ANTIOXIDANT ACTIVITY ASSAY OF ETHANOLIC EXTRACT OF SIRSAK (*Annona muricta* L) LEAVES

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Abstract

Background. *Free radicals cause cell`s damage in the body which manifestate as disease. The increase prevalence of degeneratife diseases caused by free radicals in Indonesia has motivated scientists to explore natural antioxidant compounds. Sirsak (Annona muricata L) is one of plant used as anticonvulsant, antioxidant, and anticancer.*

Objective. *This study was purposed to investigate antioxidant activity of ethanolic extract of Annona muricata L*

Methods. *This research comprised ethanolic extract of Sirsak leaves using maceration method and antioxidant in vitro examintation used 2,5; 5; 10; 20; and 40 ug/mL of DPPH. The method used for antioxidant assessment was true ability of antioxidant to capture DPPH.*

Outcome measured. *ES_{50} of DPPH technique*

Results. *The ES_{50} result of ethanolic extract on Sirsak leaves was 22.23 ± 0,64 µg/mL*

Key words : *Annona muricata, DPPH, antioxidant*

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INTRODUCTION

The diseases caused by ffree radical comprise the degeneratife illness, early aging and the general inflammation. Free radicals able to interfere the healthy cell`s nucleus then initatite mutation wich transform the cell into tumour cell or cancer (Thompson, 2004). Apart from that, free radical damages blood vessel wall because it creates inflammation which finally resulted in the coronary heart disease. It also causes the hypertension illness, stroke and diabetes mellitus (Adeyemi *et al.*, 2008).

Sirsak (*Annona muricata* L) is one of the plants that is used for detoksication, antioksidan and anti-cancer. The scientific data about the in vitro and in vivo anti-cancer activity of Sirsak is still very limited (Fang *et al*., 2009). *Annona muricata* is used as antioxidant, medicine anticonvulsant, the stamina enhancer, anti-cancer, and cytotoxic. The compounds which are contained in *Sirsak* (*Annona muricata*) are flavonoid and acetogenin .(Adewole and Ojewole, 2009).

The *Sirsak* (*A. muricata* L) has the anticonvulsant activity, anti-cancer, and antiinflammasi. The leaves extract has the anti-cancer activity HEP2 (Human epidermoid cancer cells) that shows the strong cytotoxic activity. The cytotoxic activity or anti-cancer is also due to the antioxidant effect. This is because cancer is also caused by oxidants or the free radicals (Baskar *et al*., 2007)..

The DPPH method is based on the capacity of antioxidant to hinder the free radical to donorthe hydrogen atom. The change in the purple DPPH colour became purple reddish/yellow indicate the activity of the compound antioxidant. This method uses the positive control as the standard to asertain theantioxidant activity of the sample. The antioxidant activity assay DPPH method used *1,1-difenil-2-pikrilhidra-zil* (DPPH) as the free radical. The principle is that the scavengers of hydrogen by DPPH from the antioxidant compound will change it into *1,1-difenil-2-pikrilhidrazin* (Sharma and Bhat, 2008). The scavenging activity of antioxidant toward free radicals is calculated into Electron scavenging 50 (ES_{50}) (Locatelli *et al*., 2009).

Based on the background that has been presented, the research question is outlined which is how many ES_{50} of ethanol extract of sirsak leaves in capturing DPPH. It is hoped that in the future, the phytopharmacy agency of Sirsak can be patented.

METHODS

Materials and Methods

Instrument

The instruments which were used for the antioxidant assay of ethanol extract of sirsak leaves were glass equipment, balance, electricity stove, blender, electric stirer, separation funnel, Buchner funnel, uv vis spectrofotometer.

Materials

The material that were used for the leaves ethanol extraction of sirsak leaves was quality materials technic, that was: aquadestilata, ethanol. The material for the antioxidant assay were aquadest, DPPH, and ethanol p.a.

Research Procedures

1. Plant Determination

This stage aims to validate the main material which was Sirsak (A murricata). This activity was conducted in the Farmakognosi Laboratory, Ahmad Dahlan University. Sirsak determination was conducted using the guidance of the Flora book of Java (Van steenis, 2000)

2. Extraction

Two hundred fifty gram of Sirsak was macerated using 2 litres of ethanol 70%, the maceration process was maximised using electric stirer for 3 hours, then was kept in room temperature for 24 hours. The filtrate was received used a Buchner funnel and was called as

first maseration. Extract residue resulted from the first maceration process then was macerated again using ethanol 70% totalling 1 litre, so that second and third maceration were obtained. Ekstrak liquid ethanol evaporated to obtain thick extract. This process is illustrated in figure 1.

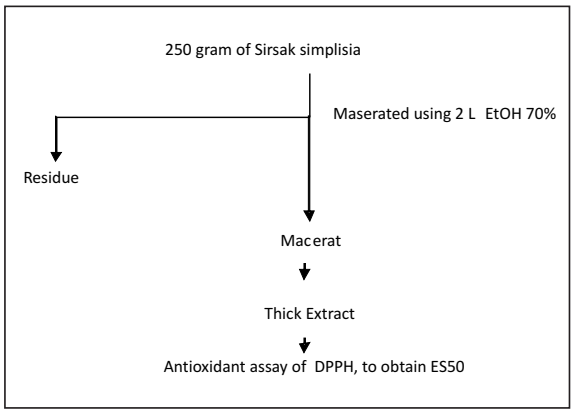

Figure 1. Ethanol Extract and antioxidant Activity Assay

3. Antioxidant assay

a. Operating time determination

Each of 1,0 ml the sample solution was stired with 1,0 ml of the DPPH solution 0.15 mM, afterwards it was observed absorbacy him for 60 minutes in long the wave 517 nm.

b. The determination of Maximum Absorbancy Wave Length.

The determination of wave length (λ) of the maximum absorption of the DPPH was carried out as follows: l,0 ml the DPPH solution 0.15 mM was combined with 1,0 ml of absolute ethanol,and was mixed homogeneous then was measured by the absorption of 400-600 nm wave lenght.

c. The determination of free radicals scavenger absorbation using DPPH method

Each of 1,0 ml the sample solutions and the solution to the standard with various concentration was mixed with 1.0 ml the DPPH solution 0.15 of mM. This mixture was kept in the dark place during operating time. Afterwards the absorbansi was measured to ascertain the maximal DPPH absorption with spectrofotometer UV-Vis. Blanko solution which was used is abolut ethanol

d. Data Analysis

The data obtained using the previous procedure was % ES_{50} and the concentration of the tested compound afterwards was analyzed using linear regression to obtain the concentration of the the radical scavenger 50 % (ES_{50}).

$$\% \text{ free radical scavenger} = \left(\frac{\text{negatif control absorbance - sampel absorbasnce}}{\text{negatif absorbance}} \right) \text{x } 100\%$$

The value of free radical percentage was regress toward the log of concentration . The value of ES_{50} which was obtained from the antilog x where Y=5.

RESULT AND DISCUSSION

The mechanism of the occurrence of the illness was often caused by the existence of the oxidant that was abundant in the body. These illnesses including hypertension, cancer, diabetes melitus, and the decline illness in the degenerative function of the other body. Up til now investigation antioxidant has been carried out by looking for the synthesis compound and from the nature material (Richards *et al.,* 2009). The need was received antioxidant especially from the nature material was caused by the source that often was available in Indonesia.

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compound and from the nature material (Schiffrin, 2010).

The main content from the leaves sirsak that played a role as antioksidan was flavonoid. This flavonoid had many double bonds and the hydroxil cluster that could play a role as free radical scavenger (Hidalgo *et al.*, 2010). After scavenge the free radical it change itself into radicals however it could stabilise itself via autoresonance. The extract was tested by the activity antioxidant by testing towards the ability of DPPH free radical scavenging. The assay was carried out by 3 times so as to be received by the ES_{50} value.

Based on the data the ES_{50} can be obtain by making the curve of log relations the level of

Table I. The result of the association of concentration and absorbacy and % of ethanol extract antioxidant first replication.

Concentration (µg/ml)	Abs	% Antioxidant	Probit
2,500	0,773	4,6072	3,36
5,000	0,718	11,3945	3,77
10,000	0,625	22,8712	4,26
20,000	0,484	40,2715	4,75
40,000	0,198	75,5656	5,71

Table II. The result of the association of concentration and absorbacy and % of ethanol extract antioxidant second replication.

Concentration (µg/ml)	Abs	% Antioxidant	Probit
2,500	0,770	4,9774	3,36
5,000	0,735	9,2966	3,66
10,000	0,637	21,3904	4,19
20,000	0,482	40,5183	4,77

Table III. The result of the association of concentration and absorbacy and % of ethanol extract antioxidant third replication

Concentration (µg/ml)	Abs	% Antioxidant	Probit
2,500	0,780	3,7433	3,25
5,000	0,729	10,0370	3,72
10,000	0,641	20,8968	4,19
20,000	0,479	40,8885	4,77
40,000	0,206	74,5784	5,67

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Table IV. The result of ES_{50} 1, 2, dan 3 replication of ethanol extract toward DPPH

Replication	ES_{50} (µg/mL)	Average	SD	%CV
1	21.57	22,23	0,64	2,88
2	22.85			
3	22.26			

vs probit. The ES_{50} value is ilustrated in the Table IV.

Based the result of ES_{50} which was 22.23 ± 0,64 ug/mL it can be concluded that the antioxidant capacity of ethanol extract of Sirsak is relatively low as the standard of antioxidant which usually has ES_{50} less than 2 µg/mL. Further research to lower the ES_{50} value by carrying out purification of the extract is recommended.

CONCLUSION

The extract of leaves ethanol sirsak has the ES50 of 22.23 ± 0,64 µg/mL towards the scavenger of the free radical DPPH.

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