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Antioxidant Activity Test for Button Mushroom *(Agaricus bisporus)* Ethanol Extract Using the FRAP Method (Ferric Reducing Antioxidant Power)

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Abstract

Button mushrooms (Agaricus bisporus) are plants that contain phenols as antioxidants. Antioxidants are electron donor or reductant compounds. These compounds have a small molecular weight but are able to inhibit the development of oxidation reactions by preventing the formation of radicals. phenol is the main antioxidant component found in Agaricus bisporus extracts. The aim of this research is to determine the antioxidant activity value of button mushroom ethanol extract (Agaricus bisporus) using the method of ferric reducing antioxidant power (FRAP). This research uses the maceration method as an extraction process using 96% ethanol. The resulting filtrate was concentrated with a vacuum rotary evaporator to produce a thick extract. Antioxidant testing was carried out using the FRAP method with a reagent solution of potassium feryricide, phosphate buffer pH 6.6 tricloroacetic acid (TCA), aquadest, and iron 3 chloride ($FeCl_3$). Samples were analyzed using a UV-Vis spectrophotometer at a wavelength of 725 nm using quercetin as a standard solution. The results showed that the antioxidant activity value of the button mushroom ethanol extract (Agaricus bisporus) with the FRAP method was 3.847 mg QE /g extract.

I. Introduction

Indonesia is an agricultural country that has very diverse agricultural commodities. One of them is in horticultural commodities, which are currently in great demand and are starting to be popular. Mushrooms have growing conditions with temperatures of 16-22ºC and 80-90% humidity. West Java is a mushroom center still in highland areas such as Lembang, Cisarua, Pangalengan, and Cipanas. This area is a very ideal area for mushroom plants. While in other areas, besides the ideal area, there is still land that can and has the potential to become a place of cultivation but is hampered by environmental factors. To be able to grow well requires an environment that is suitable for growth and development. The environmental factors that are very influential around plants are relative humidity, humidity of the growing medium, wind speed, temperature of the growing medium, and nutrients (Muchroji, 2004).

From a health perspective, the nutritional content of mushrooms and their function as medicine have been recognized by China since 2000. Mushrooms are part of traditional Chinese medicine; this is also done in Japan and Indonesia in particular. According to in history, mushrooms have also been used for medicinal purposes in Western countries, although not as much as in Asia (Sadler, 2003).

Fungi are organisms that do not have chlorophyll. Fungi are known as plant-like organisms, although their DNA is closely related to that of animals. Fungi cannot photosynthesize, they use organic components as a source of energy and carbon. Most fungi produce large numbers of spores, which are then dispersed by the wind. Fungi reproduce by spores that grow into fine threads called mycelium (mycelia) and then form fruiting bodies (Rahmawati, 2017).

White button mushrooms (*Agaricus bisporus*) are rich in protein, free amino acids, polyphenols, ergothionin polysaccharides, and vitamins. This mushroom also contains high levels of linoleic acid and the enzyme aromatase, which catalyzes sex hormones in humans. *Agaricus bisporus* has many functions, such as antioxidant, anti-bacterial, anti-inflammatory, anti-tumor, and body defense systems (Falguera *et al.*, 2011). Antioxidants are compounds that can delay, slow down, and prevent free radical oxidation reactions in lipid oxidation (Kochhar and Rossell, 1990). Antioxidants are electron-donating or reductant compounds. These compounds have a small molecular weight but are able to inactivate the

development of oxidation reactions by preventing the formation of radicals. Antioxidants are found in many foods derived from plants (Winarsih, 2007).

Free radicals are atoms or molecules that have one or more unpaired electrons in their outer orbitals. Molecules that include free radicals include superoxide anion (O^{2-}), hydroxyl (OH^-) and nitric oxide (NO). Free radicals are usually unstable and highly reactive because of their unpaired electrons, which tend to bind with electrons from other molecules. This, of course, will create new radicals for the molecules whose electrons are taken. Therefore, these free radical reactions tend to be similar to chain reactions and are highly reactive, causing chemical changes and damaging various cell components, such as proteins, fats, carbohydrates, and DNA (Betteridge, 2000).

Based on the description above, button mushroom plants (*Agaricus bisporus*) contain various chemical compounds and have various functions that are useful for human health, one of which is as an antioxidant. So that research was conducted on the analysis of the antioxidant activity of the ethanol extract of button mushroom (*Agaricus bisporus*) using the FRAP (Ferric Reducing Antioxidant Power) method. This research is expected to provide benefits to the community and can be used as an alternative herbal treatment.

II. Research Method

II.1 Preparation of Tools and Materials

Tools and materials are prepared in accordance with the needs of the research to be carried out.

II.2 Processing and Sampling

Button mushroom samples (*Agaricus bisporus*), obtained from Kalianget village, Wonosobo sub-district, Central Java district, then cleaned of dirt using running water and cut into small pieces, then dried by aerating without sunlight for ± 1 week. After drying, the sample was pulverized using a blender.

II.3 Preparation of button mushroom extract (*Agaricus bisporus*)

A total of 25 grams of button mushroom powder (*Agaricus bisporus*) were put into a maceration container, 96% ethanol solvent was added until the simplisia powder was submerged, left for 3-4 days, then filtered. After the first extraction process is complete, the pulp is macerated again with a new liquid. The results are collected and then evaporated using a Rotary Vacuum Evaporator tool until a thick ethanol extract is obtained.

II.4 Determination of antioxidant activity of quercetin standard solution by the FRAP method using UV-Vis spectrophotometer

From quercetin solution concentrations of 4, 6, 8, 10, 12, and 14 ppm, 1 mL of quercetin standard solution was pipetted to 1 mL of 0.2 M phosphate buffer (pH 6.6) and 1 mL of potassium ferricyanide [$K_3Fe(CN)_6$] 1%. The mixture was vortexed for approximately 5 minutes, then incubated at 50ºC for 20 minutes and added 1 mL of 10% TCA. Next, it was centrifuged at a speed of 3000 rpm for 10 minutes and pipetted the top layer of the solution as much as 1 mL, then mixed with 1 mL of distilled water and 0.5 mL of $FeCl_3$ 0.1%. The solution was allowed to stand for 5 minutes, and absorption measurements were taken at the maximum wavelength using a UV-Vis spectrophotometer (Chew *et al*., 2008).

II.5 Determination of antioxidant activity of ethanol extract of button mushroom (*Agaricus bisporus*) by FRAP method using UV-Vis spectrophotometer

Determination of antioxidant activity of ethanol extract of button mushroom (*Agaricus bisporus*) by FRAP method using UV-Vis spectrophotometer A total of 15 mg of ethanol extract of button mushroom (*Agaricus bisporus*) was weighed, put into a 5 mL volumetric flask,and diluted with 96% ethanol to the limit mark. So as to obtain a concentration solution of 3000 ppm. The solution was pipetted as much as 1 mL and then added 1 mL of phosphate buffer pH 6.6 and 1 mL of potassium ferricyanide [$K_3Fe(CN)_6$] 1%. The mixture was vortexed for 5 minutes, then incubated at 50 ºC for 20 minutes, then added with 1 mL of 10% TCA, then centrifuged at 3000 rpm for 10 minutes. The top layer of the solution was pipetted 1 mL into a test tube mixed with 1 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$, after which it was allowed to stand for 5 minutes. The absorbance was measured at the maximum wavelength. The FRAP value was expressed in mg quercetin equivalents/g extract (Chew *et al*., 2008).

III. Results and Discussion

This study aims to determine the value of antioxidant activity. The ethanol extract of button mushroom (*Agaricus bisporus*) using the FRAP (Ferric Reducing Antioxidant Power) method. The FRAP method was used because the method is cheap, fast, and the materials and reagents used are quite simple and do not use special tools to calculate total antioxidants. The FRAP method is the only method that directly measures antioxidants in materials, while other methods measure indirectly because what is measured is the free radical content (Mamonto, 2014).

The mechanism of the FRAP (Ferric Reducing Antioxidant Power) method is the ability of antioxidant compounds to reduce Fe^{+3} to Fe^{2+} so that the antioxidant power of a compound is analogous to the reducing ability of the compound (Halvorsen *et al.*, 2002). The reduced compound to

see the antioxidant activity is potassium ferric cyanide ($K_3Fe(CN)_6$). The activity of reducing ability is seen by measuring the absorbance of the sample. The amount of absorbance value shows the amount of reducing ability in the sample (Maryam, 2015).

This research begins with extraction with the aim of attracting secondary metabolites present in the sample. The extraction method used in this research is maceration. The selection of the maceration method. because it is the simplest method that is most widely used, as well as to prevent damage to the chemical content contained in samples that are not resistant to heat. Besides that, the maceration process is easy to do, and the tools used are easily obtained. In the maceration process on button mushrooms (*Agaricus bisporus*), the distiller used is 96% ethanol because ethanol is a solvent that can attract flavonoids optimally. This is because flavonoids are generally more soluble in water or polar solvents such as ethanol. The sample was remacerated again with 96% ethanol for 1 x 24 hours with the aim that if there were still secondary metabolites that had not been extracted in the first maceration so that they could be attracted to the second maceration, then separated between the solvent and powder by filtering, and the results of the first maceration were combined with the results of remaceration after which a vacuum rotary evaporator (Rotavapor) was used to obtain a thick extract.

The yield obtained from ethanol extracts of button mushrooms (*Agaricus bisporus*) can be seen in Table 1.

Table 1. Percent yield of 96% ethanol extract of button mushroom (*Agaricus bisporus*)

Jenis Pelarut	Amount Of Sample (mL)	Sample weight (g)	Extract yield (g)	Extract yield(%)
Etanol 96 %	550	25.644	4.127	16.093%

This study used quercetin as a comparator. Quercetin is a flavonoid that exhibits several biological activities. These activities are associated with the antioxidant properties of quercetin, among others, because it is able to capture free radicals (Morikawa *et al.* 2003). The mechanism of quercetin as a secondary antioxidant is to cutting the chain oxidation reaction of free radicals or by capture them (Winarsih, 2007). The method used in this study is the FRAP method, which uses several reagents, namely 0.2 M phosphate dapar (pH 6.6) which aims to maintain the pH of the solution, and the addition of potassium ferricyanide ($K_3Fe(CN)_6$) as an oxidizer that will react with samples that are reductant so that it will reduce Fe^{3+} ions in the solution to Fe^{2+}. Divortex for approximately 5 minutes with the aim that the sample with the added reagent can mix perfectly, then incubate at a temperature of 50ºC for 20 minutes so that the solution can be mixed perfectly. 50ºC for 20 minutes so that the solution that has been homogenized can react perfectly. The addition of TCA (trichloroacetic acid) is done so that the potassium ferricide complex formed can settle, then centrifuge at 3000 rpm for 10 minutes to accelerate the separation process and the formation of supernatant. The addition of $FeCl_3$ aims to give a color change from light yellow to dark green to blue due to the formation of Fe complexes.

The process of running the maximum wavelength was carried out on quercetin standard solution. The quercetin standard solution was made at 100 ppm, then diluted to 10 ppm, after which it was measured at a wavelength of 600-800 nm using a UV-VIS spectrophotometer. So that the maximum wavelength obtained is 725 nm with an absorbance of 0.437. After obtaining the maximum wavelength of the kursetin standard solution, continue with the measurement of the quercetin standard solution with several variations of 4, 6, 8, 10, 12, and 14 ppm concentrations at a maximum wavelength of 725 nm so that the absorbance value of the quercetin standard solution of each concentration series can be seen in Table 2. A standard curve of the relationship between concentration (C) and absorbance (A) was made so that a linear equation was obtained, which can be seen in Figure 1.

Table 2. Absorbance measurement results of quercetin standard solution at a wavelength of 725 nm

Konsentrasi (ppm)	Absorbansi (A)
4	0.179
6	0.294
8	0.378
10	0.466
12	0.574
14	0.662

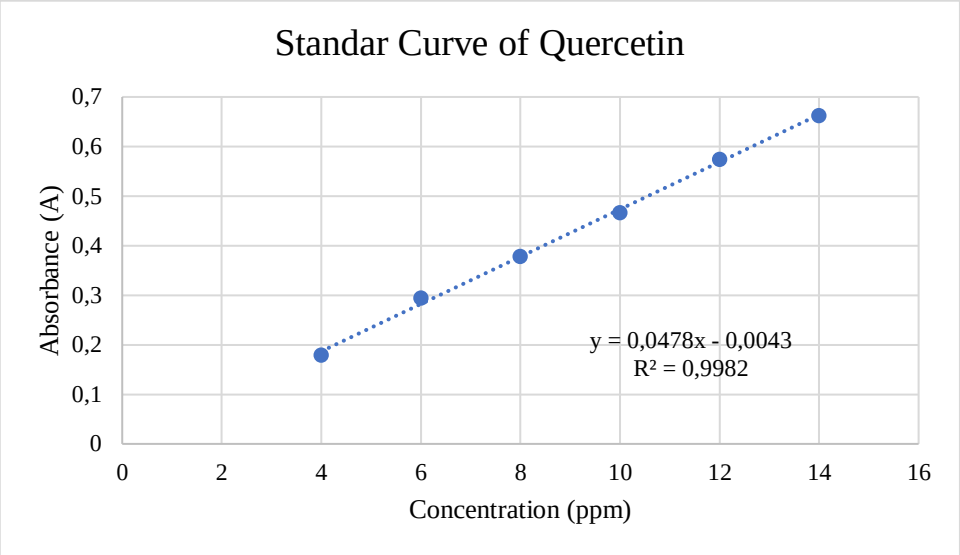

Figure 1. Standard curve of quercetin comparison solution at 725 nm wavelength

In the figure above, it can be seen that the higher the concentration of the quercetin comparison solution, the higher the value of the absorbance. The requirements for the acceptance of the correlation coefficient 0.995 (Haryanto *et al*., 2014). So that a qualified linear equation can be obtained, namely y = 0.047x - 0.004 with a value of R2 = 0.998 with a value of r = 0.998. This equation will be used to determine the antioxidant activity value of button mushroom ethanol extract (*Agaricus bisporus*). To get the absorbance value of the button mushroom ethanol extract (*Agaricus bisporus*), which will be used to calculate the antioxidant activity value of the button mushroom ethanol extract (*Agaricus bisporus*), as done by weig 15 mg of button mushroom ethanol extract (*Agaricus bisporus*) in as many as 3 replicates. After that, each weighing result is put into a 5 mL volumetric flask, and the volume is sufficient to reach the limit mark so that a concentration of 3000 ppm is obtained. The 3000 ppm concentration solution that has been made is pipetted into 1 mL and then added with 1 mL of 0.2 M phosphate dapar (pH 6.6) and 1 mL of $[K_3Fe(CN)_6]$, which aims to be an oxidizer that will react with samples that are reductants so that Fe^{3+} from potassium ferricide is reduced to Fe^{2+}. The sample was vortexed for approximately 5 minutes so that it could mix perfectly, then incubated at 50ºC for 20 minutes to ensure that it was well mixed. then incubated at 50ºC for 20 minutes to accelerate the reaction. The incubated sample was added to 1 mL of 10% TCA so that the potassium ferricide complex formed could settle, then centrifuged at 3000 rpm for 10 minutes to accelerate the separation process. After centrifuging, 1 mL of the top layer of the sample was pipetted and then put into a test tube, and 1 mL of distilled water and 1 mL of 0.1% $FeCl_3$ reagent were added to form a green-to-blue complex. The absorbance was measured at a maximum wavelength of 725 nm using a UV-Vis spectrophotometer.

The blank used for absorbance measurement is ethanol 96% ethanol was pipetted as much as 1 mL, then 1 mL of 0.2 M phosphate buffer (pH 6.6) and 1 mL of potassium ferricyanide $[K_3Fe(CN)_6]$, 1%. The mixture was vortexed for approximately 5 minutes, then incubated at a temperature of 50ºC for 20 minutes, then added 1 mL of 10% TCA. Centrifuged at 3000 rpm for 10 minutes, then pipetted the top layer of the solution as much as 1 mL, mixed with 1 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$. The solution was allowed to stand for 5 minutes, and absorption measurements were taken at a wavelength of 725 nm. Sample absorbance and antioxidant activity values obtained can be seen in Table 3.

Table 3. Absorbance measurement results and antioxidant activity values of ethanol extract of button mushroom (Agaricus bisporus) at 3000 ppm concentration

Replicate	Weight of Extract (gr)	Absorbance	Antioxidant Activity (mgQE/g extract)	Average antioxidant activity (mgQE/g extract)
1	0.01505	0.53	3.774	
2	0.01515	0.549	3.882	3.847
3	0.01516	0.55	3.887	

The antioxidant activity value of the ethanol extract of button mushroom (*Agaricus bisporus*) that has been obtained as in the table above, where in replication one, the antioxidant value obtained is 3.774 mgQE/g, then in replication two, which is 3.884 mgQE/g, and in replication three, which is 3.887 mgQE/g, so that the average value of antioxidant activity in the ethanol extract of button mushroom (*Agaricus bisporus*) is 3.847 mgQE/g extract. This means that each gram of extract has an antioxidant activity value equivalent to 3.847 mg of quercetin.

IV. Conclusions

Based on the research that has been done, it can be concluded that the antioxidant activity value of the ethanol extract of button mushroom (*Agaricus bisporus*) with the FRAP method is 3.847 mgQE/gram extract.

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