Antioxidant Activity of Pandanus Tectorius Leaves Extract and Its Fractions by WST-1 Method

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ABSTRACT

Aim: The purpose of this research was to determine the superoxide dismutase (SOD)-like activity of ethanolic extracts of *Pandanus tectorius* and its fractions of *n*-hexane, ethyl acetate, and water fraction. In addition, the research also aims to determine the secondary metabolites found in the fraction who submitted the best activity. Vitamin C is used as a positive control.

Method: Antioxidant activity was determined using the WST-1 method.

Results: The ethyl acetate fraction was showed the best SOD-like activity with IC_{50} value was 573.79 µg/mL, followed by ethanolic extract (IC_{50} = 656.60 µg/mL), water fraction (IC_{50} = 846.79 µg/mL) and *n*-hexane fraction (IC_{50} = 860.00 µg/mL) while IC_{50} value of vitamin C was 72.37 µg/mL. Secondary metabolites in the ethyl acetate fraction were flavonoid, steroid, monoterpenoid and sesquiterpenoid. Flavonoid are one of the compound based on literature known as antioxidant active compounds.

Conclusion: Ethyl acetate fraction was predicted contain flavonoids based on flavonoids investigation by UV-visible spectrophotometry,

Keywords: *Pandanus tectorius*, SOD like activity, flavonoids, WST-1 method

INTRODUCTION

Nowadays, in a modern era, the environment has undergone many changes, filled with many pollutants. These pollutants can cause the formation of a free radical [1]. Free radicals are atoms or molecules containing one or more unpaired electrons in the outer orbitals [2]. Free radicals will react with the surrounding molecules to obtain an electron pair so that it becomes stable, but the body's molecules will also turn into free radicals. This reaction will take place continuously in the body and if it is not stopped it will cause cell damage and various diseases such as cancer, heart disease, cataracts, premature aging, and other degenerative diseases. This reaction must be avoid, the body needs an important substance, namely antioxidants that are able to give electrons to free radical molecules so that the radical compounds become stable [3-4].

The compounds that have antioxidant activity are predicted to be present in plants consumed by primates. Primates are animals that have a physiological closeness with humans. Generally, human diseases are similar to diseases in primates [5]. However, primates can maintain their survival only by consuming plants in their habitat. This indicates the presence of compounds that are beneficial for these primates. One other plant that is often consumed by primates is *Pandanus tectorius*. The IC_{50} value of antioxidant activity of *Pandanus*

tectorius leaves using DPPH radical inhibition method was 36 µg/mL [6]. There are other ways to measure antioxidant activity *in vitro*, namely the superoxide dismutase (SOD) method. The principle of testing with the SOD method is that the presence of superoxide radicals will reduce WST-1 to formazan WST-1 complex. The presence of SOD activity in the sample can inhibit the reduction of the WST-1. This method has several advantages including simple, accurate, and has a high sensitivity to superoxide radicals.

MATERIALS AND METHOD

Plant material

Plant material used in this research was the crude drug of *Pandanus tectorius* leaves. The specimens collected at Pangandaran area, West Java, and determined at Department of Biology, Faculty of Mathematics and Natural Science, Universitas Padjadjaran.

Chemical materials

Ethanol, distillated water, n-hexane and ethyl acetate, chloroform, diethyl eter, magnesium powder, isoamyl alcohol, hydrochloride acid, ammonium hydroxide (Wako), DMSO (Merck®), vitamin C (Merck®), reagent kit for SOD (Dojindo Molecular Technologies®) with production code S311-10, and other chemical which are common used in phytochemical screening.

Instruments

Macerator, rotary evaporator (IKA HB® 10 Basic), water bath, separating funnel, centrifugator (SIGMA® Sartorius), micropipette 20 µL (Finnipepette®), micropipette 10-100, 100-1000 µL (Socorex®), multichannel pipette (Transferpette®-8), microplate 96-well (Sarstedt), microplate reader (Dynex Technology), monitor dan software (Revelation), Eppendorf tube, UV 254 dan 366 nm (CamagUV-Betrachter), spectrophotometer UV-Visible (Analytic Jena, Specord 200).

Extraction

A total of 540 g dried leaves of *Pandanus tectorius* was extracted by maceration method using ethanol as solvent, for 3x 24 h, then evaporated under reduced pressure until observed 37.88 g of concentrated extract.

Fractionation

A total of 29.97 g of concentrated ethanol extract was fractionated by liquid liquid extraction using water and *n*-hexane solvents. Furthermore, fractionation is carried out on the water fraction using ethyl acetate solvent. The ethyl acetate fraction obtained was 1.84 g with While the *n*-hexane fraction was obtained 3.23 g, and the water fraction obtained 16.98 g.

Phytochemical screening

Phytochemical screening was carried out to the crude drugs, extract, and all the fractions of the extract by modified Farnsworth method [7].

Antioxidant Activity Examination

Preparation of vitamin C standard solution: Vitamin C standard solution prepared in three concentration, i.e. 176.13, 88.065, and17.613 µg/mL.

Preparation of sample solution: A total of 0.1 g of sample was weight and dissolved in 1000 µL of mixtures of DMSO and aquadest. The stock solution concentration was 100.000 µg/mL.

Preparation of test solution: WST working solution, enzyme working solution, and sample solution were prepared with concentration of 10, 100 and 1000 µg/mL, respectively
Antioxidat activity measurement: Into the microplate, the test solution was inserted in according to the number and place specified (Table 1).

Table 1. Placement of Solution into Well

	sample	blank 1	blank 2	blank 3
Sample Solution	20 µl	-	20 µl	-
ddH_2O	-	20 µl	-	20 µl
WST Working Solution	200 µl	200 µl	200 µl	200 µl
Dilution Buffer	-	-	20 µl	20 µl
Enzyme Working Solution	20 µl	20 µl	-	-

Note: blank 1 was colored solution without inhibitor, blank 2 was blank of sample, and blank 3 was blank of reagent

After placement samples and blanks into wells, then microplate was incubated at 37^0C for 20 min. Absorbance were read by microplate reader and the activity of SOD was calculated [8].

$$\text{SOD activity (inhibition rate \%)} = \left\{\frac{[(A_{B1}-A_{B3})-(A_{Sample}-A_{Blank})]}{(A_{B1}-A_{B3})}\right\} \text{ x } 100\ \%$$

Antioxidant activity measurement was done by measuring SOD-like activity. The strength of SOD-like activity in the sample can be seen based on the percentage of inhibition value, whereas to see which fraction gives the best activity, the parameter used was IC_{50}.

RESULS AND DISCUSSION

Table 2. Antioxidant Activity

Sample	Concentration (µg/mL)	% Inhibition	Linear Regresion Equation	IC_{50} (µg/mL)
Vitamin C	17.61 88.07 176.13	31.75 57.04 81.48	y = 0.312x + 8 27.42 r^2 = 0.994	72.37
Ethanolic Extract	10.00 100.00 1000.00	17.11 22,88 67,80	y = 0.050x + 17.17 r2 = 0.999	656.60
n-Hexane Fraction	10.00 100.00 1000.00	25.42 31.36 54.24	y = 0.027x + 26.78 r^2 = 0.987	860.00
Ethyl acetate Fraction	10.00 100.00 1000.00	0.00 21.92 82.22	y = 0.076x + 6.392 r^2 = 0.968	573.79
Water Fraction	10.00 100.00 1000.00	0.00 0.00 60.27	y = 0.063x – 3.348 r^2 = 0.993	846.79

Along with increasing concentration, higher the percentage of inhibition (Table 2). The concentration was directly proportional to the activity, and the smallest IC_{50} was showed by ethyl acetate fraction, followed by ethanolic extract, water fraction, and the last was the *n*-hexane fraction. the ethyl acetate fraction showed the best SOD like activity. The best

antioxidant activity of ethyl acetate fraction means that in this fraction there are antioxidant active compounds. The phytochemical screening results showed that ethyl acetate fraction contained flavonoids, monoterpenoids, sesquiterpenoids, steroids, and triterpenoids. Flavonoids are one of the compounds based on literature known as antioxidant active compounds [9].

CONCLUSION

Ethanolic extract of *Pandanus tectorius* leaves an its fractions had antioxidant activity of SOD-like activity. The ethyl acetate fraction had the best activity (IC_{50}: 573.79 µg / mL) followed by ethanol extract (IC_{50}: 656.6 µg / mL), water fraction (IC_{50}: 846.79 µg / mL) and n- hexane fractions. (IC_{50}: 860.00 µg / mL). The ethyl acetate fraction was predicted contain flavonoids.

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