faculty of Pharmacy, University of Indonesia, and the Ministry of RISTEKDIKTI for the financial support through "PDUPT 2018" project.

CONFLICT OF INTEREST

The authors declare have no conflict of interest.

ABBREVIATIONS

T. hemprichii: *Thalassia hemprichii*; **DPPH**: 2,2-diphenyl-1-picrylhydrazyl; **ANOVA**: analysis of variance; **QE**: Quercetin equivalent; **GAE**: Gallic acid equivalent; **TPC**: Total phenolic content.

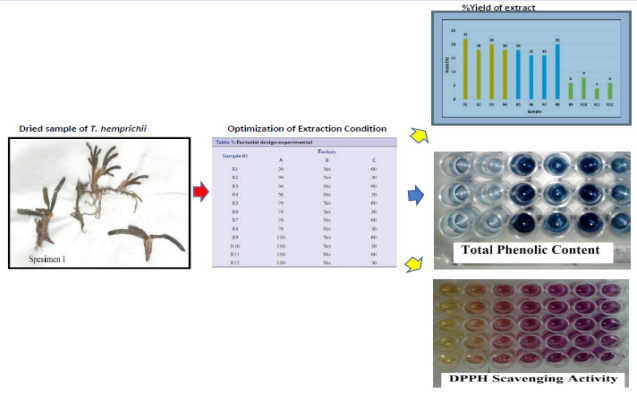
REFERENCES

1. Jeyapragash D, Subhashini P, Raja S, Abikrami K, Thangaradjou T. Evaluation of in-vitro antioxidant activity of seagrass: Signals for potential alternate source. Free Radicals and Antioxidants. 2016;6(1):77-89.
2. Hutomo M, Nontji A. Panduan Monitoring Padang Lamun. Coremap Cti Lipi. 2014. (In Bahasa)
3. Kannan RR, Arumugam R, Thangaradjou T, Anantharaman P. Phytochemical constituents, antioxidant properties and p-coumaric acid analysis in some seagrass. Food Research. 2013;54(1):1229-36.
4. Qi SH, Huang LS, He F, Zhang S, Dong JD. Phytochemical and chemotaxonomic investigation of seagrass *Thalassia hemprichii* (Ehrenb.) Aschers (Hydrochritaceae). Biochemical Systematics and Ecology. 2012;43:128-31.
5. Hawas UW, Abou El-Kassem LT. Thalassiolin D: A new flavone glycoside sulphate from the seagrass *Thalassia hemprichii*. Natural Product Research. 2017;31(20):2369-74.
6. Santoso J, Anwariyah S, Rumiantin RO, Putri AP, Ukhty N, Stark YY. Phenol content, antioxidant activity and fibers profile of four tropical seagrasses from Indonesia. Journal of Coastal Development. 2012;15(2):189-96.
7. Ignat I, Volf I, Popa VI. A critical review of methods for characterization of polyphenolic compounds in fruits and vegetables. Food Chemistry. 2011;126(4):1821-35.
8. Lopez CC, Ventura-Sobrevilla JM, Gonzalez-Hernandez MD, Rojas R, Ascacio-Valdes JA, Aguilar CN, *et al*. Impact of extraction techniques on antioxidant capacities and phytochemical composition of polyphenol-rich extracts. Food Chemistry. 2017;237:1139-48.
9. Jensen PR, Jenkins KM, Porter D, Fenical W. Evidence that a new antibiotic flavone glycoside chemically defends the seagrass *Thalassia testudinum* against zoosporic fungi. Appl Environ Microbiol. 1998;64(4):1490-6.
10. Regalado EL, Rodriguez M, Menendez R, Concepcion AA, Nogueiras C, Laguna A, *et al*. Repair of UVB-damaged skin by the antioxidant sulphated flavone glycoside Thalassiolin B isolated from the marine plant *Thalassia testidunum* Banks ex Konig. Mar Biotechnol. 2009;11(1):74. DOI:10.1007/s10126-008-9123-8.
11. Zakiah K, Anwar E, Nurhayati T. *In vitro* evaluation of antioxidant activity and anti-collagenase activity of *Thalassia hemprichii* as a potent ingredients for anti-wrinkle cosmetics. Pharmacognosy Journal. 2018;10(4). DOI: 10.5530/pj.2018.4.xx.
12. Do QD, Angkawijaya AE, Tran-Nguyen PL, Hyunh LH, Soetaredjo FE, Ismadji S, *et al*. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. Journal of Food and Drug Analysis. 2014;22(3):296-302.
13. Inggrid HM, Iskandar AR. Pengaruh temperatur pada ekstraksi antioksidan dan zat warna buah stroberi. Prosiding seminar nasional teknik kimia "kejuangan" pengembangan teknologi kimia untuk pengelolaan sumber daya alam Indonesia. 2016. (In Bahasa)
14. Wong WH, Lee WX, Ramanan RN, Tee LH, Kong KW, Galanakis CM, *et al*. Two level half factorial design for the extraction of phenolics, flavonoids and antioxidants recovery from palm kernel by-product. Industrial Crops and Products. 2015;63:238-48.
15. Bobo-Garcia G, Davidov-Pardo G, Arroqui C, Virseda P, Marin-Arroyo MR, Navarro M. Intra-laboratory validation of microplate methods for total phenolic content and antioxidant activity on polyphenolic extracts, and comparison with conventional spectrophotometric methods. 2015;95(1):204-9. SCI Research Article. Wileyonlinelibrary.com. DOI: 10.1002/jsfa.6706.
16. Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin Journal of Science and Technology. 2014;26(2):211-9.
17. Zahari A, Ablat A, Sivasothy Y, Mohamad J, Choudhary MI, Awang K. *In vitro* antiplasmodial and antioxidant activities of bisbenzylisoquinoline alkaloids from Alseodaphne corneri Kosterm. Asian Pac J Trop Med. 2016;9(4):328-32. DOI:10.1016/j.apjtm.2016.03.008.
18. Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. J Nutr Biochem. 2002;13(10):572-84. DOI:10.1016/S0955-2863(02)00208-5.
19. Sembiring EN, Elya B, Sauriasari R. Phytochemical screening, total flavonoid and total phenolic content and antioxidant activity of different parts of *Caesalpinia bonduc* (L.) Roxb. Pharmacogn J. 2018;10(1):123-7.
20. Turkmen N, Sari F, Velioglu YS. Effects of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Folin-ciocalteu methods. Food Chem. 2006;99(4):835-41.
21. Markham KR. Techniques of Flavonoid Identification. Academic Press. London. 1982;1.
22. Xiao W, Han L, Shi B. Optimization of microvawe-assisted extraction of flavonoid from Radix Astragali using response surface methodology. Sep Sci Technol. 2008:43(3):671-81.

SUMMARY

- The experimental factorial design was developed in this study with independent factors namely ethanol concentration (50%, 70%, and 100%), the use of HCl 1 N (Yes-No), and temperature (60^{0}C, 30^{0}C) to optimize the extraction condition in obtaining higher antioxidant activity and total phenolic content from *T. hemprichii* seagrass. The response variable was antioxidant activity and total phenolic content.
- Fine powder of *T. hemprichii* was extracted with the solvent for 24 h, followed by addition of HCl 1 N, and stored for 24 h at a temperature, based on the matrix described in the design. Crude extracts were then analyzed to evaluate the antioxidant activity and total phenolic content. Percentage yield of extract was also evaluated as an additional information.
- From all the factors examined, the use of HCl gave the significant influence compared with other factors, in the response of antioxidant activity with a p-value of 0.048, and in the response of total phenolic content with a p-value of 0.034 (p-value <0.05). This indicated that HCl has the most significant effect on the antioxidant activity and total phenolic content of *T. hemprichii* extract.
- The highest antioxidant activity was shown in sample R1 with IC_{50} 83.48 µg/mL. Sample R1 was extracted with 50% ethanol, added with HCl 1 N, and 60^{0}C. The sample contained total phenolic 41.03 mg GAE per gram, and 22% yield of extract.

GRAPHICAL ABSTRACT



ABOUT AUTHORS

Nely Suryani Nopi is a magister student at Faculty of Pharmacy, University of Indonesia. Her studies focused on the Pharmaceutical Science and Technology.

Effionora Anwar is a Professor at Faculty of Pharmacy, University of Indonesia. She is a member of the laboratory of Pharmaceutical formulation development. Her expertise is in food technology, pharmaceutical excipient, nutraceuticals and pharmaceutical technologies. Her publication has been presented at national, international seminars and published in various scientific journals.

Tati Nurhayati is a Professor at the Department of Aquatic Products Technology in Faculty of Fisheries and Marine Sciences. Her expertise is in the processing of fishery products and water aquatic enzyme competence.

Cite this article: Nopi NS, Anwar E, Nurhayati T. Optimization of Extraction Condition to Obtain Antioxidant Activity and Total Phenolic Content of Seagrass *Thalassia hemprichii* (Ehrenb.) Asch from Indonesia. Pharmacog J. 2018;10(5):958-62.