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Research Article

Antioxidant and Anti-Lipid Peroxidation Activities of Leaves and Seed Extracts of Gemor (*Nothaphoebe coriacea*)

Purwanto Budi Santosa[1*], Iskandar Thalib[2], Eko Suhartono[3], Maman Turjaman[4]

[1]*Banjarbaru Enviroment and Forestry Research Development Institute, Research Development and Innovation Agency, Ministry of Environment and Forestry,Banjarbaru, South Kalimantan, Indonesia*
[2]*Research Unit of Mutiara Bunda Mother and Child Hospital, Martapura of South Kalimantan, Indonesia*
[3]*Department of Medical Chemistry/ Biochemistry, Faculty of Medicine, University of Lambung Mangkurat, Banjarmasin, South Kalimantan, Indonesia*
[4]*Forest Research and Development Centre, Environment & Forestry Research Development and Innovation Agency (FORDA), Ministry of Environment and Forestry, Bogor 16610, Indonesia*

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ABSTRACT
Gemor (*Nothaphoebe coriacea*) is one of non timber forest product's (NTFPs) tree in peat swamp forest Central Kalimantan, Indonesia. It is well known that some parts of these plants have several medical benefits and contain some phytochemical constituents. The medical benefits and phytochemical constituents may be related to antioxidant and anti-lipid peroxidation activity of this part of plant extracts. Thus, our present study aimed to investigate the antioxidant and anti-lipid peroxidation activity of leaves and seed extracts of *N. coriacea*. To investigate the antioxidant activity of the leaves and seed extracts of *N. coriacea*, assay for superoxide dismutase (SOD), catalase (CAT), ascorbate dependen peroxidase (AsA peroxidase), chelating effect of ferrous iron, hydroxyl radical and hydrogen peroxide scavenging, activity were performed in this study. To investigate the anti-lipid peroxidation activity, percentage of malondialdehyde (MDA) inhibition formation was performed. The results show that the leaves extracts shows a higher activity on CAT and AsA peroxidase activity, while the seeds extracts shows a higher activity in SOD activity. Seeds extracts were found to have higher activity in chelating of ferrous iron, hydroxyl radical and hydrogen peroxide scavenging activities. The anti-lipid peroxidation activity looks slightly higher in seed than leaves extracts of *N. coriacea*. In conclusion, both leaves and seed extract of *N. coriacea* has antioxidant and anti-lipid peroxidation activities.

KeyWord*s*: Antioxidant, Antioxidant activity, Anti-lipid peroxidation activity, *Nothaphoebe coriacea*

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]. *N. coriacea* is one of Non Timber Forest Products (NTFPs) plant species found tropical peatland of Central Kalimantan[2]. *N. coriacea* including genus *Nothaphoebe* and *Lauraceae* family which the tree is naturally grown in swamp forest of Sumatra and Kalimantan[3]. *N. coriacea* has an economical value in local community livelihoods and have been exported to Taiwan, Singapore, and Japan[4]. The bark of *N. coriacea* has been exploited since 1970's and used as the main raw material for mosquito repellent manufacture, incense for rituals and raw materials for glue[4-6]. Besides for insecticide, hio, and glue based materials, some parts of *N. coriacea* known has several medical benefits. Arifin et al.[1] study showed that the twig, bark and leaves of *N. coriacea* contained several phytochemical constituents and have an anti-inflammatory activity. Our previous study showed that aqueous extract of bark and leaves of *N. coriacea* showed significant activity to inhibit the glucose metabolism alteration by Cd in liver homogenate of rats[7]. Another our previous study also showed that aqueous extract of leaves of *N. coriacea* can improve the oxidative stress status induced by cadmium in the brain of rats[8]. It has been widely accepted that oxidative stress relates to the induction of ageing, immunosupression, and the pathogenesis of many degenerative diseases such as atherosclerosis, ischemic heart diseases, diabetes mellitus, cancer, neurodegenerative diseases and others[9]. This could prevent or reduced by a compound, known as antioxidants. The term of antioxidants refers to a compound that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions and which can thus prevent or repair damage done to the body's cells by oxygen[10]. Generally, antioxidants are classified into endogenous and exogenous antioxidants. An endogenous antioxidant refers to the antioxidants that normally found in the human body or every living cells such as superoxide dismutase (SOD), catalase (CAT), or peroxidase, while exogenous antioxidants refer to antioxidants that found outside human body, such as,

**Author for Correspondence: ekoantioxidant@gmail.com*

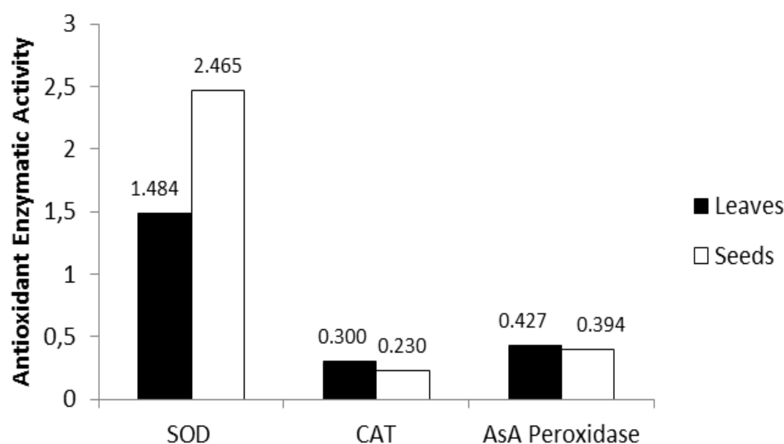

Figure 1: SOD, CAT, and AsA peroxidase activity of leaves and seeds extracts of *N. coriacea*. Each values represents as mean±SD (n=2). SOD: superoxide dismutase, CAT: catalase, AsA peroxidase: Ascorbat dependen peroxidase.

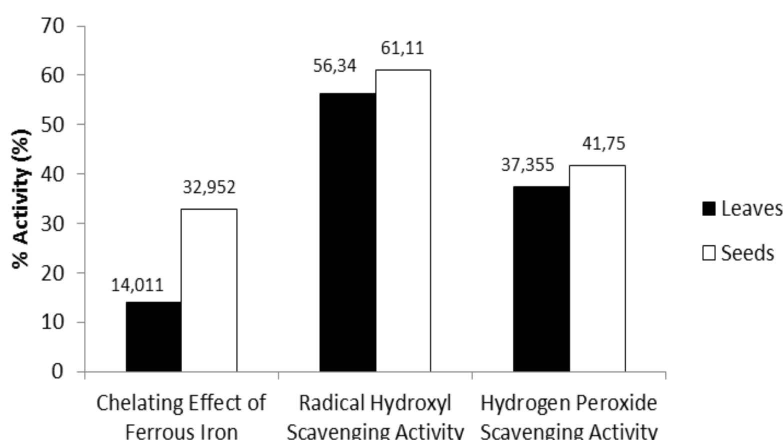

Figure 2: Chelating effect of ferrous iron, radical hydroxyl scavenging, and hydrogen peroxide scavenging activities of leaves and seeds extracts of *N. coriacea*. Each values represents as mean±SD (n=2).

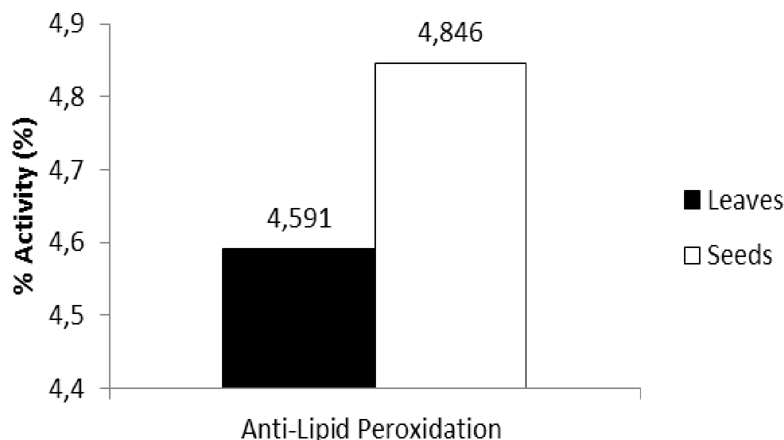

Figure 3: Anti-lipid peroxidation activities of leaves and seeds extracts of *N. coriacea*. Each values represents as mean±SD (n=2).

vitamins (A, E, β-carotene), minerals (selenium, zinc), or proteins (transferrin, ceruloplasmin, albumin)[11]. The search for raw materials containing potent antioxidants continues to attract the attention of researchers. Fruit, vegetables, legume seeds and spices are all known to be rich sources of natural antioxidants, and medicinal plants are another important source for a wide variety of natural antioxidants[12]. Although the toxicity profile of most medicinal plants have not been thoroughly evaluated, it is generally accepted that medicines derived from plant products are safer than their synthetic counterparts[13]. The medical benefits of some plants may be related to high level of antioxidant and antioxidant activity which is contained inside the parts of the plants. Thus, our present study aimed to investigate the antioxidant activity of *N. coriacea*. To investigate the antioxidant activity, SOD,

CAT, and ascorbat dependen peroxidase (AsA peroxidase), chelating effect of ferrous iron, radical scavenging abilities against hydroxyl radicals and hydrogen peroxide activities were measured. Also, in this present study inhibition of lipid peroxidation was measured.

MATERIAL AND METHODS

Samples collection

The leaves and seeds were collected from their natural habitats, mainly in the Forestry Research Area Tumbang Nusa, Central Kalimantan, Indonesia. The exat location where samples was collected according to Global Positioning System (GPS) is $3^0 27'$-3^0 59' S, 113^0 2' 36"-114^0 44' 00" E. *N. coriacea* seeds were collected on August 2015. A bulk sample of *N. coriacea* leaves was collected from sapling and poles. The collected seeds and leaves were air-dried in darkness at room temperature. Then, the dried samples were made into coarse powder using a commercial blender.

Methods of sample preparation

The applied method for the extract preparation was decoction - extracting by boiling plant material. 100 g of the shade-dried of each seeds and leaves were boiled for 30 min in 1000 ml of distilled water. Then, the mixtures were allowed to cool at room temperature and filtered through Whatman no. 5 paper. The resulting solutions then used for the experimental protocol section.

SOD activity

The SOD activity was measured by the method of Misra and Fridovich[14]. Samples was added to 0.800 ml of carbonate buffer (100 mM, pH 10.2) and 100 µl of epinephrine 3 mM. The change in absorbance of each sample was then recorded at 480 nm in spectrophotometer for 2 min at an interval of 15 sec.

CAT activity

CAT activity estimated by using Aebi's method. The first step was to prepare the stock solution by using 2 ml and 1 ml from phosphate buffer at pH 7 and H_2O_2 (30 mM) respectively. Then, 50 µl of the lysate was added to the stock solution. The ability of catalase to work a reducing factor was measured by determining the changes of absorbance at 240 nm[15].

AsA peroxidase activity

The activity of AsA peroxidase was measured according to Nakano and Asada by monitoring the rate of ascorbate oxidation at 290 nm. The reaction mixture contained 25 mm phosphate buffer (pH 7.0), 0.1 mm EDTA, 1.0 mm H_2O_2, 0.25 mm AsA, and the extract aliquot[16].

Chelating effect of ferrous iron

The chelating effect of ferrous ions was estimated by the method of Lin et al.[17] Briefly, the extracts were added to a solution of 2 mM $FeCl_2$ (0.02 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.04 mL) and the mixture was shaken vigorously and left standing at room temperature for 10 min. The absorbance of the mixture was measured at 562 nm. Chelating effect was calculated using the equation:

$$\%\ Chelating\ of\ Ferrous\ Iron = \left(1 - \frac{Absorbance\ (test)}{Absorbance\ (blank)}\right) \times 100$$

Hydroxyl radical scavenging activity

The scavenging activity for hydroxyl radicals was measured by Fenton reaction[18]. Reaction mixture contained 60 µL of 1.0 mM $FeCl_2$, 90 µl of 1mM 1,10-phenanthroline, 2.4 ml of 0.2 M phosphate buffer (pH 7.8), 150 µL of 0.17 M H_2O_2, and 1.0 ml of extract. Adding H_2O_2 started the reaction. After incubation at room temperature for 5 min, the absorbance of the mixture at 560 nm was measured using spectrophotometer. The percentage inhibition of hydroxyl scavenging activity was calculated using the following formula:

$$\%\ Hydroxyl\ Radical\ Scavenging\ Activity = \left(1 - \frac{Absorbance\ (test)}{Absorbance\ (blank)}\right) \times 100$$

Hydrogen peroxide scavenging activity

Hydrogen peroxide solution (2 mM) was prepared in 50 mM phosphate buffer (pH 7.4). Aliquots (0.1 ml) of different fractions was transferred into the test tubes and their volumes were made up to 0.4 ml with 50 mM phosphate buffer (pH 7.4) After addition of 0.6 ml hydrogen peroxide solution, tubes were vortexed and absorbance of the hydrogen peroxide at 230 nm was determined after 10 min, against a blank[13]. The abilities to scavenge the hydrogen peroxide were calculated using the following equation:

$$\%\ Hydrogen\ Peroxide\ Scavenging\ Activity = \left(1 - \frac{Absorbance\ (test)}{Absorbance\ (blank)}\right) \times 100$$

Anti-lipid peroxidation activity

The lipid peroxidation was initiated by adding the $CdSO_4$ solution to the plasma, and then the leaves and seed extracts of *N. coriacea* were added. Then the MDA levels were measured by the modified method of Ohkawa et al and Masao et al.[19]. The percentage of MDA formation inhibition was calculated using the formula:

$$\%\ Anti-Lipid\ Peoxidation\ Activity = (Abs\ control - Abs\ sample) \times \frac{100}{Abs\ control}$$

Abs control: Blank solution absorbance that contained plasma and $CdSO_4$ solution.

Abs sample: Solution absorbance that contained plasma, $CdSO_4$ solution, and the extracts.

RESULTS AND DISCUSSION

To observe the antioxidant activity of leaves and seed extracts of *N. coriacea*, the activity of SOD, CAT, and AsA peroxidase were measured. The results shown in figure 1. The results indicated that both leaves and seed extracts have an antioxidant activity. The leaves extracts show a higher activity on CAT and AsA peroxidase activity, while the seed extracts shows a higher activity in SOD activity. As far as we know, there is no such investigation of these species in all over the world especially in Kalimantan, Indonesia. However, it has long been accepted that any living organisms, including plants have developed complex systems protecting them from ROS, consisting of several enzymes and antioxidants[20]. Among these several enzymatic antioxidants, this present study mainly focused on SOD, CAT, AsA peroxidase. SOD belongs to a class of metalloproteins, which catalyze the dismutation of superoxide ($\cdot O_2$) into

molecular oxygen (O_2) and hydrogen peroxide (H_2O_2). Plant cells generally contain Cu/ZnSODs in the cytosol and chloroplasts and possibly the extracellular space, FeSODs in chloroplasts, and MnSODs in the mitochondrial matrix and peroxisomes. Both Cu/ZnSODs and FeSODs are dimers, whereas MnSODs of mitochondria are tetramers[21]. CAT was the first antioxidant enzyme to be discovered and characterized. In giving the enzyme its name, Loew noted that 'there seems to exist no plant and no animal which is without that particular enzyme. The typical CAT reaction is the dismutation of two molecules of H_2O_2 to water (H_2O) and O_2[22]. AsA peroxidase is thought to play the most essential role in scavenging ROS and protecting cells in higher plants, algae, euglena and other organisms. AsA peroxidase is involved in scavenging of H_2O_2 in water-water and ascorbate-reduced glutathione cycles and utilizes ascorbate as the electron donor. AsA peroxidase has a higher affinity for H_2O_2 than CAT and peroxidase and it may have a more crucial role in the management of ROS during stress[23]. Another antioxidant activity that investigated in this present study are chelating effect of ferrous iron, H_2O_2-scavenging, and •OH-scavenging activities. The results show in figure 2. According to figure 2, both leaves and seed extracts of *N. coriacea* have a chelating effect of ferrous iron, H_2O_2-scavenging, and •OH-scavenging activities. However, seeds were found to have higher activity in all three antioxidant activities that were measured in this present study. Ferrous ion is the most powerful pro-oxidant among the various species of metal ions. These ions could increase the formation of ROS via Fenton reaction. Minimizing ferrous ions may afford protection against oxidative damage by inhibiting production of ROS[24]. H_2O_2 itself is not very reactive, but it can sometimes be toxic to the cell because of it may give rise to •OH in the cells. Thus, the removing of H_2O_2 is very important for antioxidant defense in cell or food systems[25]. The •OH is the most reactive of the ROS and it induces severe damage in biomolecules[26]. These radicals are formed from the reaction of various hydroperoxides with transition metal ions known as Fenton reaction[27,28]. It has been reported that most of the antioxidant activity may be associated with phytochemical contents such as flavonoids, isoflavones, anthocyanins, flavones, catechins and other phenolic compounds[29]. According to Arifin et al.[1] leaves of *N. coriacea* contain flavonoid. However, there is no literature that describes the phytochemical constituents in seeds of *N. coriacea*. It has been confirmed in numerous studies that flavonoid function as antioxidants mainly by chelating metal ions and/or by scavenging free radicals[4]. ROS are related to oxidative stress and many scientific reports have shown that excessive production of ROS promote a further reaction to damage macromolecules such as lipid. This process, known as lipid peroxidation[30]. Lipid peroxidation produces a wide variety of primary or secondary oxidation products. Among the many different aldehydes which can be formed as secondary products MDA is one of the most products that have been extensively studied[31]. This present study also used MDA as a marker for anti-lipid peroxidation activities of leaves and seeds of *N. coriacea*. The results are presented in figure 3. Results from figure 3 revealed that both leaves and seed have anti-lipid peroxidation activities. The activity looks slightly higher in seed than leaves extracts of *N. coriacea*. The anti-lipid peroxidation activities of these extracts might be related to the results from figure 1 and 2. The reason why the seeds have a better activity to inhibit lipid peroxidation than leaves might be related to the antioxidant activities. Seed extracts have a higher in SOD, chelating effect of ferrous iron, hydrogen peroxide, and hydroxyl radical scavenging activities than leaves extracts. The present investigation provides conclusive in vitro evidence for the antioxidant action and anti-lipid peroxidation of leaves and seed extracts of *N. coriacea*. However, more systematic studies on Phytochemistry as well as the in vitro and in vivo assays of leaves and seed extracts of *N. coriacea* might be needed to confirm these observations.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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