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Research Article

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Comparative Study of Antioxidant Activity of Red and Green Varieties of Grape Seed (*Vitis Vinifera L.)* using DPPH Reduction Method

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ABSTRACT

Grape seed is one of proanthocyanidins sources where antioxidant activity of proanthocyanidins is 20 times greater than vitamin E and 50 times greater than vitamin C. Based on the findings, the aim of this research is to recognize the comparison of the most effective antioxidant activity of grape seed (Vitis vinifera L.) of red and green varieties with DPPH method. The principle in this research ranged from extraction by ethyl acetate followed by the process of determination of antioxidant activity assay with DPPH method in UV-Vis spectrophotometry. The results showed that antioxidant activity of green grape seed varieties is higher than red varieties with IC50 33.937 µg/mL and red varieties 60.947 µg/mL respectively.

Keywords: Antioxidants; Grape seed

INTRODUCTION

Traditional medicine is the one alternative choice to treat of desease especially with the consciousness for back to nature, even with the developments that there is a more attention for alternative health care. From a variety of research, traditional medicine has indeed been recognized its existence by the people. The benefits of medicinal plants are to cope with cancer is a real breakthrough, given the current cancer treatment is very expensive. In Indonesia, the cancer disease is in the fifth rank. Along with the use of chemotherapy to overcome cancer, led to the development of herbal products is one of options [1].

Free radical is defined as a molecule, an atom or group of atoms that has someone or more electrons are not paired on successive orbital [2,3]. The atoms or molecules are unstable and have a high reactivity [2]. There are a wide variety of free radicals as a derivative of carbon (C) and nitrogen (N), but the most studied is the oxygen radicals [3]. To fight of free radicals, the body needs a compound known as antioxidants. Antioxidants are substances that serve to protect the body from free radical attack. These antioxidants to keep the body from various diseases by means of pressing the cell damage that occurs as a result of free radical oxidation processes. Antioxidants help stop cell destruction process by giving electrons to the free radicals [3-5].

DPPH method is used because the use of this method is quite fast, accurate, and not expensive to evaluate the antioxidant activity in food and beverages [6] as well as the most widely used in the screening of antioxidant activity in medicinal plants or extracts ingredients worlds [7].

Grape seed is one of the richest sources of proanthocyanidins. Where these antioxidant power is 20 times greater than vitamin E and 50 times greater than vitamin C [5,8]. The darker the color of the skin of the grape and the higher content of flavonoid [9], as well as due to the differences in the levels of total fenolic in fruit of the vine (*Vitis vinifera* L.) varieties of red and green led distinction antioxidant activity [10].

Related to this, it is interesting to compare of the most effective antioxidant activities of grape seed (*Vitis vinifera* L.) varieties of red and green that serves as a drug ingredient.

MATERIALS AND METHODS

Sample preparation

Grape seed (*Vitis vinifera* L.) varieties of red and green that had is pollinated, then taken respectively by as much as 60 g and then for removal the fatty we use extraction with soxhletation method by using a solvent of n-heksan as many as 300 mL for 6 hours. Then extract obtained by n-heksan and residue. Then the residue already floured after removal the fatty as many as 60 g dried. The powder is then weighed as much as 50 g extracted and returned by the percolation method for 2 days with the addition of 300 mL of ethyl acetate. Then evaporated to a thick ethyl acetate extracts of grape seed extract with a heavy red 0,773 g and green grape seed 1,288 g. then further samples deposited by means of the addition of n-heksan the number is twice the initial volume is 600 mL to get procyanidin. Then stirred, and then formed the sediment [11]. Subsequent deposits taken and evaporated to a thick red grape seed extract with a weight of 0,555 g and green grape seed with a heavy 1.07 g. Then extract stored in a decicator.

Identification of Thin Layer Chromatography (TLC)

Cromatogram was sprayed with using some of the reagents the chemical components which are:

Test of alkaloids:

Spotted extract on TLC plates, then elution with eluen chloroform, methanol and water (9: 1: 1). After that, it was sprayed by using Dragendorff reactant, observed in visible light. After plate sprayed with Dragendroff reagents would show brown spots orange yellow background.

Test of flavonoids:

Aqueous extract was spotted on a TLC plate and elution with eluen with chloroform, methanol and water in comparison 9: 1: 1. Then observed spots on the UV light and sprayed with reactant $AlCl_3$ to identify of flavonoids. It contains aromatic conjugated system so that it will show a strong absorption band in the visible light and UV rays. On the analysis with TLC and the appearance of the reactant with $AlCl_3$, flavonoids would appear to be yellow in color and subject structure; flavonoids are flourecence is yellow, blue, or green under UV 366 nm [12].

Saponins test:

Aqueous extract of spotted on a TLC plate was eluted by eluen with chloroform, methanol and water (9: 1: 1). Then observed spots on the UV light and sprayed with vanilin reactant. Saponin glycosides, if it was detected with vanillin spray reagents would give of blue color to blue-violet is sometimes in the form of patches of red, yellow, dark blue, purple, green or brown on yellow rays of visible [13].

Tannins test:

Aqueous extract was spotted on a TLC plate, then eluted by eluen chloroform, methanol and water (9: 1: 1). Observed spots on the UV light and sprayed with $FeCl_3$ reactant. Tannins present in the extract if the colour is green, red, purple, blue or black and strong [12].

Determination of free radical by using DPPH (2,2- diphenyl-1-picryl hidrazyl)

Creation of a solution:

DPPH solution: DPPH solution of 50 ppm was made by means of weighing the DPPH by as much as 5,0 mg dissolved in 100 mL of absolute methanol in the pumpkin tentukur.

Aqueous samples: Stock solution of 500 ppm was made by weighing each grape seed extract (*Vitis vinifera L.*) varieties of red and green as much as 5 mg and reconstituted with absolutemethanol while stirring and homogenized then add in volume up to 10 mL.

Preparation of standard solution of quercetin

Stock solution of 100 ppm was made by weigh 1.0 mg of quercetin, and then reconstituted with absolute methanol while stirring and homogenizing, and then add in volumetric flask up to 10 mL.

Preliminary test

Determination of the maximum wavelength of DPPH. Determination of the maximum wavelength of DPPH have been done by measuring the maximum wavelength at 516 nm.

Measurement of antioxidant activity

The measurement of antioxidants power of blanko. Testing was done by means of pipette 2 mL of absolute methanol and DPPH. Incubated about 1 hour in order to obtain a perfect reaction of DPPH and samples. Then measured its absorbance.

Measurement of grape seed extract on antioxidant power (*Vitis vinifera* L.) Red and green varieties.

Testing antioxidant power has done by taken about 2,0 mL of the sample solution from a wide range of concentrations. Then each of solution was added 2,0 mL DPPH. Then it was mixed by vortex and incubated for 1 hour at a temperature 37^0C. Then measured its absorbance.

Measurement of antioxidant power of standards

Testing has been done by means of obtained 2 mL of solution concentration of quercetin. Then each of solution was added 2 mL DPPH. Mixed and incubated by vortex for 1 hour in a dark room.Then measured its absorbance. IC_{50} value was calculated by the following formula:

$$\% \text{ Inhibition} = \frac{(\text{Abs.Blank} - \text{abs.Sample})}{absorbance\ of\ the\ sample} \times 100$$

IC_{50} value is calculated using regression equations for inhibiting percentage [14].

RESULTS AND DISCUSSION

Antioxidants are substances that serve to protect the body from free radical attack. These antioxidants are to keep the body from various diseases by means of pressing the cell damage that occured as a result of free radical oxidation processes. The source of the most major antioxidants found in these fruits. So it can be inferred that the fruits are a good source of nutrients to treat and protect the skin from the aging process.

This research used grape seed (*Vitis vinifera* L.) varieties of red and green. Samples of seeds were collected, then dried to make compounds contained in it are not damaged. Samples been pollinated in order to facilitate her compounds contained in it by liquid through the percolationmethod. Grape seed (*Vitis vinifera* L.) varieties of red and green were extracted with the perkolasi method. Percolation method was chosen because it could pull the chemical components contained in the sample without destroying its compounds. In this study used the solvent ethyl acetate. Extraction is expected to separate bioactive components in the sample is dissolved in a solvent which is used in accordance with the nature of the solvent.

Test of phytochemicals was taken on grape seed extract (*Vitis vinifera* L.) Grape seed extract (*Vitis vinifera* L.) of red and green varieties were tested by TLC colorimetric method. The test aims to find out the phytochemical components in the extract. The extracts were identified of alkaloids, flavonoids, saponins and tannins for both red and green varieties.

Based on the results of the research, grape seed extract (*Vitis viniferal*.) of red and green varieties contain chemical compounds, saponins and tannins. Antioxidant activity of the quantitative assay by DPPH method was succesfull. It was used because this method is quite fast, accurate, andnot expensive to evaluate the antioxidant activity in food and beverages [6] as well as the most widely used in the screening of antioxidant activity in medicinal plants or extracts ingredients worlds [7]. The solvent was used ethyl acetate, because the ethyl acetate extracts contain lots of antioxidant.

The results showed the extract (*Vitis vinifera* l.) varieties of Green has a stronger antioxidant activity than red with the IC_{50} was about 33.973 g/mL whereas the red variety has less antioxidant activity than green which were the IC_{50} about 60.947 g/mL. As for quercetin which was used as a standard in this study had a strong antioxidant activity with IC_{50} values about (2.59 g/mL). It is less than standard value about 10g/mL for strong ranges.

CONCLUSIONS

Based on the research, it can be concluded that the antioxidant activity of the ethyl acetate extracts of grape seed (*Vitis vinifera* L.) varieties of Green is stronger than red varieties. For the ethyl acetate extracts of grape seed (*Vitis vinifera* L.) varieties of Green has a strong antioxidant activity i.e. IC_{50} value of 33.937: g/mL ethyl acetate extracts while the grape seed (*Vitis vinifera* L.) varieties of IC_{50} value of Red 60.947: g/mL.

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