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

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**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 IC₅₀ value of 72.36 μg/mL and chloroform fraction of ethanol extract with IC₅₀ value of 54.18 μg/mL.

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

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**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 extract (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
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-assisted 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

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    |

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

Figure 3. The Gallic Acid Equivalent of Total Phenolic Content of N. coriacea bark
**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/anti-radical 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 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 than 150 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-assisted showed that the yield of ethanol extract
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 IC₅₀ value of 72.36 and followed by the fractionation of ethanol extract which chloroform and ethyl acetate fractions have the IC₅₀ 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.

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