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TOTAL FLAVONOID LEVELS AND ANTIOXIDANT ACTIVITY OF KERSEN LEAF EXTRACT (*muntingia calabura* L.)

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A B S T R A C T

Kersen leaves (Muntingia calabura L.*) are plants that contain many secondary metabolites, one of which is flavonoids that can act as antioxidants. This study aims to determine the total content of flavonoids which also have the potential as antioxidants in cherry leaves using the DPPH method (2,2-diphenyl-1-picrylhidrazyl) with various filters, namely 70% ethanol, ethyl acetate and n-hexan. Testing of total flavonoid content was carried out using $AlCl_3$ reagent and measured using spectrophotometry at a wavelength of 422 nm. Antioxidant activity testing was measured at an absorption wavelength of 516 nm. The results showed the total flavonoid content of 70% ethanol filter leaf extract as much as 8.13 (mgQE/g), ethyl acetate as much as 8.32 (mgQE/g) and n-hexan as much as 18.96 (mgQE/g). antioxidant activity of cherry leaves using DPPH method (2,2-diphenyl-1-picrylhidrazyl) with filter variations. 70% ethanol extract of cherry leaves has an IC_{50} value of 7.56 µg/mL, ethyl acetate extract with an IC_{50} value of 36.05 µg/mL, and n-hexan extract with an IC_{50} value of 21.33 µg/mL. From the results obtained it can be concluded that the highest antioxidant activity is contained in 70% ethanol extract of 7.56 µg/mL.*

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INTRODUCTION

Free radicals are highly reactive molecules because they have one or more unpaired electrons. Environmental factors such as air pollution, radiation, and toxic chemicals can increase the production of free radicals in the body. Free radicals cause oxidative damage to DNA, proteins, and lipids of cells and can cause a variety of diseases, including cancer, heart disease, and neurodegenerative diseases (Liu, 2019). Antioxidants are molecules that can neutralize free radicals by donating electrons without becoming free radicals themselves, thereby reducing or preventing damage caused by free radicals (Pisoschi, *et al.*, 2020).

Kersen leaves are one of the plants that are believed to have many benefits for health. Kersen (*Muntigia calabura* L.) is a plant that grows freely anywhere and is widely found in Indonesia. Kersen trees have leaves that provide shade, so they are often overlooked and only used as shade trees. This little-known plant has many uses in human health, including as a cough reliever, headache reliever, gout, antioxidant, anticancer, and antidiabetic (Palguna dan Yustianatara, 2022). The results of Anisa and Najib's 2022 research show that kersen leaf ethanol extract contains phenols, flavonoids, tannins, alkaloids, saponins, and steroids. The phenol content obtained was 22,389 mgGAE/100g, the flavonoid content obtained was 13,375 mgQE/100g and the phenol content obtained was 13,715 mgGAE/100g.

One method to determine antioxidant activity is to use the free radical DPPH (1,1-diphenyl-2-picrylhydrazil). This method is often chosen as an antioxidant activity testing method because it is simple, easy, fast, sensitive and requires a small number of samples. The DPPH method is used to test the ability of a component to trap free radicals in a material or extract. This test method is based on the ability of antioxidants to neutralize DPPH free radicals. Measurements were made at maximum wavelengths using a UV-Vis spectrophotometer. The maximum wavelength is measured using a blank solution, namely a DPPH solution dissolved in methanol, so that DPPH absorption is obtained at the maximum wavelength. The principle of the DPPH method is to reduce the color intensity of DPPH by reducing the amount of DPPH that reacts with the sample to form DPPH. Antioxidants suppress free radicals by releasing hydrogen atoms, thereby reducing DPPH radicals. The advantage of the DPPH method is that it can be done quickly and simply (Pambudi dkk, 2021).

Based on the description above, the researcher is interested in conducting research on testing antioxidant activity using various strainer variations by looking at the IC50 value in kersen leaf extract (*Muntingia calabura* L.) using the 1,1diphenyl-2-picryldrazyl (DPPH) method. The DPPH method was used to measure antioxidant ability by using free radicals 2,2-diphenyl-1-picylhydrazyl (DPPH).

RESEARCH METHODS

Tools and Materials

The tools used in this study were a *mesh* sieve 16, a stirring rod, Erlenmeyer (*Iwaki Pyrex®Japan*), a maserator, a beaker (*Iwaki Pyrex®Japan*), a measuring cup (*Iwaki Pyrex®Japan*), a simplisia oven, a micropipette (*Nesco®*), *a rotary vaccum evaporator*

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(*Buchi*®), a UV-Vis spectrophotometer (*T60*®), a test tube (*Iwaki Pyrex*®*Japan*), analytical scales (*Chyo*®).

Bahan yang digunakan pada penilitian ini yaitu akuades (*Onemed*®*Indonesia)*, AlCl3 2% (*Merck*® German), aluminium foil, daun kersen (*Muntingia calabura* L.), DPPH (*1,1-diphenyl-2-picrylhydrazyl*), etanol 70% (*Karsavicta*®*Indonesia)*, etanol absolut (*Brand Germany*®), etil asetat (*Merck*® German), HCl pekat (*Merck*® German), natrium asetat (*Merck*®*106267)*, n-heksan (*Merck*® German), kertas saring Whatman 42 (*Cytiva*®), kuarsetin (*Sigma*®), dan serbuk magnesium (*Merck*® German).

Research Sample Processing

Kersen leaves are processed with the initial stages, namely wet sorting, washing. After the test material is clean, it is dried. Drying is carried out using a simplified oven at 40°C for 12 hours. After the drying process, dry sorting is carried out. Dried simplicia is then ground using a blender so that simplicia is obtained in powder form. It is then sifted using 60 mesh to obtain the same degree of fineness (Abidin, dkk., 2023 dan Utami, dkk., 2024).

Extraction Process

Extraction is carried out by the cascade maceration method. 250 g of simplicia is inserted into the maserator. First, the wetting or soaking process is carried out using n-hexane (let it sit for 30 minutes) then n-hexane is added until the entire simplicia is submerged (ratio 1:10). After 3x24 hours of maceration process, the maserat is filtered (filtered) to separate the residue from the filtrate. The residue is remasonized with n-hexane (until the filtrate is clear). Furthermore, the residue is remasonized by the same process sequentially using ethyl acetate and 70% ethanol. The extracted extract obtained is filtered and then evaporated by *a rotary vacuum evaporator* (Abidin, dkk., 2023).

Qualitative Analysis of Flavonoid Compounds

In a test tube each 0.5 g of n-hexane extract, ethyl acetate extract and 70% ethanol extract are added 3 mL of 70% ethanol then shaken, heated, and shaken again then filtered. The filtrate obtained in each test tube was added 0.1 g of magnesium powder and 2 drops of concentrated HCl. The formation of red, yellow or orange indicates the presence of a group of flavonoid compounds.

Determination of Total Flavonoid Levels

1. Manufacture of Quartz Stock Solution

The preparation of quartz stock solution is carried out by weighing 10 mg of quartz solids and dissolved using ethanol p.a in a measuring flask, then the volume is sufficient up to 10 mL so that a quartz stock solution with a concentration of 1000 ppm is obtained.

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2. Measurement of Quarcetin Stock Solution

Quarcetin solution with concentrations of 2, 4, 6, 8 and 10 ppm. Each was pipetted as much as 0.02 mL, 0.04 mL, 0.06 mL, 0.08 mL and 1 mL of 1000 ppm stock solution. Put it in a 5 mL measuring flask and add 0.1 mL of 2% AlCl3, 0.1 mL of sodium acetate and 3 mL of ethanol p.a. and add distilled water to 5 mL. then homogenized and the wavelength was measured using UV-Vis spectrophotometry with a wavelength of 422 nm.

3. Preparation of Kersen Leaf Extract Test Solution

The sample solution from each kersen leaf extract filter was weighed as much as 10 mg, dissolved in a measuring flask of 10 mL using ethanol p.a while stirring and homogenizing, then the volume was sufficient to 10 mL.

4. Determination of Total Flavonoid Levels of Kersen Leaf Extract

Pipetted each extract solution of 1 mL was put into a measuring flask of 5 mL, then 0.1 mL of $AlCl_3$ 2% was added, sodium acetate 0.1 mL, ethanol absolute 1.5 mL was added. Then the volume is sufficient using aquadest to the limit mark. Homogenized and incubated for 30 minutes, the solution was measured using UV-Vis Spectrophotometry at a wavelength of 422 nm.

Antioxidant Activity Testing

1. DPPH Solution Manufacturing

The preparation of DPPH 0.4 mM solution was made by weighing 7.9 mg of DPPH powder then dissolved with ethanol p.a and the volume was sufficient up to 50 mL in a flask.

2. DPPH Blank Absorption Measurement 0.4 mM

The test was carried out by pipetting 1 mL of 0.4 mm DPPH solution and sufficient volume with ethanol absolute up to 5 mL. It was then measured using a UV-Vis spectrophotometer using a wavelength of 516.80 nm.

3. Manufacture of Quartz Stock Solution

The test was carried out by pipetting 1 mL of 0.4 mm DPPH solution and sufficient volume with ethanol p.a up to 5 mL. It was then measured using a UV-Vis spectrophotometer using a wavelength of 516.80 nm.

4. Quartz Absorption Measurement

The test was carried out by pipetting from a 10 ppm stock solution. After that, it is diluted according to calculations with concentrations of 2 ppm, 4 ppm, 6 ppm, 8 ppm and 10 ppm. Then each was put into a flask and 1 mL of DPPH solution was added, then the volume was sufficient to 5 mL using ethanol p.a and then homogenized. The solution mixture was left for 30 minutes then measured using spectrophotometry with a wavelength of 422 nm.

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5. Preparation of Kersen Leaf Extract Test Solution

The preparation of sample extract stock solution was made by weighing each extract with various filters as much as 10 mg of kersen leaf extract then dissolved with ethanol p.a while homogenizing. The final volume of the pumpkin is sufficient to 10 mL until an extract solution is obtained from each strainer with a concentration of 1000 ppm.

6. Kersen Leaf Extract Test Solution Testing

Each of the stock solutions of 70% ethanol extract, ethyl acetate extract and n-hexane extract was made in five concentration series, namely 1 ppm, 4 ppm, 7 ppm, 10 ppm, and 13 ppm. The sample solution was diluted according to the calculation of concentrations of 5 µL, 20 µL, 35 µL, 50 µL and 65 µL into the flask and 1 mL of DPPH 0.4 mM solution was added and then the volume was sufficient to 5 mL. Then let it sit at room temperature for 30 minutes covered using aluminum foil. Measured using UV-Vis Spectrtophotometry with a wavelength of 516.800 nm.

RESULTS AND DISCUSSION

This research was carried out with the initial stage, namely sample processing. Sample processing is carried out with a wet sorting stage which is carried out to separate impurities or other foreign materials from the material. After that, washing is carried out on the material to remove soil and other impurities attached to the material. Drying is carried out using a simplified oven at a temperature of 40°C for 12 hours of heating. After the drying process, dry sorting is carried out to separate foreign objects such as unwanted plant parts and other impurities that are still left on the dried simplicia.

The extraction of 250 g of kersen leaf simplicia was carried out by stratified maceration using n-hexane, ethyl acetate and 70% ethanol. The maceration method is chosen to prevent the deterioration of unstable compounds to heating. The extract obtained is then evaporated with a *rotary vacuum evaporator*. The extraction process produced a yield of n-hexane extract which was 8.14%, ethyl acetate extract which was 9.50% and ethanol extract 70% which was 12.06% (Table 1).

Table 1. Extraction Results of Kersen Leaves (*Muntingia calabura* L.)

Solvent	Simplisia (g)	Extract (g)	Rendeming (%)
Ethanol 70%	250	30.017	12.06
Ethyl Acetate	250	23.764	9.50
n-Heksan	250	20.363	8.14

The extraction process occurs with the flow of solvent into the cell which causes the protoplasm to swell and the material contained in the cell will be dissolved according to its solubility. This high solubility is related to the polarity of the solvent and the polarity of the extracted compound (Vogel, 1978). The more similar the polarity of the solvent is to the

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polarity of the substance contained in the extracted material, the more components of the substance can be extracted so that there can be an increase in the yield obtained. This increase is caused by the easier binding of substances in the material by solvents. Each type of solvent has different polarity, and on the same type of solvent with different concentrations also has different polarity. The calculation of the randement was carried out to determine the ratio of the number of extracts obtained from an ingredient to the initial weight of simplisia, as well as to determine the amount of bioactivity contained in the extracted material. Factors that affect the amount of randement produced are the extraction temperature, extraction time and the concentration of the solvent used.

In this study, qualitative testing was carried out on n-hexane, ethyl acetate and ethanol extracts of 70%, where in the identification of flavonoid compounds, concentrated HCl reagents and magnesium metal were used (Table 2). The addition of magnesium and concentrated HCl metal reduces the benzopyrone nuclei contained in the flavonoid structure so that orange or red color changes occur. While the identification of phenolic compounds is carried out using the $FeCl_3$ reagent, it will react with the phenolic groups that are in the sample to form a hiau, blue, or black color indicating the presence of phenolic compounds (Harborne, 1987).

Table 2. Results of Qualitative Tests of Flavonoid and Phenolic Compounds from Kersen Leaf Extraction

Identification Test	Sample	Reagent	Result
Flavonoid Test	Daun cherries	HCl + Mg	Red Brick Solution
Phenolic Test		$FeCl_3$ Solution	Blackish Green Solution

The determination of the maximum wavelength optimization of the raw solution is carried out according to the flavonoid absorption spectrum found in the wavelength range of 400-600 nm. The maximum wavelength is indicated at 422 nm. The compound used as a standard in determining the total content of this flavonoid is quarsetin, because quartz is a flavonoid of the flavonol group that has a keto group on the C-4 atom and also an idroxy group on the neighboring C-3 and C-5 atoms (Azizah, 2014).

Based on the results of the measurement of absorbance of standard solutions at various concentrations, the calibration curve of the standard solution of flavonoid compounds obtained a linear relationship between absorbance and concentration indicated by the value of $R^2 = 0.9385$.

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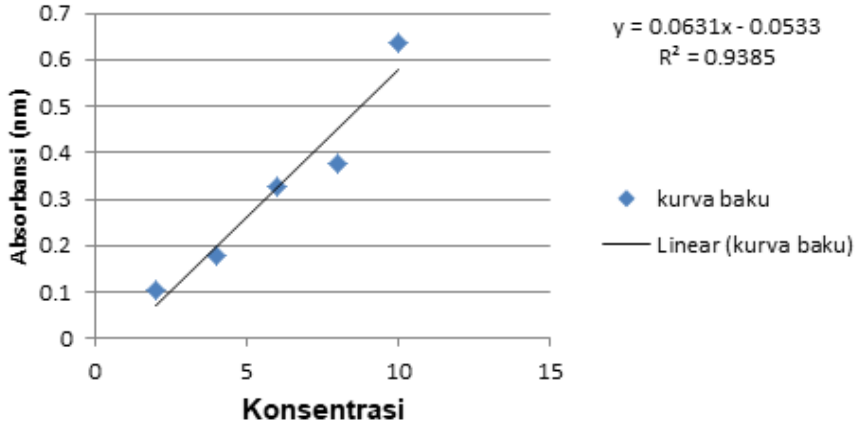

Figure 1. Quaarcetine Raw Curve

The magnitude of this linearity is close to the value or so it can be said that absorbance is a function whose magnitude is directly proportional to concentration and follows the linear regression equation as follows: y = ax ± b. After a qualitative test was carried out on the total flavonoid content, the flavonoid content was then tested on kersen leaf samples using UV-Vis spectrophotometry. The results of sample measurements can be seen in table 3.

Table 3. Kersen (*Muntingia calabura* L.) Leaf Extract Test Results

Sample	Absorbansi
Ethanol Extract 70%	0.460
Ethyl Acetate extract	0.472
n-hexane extract	0.545

The positive extract contained flavonoids in the screening test, then the total flavonoid content was tested. Measurements on total flavonoids are used with the principle of $AlCl_3$ which will form a complex because it has a C-4 keto group and then with a neighboring C-3 or C-5 hydroxyl group so that there is a visible shift from the yellow color in the solution (Ukieyanna E., 2012). Based on the results of flavonoid measurements using uv-vis spectrophotometry with a wavelength of 422 nm, the total content of flavonoids for 70% ethanol extract samples was 8.13 (mgQE/g), ethyl acetate extract samples were 8.32 (mgQE/g) and n-hexane extract samples were 18.96 (mgQE/g).

Quantitative antioxidant testing was carried out using the DPPH method. DPPH is a free radical that is stable at room temperature. The principle of this method is to measure the fading of color from DPPH radicals due to the presence of antioxidants that can neutralize free radical molecules. DPPH in its non-radical form will lose its purple color due to the

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inclusion of hydrogen atoms in unpaired atoms. The color change can be measured by absorbance using a UV-Vis spectrophotometer. The initial stage of testing the antioxidant activity of the sample was by measuring the optimization of the maximum wavelength of DPPH 0.4 mM at a wavelength of 450-550 nm. The results of wavelength optimization showed that the maximum absorbance of DPPH free radicals was at a wavelength of 516.5 nm. The antioxidant activity of each extract can be seen by the inhibition activity (%) where the very strong IC_{50} value is in the range below µg/mL, strong 50-100 µg/mL, medium 100-250 µg/mL, weak 250-500 µg/mL. The results of the antioxidant activity test of each extract can be seen in table 4.

Table 4. Results of Antioxidant Test of N-Hexane Extract of Kersen Leaves (*Muntingia calabura* L.)

Concentration (ppm)	Blanko	Absorbansi	Inhibition activity (%)	IC_{50} Value (µg/mL)
8	0.769	0.551	28.34	
20	0.769	0.378	50.84	
33	0.769	0.236	69.31	21.334307
44	0.769	0.154	79.97	
56	0.769	0.126	83.61	

Based on the results of absorbance in table 6, after making a relationship curve between the concentration of n-hexane extract of kersen leaves and (%) inhibition, the regression equation y = 1.1639x + 25.169 was obtained. From the regression equation that has been obtained, the value of x (IC_{50}) 21.334307 µg/mL is obtained. The IC_{50} value of n-hexane extract of kersen leaves obtained was classified as an antioxidant with a very strong intensity (<50 µg/mL).

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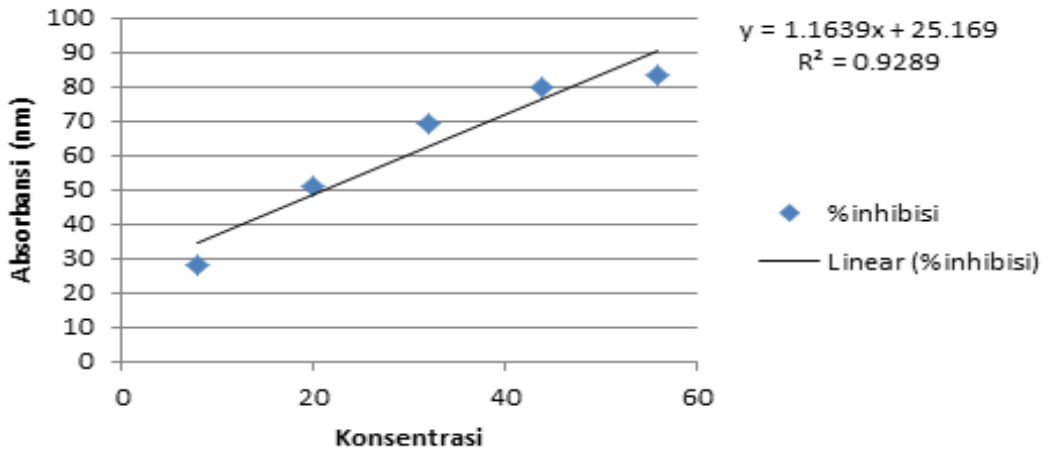

Figure 2. n-Hexane Extract Curve

Tabel 5. Results of Antioxidant Test of Ethyl Acetate Extract of Kersen Leaves (*Muntingia calabura* L.)

Concentration (ppm)	Blanko	Absorbansi	Inhibition activity (%)	IC_{50} Value (µg/mL)
10	0.880	0.630	28.40	
20	0.880	0.550	37.50	
30	0.880	0.469	47.15	36.050687
40	0.880	0.409	63.52	
50	0.880	0.355	85.65	

The results of the antioxidant test of ethyl acetate extract of kersen leaves, after making a relationship curve between the concentration of ethyl acetate extract of kersen leaves and the percent (%) inhibition, the regression equation y = 0.7852x +21.693 was obtained. From the regression equation that has been obtained, the value of x (IC_{50}) = 36.050687 µg/mL is obtained. The IC_{50} value of ethyl acetate extract of kersen leaves obtained is classified as an antioxidant with a very strong intensity (<50 µg/mL).

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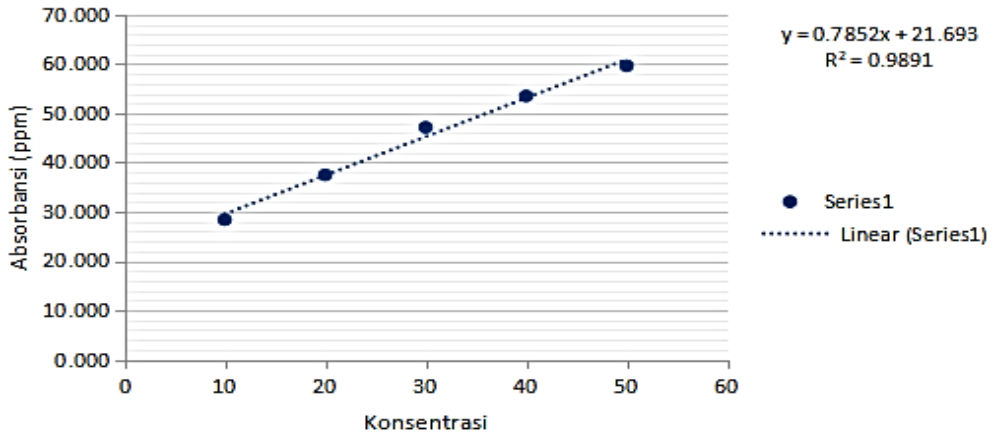

Figure 3. Ethyl Acetate Extract Curve

Table 6. Results of Antioxidant Test of Kersen Leaf Ethanol Extract

Concentration (ppm)	Blanko	Absorbansi	Inhibition activity (%)	IC_{50} Value (µg/mL)
1	0.880	0.773	12.15	
4	0.880	0.652	25.90	
7	0.880	0.517	41.25	7.568056
10	0.880	0.265	69.88	
13	0.880	0.148	83.18	

The results of the antioxidant test of kersen leaf ethanol extract, after making a relationship curve between the concentration of kersen leaf ethanol extract and the percent (%) inhibition, the regression equation y = 6.2008x + 3.072 was obtained. From the regression equation that has been obtained, the value of x (IC_{50}) = 7.568056 µg/mL is obtained. Based on the above results, the IC_{50} value of the ethanol extract of kersen leaves obtained is classified as an antioxidant with a very strong intensity (<50 µg/mL). Meanwhile, according to the results of the study according to Sitompul and Sutriningsih (2017), the IC_{50} value of ethanol extract was obtained of 79.320 µg/mL which is quite strong. The difference in IC_{50} values is suspected to be due to previous studies using 4 mg of DPPH so that the level of free radicals that must bind to antoxidants is even greater.

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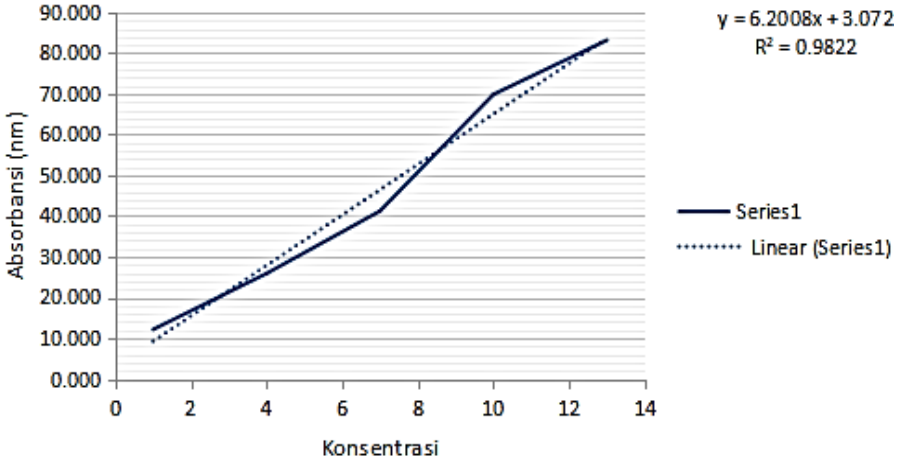

Figure 4. 70% Ethanol Extract Curve

CONCLUSION

In the measurement of the total flavonoid content using spectrophotometry, the results obtained in 70% ethanol extract were 8.13 (mgQE/g), ethyl acetate samples were 8.32 (mgQE/g) and n-Hexane samples were 18.96 (mgQE/g). Meanwhile, in the discussion and calculation of antioxidant activity, it can be concluded that 70% ethanol extract, ethyl acetate and n-hexane of kersen leaves have antioxidant activity with IC_{50} values of 7.568056 µg/mL (very strong category), 36.050687 µg/mL (very strong category) and 21.334307 µg/mL (strong category), meaning that 70% ethanol extract of kersen leaves has the greatest antioxidant activity compared to ethyl acetate extract and n-hexane extract.

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