Antioxidant Activities of Ethyl acetate Extract and Hexane Extract of Lelak (*Uvaria rufa* Blume) Leaves

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ABSTRACT ---- ***Uvaria rufa Blume is one of the main power plant that has natural antioxidants that are known to inhibit the scavenging free radicals. The present study was to evaluate antioxidant activity of ethyl acetate and hexane extracts of leaves of Uvaria rufa Blumes. Measurement of antioxidant activity carried out using the DPPH method. DPPH radical scavenging activity of Uvaria rufa Blume of hexane extract and ethyl acetate extract tested showed lower activity than that of a standard compounds: ascorbic acid (IC 50 = 3. 781 µg/ml). Extracts of ethyl acetate has strong antioxidant activity which IC_{50} values was 57.89µg/ml respectively and extracts of hexane has weak antioxidant activity which IC_{50} values was 87,292 µg/ml respectively.***

Keywords ---- Antioxidant, DPPH, Uvaria rufa, IC_{50}

1. INTRODUCTION

Antioxidants play a significant role in the prevention of diseases, and have a capacity to reduce oxidative stress by chelating trace elements or scavenging free radicals and protecting antioxidant defenses. For this reason, it is important to search for alternatives with antioxidant activity, as spices with secondary metabolites with antioxidants activity (Orozco, et al. 2013).

Antioxidant are compounds that can delay, inhibit, or prevent the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions. There are at least four general sources of antioxidant (1) enzymes, for example superoxide dismutase, glutathione peroxidase, and catalase; (2) large molecules (albumin, ceruloplasmin, ferritin); (3) smallmolecules (ascorbic acid, glutathione, uric acid, tocopherol, carotenoids, (poly)phenols); and (4) some hormones such as estrogen, angiotensin, melatonin, etc (Rohman *et al.*2006).

Uvaria rufa Blume is a plant found in tropics of Asia and Africa. It is commonly called as 'Lelak' in Kupang (Indonesia). It is traditionally used as a medicinal plant in Asia, however, for the people of Indonesia, this plant has not been popular in general as a medicinal plant, especially the Kupang district. The Leaves of *Uvaria rufa Blume* are used in Asia traditional medicine as an anti-diabetic agent, increase uterine contraction and antioxidant.

The previous study showed that ethanol extract effective as a antioxidant activity with IC_{50} 110.67µg/mL repectively (Elisma and Soeharto, 2014). However, the antioxidant activities of ethyl cetate extract and heksane extract have not been reported yet. The objectives of this study was to to evaluate antioxidant activities of ethyl acetate and hexane xtract of Uvaria rufa Blume Leaves by radical scavenging assay using DPPH (2,2- diphenyl-1-picrylhydrazyl) radical.

2. METHODOLOGY

2.1 Materials and chemicals

Lelak leafes was obtained from Kupang, East Nusa Tenggara. DPPH (2,2-diphenyl-1- picrylhydrazyl) and vitamin C were purchased from Sigma (St.Louis, MO, USA), aluminium chloride, sodium hydroxide, silica gel GF254, silica gel, ethanol, ethyl acetate, n-Heksana, chloroform were purchased from Merck (Darmstadt, Germany), and bidestilled water (Ika Pharmindo).

2.2 Preparation and fractionation of ethyl acetate extract of *Uvaria rufa* Blume leaves

A total of 150 g of dry powder leaves lelak put in a closed vessel, was added in 1125 mL of 70% ethanol is then closed , was left for 5 days protected from light while stirring occasionally. After 5 days the mixture was filtered and washed with 70% ethanol to obtain 1500 mL maserat . Move in a closed vessel and left in a cool place protected from light for 2 days, then filtered . Maserat evaporated using a rotary evaporator and concentrated using a water bath at 50° C to obtain a

viscous extract. Extract was fractionated by methods Can- Ake (2004) partitioned using 96 % ethanol -water (2 : 3) and ethyl acetate to obtain a viscous extract. .

2.3 Preparation and fractionation of hexane extract of Uvaria rufa Blume leaves

A total of 150 g of dry powder leaves lelak put in a closed vessel, was added in 1125 mL of 70% ethanol is then closed , was left for 5 days protected from light while stirring occasionally. After 5 days the mixture was filtered and washed with 70% ethanol to obtain 1500 mL maserat . Move in a closed vessel and left in a cool place protected from light for 2 days, then filtered . Maserat evaporated using a rotary evaporator and concentrated using a water bath at 50° C to obtain a viscous extract. Extract was fractionated by methods Can- Ake (2004) partitioned using 96 % ethanol -water (2 : 3) and hexane to obtain a viscous extract. .

2.4 Determination of Antioxidant activity using DPPH via free radical scavenging activity

Antioxidant activity was determined by radical scavenging activity using DPPH radical according to Zou *et. al.*, (2004). Briefly, 4 mL of testing antioxidant solution with five different concentrations was added to 5-mL volumetric flask filled with 1,0 mL of DPPH 0,5 mM in ethanol. The solution was diluted to the volume (5.0 mL) with ethanol, shaken vigorously, and kept for 30 min at room temperature in the dark. The absorbance at 517 nm was measured by spectrophotometer Shimadzu against blank of ethanol. A control containing ethanol instead of DPPH solution was also made. The experiment was done in triplicate. The antioxidant activity of the samples was calculated according to the formula:

Percentage (%) of antioxidant activity = Abs.of control / (Abs.of control − Abs.of s*ample*) x 100%

The percentage of antioxidant activity was plotted against the sample concentration (μg/mL) to obtain IC_{50}, defined as the concentration of the sample necessary to cause 50% scavenging of DPPH radical calculated by linier regression curve.

3. RESULTS AND DISCUSSION

The present study was carried out to evaluate the antioxidant effect of Ethyl acetat n hexane extract of leaves of *Uvaria rufa Blume*. In the extract of the leaves of uvaria rufa Blume there were identified flavonoids, sapponnins, tannins as table 1.

Table 1. Phytochemical screening of extracts hexane, ethyl acetate of *Uvaria rufa Blume*

Leaves Extract	Constituent
Hexane	Flavonoids
Ethyl acetate	flavonoids, saponins, tannins

The ethyl acetate extract, positively contains saponins, tannins and flavonoids, while the hexane extract containing flavonoids. Flavonoids and tannins is one group of phenol compounds . Flavonoids act as antioxidants and free radical catcher because it has a structure consisting of hydroxyl groups , which can donate hydrogen ions (Purwaningsih, 2012). Tannins compounds can inhibit the formation of active oxygen thereby inhibiting the oxidation reaction (Hernawan et al ., 2003). Saponins are active compounds that are like soap and can be detected by their ability to form foam.

Antioxidant activity measured by radical scavenging using DPPH (2,2-diphenyl-1- picrylhydrazyl) radical

DPPH method has been widely employed to evaluate the free radical scavenging effectiveness of various antioxidant substances in plant systems. The method is based on the reduction of alcoholic DPPH in the presence of a hydrogen-donating antioxidant to form the non-radical form, DPPH-H. The antioxidant activities of ethyl acetate extract and hexane extract determined by radical scavenging activity using DPPH radical was shown in table 2 and 3.

Table 2: DPPH Radical Scavenging Assay of Hexane of *Uvaria rufa* Blume leaves.

Concentration (ppm)	% RSA	IC_{50} value (µg/ml)
25	18.76	**87,292**
50	18.87	
75	22.76	
100	21.10	
125	23.97	
150	26. 80	

Table 3: DPPH Radical Scavenging Assay of Ethyl acetat fraction of *Uvaria rufa Blume* leaves.

Concentration (ppm)	% RSA	IC_{50} value (µg/ml)
25	21.88	**57.89**
50	40.79	
75	51.15	
100	76.51	
125	80.86	
150	84.01	

When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance. The antioxidant activity of the extracts is shown in table 2 and table 3. The experimental data reveals that the Ethyl acetate extract have more scavenging activity than the hexane extracts. At the maximum concentration the scavenging activity of the free radicals are Ethyl acetat extract > Hexane extract. IC_{50} is a value indicating the magnitude of the concentration of a substance, which can reduce DPPH radicals by 50 %. The smaller the IC_{50} means higher antioxidant activity. The results showed that the ethyl acetate extract had lower IC_{50} value compared with n - hexane extracts so that the ethyl acetate extract has anti-oxidant properties which is higher compared to the hexane extract

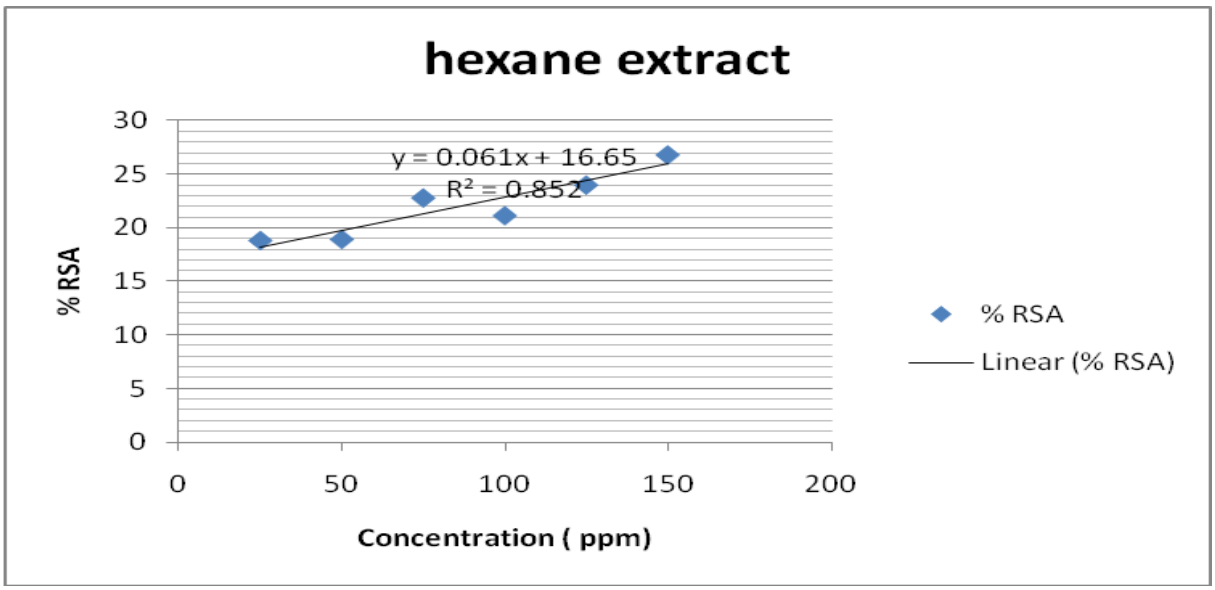

Figure 1. DPPH Radical Scavenging Assay of *hexane Extract*

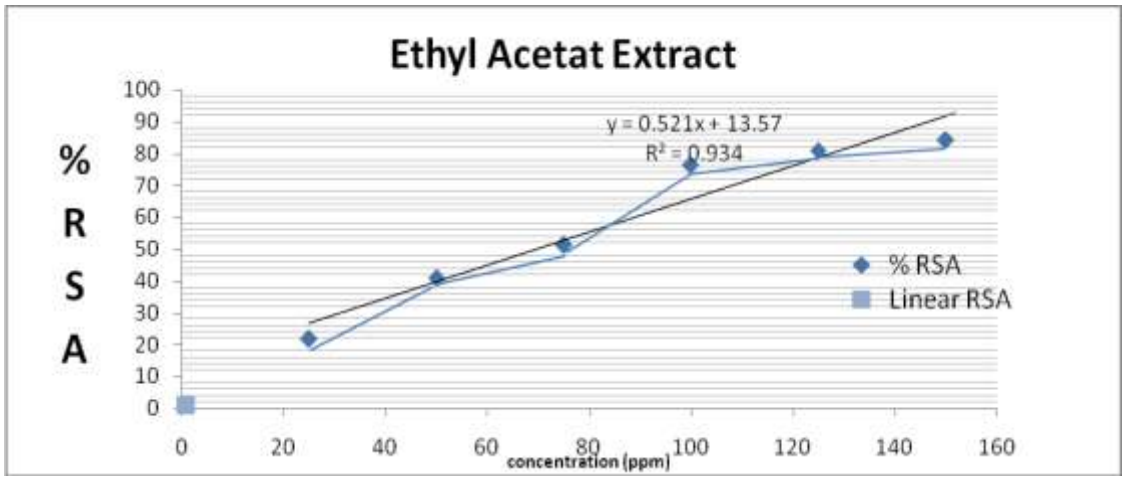

Figure 2: DPPH Radical Scavenging Assay of *Ethyl Acetate Extract*

The study shows that % RSA increases gradually with increase with concentration of samples. The concentration at which the %RSA value i.e. the inhibition value reaches 50% is called the IC_{50} value. The lower IC_{50} value indicated high antioxidant value in analyte. The IC_{50} values in Ethyl Acetat and Hexane Extracts of *Uvaria rufa* Blume *leaves* were found to be 57.89 µg/mL; 827, 292 µg/mL respectively.

The experimental data reveals that the Ethyl acetate extract have more scavenging activity than the hexane extracts. At the maximum concentration the scavenging activity of the free radicals are Ethyl acetat extract > Hexane extract. IC_{50} is a value indicating the magnitude of the concentration of a substance, which can reduce DPPH radicals by 50 %. The smaller the IC_{50} means higher antioxidant activity. The results showed that the ethyl acetate extract had lower IC50 value compared with n - hexane extracts so that the ethyl acetate extract has anti-oxidant properties which is higher compared to the hexane extract

Table 4. Value of IC_{50}

Leaves Extract	Value Of IC_{50} ± SD	Antioxidant activity
Extract Of Heksane	87.292,7995 ± 95.657,624 ppm	Weak
Extract Of Ethyl acetate Of *Uvaria rufa* Blume Leaves	57,8852 ± 2,2331 ppm	Strong
Vitamin C	3,781 ± 0,410 ppm	Very Strong

Weak anti- oxidant activity is due to active compounds that can be extracted by n - hexane compounds which are non -polar only partially soluble.

4. CONCLUSION

The results showed that the ethyl acetate extract had lower IC_{50} value compared with hexane extracts so that the ethyl acetate extract has anti-oxidant properties which is higher compared to the hexane extract. In this study, it was found the ethyl acetate fraction from uvaria rufa Blume leaves showed strong antioxidant activity in DPPH test.

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