Total Phenol, Flavonoid Levels and IC_{50} in Local Grape (*Vitis vinifera L*) Skin Waste Wine

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Abstract. Grape skin is a waste produced from *wine* making derived from grapes. Currently the use of grape skins is still limited as a natural fertilizer. This research is study to determine the total levels of phenols, flavonoids both as antioxidants and IC_{50} in the grape skins from the waste of *wine* making. The study was determination quantitative of total phenolics with the *folin-ciocalteu* method expressed as mg of *gallic acid equivalent* (GAE), total flavonoids content by $AlCl_3$ method expressed as *Quercetin equivalen* (QE) and antioxidant activity can be determined by using the DPPH test. The results stated that the total phenol, flavonoid and IC_{50} levels of grape skins were 168.55 (mg / 100gr) ; 61.10 (mg / 100 gr) and 1,065.19 ppm. The presence of total phenols, flavonoids and IC_{50} in grape skins is relatively high, so the use of grape skins from the process of making wine makes attention.

Keywords: Grape skin, Wine, Total phenol, Flavonoid, Antioxidant, IC_{50}

1 Introduction

Grape (*Vitis vinifera L*) is one of the fruits containing large amounts of phytochemicals or antioxidants that are beneficial to health[1]. Antioxidants in grapes (*Vitis vinifera L*) are flavonoids and non flavonoids. Flavonoids consist of quercetin, prosiadine, catechins, resveratrol and anthocyanins. Subsequent non flavonoids such as fiber.

Grapes (*Vitis vinifera L*) have 60-70% polyphenols[2]. Almost all polyphenols from nature are pigments usually yellow, purple or red, which are capable of absorbing ultraviolet (UV) radiation. Radiation that can be absorbed by polyphenols covers the entire wavelength of the UV spectrum and part of the UV A and UV C spectrum. Topical use can have a photoprotective effect on the skin[3].

Fruit of the (*Vitis vinifera L*) plant is a part that is often used either for direct consumption, or as one of the raw materials for food or beverage products, such as raisins, wine, fruit juice and others. The contents of grapes (*Vitis vinifera L*) are carbohydrates, proteins, minerals, vitamins, food fiber, and phytochemicals. Flavonoids, saponins and polyphenols are phytochemical compounds. Flavonoids are antioxidants that work as a cancer prevention and also have antimicrobial effects[4]. Polyphenols are also antioxidants, in grapes (*Vitis vinifera L*)known as resveratrol which inhibits enzymes that can stimulate the growth of cancer cells and suppress the immune response.

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Bali is one of the grape(*Vitis vinifera L*) producing regions known by the name of local grape (*Vitis vinifera L*). The taste of Balinese grape (*Vitis vinifera L*) is sweet and there is also a sour, but the Balinese grape has the content grape (*Vitis vinifera L*) has antioxidant content that is not inferior to the grape outside. The grape (*Vitis vinifera L*)producing area in Bali such as Buleleng is a region of north Bali.

Utilization of grapes (*Vitis vinifera L*) not only as fresh fruit, eat fruits that are directly consumed, but other uses of grapes are used in the production of refreshing drinks, including *wine*. *Wine* is an alcoholic beverage made from grape (*Vitis vinifera L*) juice, through the fermentation process of sugar that is available using yeast. There are several types of *wine*, namely Red *Wine*, White *Wine*, Rose *Wine*, Sparkling *Wine*, Sweet *Wine*, and Fortified *Wine*. The difference from this *wine* is the manufacturing process. White *wine* is *wine* made using the basic ingredients of green grapes (*Vitis vinifera L*) or black grapes (*Vitis vinifera L*) whose skin is peeled or separated from the juice, in contrast to red *wine*, where the skin on grape seeds is not removed and blended to become juice.

The process of making white *wine* in the home industry scale is different from one person to another. This process begins with the process of destroying or crushing the grapes (*Vitis vinifera L*) by adding water and proceed by adding sugar with a certain ratio. After the sugar is mixed with the *wine* solution, the process is continued by heating the mixture until it boils and proceed with the filtering process. The result will be produced pulp in the form of grape (*Vitis vinifera L*) skins and grape (*Vitis vinifera L*) seeds. In the other white wine industry, there is also waste in the form of pulp separated from the grape (*Vitis vinifera L*) juice before the *wine* mixture is added with sugar. Balinese grape (*Vitis vinifera L*), the fruit is not only used for fresh fruit but also used to make *wine*.

Grape (*Vitis vinifera L*) skins are currently untapped, so hoarding occurs around the home industry of making white *wine*. This situation has an impact on environmental pollution that occurs in the form of air pollution, where the odor caused by the accumulation of grape (*Vitis vinifera L*) skin that is not utilized, because it considers the skin is not useful.

Reactive oxygen species (ROS) and free radicals can cause damage to normal body cells. Further damage will occur to DNA, protein and other macromolecules. This damage is the beginning of various diseases, especially heart disease and cancer. There are many studies that prove that because this disease is mediated by oxidative stress and disrupts the balance between prooxidants and antioxidant factors, so antioxidants can play an important role in preventing or slowing the development of this condition[5]. One way to overcome these health problems is to consume foods that contain high antioxidants.

Grape (*Vitis vinifera L*) skin is a material that contains high antioxidants. Antioxidant properties in grape (*Vitis vinifera L*) skins are caused by various components present in grape (*Vitis vinifera L*) skins, including flavonoid components, phenolics, vitamin C, amino acids and catalase enzymes. Seeing the antioxidant potential of grape (*Vitis vinifera L*) skin which is a waste of *wine* making, the antioxidant content in the form of total phenol and its flavonoid levels were investigated. The further impact of the use of antioxidants in the skin of waste grapes (*Vitis vinifera L*) is seen antioxidant activity that is known from the IC_{50} value.

2 Method

This study is an experimental study to determine the total content of phenols, flavonoid levels and IC50 of grape (Vitis vinifera L) skin from wine waste.

2.1 Materials and Tools

The main ingredients used in this study are 250 mesh grape (*Vitis vinifera L*) skin Balinese powder, quercetin, DPPH (*2,2-diphenyl-1-picrylhydrazyl*), gallic acid, ethanol, glacial acetic acid 5%, $AlCl_3$ 2%, HCl 25%, hexamethylenetetramin (HMT) 0.5%, acetone, ethyl acetate, *Folin-Ciocalteu* reagent, and Na_2CO_3.

The main tools used are Scale AND GR-200 series analytical balance, HITACHI UV / VIS Spectrophotometer U2800 BRUKER, and EPOCH Microplate Spectrophotometer. Other tools are glasses; upright cooler; Thermo Scientific micropipets 10 µl, 100 µl, and 1000 µl; the Falcon microplate, and the BRANSON B1510 sonicator.

2.2 Research Procedure

Sample and preparation of grape (Vitis vinifera L) skin extract. Grape (Vitis vinifera L) skin is dried by aerating it for 2 days. Furthermore, mashed by blending to form a powder with a size of 250 mesh. Grape (Vitis vinifera L) skin powder extracted by maceration with ethanol 96% (1:10). Maceration was carried out for 72 hours, and filtered to obtain the filtrate. The filtrate was concentrated by evaporator at 50°C and viscous extract obtained[6].

Total phenols analysis. A total of 10 mg extract was dissolved in 25 mL 96% ethanol. Two ml of the solution is put into a test tube then added 5 ml of aquabidest, and 0.5 ml of Folin-Ciocalteau reagent 50% then shaken with vortex. The mixture was incubated at room temperature for 5 minutes then added 1 ml of 5% Na2CO3, stirred, then incubated at room temperature for 60 minutes. Absorbance of the solution was measured using a spectrophotometer at a wavelength of 725 nm. The standard used is gallic acid with various concentrations (10, 30, 50, and 70 µg / ml)[7].

Flavonoid compounds. As much as 0.2 g of extract, 1 ml of heksamethtetraamin (HMT) 0.5%, 20 ml of acetone and 2 ml of HCl 25% was inserted into the Erlenmeyer flask 250 ml. The mixture is hydrolyzed by a flux at 100 °C for 30 minutes. Filtrate hydrolyzed results are filtered with a filter paper to a 100 ml notch flask and then added acetone until the impressions. A total of 20 ml of filtrate solution, 20 ml of water, 15 ml of ethyl acetate is inserted into the separating funnel. The liquid on the top layer is inserted into the 50 ml notch flask. The work is done 3 times with the same solvent to the separating funnel. A total of 10 ml of the notch flask was inserted into a 25 ml and added 1 ml AlCl3 2% and 5% glacial acetic acid to the impressions. The solution was incubated at room temperature for 30 minutes. Absorption of the solution is measured using a spectrophotometer at a wavelength of 425 nm. The standard used is kuersetin with various concentrations (0.1; 0.5; 1; 3; 5; and 10 µg/ml)[7].

Antioxidant activity analysis which includes the DPPH test or IC50 test. Grape (Vitis vinifera L) skin extract is dissolved in ethanol 96% with concentrations of 400, 200, 100, 50, and 25 µg/ml. A total of 100 µl of each concentration is inserted into well on the microplate, then added 100 µl DPPH 125 µM. A total of 200 µl of ethanol is inserted into the well as Blanko. Kuersetin is used as a positive control with 5 concentrations; 1.5; 1.25; 0.63 and 0.31 µg/ml. Subsequently, incubated at room temperature in a dark state for 30 minutes. Absorption is measured using a microplate reader at a wavelength of 517 nm. Antiradical activity was calculated by the DPPH method in which the sample was reacted with a DPPH solution. Antiradikal activity is shown in systems whose color changes from purple to yellow. The change

in color of the solution indicates DPPH free radical scavenging activity and can be measured by the difference in absorbance produced in the sample compared to the control[8].

3 Results and Discussion

The antioxidant of grape skin estract as phenol, flavonoid. Phenol compounds have a very important role in providing antioxidant benefits to fruits and vegetables. The content of phenol compounds is most commonly found in the skin, stem, leaf and seed of grapes. Flavonoid is a compound derived from phenol, also a secondary metabolite. In general, flavonoids are organic compounds consisting of 15 carbon atoms with two aromatic rings connected by three carbons that can form a third ring. The hydroxyl group found in flavonoids is a place to attach various sugars that can increase the solubility of flavonoids in water.

Flavonoids are synthesized by the same precursors (phenylalanine, which is an aromatic amino acid) through the biosynthetic pathway which is typical of cyclic acid only in plants.

Phenolic compounds are secondary metabolites, mainly located in the epidermal layer of grape (*Vitis vinifera L*) skin known as important bioactive compounds, as well as having strong antioxidant activity[9]. Determination of antioxidant content and bioactivity in grape (*Vitis vinifera L*) skins as a waste of wine making is important for public knowledge in order to provide information about possible health benefits and bioactivity. Many of these benefits are related to antioxidant compounds found in grapes (*Vitis vinifera L*).

Resveratrol is found in many parts of the skin and grape seeds. Fresh wine Skin has resveratrol content of 40 mg of perliter extract. Resveratrol is also widely found in wine processed products namely wine. Resveratrol found in grapes can increase blood flow in the brain, so as to reduce stroke disease, prevent cancer, inhibit benzopyrene compounds, which are compounds that can cause cancer, as well as inhibit Tumor cell growth.

In this study, total phenol, flavonoid and IC_{50} obtained from grape (*Vitis vinifera L*) skins were 168.55 (mg / 100 gr GAE); 61.10 (mg / 100 gr) and 1,065.19 ppm. This analysis aims to determine the total levels of phenols and total flavonoids in the extract obtained from the standard curve equation. Phenols obtained are greater than the total levels of flavonoids because flavonoids are a class of phenol compounds.

The difference in antioxidant activity obtained is influenced by the levels of total phenols and total flavonoids. Phenol and flavonoid compounds have a linear contribution to antioxidant activity, so the higher the level, the better the antioxidants.[10] High total phenolic levels in local grape (*Vitis vinifera L*) skin extracts are thought to have an important role as antioxidants. Besides flavonoids, other phenolic components such as tannins are known to have antioxidant activity. In addition, other secondary metabolites in the bark as well as alkaloids and terpenoids also contribute as an antioxidant. The phenolic components (flavonoids and tannins), alkaloids, terpenoids, and organic sulfur components act as natural antioxidants[10,11].

Antioxidant activity is not always correlated with phenol or flavonoid levels. This can be due to several factors such as differences in active components in plants, synergistic effects or antagonistic effects between active components contained, research conditions, and methods used can affect antioxidant activity in plants[12,13].

The DPPH method was chosen in testing this antioxidant activity because it has an easy and fast procedure for evaluating the activity of radical capture of nonenzymatic antioxidants. The DPPH radical is a stable radical and has a maximum absorption at a wavelength of 517 nm. The testing principle is the transfer of electrons and the transfer of hydrogen atoms between

antioxidants and DPPH radicals, so that DPPH *(Diphenyl Pikril Hidrazil)* will be reduced to DPPH-H *(diphenyl picril hydrazine)* and the color changes from purple to yellow[14,15].

The content of phenols in grape (*Vitis vinifera L*) skins (168.55 ± 5.07 mg / 100 gr GAE) in this study is high, while the flavonoid content (61.10 ± 2.05 mg / 100 g) is classified as moderate when compared with some other studies with the test sample part of grape skin[16].

4 Conclusion and Suggestion

4.1 Conclusion

Grape (*Vitis vinifera L*) skin has phenol levels of 168.55 (mg / 100 gr GAE), while the flavonoid levels are 61.10 (mg / 100 gr). IC_{50} value of grape (*Vitis vinifera L*) skin with DPPH method is 1,065.19 ppm.

4.2 Suggestion

This research can be continued with the analysis of phytochemical compounds and total tannin content and identification of compounds in grape skin extracts so that the wine compounds are known to play a role as an antioxidant

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