Prisma Sains: Jurnal Pengkajian Ilmu dan Pembelajaran Matematika dan IPA IKIP Mataram
https://e-journal.undikma.ac.id/index.php/prismasains/index
e-mail: prismasains.pkpsm@gmail.com

July 2022. Vol. 10, No. 3
p-ISSN: 2338-4530
e-ISSN: 2540-7899
pp. 658-672

Determination of Flavonoid Concentration, Phenolic Concentration, and Antioxidant Activity of *Allium cepa L* Extract

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Received: June 2022; Revised: July 2022; Published: July 2022

Abstract

Free radicals are unstable molecules caused by several electrons in the molecule that does not have a partner. Free radicals can potentially trigger the onset of various degenerative diseases. One of the efforts that can be made to ward off the disease is by consuming antioxidant compounds. Antioxidant compounds can be found in plants with the genus Allium, such as Allium cepa L. This study aims to determine antioxidant activity, flavonoid concentration, and phenolic concentration in *Allium cepa L* extract. The DPPH method (2-2-diphenyl-1-picryhydrazil) is used to determine antioxidant activity, calorimetrically determined flavonoid consensuality with $AlCl_3$, and the Follin-ciocalteu method is used to determine the phenolic consensual consensuality in the sample. The multilevel maceration method in sample extraction uses different polarity solvents, dichloromethane, ethyl acetate, and 96% ethanol. Based on the research data, flavonoid and phenolic compounds were found in 96% ethanol extract but in dichloromethane and ethyl acetate extracts were not found. The concentration of flavonoid ethanol extract is 96% by 4.125 mgQE/gr extract. Phenolic concentration in ethanol extract was 96% by 1.071 mgGAE/gr extract. The IC_{50} value in ethanol extract is 96% which is 201.221 ppm. These results show that the antioxidant activity of *Allium cepa L* is classified as moderate, with IC_{50} values being between the range of 100-250 ppm.

Keywords: antioxidant activity, Allium cepa L, concentration phenolics, concentration flavonoids

How to Cite: Lu'ma, A., & Anggarani, M. (2022). Determination of Flavonoid Concentration, Phenolic Concentration, and Antioxidant Activity of Allium cepa L Extract. *Prisma Sains : Jurnal Pengkajian Ilmu dan Pembelajaran Matematika dan IPA IKIP Mataram, 10*(3), 658-672. doi:https://doi.org/10.33394/j-ps.v10i3.5394

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INTRODUCTION

In the human body, there are no antioxidant reserves that function to ward off free radicals, so the body needs radical prevention compounds from outside the body that can prevent exposure to excess free radicals. The effect caused when the body is exposed to excess free radical exposure is the occurrence of damage to cells in the body. One way that can be done to protect the body from exposure to radicals is by consuming synthetic (artificial) antioxidant substances that have been widely circulated in the market. The compound can repair and prevent cell damage in the body (Rahayu et al., 2015).

Free radicals can be interpreted as an unstable molecules in which one or several electrons do not have a pair on the outermost located orbital (Halliwel & Gutteridge, 2007). Various degenerative diseases can develop caused by an increase in the level of free radicals in the human body. Degenerative diseases are defined as persistent diseases characterized by decreased cell function and organ function in the human body (Notoatmodjo, 2007). Degenerative diseases are caused by the development of hydroxyl radicals in biochemical mechanisms in the body. However, the antioxidants available in the body cannot prevent the high concentration of these free radicals (Atun, 2006).

The body needs an important substance, especially antioxidants, because it can act as a body protector from exposure to free radicals. In addition, antioxidant substances serve to reduce side effects due to exposure to free radicals (Dontha, 2016). As a protection for the body from the dangers of free radical exposure, there is a compound, namely antioxidants, that can act as antitoxins and can ward off free radicals, meeting the lack of electrons from free radicals to prevent chain reactions in the body (Runtuwene et al., 2016).

Antioxidant compounds are compounds that function as inhibitors of the development of free radicals that occur in the body and prevent damage to cells in the body (Cahyaningsih et al., 2019). Based on the source of their acquisition, antioxidant compounds are divided into two parts, antioxidants obtained from natural ingredients or so-called natural antioxidants and synthetic antioxidants or artificial antioxidants. The synthetic antioxidants produced have been thoroughly tested for their toxicity response. However, it is known that some of these compounds can be dangerous when used for a long time, and there are some caveats to the use of toxicological information (Salamah & Widyasari, 2015). In the natural antioxidant group, the most crucial content of phenolic compounds is phenolic acids and flavonoid compounds (Salamah & Widyasari, 2015).

The use of synthetic antioxidants that have been produced, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), as well as tertbutylhydroxy quinone (TBHQ), has been restricted to use as a food product because the ingredient is considered to contain substances that can be one of the cancer-causing impacts or called carcinogenic. This triggered various studies conducted to observe and identify new sources of antioxidants obtained from natural ingredients and are expected to be able to replace synthetic antioxidants (Samin et al., 2013)

Chemically, the content of natural antioxidant substances sourced from natural materials (plants) comes from derivatives of polyphenol compounds. The compound is a flavonoid compound that has a function to capture free radicals (lipid peroxide radicals, superoxide anions), as a singlet oxygen suppressor, as metal chelate, and function as a reductor (Khaira, 2010)

Phenolic compounds in certain plants such as flavonoids, coumarin derivatives and other phenolic compounds are known to play a role in preventing oxidative stress that occurs in the human body. Phenolic compounds can play a role in maintaining the balance between oxidants and antioxidants (Prasonto et al., 2017). Flavonoid compounds play a direct role in reducing oxygen free radicals. For example, an oxide compound, namely superoxide, results from the enzyme xanthine oxidase reaction. In addition to functioning as antioxidant substances, flavonoid compounds have functioned as anti-thrombinogenic, anti-atherosclerosis, antitumor, anti-inflammatory, antiosteoporosis, and antiviral (Simanjuntak, 2012).

Phenolic compounds can fight adverse effects caused by free radicals and are known to reduce the risk of cancer growth, stroke, heart disease, atherosclerosis, osteoporosis, irritation, as well as other neurodegenerative diseases that can be associated with oxidative stress (Ness & Powles, 1997) (Watson, 2003). Phenolic compounds have multifunctional properties, such as acting as a reductant because they can counteract radicals, singlet oxygen questionnaires, and metal chelators (Pratt, 1992).

Natural antioxidants can be found in plant materials, one of which is in the genus *Allium*, since the main components contained in the genus Allium are polyphenol and organosulfur compounds (Werdhasari, 2014). *Allium cepa L*, often referred to as onion Bombay, is a type of onion that is widely developed, used as a flavoring or food, with a large round shape and thick flesh. It is named an *Allium cepa L* because the onion comes from the city of Mumbai in India, which traders brought to trade in Indonesia. There are several types of onions Bombay, including yellow or brown onions, white onions, and Allium cepa Ls Bombay. In this study, researchers chose *Allium cepa L* because there is still little information related to the potential of Allium cepa Ls as antioxidants, and based on the elaboration by

Cheng et al (2013), it is known that in *Allium cepa L*, there are many flavonoid compounds and polyphenol compounds and shows that there is the antioxidant activity that can play an antidote to free radicals.

The results of another study, namely, Ladeska & Rindita (2020), stated that the results obtained from testing bioactive compounds showed that in onion bulbs bombay, several compounds functioned as antioxidants. These compounds are phenolics, flavonoids, triterpenoids, and saponins. The concentration of flavonoid levels and phenolic concentrations found in the content of *Allium cepa L* extract with ethanol solvents, respectively amounted to 1.4868 ± 0.1260 mgQE / g and 103.4727 ± 3.0951 mg GAE / g. The DPPH method (1,1-diphenyl-2-picrilhydrazyl) is used to measure antioxidant activity against ethanol extract of 70% (*Allium cepa L*. Obtained the ic50 antioxidant activity value of 65.3198 ppm and is a strong antioxidant group to be able to ward off free radicals.

Based on the description of the results of previous studies, researchers are interested in conducting a study on the antioxidant activity of one type of onion, namely *Allium cepa L*. In previous research, it has been found that there is antioxidant content in white onions Bombay, but there is still little information about the antioxidant activity in *Allium cepa L*. So researchers want to find innovations regarding the potential of *Allium cepa L* as antioxidants. The parameters to be measured are the content of bioactive compounds of Allium cepa Ls, the antioxidant activity of *Allium cepa Ls*, determination of phenolic concentration levels of *Allium cepa L* extract, and determination of flavonoid concentration levels of Allium cepa L extract.

METHOD

Tools

Used several tools in the study, namely measuring flasks 10 mL, 25 mL, and 100 mL, test tubes, volume pipettes, drip pipettes, micropipets, herma beakers 50 mL and 600 mL, glass vials, dark glass bottles, glass spatulas, filter paper, blenders, 100 mesh sieves, analytical balances (Denver Instrument SI-234), bunchner funnels, vacuum rotary evaporator (Buchi), UV-Visible spectrophotometer (Shimadzu UV-1800) and vacuum pump (Drech Schieber Vacuum Pumpee DSEZ).

Material

Several ingredients were used in the study, namely, Allium cepa L obtained from Jombang Market, dichloromethane, ethyl acetate, 96% ethanol, chloroform, ammonia, H2SO4, HCl 6N, Mg powder, 10% gelatin, anhydrous CH_3COOH, $FeCl_3$, NaOH, gallic acid (Merck), ethanol p.a., methanol p.a., Folin Ciocalteu (Merck) reagents, quercetin (Sigma), Na_2CO_3, $AlCl_3$, CH_3COOK, DPPH solids (Sigma), and aquades.

Sample Preparation

Samples of Allium cepa Lused were obtained from Jombang Market, East Java. A total of 10 kg of Allium cepa L are peeled and cleaned of dirt, cut to a thickness of ± 1 mm, and then placed in the sun to dry indirectly. Samples that have been dried were ventilated at 110^0C for 30 minutes to ensure that there was no moisture content contained in the sample. Samples are weighted using an analytical balance until the sample weight stabilizes. Dried Allium cepa L was put in a blender for the grinding process to obtain a dry powder of Allium cepa L. Then, a 100 mesh sieve is used for the filtering process to obtain a fine powder of Allium cepa Ls Bombay.

Making *Allium cepa L* Extract

Allium cepa L samples were extracted using a multilevel maceration method at the Biochemistry Laboratory of Surabaya State University. Five hundred grams of fine powder of Allium cepa Ls were soaked in 2500 mL of dichloromethane (non-polar) solvent for 24 hours, and then the sample was filtered using a vacuum pump. The residues resulting from

the filtration process are re-dried and soaked with ethyl acetate (semi-polar) solvents. The same treatment is also applied to ethanol (polar) solvents. The filtrate obtained from the three solvents was evaporated with a vacuum rotary evaporator device, and a viscous extract from the sample was obtained. The third amendment of the extract is determined using the equation:

$$\%\ Rendemen = \frac{heavy\ viscous\ extract}{powder\ weight} \times 100\%$$

Bioactive Compound Test

Flavonoid Identification

.Put 1 mL of *Allium cepa L* extract in a test tube and add 70% ethanol to as much as 3 mL. The test tube is heated using a water bath, and then the filtration process is carried out using filter paper and obtained filtrate and residue. Then the filtrate resulting from the filtering process was added with 0.1 grams of Mg powder and two drops of HCl 6 N. Test results that showed positive flavonoids were the change in the solution color to orange, yellow, or red (Harbone, 1987).

Saponin Identification

Put 1 mL of Allium cepa L extract in a test tube and add 10 mL of aquades. Then it is heated using a water bath. The solution is cooled and shaken. A positive saponins test showed the appearance of stable foam (Harbone, 1987).

Identification of Steroids and Triterpenoids

1 mL of *Allium cepa L* extract was included in a test tube, and 3 mL of 70% ethanol, 2 mL of 98% H_2SO4, and 2 mL of anhydrous CH_3COOH. The test results that showed positive steroids were the change in color from the solution to green or blue. The test results that showed positive triterpenoids were the change in color from the solution to red-brown or purple (Harbone, 1987).

Phenolic Identification

Put 1 mL of *Allium cepa L* extract in a test tube and then add 1 mL of NaCl 1% and 1 mL of 10% gelatin. Phenolic positive tests were shown in white deposits (Harbone, 1987).

Quinone Identification

0.5 mL of *Allium cepa L* extract is put in a test tube and then heated using a water bath. Next, three drops of NaOH 1 N were added. A positive quinone test showed the solution's color change from yellow to red (Robinson, 1995).

Determination of Antioxidant Activity

Testing the antioxidant activity contained in the viscous extract of Allium cepa L with the DPPH method refers to the procedure of Tukiran et al. (2020). Dissolved as much as 0.05 grams of thick extract using a 50 mL measuring flask with methanol p.a. A mother liquor with a concentration of 1000 ppm is produced. In the next step, a test solution is made with five concentration variations, namely 100, 150, 200, 250, and 300 ppm.

Put 2 mL of extract solution *Allium cepa L* of various concentrations into vials of dark vials and 2 mL of 0.004% DPPH solution. After that, the solution is incubated in a *styrofoam* box for 30 minutes. To determine the absorbance of the sample solution, measurements were made using the UV-Vis spectrophotometer instrument at a wavelength of 515 nm. In this study, the measurement of the absorbance of the blanko solution was used in the calculation of the inhibition percent by the equation:

$$\%\ inhibisi = \frac{A_{blanko} - A_{sampel}}{A_{blanko}} \times 100\%$$

Sample concentration and percent inhibition will be used to determine regression equations that function in determining antioxidant activity and looking for IC_{50} values from sample extracts (Tukiran et al., 2020)

Determination of Flavonoid Concentrations

The flavonoid concentration of Allium cepa L extract was determined by chlorometry using aluminum chloride, referring to the procedure by Pallab et al. (2013). Quercetin is used as a standard solution for manufacturing calibration curves in this method. A total of 0.01 grams of viscous extract of Allium cepa L was diluted with ethanol solvent p.a. using a 10 mL measuring flask. Then as much as 0.5 mL of diluted extract solution is put in a vial vials and 1.5 mL of ethanol p.a.; 0.1 mL $AlCl_3$ 10%; 0.1 mL CH_3COOK 1 M and 2.8 mL aquades. The next step allowed to stand for a time of 30 minutes. The absorbance of the sample solution was determined using a UV-Vis spectrophotometer at a wavelength of 435 nm. Treatment with the same procedure is also applied to the standard quercetin solution. Then a calibration curve is determined with coordinates (X) indicating the concentration of the standard solution and coordinates (Y) indicating the absorption value of the absorbance. So that a linear regression equation is obtained, which is used as a determinant of extract levels. Flavonoid concentrations in *Allium cepa L* extract are reported in mg QE/g units of extract.

Determination of Phenolic Concentration

The phenolic concentration of Allium cepa L extract was determined using the Folin-Ciocalteau test method, which refers to Safdar et al. (2017). In this method, the manufacture of calibration curves uses a standard solution of gallic acid. 0.01 grams of viscous extract of *Allium cepa L* diluted with ethanol pa. using a 10 mL measuring flask. Then 0.5 mL of dilution extract solution is taken, and 1.5 mL of 10% Folin-Ciocalteau reagent is added and left in 3 minutes.

Furthermore, 1.2 mL of Na_2CO_3 7.5% was added and then left back within 30 minutes. A UV-Vis spectrophotometer was used to measure the absorbance of the sample solution at a wavelength of 758 nm. Treatment with the same stage is applied to the solution of gallic acid as standard. Then a calibration curve is determined with coordinates (X) showing the concentration of the standard solution and coordinates (Y) indicating the absorption value of the absorbance. So that a linear regression equation is obtained, which is used as a determinant of extract levels. Phenolic concentration in Allium cepa L extract is expressed in mg GAE/g extract units

RESULTS AND DISCUSSION

Sample Extraction

Fresh *allium cepa L* (Figure 1.a) is peeled and separated from impurities, then cut to a thickness of ± 1 mm and then placed in the sun to dry indirectly. The purpose of the process is to reduce the water content contained in Allium cepa L and inhibit microbial growth in the sample so that the sample can be stored for a long time. The dry sample from drying is then ground using a blender until it becomes powder (Figure 1.b) for use in the following process, namely extraction. Samples that have become powders can expand the active side so that the extraction process can be faster to bind to the bioactive compounds contained in the sample (Suryani et al., 2016).

Figure 1. (a) Allium cepa L Fresh, (b) Powder *Allium cepa L*

The extraction process at this study stage was carried out using a multilevel maceration extraction method involving three solvents. Three solvents with different polar properties are used: dichloromethane solvents are non-polar, ethyl acetate solvents are semi-polar, and ethanol solvents are 96% polar. The results obtained from the extraction of stratified maceration are judged to be purer than those of partial maceration. The use of three types of solvents with differences in their polarity properties is expected to be able to extract compounds of unknown polarity. The principle of stratified maceration is like dissolved, which means that compounds with polar properties will be attracted to polar solvents and vice versa (Pratiwi et al., 2010). Differences in the type of solvent will also affect the yield of extracts, qualitative observation of flavonoids and phenolics, as well as the determination of flavonoid and phenolic consensual constituencies (Firdiyani, Agustini, et al., 2015).

The maceration result of *Allium cepa L* extract with dichloromethane solvent is yellow (++), ethyl acetate solvent is yellow (+), and ethanol solvent is 96% brown. The yellow color of dichloromethane extract is more intense than ethyl acetate extract because more bioactive compounds attract dichloromethane solvents. The following process that is carried out is that the Maserati is filtered using a vacuum pump so that the filtrate is separated from the residue. The filtrate obtained is evaporated first using a Rotary Vacuum Evaporator tool. This has the purpose of separating a fairly concentrated extract from the solvent. From the evaporation process, a thick extract of dichloromethane is yellow-brown, and a thick extract of ethanol is yellow-brown. In ethyl acetate solvents, no viscous extract is obtained because the extract obtained is quite small and in the form of a yellow-brown liquid.

The results of the calculation of the yield of Allium cepa L extract by the multistage maceration method using a type of solvent whose polarity properties are different are presented in Table 1.

Table 1. Yield of *Allium Cepa L* Extract Amendment

Solvent	Powder Weight (g)	Solvent Volume (mL)	Extract Results (g)	Extract Amendment (%)
Dichloromethane	500	2500	11.3538	2.27076
Ethyl acetate	500	2500	-	-
Ethanol 96 %	500	2500	64.6287	12.92574

Based on the data presented in the Table. 1, the yield with the highest yield is found in the ethanol extract of Allium cepa L. This shows that ethanol solvents with polar properties can extract more bioactive compounds compared to dichloromethane solvents (Suryani et al., 2016).

In the molecular structure of ethanol, there is an OH group that can dissolve polar molecules, as well as an alkyl CH3CH2 group that can bind to non-polar molecules (Aziz & Fresca, 2009). Dichloromethane extract has the lowest soaking because there is still a hydrogen bond between the compounds in the sample and the hydroxy group.

Testing of Bioactive Compounds

Analysis of bioactive compounds is a qualitative observation of the bioactive components contained in a sample extract (Kristanti et al., 2008). Testing of bioactive compounds is carried out qualitatively by determining whether or not there is a content of bioactive compounds in the sample that can potentially become antioxidants. The test results of the content of bioactive compounds of Allium cepa L extract with three solvents, namely dichloromethane, ethyl acetate, and ethanol, are shown in Table 2.

Table 2. Test Results of Bioactive Compounds of *Allium cepa L* Extract

Bioactive Compounds	Solvent Extractor			Information
	Dichloromethane	Ethyl Acetate	Ethanol	
Flavonoids	-	-	+	Yellow solution
Saponins	+	+	+	Formed stable foam
Steroids	-	-	-	Green solution

Bioactive Compounds	Solvent Extractor			Information
	Dichloromethane	Ethyl Acetate	Ethanol	
Triterpenoids	+	-	+	Brownish-colored solution
Phenolic	-	-	+	Solution is white, white precipitate
Quinones	+	+	+	Yellow solution

Ket: The sign (+) indicates the presence of the content of the bioactive compounds tested in the extract; sign (-) indicates the bioactive compound tested was not detected on the extract.

Table 2 shows that the extract of dichloromethane Allium cepa L extract can contain bioactive compounds. These compounds are saponins, triterpenoids, and quinones. Allium cepa L ethyl acetate extract contains bioactive compounds in the form of saponins and quinones. Allium cepa L ethanol extract contains bioactive compounds including flavonoids, saponins, triterpenoids, phenolics, and quinones. This is in accordance with the research of Ladeska and Rindita (2020), which states that the test results of bioactive compounds from onion bulbs contain bioactive compounds in the form of flavonoids, phenols, triterpenoids, and saponins.

Flavonoid compounds have bioactive properties, namely as antioxidants, by inhibiting oxidation. Phenolics have bioactive properties as antioxidants because they can contribute H atoms to free radicals (Dhurhania & Novianto, 2019). Testing of flavonoids and phenolics in the study resulted in positive tests on allium cepa L ethanol extract and negative on dichloromethane and ethyl acetate extracts.

Flavonoids dissolve in polar solvents due to hydroxyl groups attracted by polar solvents such as ethanol and are insoluble in non-polar compounds. Phenolics are polar compounds due to glycosides, namely the bonds of sugars with phenolics in cell vacuoles (Marliana et al., 2005).

Based on the testing of bioactive compounds of Allium cepa L extract with dichloromethane solvents which include solutions with non-polar properties, ethyl acetate solvents which include solutions with semi-polar properties, and 96% ethanol solvents which include solutions with polar properties, then 96% ethanol extract has the potential to be an antioxidant because positive test results were obtained on testing flavonoid and phenolic compounds. This is in accordance with the statement of Salamah (2015), that in the natural antioxidant group the most important content of phenolic compounds is flavonoids and phenolic acids. The results obtained at the testing stage of bioactive compounds will be used as a reference for the next testing procedure, namely the determination of antioxidant activity.

Antioxidant Activity

At this stage of testing, the DPPH method (2,2 diphenyl-1-picrichidrazil) was used to determine the intensity of antioxidant activity of Allium cepa L extract. Antioxidants include a compound that prevents exposure to free radicals that inhibit the performance of cells in the body (Cahyaningsih et al., 2019). The principle of testing at this stage is that DPPH free radicals will attract hydrogen contained in antioxidant compounds. The occurrence of this process is indicated by the change in the color of the solution on the DPPH, which initially had a deep purple color turning yellow. In determining the intensity of antioxidant strength, a benchmark for discoloration of DPPH solution was used to measure color intensity using a UV-Vis spectrophotometer instrument at a wavelength of 517 nm (Tukiran et al., 2020). The strength level of antioxidant activity judging by the IC_{50} values is presented in Table 3.

Table 3. Antioxidant Activity Strength Based on IC50 Value (Syarif et al., 2016)

Antioxidant Power	IC_{50} Rated
Weak	range from 250 to 500 ppm
Keep	range from 100 to 250 ppm
Strong	range from 50 to 100 ppm
Very powerful	less than 50 ppm

DPPH is a compound with free electrons that react with antioxidants (RH). The change in the color of the solution is caused by antioxidants that give one hydrogen atom to DPPH, causing a change from a deep purple color to a yellow color. The reaction of the testing process between DPPH (2,2 diphenyl-1-picrichydrazyl) with antioxidants is presented in Figure 2 (Liang & Kitts, 2014).

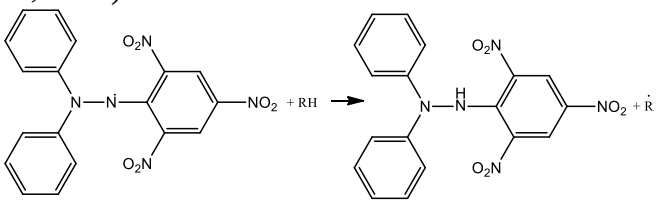

Figure 2. The reaction of DPPH (2,2 diphenyl-1-picrichydrazyl) with Antioxidants

At this stage, a linear regression equation is used to determine the IC50 value of the tested sample. The linear regression equation indicates a relationship between the sample solution concentration and the percent inhibition. Percent inhibition is plotted with the concentration of the sample extract so that a linear regression equation is obtained to calculate the IC50 value. The linear regression equation is used to determine antioxidant activity, where the value of y = 50, so that the value of x is obtained as IC50 (µg/mL) or ppm. IC50 shows the magnitude of the concentration value of Allium cepa L extract (µg / mL or ppm), which can prevent free radical exposure by 50%. If a low IC50 value is obtained, the sample used in the study has high antioxidant activity and vice versa (Izzati et al., 2012).

The linear regression equation obtained as well as the results of determining the IC_{50} value of dichloromethane extract and Allium cepa L ethanol extract are presented in Table 4. At the same time, the graph states the relationship that occurs between the sample concentration of dichloromethane extract and ethanol extract with inhibition pesen is presented in Figure 3.

Table 4. IC_{50} Value of Dichloromethane Extract and Allium cepa L Ethanol Extract

Sample Extract	Linear Regression Equation	IC_{50} rated (ppm)
Dichloromethane Extract	y = 0,1186x – 6,552 R^2 = 0,9896	476,829
Ethanol Extract 96 %	y = 0,2799x – 6,322 R^2 = 0,9899	201,221

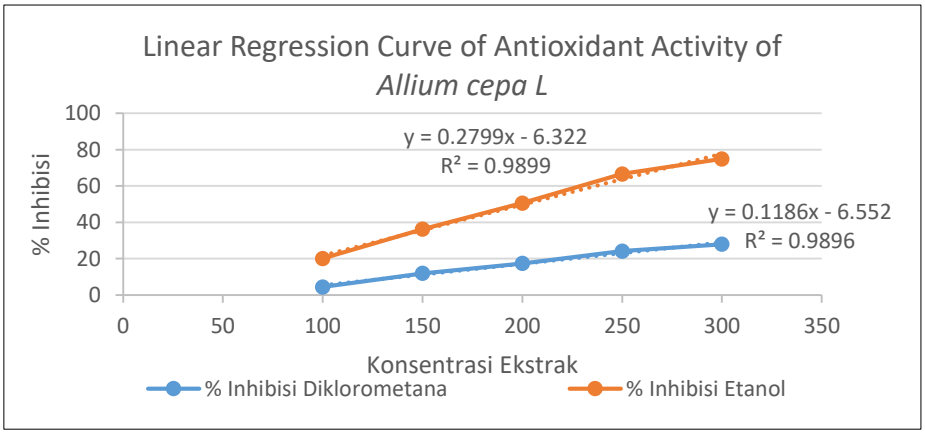

Figure 3. Linear Regression Curve of Antioxidant Activity of *Allium cepa L*

Based on the presentations from Table 4 and Figure 3, the IC50 value of the 96% ethanol extract was 201,221 ppm. The antioxidant activity is classified as moderate because it ranges from 100-250 ppm (Syarif et al., 2016). The IC50 value in dichloromethane extract was 476,829 ppm. The antioxidant activity is included in the weak group because it ranges between 250-500 (Syarif, Aktsar, Oskiana, & Malik, 2008). The antioxidant activity contained in the extract of dichloromethane can be caused by the presence of tocopherol, which is an antioxidant that has non-polar properties. Tocopherol belongs to the class of antioxidant compounds with fat-soluble properties and is among the best in the fat oxidizing group.

Judging from the calculation of the IC50 value, it is proven that in allium cepa L extract, there is antioxidant activity. However, the test results show that extracts with ethanol solvents have better strength in preventing exposure to free radicals than the test results of dichloromethane extracts. This is because there is a content of bioactive compounds in the extracted 96% Allium cepa L ethanol extract, especially flavonoid and phenolic compounds where both compounds are bioactive compounds that have the potential to be antioxidants. Flavonoids and phenolics are bioactive compounds with polar properties, so the two compounds will be more attractive to ethanol as polar solvents. The results of other studies regarding the antioxidant activity of Allium cepa L extract are Ladeska and Rindita (2020), which state that in determining antioxidant activity, an IC50 value was obtained in onion extract (Allium cepa L.) with a 70% ethanol solvent of 65.3198 ppm. The DPPH method (1,1-diphenyl-2-picrilhydrazyl) is used to determine antioxidant strength. The difference in the results obtained can be due to several environmental factors, including the plant environment, plant seed sources, planting methods, and climatic conditions, so that it can affect the content of bioactive compounds in plants (Kuntorini et al., 2010).

Flavonoid concentration

Flavonoid compounds function as antioxidants because there is a hydroxyl group bond with an aromatic ring carbon which causes flavonoids to play a role in warding off free radicals (Selujeng & Budgeti, 2021). Chlorometric testing with the AlCl3 method is used in determining the concentration of flavonoids in Allium cepa L and quercetin extracts as the standard. Quercetin includes flavonoids in the flavonol group, hydroxyl groups in adjacent C-3 and C-5 atoms, and a ketone group in the C-4 atom (Wirasti, 2019). Potassium acetate addition treatment was carried out to determine the presence or absence of a 7-hydroxy group in the tested sample. The principle of the approval method at this stage is the presence of an acid complex with an orthohydroxyl group of flavonoid compounds from the addition of $AlCl_3$ and CH_3COOK, so that yellow color is formed. The reaction at the testing stage of flavonoid concentration is shown in Figure 4 (Fachriyah et al., 2020).

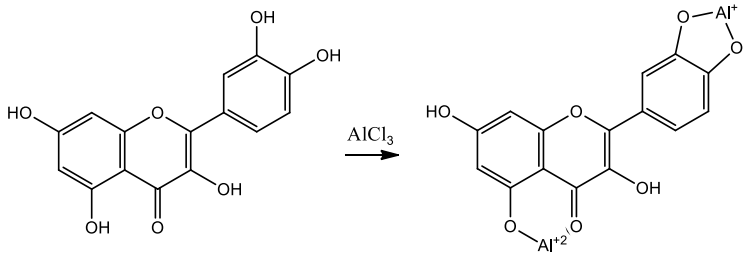

Figure 4. Reactions to the Determination of Flavonoid Consensual with AlCl3

Based on the results of testing of bioactive compounds that have been carried out, the flavonoid content in the sample was only found in the 96% allium cepa L ethanol extract, but in the testing of dichloromethane extract and ethyl acetate extract was not found. This is because flavonoids are compounds of the polyphenol group and are found to be bound to sugars to form glycosides so that they are polar (Suryani et al., 2016).

Quercetin as a standard solution with a wavelength of 439 nm was used to determine flavonoid concentration. The measurement uses a Uv-Vis spectrophotometer instrument. The calibration curve for a standard solution of quercetin is presented in Figure 5.

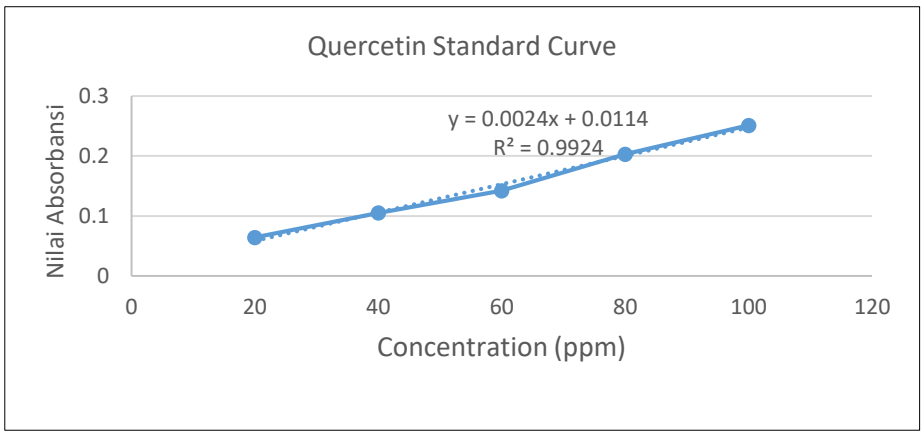

Figure 5. Quercetin Standard Solution Calibration Curve

Based on the presentation of Figure 5, in the quercetin calibration curve as a standard solution, a linear regression equation is obtained, namely y = 0.0024x + 0.0237 and R2 = 0.9924. A linear regression value close to 1 on the curve above indicates a relationship between the concentration of the tested solution and the absorbance value. The linear regression equations obtained are used to determine flavonoid concentration of Allium cepa Lyang extract presented in Table 5.

Table 5. Results of Determining the Concentration of Flavonoids of Allium cepa L Extract

Solvent	Replication	Absorbance	Concentration (mg/mL)	Consentaration of Flavonoids (mgQE/g extracts)	Average Concentration of Flavonoids (mg QE/g extract)
Ethanol	1	0,2071	0,0815	4,075	4,125
	2	0,2104	0,0830	4,150	
	3	0,2105	0,0830	4,150	

Based on the calculation data obtained in Table 5, the concentration level of flavonoids in the extract of allium ethanol cepa Lsebesar is 4.125 mg QE /g extract. Flavonoids dissolve with polar ethanol solvents. This is due to the presence of hydroxyl groups that are attracted by solvents with polar properties such as ethanol and cannot dissolve with solvents of a non-polar nature. Flavonoids have the potential to be antioxidant compounds, caused because, in the structure of flavonoid molecules, there is a hydroxyl group that acts as a hydrogen donor so that these molecules can inhibit cell damage due to free radicals (Simanjuntak, 2012).

The research results by Ladeska and Rindita (2020), stated that onion bulb extract with 70% ethanol solvent has a flavonoid concentration content of 1.4868 ± 0.1260 mgQE / g. The difference in results obtained can be caused by several environmental factors, including the plant environment, plant seed sources, planting methods, and climatic conditions, which can affect the content of bioactive compounds in plants (Kuntorini et al., 2010).

Fenolik concentration

In phenolic compounds, there is a hydroxyl group in phenol compounds that have the potential to be antioxidants (Selujeng & Anggarani, 2021). This group will give hydrogen atoms to free radical compounds that do not have a partner so that they can inhibit the oxidation process (Miguel, 2017). Determination of phenolic concentration levels at this stage is carried out to calculate the levels of phenolic condensation contained in the sample

extract that has the potential to become antioxidants. The method used, namely Follin-Ciocalteau, is reacted with gallic acid, a phenolic acid class standard (Sam, A, & Handayani, 2016). The principle of the method used is that phenolic ions will reduce phosphotungstic acid and phosphomolybdic acid in the alkaline atmosphere to form a blue molybdenumtungsten complex (Wilujeng & Budgeti, 2021). The higher the concentration of phenolic compounds, the more saturated the blue color in the solution and the absorbance value is higher. This is due to the increasing number of phenol ions that reduce fophomolybic acid and phosphotungstate so that more molybdenum tungsten complexes are formed (Andriani & Murtisiwi, 2018). The reactions that occur in the phenolic testing of condensation are presented in figure 6 (Mukhriani et al., 2019).

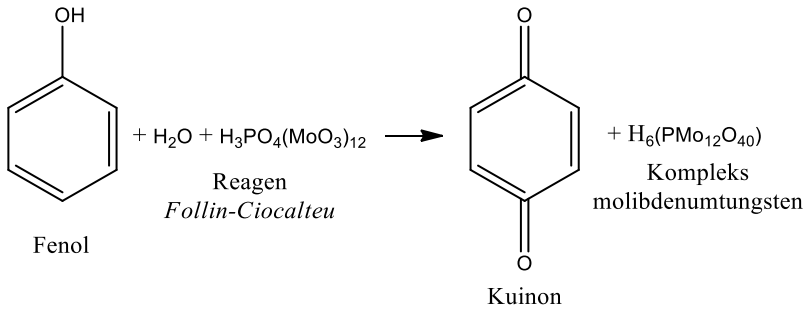

Figure 6. Reactions to Phenolic Determination with Follin-Ciocalteau

Based on the results of the testing of bioactive compounds that have been carried out, the phenolic content in the sample was only found in the ethanol extract of Allium cepa L, in the results of extraction with dichloromethane solvents and ethyl acetate solvents were not found. This is because phenolic is a polar compound due to the presence of glycosides, namely the bond of sugars with phenolics in cell vacuoles (Marliana et al., 2005), so the phenolic content is only found in polar ethanol solvents.

Determination of phenolic concentration in allium cepa L ethanol extract using a standard solution, namely gallic acid with a wavelength used of 758 nm. The determination of these levels uses a Uv-Vis spectrophotometer instrument. The calibration curve for the gallic acid that acts as the standard solution is presented in Figure 7.

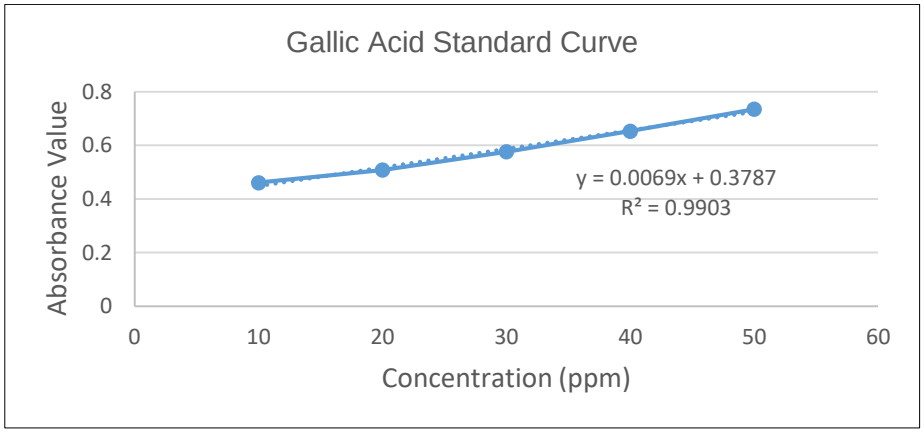

Figure 7. Calibration Curve of Gallic Acid Standard Solution

Based on the presentation of the calibration curve data in Figure 7, a standard solution linear regression equation is obtained, namely y = 0.0069x + 0.3787 and R2 = 0.9903. A linear regression value close to 1 on the curve above indicates a relationship occurring between the concentration of the solution being tested and the absorbance value. The equations that have been obtained are used for the determination of phenolic concentration of Allium cepa L which extract is presented in Table 6.

Table 6. Results of Determination of Phenolic Consentaration of Allium cepa L Extract

Solvent	Replication	Absorbance	Concentration (mg/mL)	Phenolic Consensual (mg GAE/g extract)	Average Phenolic Concentration (mg GAE/g extract)
Ethanol	1	0.5191	0.0203	1.015	1.017
	2	0.5191	0.0203	1.015	
	3	0.592	0.0204	1.020	

Table 6 shows data on the determination of phenolic concentration levels in the extract of Allium cepa Lsebesar ethanol 1,017 mg GAE/g extract. Phenolic compounds contained in Allium cepa L extract include the polar compound group because they can dissolve with solvents with polar properties, namely ethanol. These results are in accordance with the principle of like dissolved like, where compounds whose nature is polar will be attracted to polar solvents and vice versa (Pratiwi et al., 2010).

The results of research by Ladeska and Rindita (2020) stated that onion bulb extract with 70% ethanol solvent has a phenolic concentration content of 103.4727 ± 3.0951 mg GAE / g. Differences in research results can be caused by several environmental factors, including the plant environment, plant seed sources, planting methods, and climatic conditions, that can affect the content of bioactive compounds in plants (Kuntorini et al., 2010).

Based on the acquisition of research data, it was found that the extract from Allium cepa Lberpotency as a natural antioxidant is characterized by the presence of flavonoid and phenolic concentrations in the extract. However, the IC50 value obtained in testing the antioxidant activity of Allium cepa Lsebesar is 201,221 ppm, so the ability of Allium cepa L to dampen free radicals is still classified as moderate.

Flavonoid concentration and phenolic concentration are directly proportional to the antioxidant activity of Allium cepa L extract. If the concentration flavonoid levels and the phenolic concentration levels in the sample are higher, then the antioxidant activity of a sample is stronger. The results are in accordance with Syamsu et al (2019), where the ability of antioxidant activity will be stronger in counteracting free radicals if flavonoid or phenolic concentration in a compound is higher.

The results of another study on the antioxidant activity of onions were carried out by Ladeska and Rindita (2020), which stated that in determining antioxidant activity, an IC50 value was obtained from onion extract (Allium cepa L.) with a 70% ethanol solvent of 65.3198 ppm. Determination of antioxidant activity in the research of Ladeska and Rindita (2020) was carried out using the DPPH method (1,1-diphenyl-2-picrilhydrazyl). The difference in test results can be due to several environmental factors, namely the plant environment, plant seed sources, planting methods, and climatic conditions. It can affect the content of bioactive compounds in plants (Kuntorini et al., 2010).

CONCLUSION

Based on research data, it can be seen that Allium cepa L extract is potent as an antioxidant. The highest antioxidant activity was found in the extract of Allium cepa L ethanol with an IC50 value of 201,221 ppm as well as flavonoid concentrations and phenolic consensual constituents of Allium cepa Lberturut-cohesive extract of 4,125 mg QE/g extract and 1,017 mg GAE/g extract. From the data results, allium cepa L ethanol extract is potent as an antioxidant. However, its antioxidant activity is classified as moderate because it ranges from 100 to 250 ppm.

RECOMMENDATION

Finally, the author recommends that the overall development of Allium cepa L tubers be carried out, which have the potential to become antioxidants to reduce exposure to free

radicals that are harmful to health. This is because there is still a lack of public knowledge about the antioxidant content in Allium cepa L. In determining antioxidant activity, it can be done by other methods such as the FRAP (Ferric Reducing Antioxidant Power) or FIC (Ferrous Ion Chelating) methods that have not been carried out in this study.

ACKNOWLEDGMENT

The author expresses his gratitude to various parties, especially supervisors who have provided input and suggestions regarding research to report writing, laboratories that provide permits in research activities, and friends and parties involved in research administration.

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