ALKIMIA : Jurnal Ilmu Kimia dan Terapan

ARTICLES

View Article Online
View Journal | View Issue

OPEN ACCESS

Ultrasonic Extraction of *Nothaphoebe Coriacea* Bark and Its Antioxidant Evaluation

Salsabila Aqila Putri[a], Ratih Febrianti[a], Khoirun Nisa[b] and Sunardi[a,c*]

Abstract. *Nothaphoebe coriacea* (in Kalimantan, Indonesia, commonly known as gemor) is a native wetland-plants that can grow well in swampy areas. This tree is distributed throughout South and Central Kalimantan, Indonesia. The bark of *Nothaphoebe coriacea* is used for bioactive compound source for larvacide, mosquito repellent, *hio* and glue. The objective of this research was to investigate the ultrasound-assisted extraction methods for the extraction of antioxidants compounds of *Nothaphoebe coriacea.* The barks were subjected to ultrasound-assisted ethanolic and hexane extraction followed by fractionation (using ethyl acetate, chloroform, and hexane) and the antioxidant activity test was performed. The activity of antioxidant was carried out through a DPPH method. The highest antioxidant activities are shown in ethanol extract with IC_{50} value of 72.36 µg/mL and chloroform fraction of ethanol extract with IC_{50} value of 54.18 µg/mL.

Keywords : *Nothaphoebe coriacea*; antioxidant; bark; ultrasonic extraction; wetland-plant

[a]*Department of Chemistry, Mathematics and Natural Science Faculty, Lambung Mangkurat University, Banjarbaru 70714, Indonesia.*
[b]*Research Division for Natural Products Technology, Indonesian Institute of Sciences, Gunungkidul 55861, Indonesia.*
[c]Wetland-Based Materials Research Center, Lambung Mangkurat University, Banjarbaru 70714, Indonesia .
Correspondence and requests for materials should be addressed to Sunardi, S.
(email : sunardi@ulm.ac.id)

Introduction

Kalimantan is one of the large islands in Indonesia that has a variety of plants. One of the plants that can be found in Kalimantan is gemor (*Nothaphoebe coriacea*) [1]. The genus of gemor tree is *Nothaphoebe* of the family *Lauraceae*. The species has been commonly identified as belonging to the genus *Alseodaphne* in the same family. The bark of *N. coriacea* is used as material for insecticide, *hio* and glue [2]. Besides for insecticide, *hio*, and glue based materials, some parts of *N. coriacea* known has several medical benefits [1]. *N. coriacea* contain phytochemical components in bark, twig, and leaves such as alkaloid, steroid, flavonoid, saponin, triterpenoid, tanin, and phenolic compounds [3]–[5]. The high substance of phenolic and flavonoid in bark and leave *N. coriacea* is commonly used as anti-mosquito, then it also prospective to be used as a drug and for antioxidants source [3][4].

N. coriacea leaves and seeds extracts had the activity of •OH radical which *N. coriacea* seeds extract had the higher antioxidant activity than *N. coriacea* leaves. The percentage of radical scavenging activity of *N. coriacea* leaves and seeds extracts are 56.34% and 61.11%, respectively [1]. Based on these data, there would be a possibility of *N. coriacea* bark has a great antioxidant activity as well. However, this research has not been carried out on *N.coriacea* bark.

The bioactive compounds of *N. coriacea* bark which has a possibility as antioxidant agent is be able to extract it with solid-liquid method. Sonication is one of the recommended extraction methods for extracting the organic compounds. It is a fast and effective method since simple equipment is required and liquid-solid separations can usually be achieved in a short time in a low temperatures and using diluted reagents and low temperatures [6] .

Antioxidant assay was performed using a free radical method DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate). The principle of free radical is based on electron-transfer and when antioxidant molecule is present, it is reduced for giving rise to colorless ethanol solution. DPPH methods is one of rapid and easy method to evaluate antioxidant activity by using spectrophotometry UV-Vis [7]–[9]. The maximum absorption of free radical DPPH with an odd electron gives at 517 nm [10]. The objective of this research was to investigate the ultrasound-assisted extraction methods for the extraction of antioxidants compounds of *Nothaphoebe coriacea* bark using some different organic solvents.

Experimental

Materials and Chemicals. Organic solvents include ethanol 96%, n-hexane, chloroform, ethyl acetate, Folin-Ciocalteu's phenol reagent, sodium carbonate, gallic acid (anhydrous), 2,2-diphenyl-1-picrylhydrazyl, and methanol.

Methods.

Preparation of N. coriacea Bark Extract. Bark of *N. coriacea* of 500 g was extracted in 1.5 L ethanol at room temperature for 1.5 hours by immersing the erlenmeyer flask in the ultrasonic bath (Elmasonic, 220-240 V, 50/60 Hz, and 550 W). The *N. coriacea* bark extract was concentrated using rotary evaporator. The residue in the erlenmeyer flask was re-extracted twice follow the same procedure. Later, the ethanol crude exract (4.63 g) from *N. coriacea* bark was fractionated using separatory funnel, as performed according Hossain *et al*. [11]. It was fractionated continuously with different organic solvent such as chloroform (40 mL), ethyl acetate (40 mL), and hexane (40 mL). All crude fractions were filtered individually using Whatman No. 41 filter paper to eliminate particles. The particle free crude extract was concentrated using waterbath (Memmert). The residue left in the separatory funnel was re-fractionated twice [12].

Determination of Total Phenolic Content. Sample of 0.25 mL was dissolved in 3.75 mL aquadest and 0.25 mL of Folin-Ciocalteu. The mixture sample was shaken for a few minutes, incubated for 8 minutes, and added 20% (b/v) of sodium carbonate solution. The mixture was homogenized and incubated for 2 hours. Gallic acid solutions of 1000 ppm were prepared as standard solution for a calibration curve and the absorbance measured at 765 mm using a spectrophotometer UV-Vis. The phenolic content are showed as gallic acid corresponding per gram (GAE/g) [8][9].

Antioxidant Activity. The DPPH of 4 mg was prepared by dissolving in 10 mL methanol. A stock sample solution of 1000 ppm was prepared, and the concentration of the stock solution were varied at 96-wells Elisa plate (800, 400, 200, 100, 50, 25

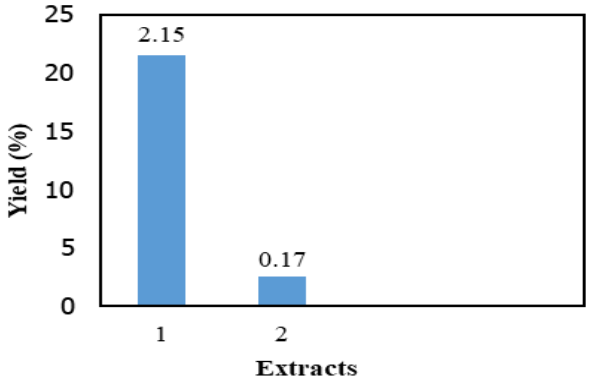

Figure 1. Yield of *N. coriacea* bark extract in (1) ethanolic, and (2) hexane solvent

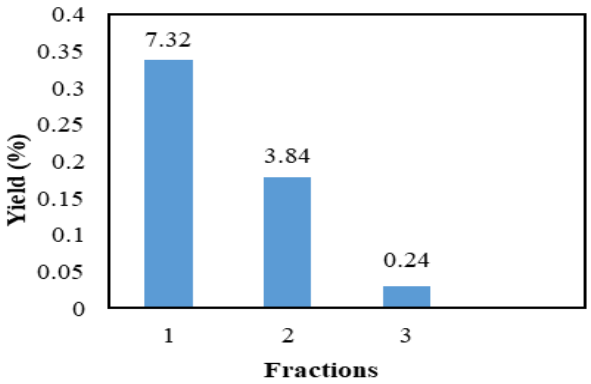

Figure 2. Fraction Yield of *N. coriacea* Bark Etanol Extract in (1) ethyl acetate, (2) hexane, and (3) chloroform

ppm). Then, 20 µL DPPH solution was added to the each of 96-wells. The solution was incubated for 30 minutes in the absence of light and shaken for 5 seconds. The absorbance was measured at 517 nm by using ELISA reader [13] .

Result and Discussion

Extraction of *N. coriacea*. The extraction of *N. coriacea* bark was performed using ultrasound-assited ethanolic and hexane solutions without temperature effect. It is important to use various solvents of different polarities to obtain the extract of different phenolic compounds from plants. When extraction the phytochemical content, the solvent will properly dissolve the solute by choosing the similar polarity with the sample [13]. The selection of ethanol as extraction solvent in this study because it is less-toxic and safer as compared to other organic solvent, such as methanol and acetone [14]. The other organic solvents for extraction and fractionation such as hexane, choloroform, and ethyl acetate are chosen based on different degree of polarity.

The extraction yield of *N. coriacea* bark by ethanolic and hexane solvents are given in Figure 1. It shows that 1 kg *N. coriacea* contains 2.15% of ethanol extract while 1. kg *N. coriacea* only contains 0.17% of hexane extract. Later, the ethanolic extract was fractionated using some organic solvents to obtain compounds with their solubility in solvents of differing polarities while the hexane extract did not need to be fractionated because it had been extracted using a low polarity solvent. The yield of *N. coriacea* bark fractions are given in Figure 2.

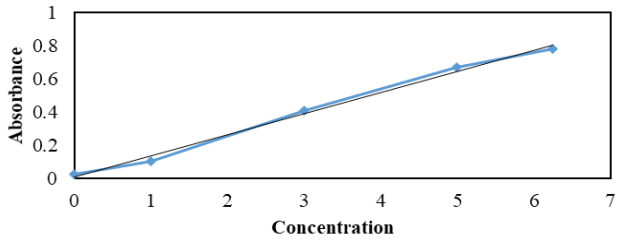

Figure 3. The Gallic Acid Equivalent of Total Phenolic Content of *N. coriacea* bark

Total Phenolics. The total phenolic content of *N.coriacea* bark extract was measured using Folin–Ciocalteu reagent. It depends on the oxidation of phenolic compounds and the phosphomolybdic–phosphotungstic acids in folin ciocalteu reagent. Gallic acid equivalent indicate the total phenolic compounds [15]. The total phenolic compounds of the extract and fraction are shown in Table 1 and 2, while the gallic acid equivalent is given in Figure 3. The phenolic compounds and most other reported bioactive compounds are generally more soluble in polar solvents [16]. Table 1 showed that the highest total phenolic compounds are found in ethanol extract of 95.51 mg/g GAE. Therefore, the ethanol extract has the higher phenolic content than hexane extract. In addition, the fractionation data also showed that the total phenolic content was higher in the fraction at more polar solvents, this was seen in the ethyl acetate fraction of 150.97 mg/g GAE.

Table 1. The Total Phenolic Content in Extract of *N. coriacea* bark

Extract	mg/g GAE
Ethanol Extract	95.51
Hexane Extract	15.67

Table 2. The total Phenolic Content in Fraction of *N. coriacea* Bark Ethanol Extract

Fraction of Ethanol Extract	mg/g GAE
Ethyl Acetate Fraction	**150.97**
Chloroform Fraction	**73.25**
Hexane Fraction	**43.84**

Antioxidant Activity. The hydrogen atom or electron donation capabilities of the equivalent extracts and standard solutions were measured from the bleaching of purple solution of DPPH in methanol [17]. DPPH method is widely used to evaluate the antioxidant/ antiradical activity of synthetic phenolic compounds as well as natural plant extracts [18]. Linear regression in Figure 4 is used to determine the IC_{50} -value of the samples, respectively. The IC_{50} values indicate the concentration of antioxidants to prevent 50% of free radicals' activity. The antioxidant activity of various solution extracts and fractions of *N. coriacea* bark are shown in Figure 5 and 6. The maximum antioxidant activity of *N. coriacea* bark is given in ethanol extract which has IC_{50} value of 72.36 ppm. It showed that *N.coriacea* bark extract has antioxidant activity as good as leave and seed extract that reported by Santosa *et al*. [1] which IC_{50} of leave and seed extract are 56.31% and 61.11%, respectively. In addition, the fractionation of *N.coriacea* bark extract showed that chloroform fraction has the highest antioxidant activity than the other fractions with IC_{50}- value of 54.18 ppm.

A natural compound have strong antioxidant when the IC_{50} value is less than 50 ppm, a

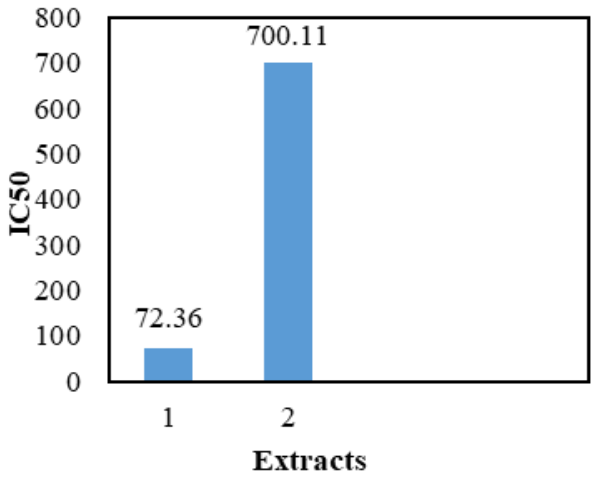

Figure 5. IC_{50} Value of *N. coriacea* bark extract in (1) Ethanolic, and (2) Hexane Solvent

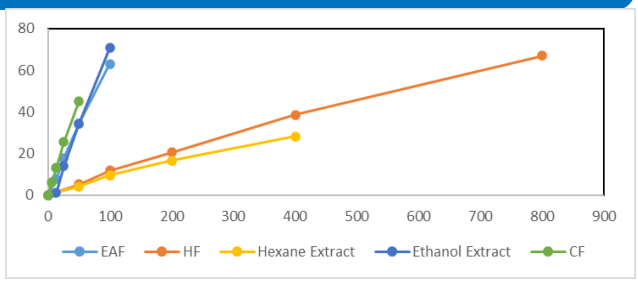

Figure 4. Graphic of the Correlation between Concentration and Inhibition (%) of Ethyl Acetate Fraction of Ethanol extract (EAF), Hexane Fraction of Ethanol Extract (HF), Hexane Extract, Ethanol Extract, and Chloroform Fraction of Ethanol Extract (CF)

powerful antioxidant for IC_{50} value in the range of 50-100 ppm, moderate antioxidants if IC_{50} value range between 100-150 and weak antioxidants if IC_{50} value is higher than150 ppm [19]. The result indicate ethanolic extract of *N. coriacea* bark has a powerful antioxidant activity, while chloroform and ethyl acetate fraction of *N. coriacea* bark extract also has a powerful antioxidant activity. The higher antioxidant activity of ethanolic extract than hexane is suspected that ethanolic extract comprised compounds that play activity role as antioxidant and the good antioxidant activity of both fractions shows that the compounds contained in this fraction can be active as antioxidant. Later, it shows that the antioxidant activity is influenced by the presence of phenolic content in a plant.

CONCLUSION

N. coriacea bark was extracted using ultrasound-assited showed that the yield of ethanol extract

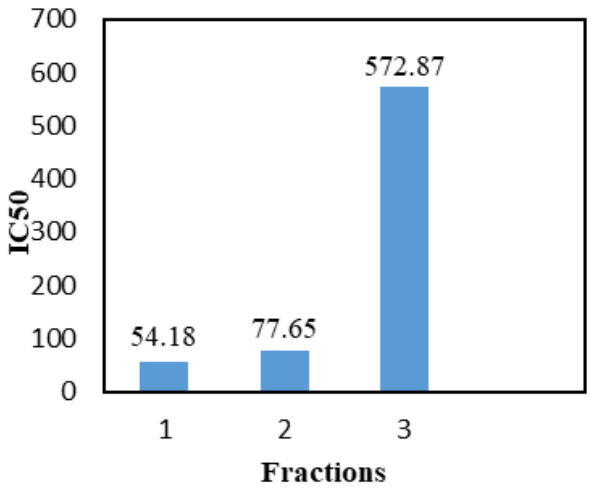

Figure 6. IC_{50}- Value of *N. coriacea* bark fraction in (1) Chloroform, (2) Ethyl Acetate, and (3) Hexane Solvent

was 2.15% with the total phenolic contents were 95.51 mg/g GAE. *N.coriacea* bark extract has a good antioxidant activity. It is shown in ethanol extract with its IC_{50} value of 72.36 and followed by the fractionation of ethanol extract which chloroform and ethyl acetate fractions have the IC_{50} value of 54.18 ppm and 77.65 ppm, respectively. This antioxidant activity has a correspond with the present of phenolic content in *N. coriacea* bark.

References

[1] Santosa, P. B., Thalib, I., Suhartono, E., & Turjaman, M. Antioxidant and anti-lipid peroxidation activities of leaves and seed extracts of gemor (Nothaphoebe coriacea). *Int. J. Pharmacogn. Phytochem. Res*. **8** (7). 1149–1153. (2016).

[2] Adinugroho, W. C., Sidiyasa, K., Rostiwati, T., & Syamsuwida, D. Ecological Conditions and Distribution of Gemor Tree Species in Central and East Kalimantan. *Indones. J. For. Res*. **8** (1). 50–64. (2011). DOI: 10.20886/ijfr.2011.8.1.50-64.

[3] Arifin, Y. F., Hamidah, S., Panjaitan, S., & Suhartono, E. In Vitro Anti-Inflammatory Activities of Red Gemor (Nothaphoebe cf Umbelliflora). *J. Med. Bioeng*. **4** (4). 312–317. (2015). DOI: 10.12720/jomb.4.4.312-317.

[4] Firmanul Arifin, Y., Hamidah, S., Satriadi, T., & Muhammad Hatta, G. Habitat and Phytochemical Analysis of Gemor (Nothaphoebe sp.) on Peat-swamp Forest Areas, Central Kalimantan. *Environ. Ecol. Res*. **5** (4). 269–275. (2017). DOI: 10.13189/eer.2017.050403.

[5] Kurniawan, Y. S., Priyangga, K. T. A., Krisbiantoro, P. A., & Imawan, A. C. Green Chemistry Influences in Organic Synthesis : a Review. *J. Multidiscip. Appl. Nat. Sci*. **1** (1). 1–12. (2021). DOI: 10.47352/jmans.v1i1.2.

[6] Bendicho, C. & Lavilla, I. *Ultrasound Extractions*. (2018).

[7] Garcia, E. J., Cadorin Oldoni, T. L., de Alencar, S. M., Reis, A., Loguercio, A. D., & Miranda Grande, R. H. Antioxidant activity by DPPH assay of potential solutions to be applied on bleached teeth. *Braz. Dent. J*. **23** (1). 22–27. (2012). DOI: 10.1590/S0103-64402012000100004.

[8] Amrulloh, H., Fatiqin, A., Simanjuntak, W., Afriyani, H., & Annissa, A. Antioxidant and Antibacterial Activities of Magnesium Oxide Nanoparticles Prepared using Aqueous Extract of Moringa Oleifera Bark as Green Agents. *J. Multidiscip. Appl. Nat. Sci*. **1** (1). 44–53. (2021). DOI: 10.47352/jmans.v1i1.9.

[9] Amrulloh, H., Fatiqin, A., Simanjuntak, W., Afriyani, H., & Annissa, A. Bioactivities of nano-scale magnesium oxide prepared using aqueous extract of Moringa Oleifera leaves as green agent. *Adv. Nat. Sci. Nanosci. Nanotechnol*. **12** (1). 015006. (2021). DOI: 10.1088/2043-6254/abde39.

[10] Ganguly, D., Chandra Santra, R., Mazumdar, S., Saha, A., Karmakar, P., & Das, S. Radioprotection of thymine and calf thymus DNA by an azo compound: mechanism of action followed by DPPH radical quenching & ROS depletion in WI 38 lung fibroblast cells. *Heliyon*. **6** (5). (2020). DOI: 10.1016/j.heliyon.2020.e04036.

[11] Hossain, M. A., Al-Hdhrami, S. S., Weli, A. M., Al-Riyami, Q., & Al-Sabahi, J. N. Isolation, fractionation and identification of chemical constituents from the leaves crude extracts of Mentha piperita L grown in Sultanate of Oman. *Asian Pac. J. Trop. Biomed*. **4**. S368–S372. (2014). DOI: 10.12980/APJTB.4.2014C1051.

[12] El-Malah, M. H., Hassanein, M. M. M., Areif, M. H., & Al-Amrousi, E. F. Utilization of Egyptian Tomato waste as a potential source of natural antioxidants using solvents, microwave and ultrasound extraction methods. *Am. J. Food Technol.* **10** (1). 14–25. (2015). DOI: 10.3923/ajft.2015.14.25.

[13] Shakila, K. Bioactive Flavonids of Spinifex littoreus (Burm. f.) Merr - Investigation By Elisa Method. *J. Stress Physiol. Biochem*. **16** (4). 107–112. (2020).

[14] Chew, K. K., Ho, C. W., Khoo, M. Z., Ng, S. Y., Thoo, Y. Y., & Wan Aida, W. M. Effect of ethanol concentration, extraction time and extraction temperature on the recovery of phenolic compounds and antioxidant capacity of Orthosiphon stamineus extracts. *Int. Food Res. J*. **18** (4). 1427–1435. (2011).

[15] Kaleem, M., Ahmad, A., Amir, R. M., & Raja,

G. K. Ultrasound-assisted phytochemical extraction condition optimization using response surface methodology from perlette grapes (Vitis vinifera). *Processes*. **7** (10). (2019). DOI: 10.3390/pr7100749.

[16] de O. Silva, M., Honfoga, J. N. B., de Medeiros, L. L., Madruga, M. S., & Bezerra, T. K. A. Obtaining Bioactive Compounds from the Coffee Husk (Coffea arabica L.) Using Different Extraction Methods. *Molecules*. **26** (1). (2020). DOI: 10.3390/molecules26010046.

[17] Hidayat, F. & Fatmawati, S. Antioxidant Evaluation of Ganoderma lucidum Extracts. *IOP Conf. Ser. Mater. Sci. Eng*. **588** (1). (2019). DOI: 10.1088/1757-899X/588/1/012042.

[18] Shalaby, E. A. & Shanab, S. M. M. Comparison of DPPH and ABTS assays for determining antioxidant potential of water and methanol extracts of Spirulina platensis. *Indian J. Mar. Sci*. **42** (5). 556–564. (2013).

[19] Muttakin, M., Zulfajri, M., & Mariati, M. Antioxidant Activity from SyzygiumCumini (L.) Skeels. *J. Phys. Conf. Ser*. **1232** (1). (2019). DOI: 10.1088/1742-6596/1232/1/012009.