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Antioxidant Activities of *Cyperus rotundus* L. Rhizome and *Areca catechu* L. Seed

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***Abstract*— The aim of this study was to extract the active antioxidant compounds from *Cyperus rotundus* L. rhizome and *Areca catechu* L. seed using water and ethanol (50%), and determine the total polyphenol contents, activity of free radicals scavenging using DPPH (1,1-diphenyl-2-picrylhydrazyl) and ferric reducing power of the extract of those materials. The result showed that both *C. rotundus* rhizome and areca seed extracted using ethanol contain a higher amount of polyphenols than water extracts. Total polyphenol extract of *C. rotundus* rhizome had a positive correlation with ferric reducing power, in which the ethanol extract that contain a high amount of polyphenols also exhibit high ferric reducing power. However, both ethanol and aqueous extracts of *C. rotundus* rhizome had the same ability to scavenge DPPH free radicals. On the other hand, there were different trends in the antioxidant activity of areca seed extract. The ethanol extract of areca seed contain a higher amount of total polyphenols than the aqueous extract, but it showed lower antioxidant activity to scavenge DPPH free radicals. However, both ethanol and aqueous extracts of areca seed showed the same level of ferric reducing power.**

***Keywords*— *Cyperus rotundus* L; *Areca catechu* L; antioxidant activity; total polyphenol contents.**

I. INTRODUCTION

Free radical reactions occur both in the human body and foods. Free radicals formed during normal metabolic processes can damage biological structures such as proteins, lipids and DNA and cause a variety of human chronic diseases. Moreover, those molecules induce lipid peroxidation leading the deterioration of foods. Many synthesis antioxidants have been used to inhibit the lipid peroxidative damage in foods and defend the human body against diseases [1], [2]. However, the use of the synthetic antioxidants has potential health risks and toxicity [3]- [5]. Therefore, the search for antioxidants from natural sources has attracted much attention and great effort has been made to identify compounds that can act as suitable antioxidants to replace synthetic ones.

Plants are the potential sources of natural antioxidants because they contain many phytochemicals such as flavonoids with various bioactivities, including antioxidant. The antioxidative effect of flavonoids has long been recognized. They have been reported to retard lipid peroxidation, to scavenge free radicals and active oxygen, to chelate iron ions, and to inactivate lipoxygenase [6], [7]. Many studies have reported that extracts from natural products, such as fruits, vegetables and medicinal herbs contain antioxidants that can prevent lipid peroxidation in food and help the human body to reduce oxidative damage [8] - [14].

Cyperus rotundus L. and *Areca catechu* L. are plants widely used in traditional medicine throughout the world for treatment of various diseases. *C. rotundus*, belonging to *Cyperaceae* family, is reported to have many pharmacological activities including anti-inflammatory, antipyretic, antidiabetic, analgesic activities, etc [15], and could be a potential source of natural antioxidant as well [16]. On the other hand, *A. catechu* is reported to possess antioxidant, anti-inflammatory, antidepressant, anthelmintic, psychoactive, etc [17].

Although the antioxidative activities of *C. rotundus* and *A. catechu* were recognized by some studies, further research on the efficient solvent kind used for the extraction is required. It is reported that the antioxidant activity of certain compounds depends on the solvent used [18]. Solvent polarity level will determine extraction result and antioxidant activity contained in the extract [19]. Therefore, the purpose of this study is to extract the active antioxidant compounds from *C. rotundus* and *A. catechu* grown in Indonesia, using two different solvent types; water and ethanol, and measure total polyphenol contents, free radical scavenging activity using DPPH (1,1-diphenyl-2-picrylhydrazyl) and reducing power of those extracts.

II. MATERIALS AND METHODS

A. Materials

Fresh samples of *Cyperus rotundus* L. rhizome and *Areca catechu* L. seed were obtained from Penyeurat village, Banda Aceh, Indonesia. Chemicals used were ethanol, water, Folin-Ciocalteu phenol reagent, gallic acid, sodium carbonate (Na_2CO_3), potassium ferricyanide [$(K_3Fe(CN)_6$], trichloroacetic acid, ferric chloride ($FeCl_3$), phosphate buffer, and 1,1-diphenyl-2-picryhydrazyl free radical (DPPH).

B. Methods

1) Extraction, The samples were ground using a domestic dry blender. Extraction of samples were performed according to the method of [2] and [20]. Each sample (2,5 g) was extracted using 25 ml of water (1:10 w/v). The mixture was allowed to stand at room temperature for 1 hr in the dark, then the mixture was centrifuged at 2000 rpm for 5 minutes. The obtained extract was filled in sealed small bottles and stored in a refrigerator at 4°C until ready for analysis [2]. For extraction using ethanol, each sample (2,5 g) was extracted with 25 ml of solvent (ethanol 50%) (1:10 w/v). Then the mixture was stirred for 60 s using a vortex and centrifuged at 2000 rpm for 5 minutes [20]. The obtained extract was filled in sealed small bottles and stored in a refrigerator at 4°C until ready for analysis.

2) Total polyphenol contents determination. The total polyphenol contens of the extracts was measured using the Folin-Ciocalteu assay according to the method described by [21]. A 0,1 ml extract was mixed with 0,1 ml of aquadest and 0,1 ml of Folin-Ciocalteu reagent 50%. The mixture was stirred for 3 minutes using a vortex and added 2 ml of Na_2CO_3 2%. Then the solution was shaken by using a vortex and allowed to stand for 30 minutes in the dark. The absorbance of the reaction mixture was read at λ = 750 nm. The total polyphenols contents of the extract was expressed as mg gallic acid equivalents per g sample.

3) Antioxidant activity determination using DPPH free radical scavenging method. The DPPH free radical scavenging activity of sample was assayed using Spectrophotometer (UV-Vis 1700 Pharma Spec, Shimadzu) according to the method of [22], which modified. Briefly, a 0,1 mM solution of DPPH in ethanol was prepared. Each extract (1 ml) was added to 2 ml of ethanolic DPPH solution until the color of sample became purple. Then, the mixture was shaken using a vortex and left to stand at room temperature for 30 minutes in a dark place. Furthermore, it was stirred again using a vortex. The absorbance of the solution was measured at 517 nm. The degree of decoloration of the solution indicates the scavenging efficiency of the added substance. The free radical scavenging activity was calculated as a percentage of DPPH decoloration using the following equation:

$$\text{Free radical scavenging activity} = \frac{100 \text{ x (1-absorbance of sample)}}{\text{Absorbance of reference}}$$

4) Reducing Power Determination. The reducing power of sample extracts was determined according to the method of [23]. Sample extracts were mixed with phosphate buffer (2,5 ml, 0,2 M, pH 6,6) and potassium ferricyanide [$(K_3Fe(CN)_6$] (2,5 ml, 1%). The mixture was shaken using a vortex and incubated at 50°C for 20 min. Then it was cooled. Trichloroacetic acid (2,5 ml, 10%) was added to the mixture, which was then stirred using a vortex and centrifuged at 3000 rpm for 10 min. The solution (2,5 ml) was mixed with distilled water (2,5 ml) and ferric chloride ($FeCl_3$) (0,5 ml, 0,1%). Then the mixture was shaken again using a vortex and the absorbance of the solution was measured at 700 nm.

5) Statistical Analysis. The research was conducted using randomized complete block design with two treatments (the source of natural antioxidant and the type of solvent for the sample extraction) and two replications. The obtained data were then statistically analyzed using Analisys of Variance (ANOVA). If the test result indicates significant differences between the treatments, it will be proceed with the Least Significant Difference Test (LSD).

III. RESULTS AND DISCUSSION

A. Total polyphenol contents

Extraction was performed using two kinds of solvent with different polarity, to obtain every active component in the *Cyperus rotundus* rhizome and *Areca catechu* seed as a potential antioxidant compounds. The level of polarity will determine extraction result and antioxidant activity contained in the extract.

The Folin-Ciocalteu phenol reagent is used to obtain a crude estimate of the amount of phenolic compounds present in the plant extracts. The result of the total polyphenol analysis (Figure 1) shows that both ethanol extracts of *Cyperus rotundus* rhizome and areca seed (47.18 and 55.03 mg gallic acid equivalent per g sample, respectively) contain a higher amount of polyphenols than aqueous extracts (24.20 and 19.35 mg gallic acid equivalent per g sample, respectively). This indicates that the semi polar solvent (ethanol) was more effective to extract the phenolic compounds of *C. rotundus* rhizome and areca seed.

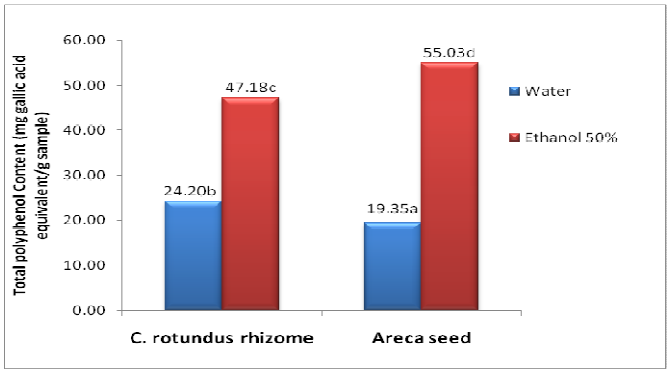

Fig. 1 The Interaction effect of natural antioxidant extracts and solvent types on total polyphenol content (values followed by the same letter indicate no significant differences)

B. Antioxidant activity

1) DPPH free radical scavenging activity

The measurement of DPPH free radical scavenging activity is based on the DPPH radicals reduction in ethanol which causes an absorbance drop at 515 nm [2]. The solution color changes from purple to yellow. This change occurs when DPPH was captured by antioxidants which remove H atoms to form a stable DPPH-H [24].

The DPPH free radical scavenging activity of *C. rotundus* rhizome and areca seed are shown in Figure 2. It indicates that the type of solvent gave a different antioxidant activity of extracts. According to [19], the polarity will determine the extraction result and antioxidant activity contained in the extract. Aqueous extract of areca seed showed higher antioxidant activity (76.64%) than ethanol extract (66.82%). However, solvent type has no effect on the antioxidant activity of the rhizome of *C. rotundus*. Both water and ethanol extracts of *C. rotundus* rhizome exhibited the same level of the antioxidant activities (64.67% and 64.16%, respectively).

Generally, extracts that contain a high amount of polyphenols also show high antioxidant activity. In contrast, ethanol extract of areca seed had higher total polyphenol content than water extract, but it showed lower DPPH free radical scavenging activity than aqueous extract. It could be due to the presence of compounds not reactive to DPPH. Antioxidant compounds such as polyphenols may be more efficient as reducing agents for ferric iron but some may not scavenge DPPH free radicals as efficiently because of steric hindrance [2].

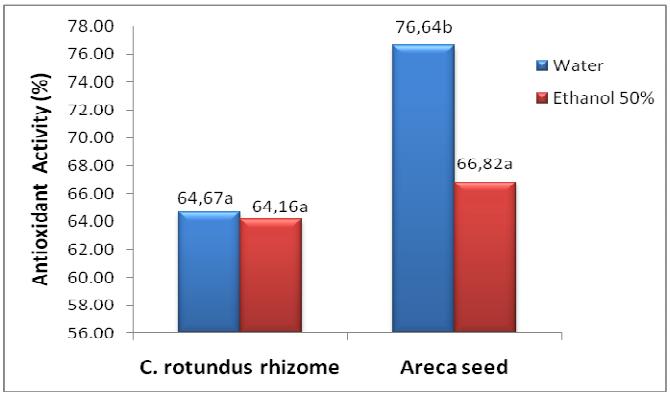

Fig. 2 The interaction effect of natural antioxidant extracts and solvent types on antioxidant activity (values followed by the same letter indicate no significant differences)

2) Reducing Power

Level-3 Heading: A level-3 heading must be indented, in In the reducing power measurement, the reductant (antioxidant) in the sample will reduce Fe^{3+} ions (potassium ferricyanide complex [$(K_3Fe(CN)_6]$) to the ions Fe^{2+} (ferrous form)). Therefore, Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increased absorbance indicated an increase in the reducing power [1], [23].

As shown in Figure 3, ethanol extract of the rhizome of *C. rotundus* exhibited a higher reducing power than water extract. This result correlated positively with the total polyphenol content. High content of total polyphenols showed a high reducing power of the rhizome of *C. rotundus* extract. However, there was no difference in the reducing power of the areca seed. Both water and ethanol extracts of the the areca seed have a same level of the reducing power. The results reveal that both *C. rotundus* rhizome and areca seed are electron donors and could react with free radicals, convert them to more stable products, and terminate radical chain reaction.

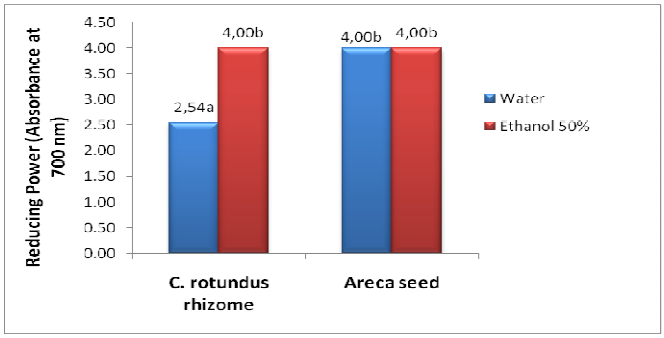

Fig. 3 The interaction effect of natural antioxidant extracts and solvent types on reducing power (values followed by the same letter indicate no significant differences)

IV. Conclusions

The results obtained in the present study indicated that both *Cyperus rotundus* rhizome and *Areca catechu* seed extracted using ethanol contain a higher amount of polyphenols than aqueous extracts. Total polyphenol extract of *C. rotundus* rhizome had a positive correlation with ferric reducing power. However, both ethanol and water extracts of *C. rotundus* rhizome had the same ability to scavenge DPPH free radicals. In contrast, there were different trends in the antioxidant activity of areca seed extract. The ethanol extract of areca seed contain a higher amount of total polyphenols than the water extract, but it showed lower antioxidant activity to scavenge DPPH free radicals. However, both ethanol and aqueous extracts of areca seed showed the same level of ferric reducing power. Further research is required to isolate and identify the antioxidative components in *C. rotundus* and areca seed.

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