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The potential antioxidant activity of ethanolic extract of Aceh ant-plant (*Mymercodia sp*) on the free radical DPPH(1,1-Diphenyl-2-pikrylhidrazil)

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Abstract. This research aims to investigate the potential antioxidant activity of ethanolic extract of Aceh ant-plant (*Mymercodia* sp) on the free radical DPPH (1,1-diphenyl-2-pikrylhidrazyl). The ant-plant was extracted using maceration technique with the ethanol as solvent. The extract was made in various concentrations: 5, 10, 25, and 50 ppm. Then DPPH solution was added to it. Absorbance reading was conducted with spectrophotometry at the wave length of 517 nm after 30 minutes. The result of research shows the potency of ant-plant (*Mymercodia* sp) as an antioxidant free radical scavenging ethanolic extract with IC_{50} value of 14.73 ppm. The ascorbic acid as the control showed IC_{50} value of 7:32 ppm.
Keywords: Antioxidant, Aceh ant-plant, Ethanolic extract, DPPH

Introduction

Plant is one of the traditional ingredients that have been known since time immemorial. In the last decade the use of traditional medicine is increasing, one of the reasons is the increased confidence in the benefits and uses of medicinal plants in health care (Christine, 1985). Selection of plants in order to find the new bioactive compounds from natural materials can be conducted through etnofarmacology and chemotaxonomic approach. Etnofarmacology is intended as a search based on the usage of natural materials by a particular ethnics for treatment, while the chemotaxonomic approach is done through a search based on the relationships between plants with the assumption that plants contain the same chemicals or at least have the same framework or the same core on their active compound (Anonymous, 1997; Simanjuntak, 2003).

One of the plants that have the potential as a source of bioactive chemical compounds is genus *Myrmecodia* or ant nest-plant. This plant was popular in the early 2000s, introduced by Subroto, A and Hendro (2006). They published the ant nest-plant taken from Papua (*Myrmecodia pedans*). The report mentioned that the plant has been used empirically in the local community as a drug for the treatment of chronic diseases such as diabetes mellitus, gout, hypertension and endurance enhancer. The report also revealed that the active compounds contained in the ant nest-plant were antioxidantscompounds including flavonoids, tannins, and polyphenols. In addition the plant wasalso enriched by minerals.

The ant-nest plant is not only can be found in Papua, but also in other areas with tropical forests such as Toraja, Kalimantan and Sumatra, including Aceh forest. Normally, the local communities name the plant by the name of the region, so that the ant nest found in Aceh is known as ant-nest Aceh. Based on this background the author intends to conduct a research on ant-nest plant found in Aceh in order to proof scientifically the use of ant nest found in Aceh as an alternative medicine for the people in Aceh, especially for cancer treatment.

The study was started by the extraction of active compounds using maceration with ethanol as the solvent. Then the identification of chemical content was carried out. The antioxidant activity was evaluated using the DPPH (1,1-diphenyl ,2-pikrilhidrazil) method. This method is a common method used in the determination of the antioxidant potency of natural medicine. Moreover, this method is relatively easy to carried out and inexpensive compared to the other method (Szabo et al., 2007).

Materials and Methods

Research sites

This research was conducted at the Laboratory of theChemistry Natural Products, Research Center for Chemistry LIPI Serpong and Faculty of Veterinary Laboratory, Unsyiah.

Materials and equipment

The plant is a local ant-nest (Aceh) which was obtained from the local market, Lambaro-Aceh Besar. Extraction using 70% ethanol, phytochemical screening using mercury chloride, potassium iodide, 1% ferry chloride, hydrochloric acid, magnesium powders, concentrated sulfuric acid, chloroform, ammonium hydroxide, acetic acid anhydride, Meyer and Dragendorff reagents. Color fading for the determination of antioxidant activity using DPPH. Standard antioxidant was ascorbic acid. The equipments used in this study are a maceration equipment, rotary evaporator (Buchi rotary evaporator R-215, Germany), UV-VIS spectrophotometer (U Hitaci 2000, Japan), micro pipette (Eppendrof), refrigerators, incubators containers, tubes of various sizes (pyrex) and a test tube rack

Preparation of simplicia

Ant nests are collected and cleaned from attached dirt (moss), then thinly sliced and dried in the sun ± 3 days. The dried materialswas then smoothed by grinding machine. Next, the obtained powder was collected and stored in a tightly closed container.

Phytochemical screening

The identification of secondary metabolites was conducted using modified Simes method, *et al* (1995),explained as follows: the condensed ethanol extract obtained from ± 5 g wet sample which was added with 5-10 mL distilled water and $CHCl_3$, strongly shaken and allowed to form two layer and then separated. Water layer is used to test flavonoids, phenolic and saponin. The determination of flavonoid, saponins, tannins and sterols/ terpenoids are as follows (Ciulei. 1984)

Extraction

The dried ant-nest that has been smoothed was blended dried and weighed as the powder. This powder was extracted by maceration using 70% ethanol for 3 x 24 hours. The solvent was then evaporated with a rotary evaporator and concentrated ethanol extract obtained. The remained solvent and water were evaporated in water bath to dry, and then weighed.

Antioxidant activity test

The extract (2.0 mg)was dissolved in 2 mL of methanol and then shaken until homogeneous. Then The mastersolution of test material waspipetted (10, 20, 50 and 100 mL) and put into 4 test tubes. Into each tube was added methanol to make volume of 1600 µL, then added with 400 mL DPPH so that the total volume to 2 µL, shaken until homogeneous. Then they were incubated at 37 °C for 30 minutes.

Calculation

The IC_{50} values were statistically determined by simple linear regression equation (the x-axis is the series of concentration, whereas y-axis is percentage of inhibition)(Blois, 1958). this calculation was undertaken with SPSS version 16.01.

Results and Discussion

Ant-nest plant was thinly sliced and dried in the direct sunlight to dry, then mashed. Extraction of chemical constituents of plants was carried out with maceration, the process in which sample was immersed in 70% ethanol for 3 days with occasional shaking and then was filtered.The rest of solvent contained in obtained sample was evaporated in *vacuo* in order to prevent the damage of termolabile compounds. Extracts dried in water bath at 40 °C and then weighed. The obtained result was 10.5 g.

Preliminary test of the ethanol extract was the phytochemical screening of active compounds contained in the plant. The test included the identification of classes of compounds alkaloids, phenolics, flavonoids, saponins, steroids and terpenoids. Phytochemical screening results indicated the presence of phenolics, saponins, steroids and terpenoids (Table 1)

Table 1. Phytochemical screening results ant-nest Aceh

No	Classes of compounds	Methode	Results
1	Alkaloids	Culvenor-Fitgerald	Negative
2	Flavonoids	Sianidin test	Negative
3	Phenolics	Sianidin test	Positive
4	Saponins	Sianidin test	Positive
5	Steroids	Simest test	Positive
6	Terpenoids	Simest test	Positive

The next test was evaluation of antioxidants activity with DPPH (1,1-diphenil-2-pikrilhidrazil). DPPH was a stable free radical compounds. When it was reacted with extracts or pure compounds from plants that contain antioxidants, It would catch the hydrogen released by antioxidants. The DPPH free radicals (purple) would transform into 1,1-diphenyl-2-pikrilhidrazin (yellow). The principle of this method is the determination of antioxidant activity of compounds by measuring the absorbance of the reaction products (1,1-diphenyl-2-pikrilhidrazin). Antioxidant activity was obtained from the regression

equation with concentration as the independent variable and the percentage of inhibition as an independent variable.

The antioxidant activity of the samples expressed as IC_{50}. The IC_{50} values was determined with SPSS version 16.01 for Windows. Tthe regression equations obtained in this study was y = 1.61x-16:09 with r2 = 0931. From the regression equation, it was obtained that IC_{50} of ethanol extract was 14.73 mg / mL (Table 2 and Figure 1).

Table 2. The result of antioxidant test of ethanol extract ant-nest Aceh

No	Sample	Concentration (ppm)	% Inhibition	IC-$_{50}$	Antioxidant Activity
1	Ascorbic acyd	20	96.19	7.32	Very strong
		15	92.34		
		10	70.90		
		5	32.65		
2	Ant-nest	50	90.98	14.73	Very strong
		25	69.05		
		10	31.48		
		5	17.70		

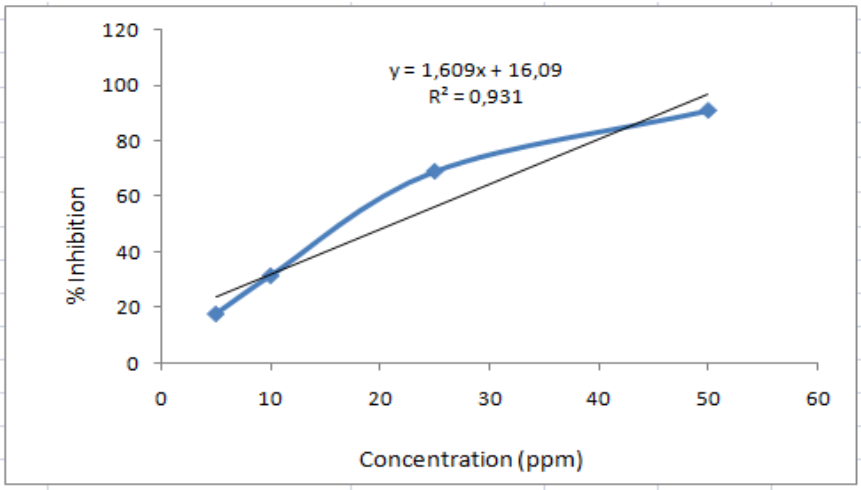

Figure 1. Graphs and linear regression of antioxidant activity of extract ant Aceh

The calculation result of the inhibition activity of the sample at a concentration of 5 ppm showed that the antioxidant activity of 17.7%. At a concentration of 10 ppm, the sample showed the ability to reduce the free radical DPPH of 31.48%. At a concentration of 25 ppm, It showed the ability to reduce free radical DPPH was 69.05% and the concentration of 50 ppm, It showed the ability to reduce free radical DPPH was 90.98%.

After the addition of DPPH, there was an immediate reaction shown as the colour chane from purple to yellow in each concentrations of sample without started by shaking. This phenomenon indicated that the ethanol extract ant-nest had a very strong antioxidant activity. According to Jun *et.al*, 2003, antioxidant activity is considered as very active when IC_{50} values less than 50 ppm; active when IC_{50} values is 50-100 ppm, moderate when $IC_{50}$is 101-250 ppm, and weak when IC_{50} values is 250-500 ppm , and not active if IC_{50} values is greater than 500 ppm. The result (IC_{50}) obtained in this study was 14.73 mg / mL. Although it was weaker than the antioxidant, vitamin C (IC_{50} 7.32 mg / mL), the sample is classified into a very active material (<50 mg / mL). These results indicated that the active compounds (that could be isolated in the future) could be more potent than this extract, or might be even better than vitamin C.

Conclusion

The ethanol extract of Aceh ant-nest had the very active antioxidant activity with IC_{50} of 14.73 mg / mL. This antioxidant activity was due to the presence of active compounds that can trap free radicals.

Suggestion

The isolation and identification of the active compounds contained in the plant and further test as anticancer could be conducted for future works.

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