RESEARCH ARTICLE

IN-VITRO STUDY OF ANTIOXIDANT ACTIVITIES FROM ETHANOL EXTRACTS OF AKAR KUNING (*Arcangelisia flava*)

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ABSTRAK

Penelitian ini bertujuan untuk mengetahui aktivitas antioksidan dari ekstrak batang Akar Kuning (*Arcangelisia flava)* dari Kalimantan Tengah menggunakan metode 1,1-difenil-2-pikrillhidrazil (DPPH). Penelitian ini meliputi ekstraksi simplisia dan uji aktivitas antioksidan dengan metode DPPH. Berdasarkan analisis aktivitas antioksidan pada ekstrak etanol dari batang tanaman akar kuning diperoleh nilai IC_{50} sebesar 136,81 ppm. Nilai IC_{50} ini menunjukkan kekuatan antioksidan yang terkandung dalam batang tanaman akar kuning termasuk dalam kategori sedang. Penelitian lebih lanjut tentang aktivitas antioksidan pada fraksi batang kuning perlu dilakukan untuk menentukan komposisi senyawa antioksidan dalam setiap pelarut.

Kata kunci: *Arcangelisia flava*, antioksidan, DPPH

ABSTRACT

This study aims to determine the antioxidant activity of akar kuning (*Arcangelisia flava*) stem extract from Central Kalimantan using 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. This research includes extraction of simplicia and test of antioxidant activity by DPPH method. Based on the analysis of antioxidant activity on ethanol extract from yellow root plant stem IC_{50} values of 136.81 ppm were obtained. This IC_{50} value indicates the antioxidant strength of the yellow root plant stems including in the moderate category. Further research on antioxidant activity in the fraction of akar kuning stems needs to be done to determine the composition of the antioxidant compounds in each solvent.

Keywords: *Arcangelisia flava*, antioxidant, DPPH

INTRODUCTION

Akar kuning or kayu kuning (*Arcangelisia flava*) is a medicinal plant from Central Kalimantan which is known to have antifungal activity, especially from the stem which containing berberine[1,2,3]. Akar kuning grow a lot in forest areas in Kalimantan, including in Central Kalimantan. For the Dayak tribe, akar kuning is used to treat various diseases such as phlegm, scabies, ringworm, malaria, fever, and several bacterial infections[2,4]. Previous studies showed that the antimicrobial activity of the yellow root stem was predominantly influenced by the presence of berberine in extracts and polar fractions[2].

Figure 1. Stem of akar kuning[1]

In other regions in Indonesia such as in Sumatra, akar kuning are also used to treat jaundice and diarrhea. Currently, the use of akar kuning in new communities is limited to the use of a decoction of water to drink[1]. Besides containing berberine, akar kuning is also known to provide various other secondary metabolites such as saponins, flavonoids, polyphenols, glycosides, and other alkaloids. One of the similarities in the features of some of these metabolites is that almost all have the potential to be used as antioxidant compounds[5].

Antioxidant compounds from natural ingredients consist of various groups of compounds such as tocopherol, lecithin, phosphatide, sesamol, gossypol, carotene, tannic acid, gallic acid, ferulic acid, and quercetin. While for synthetic antioxidants are antioxidants obtained from the synthesis of chemical compounds, such as butyl hydroxy anisole (BHA), butyl hydroxy toluene (BHT), terbutyl hydroxyquinone (TBHQ), diphenilpicrylhydrazil (DPPH), and dinitrophenylhydrazine (DNPH)[6,7]. These antioxidant compounds are needed by the body to deal with oxidative stress, which can trigger various diseases such as cancer, diabetes mellitus, and atherosclerosis[8].

Various antioxidant compounds can be found in the form of secondary metabolites of medicinal plants, where plants are known as the most significant source for the discovery of new antioxidant compounds[9]. These secondary metabolites often have various uses, such as being used as traditional medicine to treat multiple diseases in humans including diseases caused by oxidation processes at the cellular level. The cellular oxidation process is closely related to various degenerative diseases, including those related to the aging[5].

Determination of antioxidants contained in a plant sample can be done through several methods. One standard test used to analyze the content, and antioxidant concentration is to use a technique based on the reduction of DPPH reagent. DPPH is a free radical that is stable at room temperature, which produces a purple solution in methanol[10].

METHOD

Collection of akar kuning stems

Akar kuning stems are collected and separated from the dirt that may stick and other impurities.The portion of the stem that has been collected is then transferred into a dry and clean storage container so that it is not overgrown with mold.

Simplicia Preparation

Akar kuning stems are washed using clean running water, then reduced in size using appropriate equipment such as a knife to form a sharp horn. The haxel is then dried under the morning sun until it dries. The dried haxel is smoothed using a blender until it is smooth enough but not until it becomes a fine powder.

Simplicia Extraction

The 5 kg simplicia extracted using the solvent used was ethanol 96% pro analysis which was added to soak all the simplicia. The maceration vessel is then closed and left to stand for 24 hours with stirring occasionally. The replacement of the extraction solvent is done three times using the same simplicia until the extract obtained is clear. The maceration solvents that have been collected are then put together and filtered and then evaporated using a rotary evaporator at 40°C until a thick extract from simplicia is obtained. The concentrated extract was then weighed to determine the yield of the extract.

Antioxidant Test with DPPH Method

Control solution using DPPH solution concentration of 0.4 mM by dissolving 4 mg of DPPH powder in methanol p.a. on a 25 mL volumetric flask.Absorbance is read at the maximum theoretical wavelength. The absorbance of the solution is called absorbance control.

The sample solution was made by weighing the extract and dissolved with methanol p.a. and variations in concentration are made.Measurement of sample absorbance: 1 mL of 0.4 mM DPPH solution was put into a 5 mL volumetric flask, then added with a sample solution of 4 mL. The solution is allowed to stand for 30 minutes then read the absorbance at the maximum wavelength.

% Inhibition and IC_{50} calculation: Made the standard curve equation y = bx + a with the x-axis in the form of the concentration of the sample solution and the y-axis in the way of% inhibition of the sample. Furthermore, in the standard curve equation, the variable y is substituted with 50 so that the value of variable x is obtained which is a concentration that can capture 50% of free radicals for 10 minutes. The % inhibition is calculated by the formula:

$$\%\ inhibition\ = \frac{(A-B)}{A} X\ 100\%$$

A = Control (DPPH) absorbance

B = Sample or reference absorbance

The IC_{50} value is calculated by the formula:

$$IC50\ = \frac{(50-a)}{b}$$

a = *slope*

b = *intercept*

Measurement of antioxidant activity was carried out by the DPPH method. DPPH acts as a free radical that can bind to the H atom from the sample extract so that it becomes pikrihidrazine, and causes DPPH purple discoloration to be yellow. The antioxidant activity of the sample through the DPPH method can be determined through% inhibition[11]. The results of % inhibition of extract samples are shown in table 1:

RESULTS AND DISCUSSION

Table1. Percentage of sample inhibition

Sample concentration (ppm)	Absorbance (Å)				%Inhibition
	A	B	A-B	(A-B)/A	
25	0.76	0.655	0.105	0.138	13.816
50	0.76	0.545	0.215	0.283	28.289
75	0.76	0.506	0.254	0.334	33.421
100	0.76	0.481	0.279	0.367	36.711

Description: A = Absorbance Control, B = Sample Absorbance

Table 1 shows the results of measurements of absorbance and% inhibition of akar kuning stem extract. Based on Table 1, it can be seen that from the results of the measurement of antioxidant activity in akar kuning stem extract, it shows that along with the increase in extract concentration, the absorbance value produced decreases. The greater the concentration of the solution, the more antioxidant compounds that become hydrogen or electron donors on DPPH radicals, so that the color of the test solution changes which causes the absorbance produced to be smaller. There is a proportional relationship between the increase in extract concentration and the% inhibition value. The greater the concentration of the solution, the higher the % inhibition value[12].

Based on the data in Table 1, a sample concentration curve can be made against the% inhibition as shown in Figure 2.IC_{50} values can be determined through the values of a and b from the linear equation of the concentration curve and% inhibition.The linear regression equation of the concentration and% inhibition curves in Figure 2, obtained y = 7.3816x + 9.6053 and the R value is 0.9423. The value of a

= 9.6053 and b = 0.29526 is obtained from this equation and from the calculation obtained IC_{50} value 136.81 ppm. This means that the concentration of extract needed to inhibit 50% of free radicals is 136.81 ppm. As a comparison, ascorbic acid which is the most common antioxidant compound with IC_{50} of 19.32 ppm (Muharni *et al*., 2013), while in other studies with IC50 values 24.63 ppm[13].

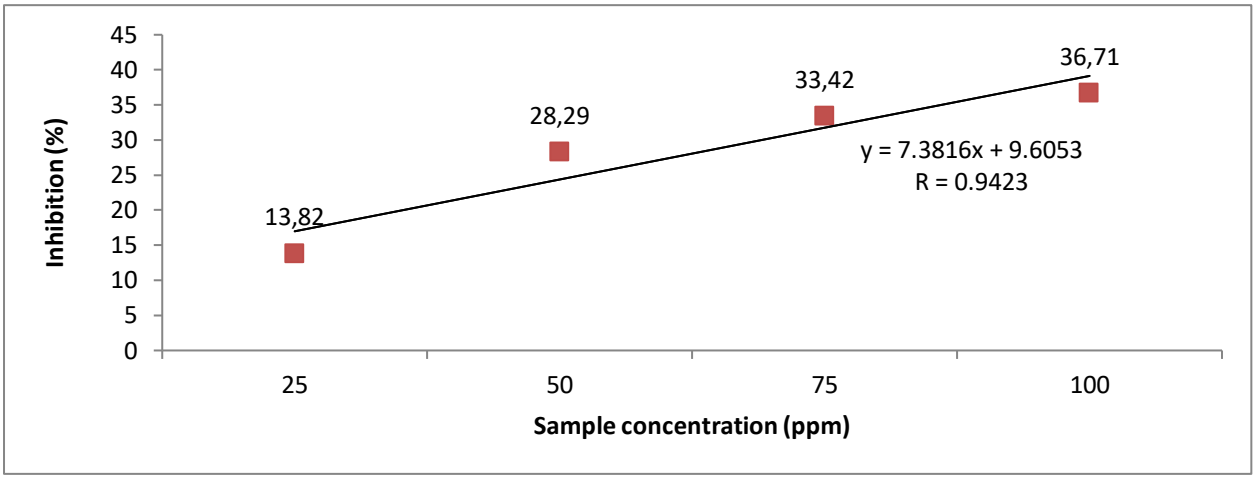

Figure 2. Sample concentration curve with% inhibition

IC_{50} value is defined as the amount of concentration of test compounds that can reduce/inhibit free radicals by as much as 50%. The smaller the IC_{50} value the higher the free radical reduction activity. A substance has antioxidant properties if the IC_{50} value is less than 200 ppm. If the IC_{50} value obtained ranges from 200-1000 ppm, then the substance is less active but still has the potential as an antioxidant (Molyneux, 2004). A compound is said to be an antioxidant is very strong if the IC_{50} value is less than 50 ppm, strong (50-100), moderate (100-150), and weak (151-200). The smaller the IC_{50} value the higher the antioxidant activity[10].

CONCLUSION

Based on the test of antioxidant activity on ethanol extract from akar kuning plant stem IC_{50} value of 136.81 ppm was obtained. This IC_{50} value indicates the antioxidant strength of the akar kuning plant stems including moderate. Further research on antioxidant activity in the fraction of akar kuning plant stems needs to be done to determine the composition of the antioxidant compounds in each solvent.

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