Radical scavenging activity and total phenolic content of seven tropical plants

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Abstract. Seven tropical plants were extracted and evaluated for their radical scavenging activity and Total Phenolic Content (TPC). Total phenolic content was determined by Folin-Ciocalteu reagent equivalent to gallic acid. The plant extracts were obtained by sonication using ethanol as the solvent. Four plant extracts were found as active antioxidant in this work. The research revealed that Schleicera oleosa bark extract had the highest DPPH radical scavenging activity with (IC₅₀) 6.59 ± 0.013 µg/ml, followed by Anacardium occidentale bark extract 8.07 ± 0.12 µg/ml, Anacardium occidentale leaves extract 9.65 ± 0.05 µg/ml, and Muntingia calabura leaves extract 27.12 ± 0.5 µg/ml. Positive correlation between free radical scavenging activities and Total Phenolic Content (TPC) in plant extracts was observed. Schleicera oleosa contained a highest Total Phenolic Content (TPC) 451.071 ± 3.3 mg/gr GAE, followed by Anacardium occidentale bark extract 327.6 ± 2.7 µg/ml, Anacardium occidentale leaves extract 307.08 ± 0.55 µg/ml, and Muntingia calabura leaves extract 288 ± 0.5 µg/ml. The result suggest that these tropical plants can be used as resources for natural antioxidant.

1. Introduction
Metabolism on living organisms are naturally produce Reactive Oxygen Species (ROS) like superoxide, hydroxyl, and peroxyl radicals. These are natural by products metabolism with critical roles in cell signalling [1,2]. The overproduction of ROS can cause biomolecule oxidation and damage the cell culture as a result, contributing to various diseases. For that reason, antioxidant can play role as a defence mechanism to avoid the lipid peroxidation that can damage cells on living organisms [3]. Not only for living organisms, antioxidant can also be applied for food protection [4].

Vegetables, fruits, herbs, and spices may contain a wide variety of free radical scavenging content, like phenolic content, nitrogen content, vitamins, and terpenoids, which are high in antioxidant capacity. Nowadays, for primary health care, plant materials have a role as therapeutic traditional medicine to treat many diseases [5]. Many of these plants have excellent properties for phytochemicals and also contain potential antioxidant activities. Phenolic content are secondary metabolites in plants as a key role in pigmentation, reproduction, growth, and resistance to pathogen [6]. These secondary metabolites have been provided as anti-allergic, anti-inflammatory, antioxidant, antiviral, and anticarcinogenic [7,8].
Plant polyphenols with antioxidant capacity could scavenge reactive chemical species as well as decrease oxidative damage. Thus, an antioxidant can delay the oxidation process and inhibited free radicals formation or interfere with the reaction of free radical chain propagation [9]. However, synthetic antioxidants can be accumulated, potential to cause cancer, and damage human organ in previous research. Therefore, natural antioxidants extracted from plants are highly suggested in food applications for safety, and their prospectively nutritional and have therapeutic effects [9,10].

The most famous method to determine the antioxidant capacity is DPPH scavenging assay. DPPH is a stable radical that reacts with compounds that can donate a hydrogen atom. This assay is based on the scavenging of DPPH through the addition of radical species or an antioxidant that decolorizes the DPPH solution [11]. This study aimed to evaluate radical scavenging activity and total phenolic content of seven tropical plants indigenous from Alor Island. Moreover, the potential use of Alor flora for their antioxidant activities is discussed. As the result, it would provide an incentive for the biodiversity conservation in the Alor Island.

2. Materials and methods

2.1. Materials
All of the tropical plants taken from Alor, Nusa Tenggara Timur, Indonesia in August 2019. There were bark of Schleicher oleosa, Anacardium occidentale, and Artocarpus heterophylus and also the leaves of Anacardium occidentale, Muntingia calabura, Piper aduncum, Moringa oleifera, and Vernonia amygdalina. The chemicals used in this work were 1,1-diphenyl-2-picrylhydrazyl (Sigma-Aldrich), Gallic acid (Sigma-Aldrich), Ascorbic acid (Merck), Folin-Ciocalteu reagent (Merck), ethanol (Merck), and Sodium carbonate (Merck).

2.2. Preparation of ethanol extract
The plants were dried, powdered and extracted with ethanol (1:5 w/w). The powdered plants were extracted by sonication method for one hour, then repeat for three times sonication. The solvent was evaporated used rotary evaporator (Buchi), equipped with a vacuum pump until the plant extract was obtained. The evaporation of solvent occurred on vacuum conditions below its temperature point. The ethanol solvent can be carried out below 50°C [12].

2.3. Total phenolic content evaluation
Quantitative analysis of total phenolic contents of plant extracts was determined using Folin-Ciocalteu reagent. Plant extract stock solution was made with concentration 1000 µg/mL. The Folin-Ciocalteu reagent add to 500 µL of plant extracts and was mixed thoroughly, then add 1.5 mL of 20% sodium carbonate. The final volume was made until 10 mL with pure water, and incubated for 2 hours. After 2h of incubation, the absorbance of the samples at was measured 765 nm. Total phenolic content of plant extracts was presented. The gallic acid stand equivalent concentration was used as the concentration of total phenolic groups in the plant extracts. The calibration curves of gallic acid were made by plotting the various concentrations (20, 45, 60, 80, 100, 125 and 150 µg/mL) versus absorbance. Total phenolic was represented as milligrams of gallic acid per gram of dry weight plant extracts [12].

2.4. Radical scavenging activity assay by DPPH method
The radical scavenging activity of plant extracts were determined using 1,1-diphenyl-1-picrylhydrazyl (DPPH) using 96 well microplate. The stock solution (1000 µg/mL) was prepared by dissolving extract in ethanol at various concentrations and treated with DPPH (1 mM in methanol) and left to stand for 30 min at 25°C in the dark place. Absorbance was measured at 517 nm using an Elisa Reader (Thermo-scientific). The percentage activity of the samples to scavenge the DPPH radical was calculated using equation (1).
Radical scavenging activity = \left( \frac{A_0 - A_1}{A_0} \right) \times 100\% \tag{1}

where $A_0$ is absorbance of the control and $A_1$ is absorbance in the presence of the sample. The inhibitory concentration (IC$_{50}$) of the samples was calculated using regression analysis from a graph plotting scavenging activity against concentration. The assays were carried out in triplicate where $A_0$ is the absorbance of the standard (mixture methanolic DPPH), and $A_1$ is the absorbance of the sample. The IC$_{50}$ value denoted as the concentration of that inhibited 50% DPPH radicals [13,14].

3. Results and discussions

3.1. Antioxidant activity of plant extract

The tested tropical plants are indigenous from Alor Island, Nusa Tenggara Timur, Indonesia. This study is addressed to provide prominent information for the biodiversity conservation in the Alor Island.

The antioxidant capacity of the ethanolic extracts of the *Schleichera oleosa*, *Anacardium occidentale*, and *Artocarpus heterophylus* bark extract and the leaves extract of *Anacardium occidentale*, *Muntingia calabura*, *Piper aduncum*, *Moringa oleifera*, and *Vernonia amygdalina* was calculated using DPPH radical scavenging activity with ascorbic acid as a positive control. The results of the free radical scavenging activity assay and total phenolic content of the extracts are presented in table 1, figure 1, and figure 2.

| Plant name             | Part used | DPPH Scavenging (IC$_{50}$, µg/ml) | Total polyphenols (mg/g GAE) |
|------------------------|-----------|-----------------------------------|------------------------------|
| *Schleichera oleosa*   | Bark      | 6.59 ± 0.01                       | 451.07 ± 3.35                |
| *Anacardium occidentale* | Bark    | 8.06 ± 0.12                       | 327.60 ± 2.79                |
| *Anacardium occidentale* | Leaves  | 9.65 ± 0.05                       | 307.09 ± 0.56                |
| *Muntingia calabura*   | Leaves    | 27.12 ± 0.01                      | 288.15 ± 0.56                |
| *Artocarpus heterophylus* | Bark    | 323.49 ± 0.72                     | 90.33 ± 0.23                 |
| *Vernonia amygdalina*  | Leaves    | 373.49 ± 0.29                     | 84.26 ± 0.29                 |
| *Piper aduncum*        | Leaves    | 499.23 ± 5.40                     | 57.53 ± 0.14                 |
| *Moringa oleifera*     | Leaves    | 450.61 ± 10.5                     | 52.49 ± 0.19                 |

*Ascorbic acid*  

Standard

The scavenging assay of DPPH has been commonly used for antioxidant activity determination on natural compound. This method is easy, rapid, and sensitive [10]. The principle of DPPH scavenging assay is the interaction between antioxidant with DPPH as free radical by electron transfer or hydrogen radical, then the radical from DPPH will be neutralize by this process. Free radicals have unpaired electrons in outer orbitals that highly reactive. They are binding to cell electrons in cellular molecules, developing in a chain reaction and induces new free radicals [15,16]. The solution would change from dark purple to bright yellow if all the electrons in DPPH become paired [17]. The quantification of DPPH scavenging were performed after incubation for 30 min to admit the reaction of DPPH as free radical with the samples.
Figure 1. Antioxidant activity of *Schleichera oleosa*, *Anacardium occidentale* bark extract and leaves extract, *Muntingia calabura*, *Artocarpus heterophylus*, *Vernonia amygdalina*, *Piper aduncum*, *Moringa oleifera*, and ascorbic acid.

Figure 2. Total polyphenol content of *Schleichera oleosa* bark extract, *Anacardium occidentale* bark extract and leaves extract, *Muntingia calabura* leaves extract, *Artocarpus heterophylus* leaves extract, *Vernonia amygdalina* leaves extract, *Piper aduncum* leaves extract, *Moringa oleifera* leaves extract, and Ascorbic acid.

The determination of antioxidant activity values of seven tropical plants was performed. The radical scavenging capacity of plant extracts ranged from 6 to 500 µg/mL compared with ascorbic acid as antiradical standard. Among the plant extracts, the highest radical scavenging capacity was shown by the bark extracts of *Schleichera oleosa* with an IC₅₀ of 6.59 µg/mL. This antioxidant activity result
was stronger than ascorbic acid as antiradical standard. Thind et al (2010) reported that aqueous extracts of Schleichera oleosa bark showed a good antioxidant activity with an IC\textsubscript{50} of 30 µg/ml [18]. Anacardium occidentale bark extract and Anacardium occidentale leaves extract also had high antioxidant capacity with IC\textsubscript{50} of 8.06 µg/mL and 9.65 µg/mL, respectively followed by Muntingia calabura leaves extract with IC\textsubscript{50} of 27.12 µg/mL. The antioxidant activity of Anacardium occidentale leaves ethanolic extract was similar with that of ethanolic extract prepared from Indian region which reported by Jaiswal et al [17]. Secondary plant metabolites are classified according to their chemical structures into various classes. The biosynthesis and accumulation of secondary metabolites can be affected by several factors including genetic, ontogenic, morphogenetic and environmental condition. Antioxidant activity is classified into 5 groups: highly active (<50 µg/mL), active (50 – 100 µg/mL), moderate (101 – 250 µg/mL), weak (250 – 500 µg/mL) and inactive (>500 µg/mL) [19]. Based on this category, Schleichera oleosa, Anacardium occidentale, and Muntingia calabura extracts be included as highly active antioxidant. Meanwhile, another extract could be classified in weak category of antioxidant capacity. They are Artocarpus heterophyllus bark extract (IC\textsubscript{50} 323.49 µg/ml), Vernonia amygdalina leaves extract (IC\textsubscript{50} 373.49 µg/ml), Piper aduncum leaves extract (IC\textsubscript{50} 499.23 µg/ml), and Moringa oleifera leaves extract (IC\textsubscript{50} 450.61 µg/ml). The difference of IC\textsubscript{50} values among seven ethanolic tropical plant extracts can be related to differences in phenolic content of the extracts. Phenolic compounds capable of donating a hydrogen atom are known to be more effective in scavenging radicals [6]. The free radical from DPPH binding with the phenolic compound from the ethanolic extract, hence it becomes stable molecule.

The total phenolic content of the seven ethanolic plant extracts was measured using Folin-Ciocalteu’s reagent. The results are presented as milligrams of gallic acid equivalent (GAE) per gram of dry extract. The mean values of total phenolic content varied from 52.49 to 451.07 mg/g (gallic acid equivalent). Among the extracts, the highest phenolic content was observed in the extracts of Schleichera oleosa with 451.07 mg/g, followed by followed by Anacardium occidentale bark extract 327.6 mg/g, Anacardium occidentale leaves extract 307.08 ± 0.55 mg/g, and Muntingia calabura leaves extract 288.15 mg/g, Artocarpus heterophyllus bark extract 90.33 mg/g, Vernonia amygdalina leaves extract 84.26 mg/g, Piper aduncum leaves extract 57.53 mg/g, Moringa oleifera leaves extract 52.49 mg/g.

3.2. Correlation between antioxidant activity and polyphenol content

The correlation of correlation antioxidant activities of plant extracts and their total phenolic content was plotted (figure 3).

![Figure 3. Coefficient correlation of antioxidant activity and total phenolic content.](image-url)
Total phenolic content (x) of the extracts (figure 3) had a correlation with the antioxidant activity of at 1000 µg/mL (y), with coefficient of $R^2 = 0.8884$ ($y=-0.6609x + 347.61$). This research reveals that total phenolic content and antioxidant activity of the plant extracts had a positive correlation and also suggests that 88% of the antioxidant activity of the plant extract results from the contribution of phenolic compounds. Free radical scavenging and antioxidant activity of phenolic compound primarily depends on the number and position of hydrogen-donating hydroxyl groups on the aromatic ring of the phenol molecules [21]. This could be concluded that the antioxidant activity of the plant extracts is not limited to phenolic compounds. The antioxidant activity may also come from the presence of secondary metabolites that potential as antioxidant agents, such as volatile oils, carotenoids, and vitamins. The antioxidant activity of phenolic compound is primarily due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen [6]. Furthermore, the results previously presented agree with phenol compounds being major contributors to the antioxidants of plants, which is in related with the literature [22].

4. Conclusion
Four plant extracts were found as highly active antioxidant in this research. They are Schleicera oleosa (bark), Anacardium occidentale (bark), Anacardium occidentale (leaves), and Muntingia calabura (leaves). Total Phenolic Content and free radical scavenging activities had a positive correlation. Schleicera oleosa contained a highest Total Phenolic Content, followed by Anacardium occidentale bark extract, Anacardium occidentale leaves extract, and Muntingia calabura leaves extract associated to their antioxida nt activity. The result suggest that these four tropical plants can be used as resources for natural antioxidant.

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