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Chemometric Analysis of Phytochemical Content and Antioxidant Activity of Ethanol Extract 70% and Ethyl Acetate of Kersen Leaf (*Muntingia calabura* L.)

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Abstract: This study analyzed the phytochemical content and antioxidant activity of kersen leaf extract (*Muntingia calabura* L.) obtained with 70 % ethanol and ethyl acetate through a chemometric approach. Extraction is carried out by maceration, total flavonoids are determined by UV-Vis spectrophotometry with quercetin, while total phenolics with gallic acid. Antioxidant activity was tested using the DPPH method and the IC_{50} value was calculated. The results showed that the total flavonoid content in 70% ethanol extract was 3.374 ± 0.082 mg EK/mL, while in ethyl acetate extract it was 2.726 ± 0.014 mg EK/mL. The total phenolic content in 70% ethanol extract was 152.34 ± 5.71 mg EAG/mL, while in ethyl acetate extract it was 262.78 ± 6.27 mg EAG/mL. The highest antioxidant activity was obtained from ethyl acetate extract with an IC_{50} value of 34.45 ± 1.23 µg/mL, while ethanol extract 70% showed an IC_{50} value of 42.35 ± 7.22 µg/mL. Chemometric analysis using *Principal Component Analysis* (PCA) showed a relationship between the 70% ethanol flavonoid content and ethyl acetate on the antioxidant activity of the DPPH method.

Keywords: *Muntingia calabura* L.flavonoids; phenolics; chemometrics; DPPH

1. Introduction

Indonesia is a country rich in biodiversity, including in terms of medicinal plants. According to the results of Basic Health Research in 2010, it was recorded that the prevalence of Indonesian people over the age of 15 who have consumed traditional medicine, as much as 59.12% spread across various regions in Indonesia (Adiyasa & Meiyanti, 2021). One of the plants that is starting to attract attention and has the potential as a source of natural materials with benefits in health research is kersen leaves *(Muntingia calabura* L.*)* (Panaungi & Hasma, 2022). This plant is often found in various tropical regions, including Indonesia, and has long been used traditionally to treat a variety of health problems (Rahmadani *et al.*, 2022).

Cherry leaves *(Muntingia calabura* L.*)* It is known to contain a number of phytochemical compounds that have the potential to be therapeutic agents. Phytochemicals are compounds produced by plants that have biological activity (Widodo) *et al.*, 2019), including antioxidant, antimicrobial, anti-inflammatory, analgesic, anti-cancer, and antiplatelet (Fang *et al.*, 2022). Annisa & Najib (2022)) stated that the phytochemical content contained in kersen leaves includes flavonoids of 13.375 mg EK/g, tannins of 13.715 mg EAG/g. Puspitasari (2017) also stated that the total phenolic content of kersen leaf extract with 70% ethanol solvent was obtained at 1,163 mg of EAG/mL extract. This phytochemical content provides the potential for kersen leaves to be developed as a natural ingredient that is useful in the prevention and treatment of various diseases (Vonna *et al.*, 2021).

One of the important activities of phytochemicals in plants is antioxidants. Antioxidants have the ability to protect the body from oxidative damage triggered by free radicals that are a major factor in various degenerative diseases, such as cancer, diabetes, and premature aging (Besan *et al.*, 2015, Jang & Lee, 2023). The antioxidant activity of plant extracts is often measured using various laboratory methods, one of which is by the DPPH test (*1,1-diphenyl-2-pikrilhydrazil*), which is one way to measure the ability of compounds to reduce or neutralize free radicals (HDT, 2023). Munteanu & Apetrei (2021) proves that the application of

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antioxidant activity tests using the DPPH method in this study is because it has several advantages, including low cost, easy to conduct, and high reproducibility.

Several studies have been conducted to identify the antioxidant activity of kersen leaf extract using the DPPH method (Ocean *et al.*, 2023), but no one has studied the relationship between the phytochemical content and the antioxidant activity of kersen leaf extract. In addition, the analysis of phytochemical content and antioxidant activity involves complex data processing, which requires a proper scientific approach in order to reveal a valid correlation between phytochemical components and the demonstrated biological effects (Kucharska-Ambrożej & Karpinska, 2020).

Chemometric analysis can be used as an effective method in exploring the relationship between phytochemical content and antioxidant activity of kersen leaf extract (Risal, 2020). Chemometrics is a discipline that uses statistical and mathematical approaches to analyze complex and multidimensional data, such as data from the extraction of phytochemical compounds and measurements of antioxidant activity (Risal, 2022). Chemometric analysis can provide more in-depth information about the correlation between the various phytochemical compounds contained in kersen leaf extract and the level of antioxidant activity (Arina *et al.*, 2022). Based on this background, the researcher is interested in conducting further research on the chemometric analysis of the phytochemical content and antioxidant activity of 70% ethanol extract and ethyl acetate of kersen leaf *(Muntingia calabura* L.*)*.

2. Research Methods

This research was conducted experimentally at the ITEKES Cendekia Utama Pharmaceutical Laboratory with a quantitative descriptive approach. The material in the form of fresh kersen leaves is collected from the Blora area, Central Java, then dried in the shade and ground into powder. Simplicia powder is then extracted using two types of solvents: 70% ethanol and ethyl acetate. The extraction process was carried out by the maceration method for three times 24 hours with a ratio of simplicia and solvent of 1:10. Flavonoid levels were determined by UV-Visible spectrophotometry method using aluminum chloride reagents at a wavelength of 373 nm, using quercetin as the standard. Phenolic levels were determined by the *Folin-Ciocalteu* method at a wavelength of 760 nm, using gallic acid as the standard. Antioxidant activity was evaluated using the DPPH method by measuring the decrease in absorbance at a wavelength of 515 nm. The IC_{50} is calculated from a graph of the relationship between the concentration of the sample and the percentage of inhibition. All test results were then statistically analyzed with Minitab version 19 software using *Principal Component Analysis* (PCA).

3. Results and Discussion

Research Results

Plant Determination Results

Cherry leaves (*Muntingia calabura* L.) used in this study was determined at the Biology Laboratory of Ahmad Dahlan University with the number 064/Lab.Bio/B/I/2025. The determination results showed that the analyzed specimens had morphological characteristics that were consistent with (*Muntingia calabura* L.) based on references from the Flora of Java (Steenis, 1958). The results of the determination carried out are as follows:

1b – 2b – 3b – 4b – 6b – 7b – 9b -10b – 11b – 12b – 13b – 14a – 15a – 109b – 119b – 120b – 128b – 129b – 135b – 136b – 139b – 140b – 142b – 143b – 146b – 154b – 155b – 156b – 162b – 163b – 167b – 169b – 171b – 177b – 179a – 180b – 182b – 183b – 184b – 185b – 185b – 186b
Tiliaceae
1a Muntingia
Muntingia calabura L.

Results of Determination of Moisture Content of Simplisia Powder

Table 1. Yield of Kersen Leaf Extract

Material	Powder weight	Replication I	Replication II	Replication III	Average	Information
Cherry leaves	1 g	3,97%	3,97%	3,97%	3,97%	Qualify

Extract Results

Table 1. Kersen Leaf Extract Results

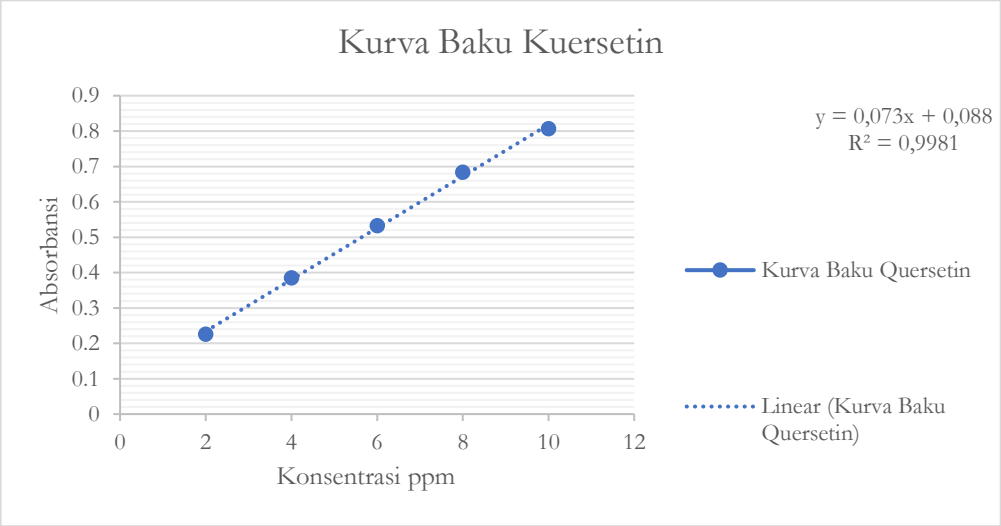
Solvent	Powder Weight	Extract Weight	Extract Yield	Information
Ethyl Acetate 2L	200 g	30,162 g	15,081%	Qualify
Ethanol 70% 2L	200 g	23,094 g	11,547%	Qualify

Ethanol-Free Test Results

Table 3. Ethanol-Free Test Results

Extract	Reagents	Result	Conclusion
Ethanol 70% Cherry Leaves	Extract + CH3COOH + H2SO4	No ester smell	Ethanol-Free
According to the Indonesian Pharmacopoeia Edition VI (2020), the requirement for an ethanol-free test is that there is no ester odor			

Total Flavonoid Yield

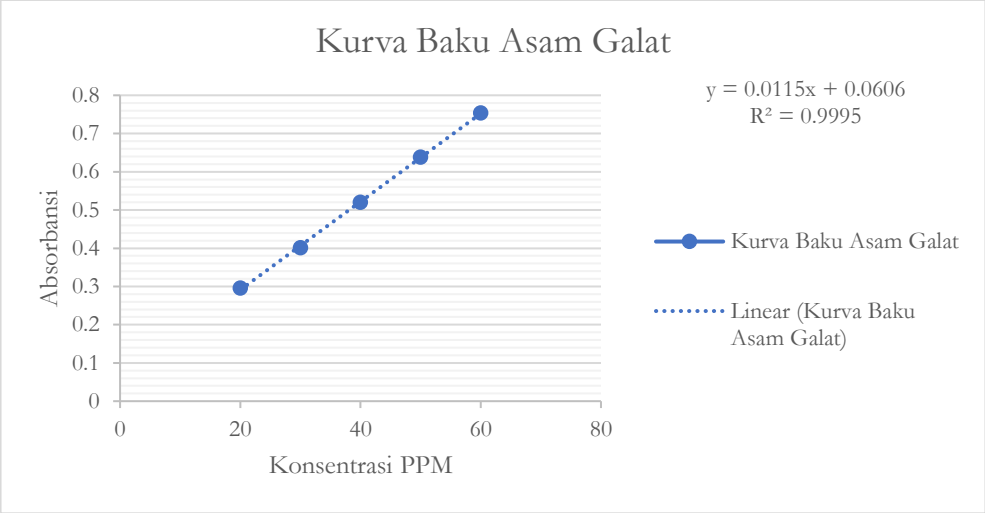


Picture 1. Flavonoid Comparator Quercetin Calibration Curve

Table 4. Total Flavonoid Yield

Solvent	Replication	Absorbance Value	Flavonoid Content (mg EK/ml)	Average flavonoid content ± SD
Ethyl Acetate	1	0,286	2,712	2.726 ± 0.014
	2	0,287	2,726	
	3	0,288	2,739	
Ethanol 70%	1	0,335	3,383	3,374 ± 0.082
	2	0,340	3,452	
	3	0,328	3,287	

Total Phenolic Results



Picture 2. Phenolic Comparator Gallic acid Calibration Curve

Table 5. Total Phenolic Results

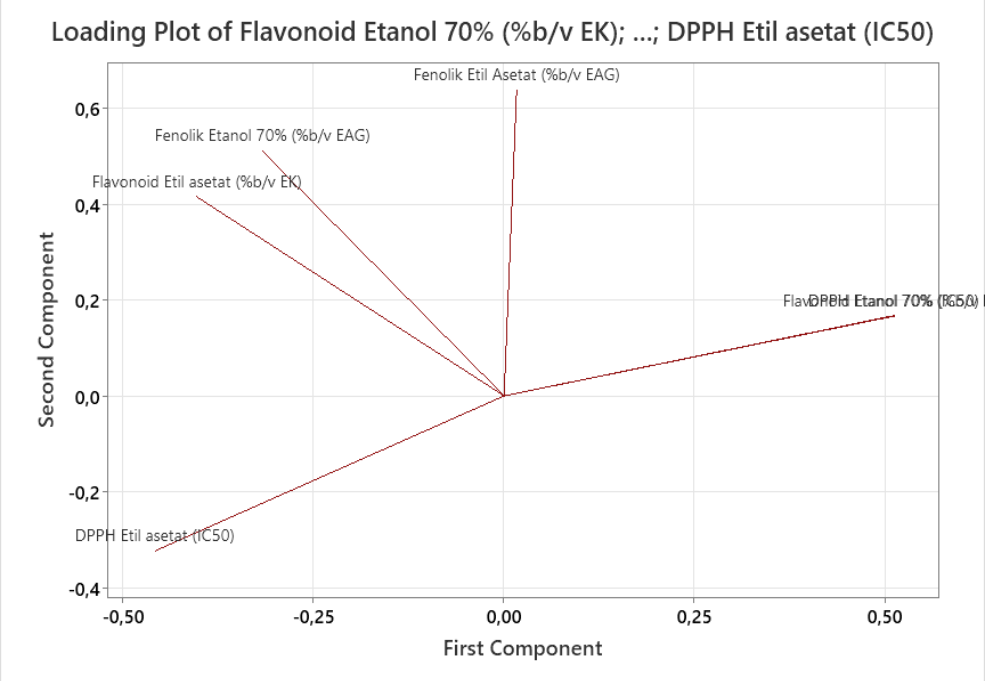
Solvent	Replication	Absorbance Value	Phenolic content (mg EAG/ml)	Average phenolic content ± SD
Ethyl Acetate	1	0,356	256,69	262.78 ± 6.27
	2	0,369	268,00	
	3	0,364	263,65	
Ethanol 70%	1	0,228	145,39	152.34 ± 5.71
	2	0,238	154,08	
	3	0,242	157,56	

Results of Antioxidant Activity

Table 6. Results of Antioxidant Activity of the DPPH Method

Extract	IC_{50} value (µg/ml)			Average (µg/ml) ± SD	Antioxidant Categories
	Rep 1	Rep 2	Rep 3		
Ethanol 70% Cherry Leaves	43,15	49,14	34,76	42.35 ± 7.22	Very Powerful
Ethyl Acetate Cherry Leaf	34,69	33,11	35,54	34.45 ± 1.23	Very Powerful
Kuersetin	3,68	3,67		3.675 ± 0.0071	Very Powerful

Chemometric Analysis Results
Loading PLot



Picture 3. Loading Plot

Discussion
Plant determination

The kersen plant (*Muntingia calabura* L.) used in this study has gone through the process of determination or taxonomic identification at the Biology Laboratory, Ahmad Dahlan University, Yogyakarta. This aims to ensure the accuracy and correctness of the plant species used in the research, with identification number 064/Lab.Bio/B/I/2025. This determination is based on the key of taxonomic determination that leads to species with specific characteristics. The results of the determination showed that the plant was *Muntingia calabura* L. From the Tiliancea family (Muntingiaceae). This determination is taxonomically valid and according to the morphological character of the observed plant.

Ethanol-Free Extraction and Testing

Extraction using the maceration method is carried out because it is able to prevent damage to active substances caused by heating (Ministry of Health of the Republic of Indonesia, 2000). Kersen leaf plant powder is irrigated using 70% ethanol and ethyl acetate

solvents. Ethanol is 70% effective in extracting polar flavonoid compounds, while ethyl acetate is more effective in extracting semi-polar phenolic compounds (Khairunnisa et al., 2022). Based on the results obtained, as many as 200 g of kersen leaf powder extracted using ethyl acetate solvent produced 30.162 g of thick extract with a yield of 15.08%. Extraction with ethanol solvent 70% of the same amount of powder yielded 23,094 g of viscous extract with a yield of 11,547%. Factors that can affect yield are the length of extraction and the accuracy of the length of time used to extract (Apriliana) *et al.,* 2020).

Ethanol-free testing is important because the presence of organic solvents can affect the safety of the extract for further use (Aulani, 2019). Based on the results of organoleptic observations, 70% ethanol extract of kersen leaves showed no ester odor after the addition of reagents indicating that the extract was free of ethanol solvent residues, in accordance with the requirements of the Indonesian Pharmacopoeia Edition VI by BPOM RI (2020).

Determination of Phytochemical Levels

Total Flavonoids

The determination of total flavonoid levels was carried out using the UV-Vis spectrophotometry method with quercetin as standard. Measurements were made at the maximum wavelength obtained, which was 373 nm with an observation time (Operating Time) of 17 minutes after the reaction. The determination of the quercetin standard curve is made with a series of 2, 4, 6, 8, 10 ppm levels. The determination of flavonoid levels is based on the calibration curve shown in figure 4.1, with the regression equation Y = 0.073x + 0.088 and the determination coefficient R2 = 0.9981 this figure is close to 1 which indicates a linear calibration curve and there is a relationship between the concentration of quercetin solution and the absorption value. This linear regression equation is used to calculate the total flavonoid content in the ethanol extract of 70% kersen leaves.

The determination of total flavonoid levels was carried out three times replicated in each solvent for data accuracy. Based on the results of this study, the average total flavonoid content of ± SD of kersen leaf extract with ethyl acetate solvent was 2.726 ± 0.014 mg EK/ml of extract, meaning that in each ml of kersen leaf extract there are flavonoids equivalent to 2.726 mg of quercetin. Obtained 3.374 ± 0.0828 mg EK/ml of extract obtained the average total flavonoid content of kersen leaf extract with 70% ethanol solvent, meaning that in each ml of kersen leaf extract there are flavonoids equivalent to 3.374 mg of quercetin.

In a previous study by Annisa (2022), the total flavonoid content of kersen leaf extract with ethanol solvent was reported to be 13.375 mg QE/g extract. The flavonoid content in the 70% ethanol solvent was 3.374 ± 0.0828 mg QE/mL extract. These differences in results can be due to variations in extraction methods, extract solvents, and extraction duration (Velisdeh et al., 2021).

Based on the results of the study, ethanol solvents are 70% more effective in extracting flavonoids from kersen leaves than ethyl acetate solvents. These results show that the type of solvent has an effect on the amount of flavonoids extracted.

Total phenolics

The determination of total phenolic levels was carried out using *the Folin Ciocalteu method* with gallic acid as the standard. Absorbance readings were carried out at the maximum wavelength obtained which was 760 nm, with an observation time (Operating Time) of 25 minutes after the reaction took place. Gallic acid solution was measured with concentrations of 20 ppm, 30 ppm, 40 ppm, 50 ppm, and 60 ppm, then the absorbance of the standard solution was obtained at each concentration. A linear line was obtained which would later be used to determine the total phenolic level The determination of phenol levels was based on a calibration curve, and the regression equation y = 0.0115x + 0.0608 and the value of the determination coefficient R2 = 0.9995 which means that 99.9% of the absorption was influenced by concentration, as shown in figure 4.2.

Based on the results of the study, the average total phenolic content of ± SD in kersen leaf extract using ethyl acetate solvent was 262.78 ± 6.27 mg of EAG/ml of extract, which shows that each ml of phenolic content is equivalent to 262.78 mg of gallic acid per gram of extract. Kersen leaf extract with 70% ethanol solvent yielded a total phenolic content of 152.34 ± 5.71 mg of EAG/ml of extract, which means that each ml of extract contains phenolic compounds equivalent to 152.34 mg of gallic acid.

In a previous study by Puspitasari (2017), the total phenolic content of kersen leaf extract with 70% ethanol solvent was obtained at 1,163 mg of EAG/mL extract. The 70% ethanol extract in this study produced 152.34 ± 5.71 mg EAG/mL and ethyl acetate extract produced

262.78 ± 6.27 mg EAG/mL. This value is much higher than previous studies, which can be due to differences in solvent types, and extraction conditions (Ezez & Tefera, 2021). Based on these results, ethyl acetate solvent is more effective in extracting phenolic compounds from kersen leaves than 70% ethanol.

Results of Antioxidant Activity of the DPPH Method

This test aims to determine the antioxidant activity of two types of kersen leaf extract (Muntingia calabura L.), namely 70% ethanol extract and ethyl acetate extract, which was tested using the DPPH (2,2-diphenyl-1-picrylhydrazyl method). This method measures the ability of the compound to capture free radicals through a decrease in the absorbance of the DPPH solution at the maximum wavelength obtained which is 515 nm and with an operating time of 37 minutes. The parameter used to assess the strength of antioxidant activity is the IC_{50} value, which is the concentration required to dampen 50% of DPPH radicals. The lower the IC_{50} value, the stronger the antioxidant activity of a compound or extract. Based on the general classification used in the DPPH test, antioxidant activity is divided into several categories, namely: very strong (IC_{50} < 50 μg/mL), strong (IC_{50} 50-100 μg/mL), moderate (IC_{50} 101-150 μg/mL), and weak (IC_{50} > 150 μg/mL). Based on this classification, both kersen leaf extracts have very strong antioxidant activity, because the IC_{50} value is below 50 μg/mL (Sami et al., 2017). The results of the antioxidant activity test of both extracts as well as the benchmark standard (quercetin) are presented in Table 4.8.

In figure 4.8, it is known that the average IC_{50} value for ethyl acetate extract is 34.45 ± 1.23 μg/mL, while 70% ethanol extract has an IC_{50} value of 42.35 ± 7.22 μg/mL. Although both fall into the same category, ethyl acetate extract has a lower IC_{50} value. This can be due to ethyl acetate as a semi-polar solvent that is more selective in extracting antioxidant compounds such as flavonoids and phenolics, which are known to contribute greatly to free radical scavenging activity. Ethanol extracts of 70% showed higher IC_{50} values with greater variation between data (SD = ±7.22 μg/mL). This is due to the nature of ethanol as a polar solvent that can extract compounds in various polarity groups, so the resulting extract contains a more complex mixture of active and inactive compounds.

In a previous study by Puspitasari (2017), kersen leaf ethyl acetate extract was reported to have an IC_{50} value of 53.254 μg/mL, while in this study a value of 34.45 ± 1.23 μg/mL, which means that the antioxidant activity is stronger. This can be caused by differences in extraction conditions, material variations, and post-harvest and storage conditions, e.g. drying and pre-extraction treatment that affect the stability of phenolic compounds and flavonoids.

As a positive control, quercetin was used to compare the effectiveness of the extract with pure antioxidant compounds. The results showed that quercetin had an IC_{50} value of 3.675 ± 0.0071 μg/mL with a very strong category compared to the two kersen leaf extracts. This value is in accordance with the literature that states that quercetin is a powerful flavonoid with a polyphenol structure that is very effective in neutralizing free radicals through the donation of electrons or hydrogen atoms (Carrillo-Martinez et al., 2024). Quercetin is used as a positive control because it is one of the main flavonoid compounds contained in kersen leaves, and the selection of quercetin as a comparator is based on the similarity of its chemical structure to the antioxidant compounds in the extract, as well as its good solubility in semipolar solvents (Cunico et al., 2020).

Chemometric Analysis

The analysis of the relationship between flavonoid levels, phenolics, and antioxidant activity was carried out using *the Principal Component Analysis* (PCA) method with Minitab software. PCA is used by converting a number of interrelated variables into several *principal components that* are not correlated with each other. This helps to summarize important information from the entire data by using fewer new variables, but still covering most of the variation in the data (Risal, 2022).

Two vectors that form an angle of less than 90° show a positive correlation, and two vectors that form an angle of more than 90o show a negative correlation. Flavonoids of 70% ethanol and DPPH of 70% ethanol (IC_{50}) have overlapping vectors that show a very strong positive correlation, meaning that the higher the flavonoid content, the better the antioxidant activity (the lower the IC_{50}). Phenolic ethanol 70% and DPPH ethanol 70% have a vector angle of more than 90° indicating a negative correlation. Ethyl acetate flavonoids and DPPH ethyl acetate have a vector angle of less than 90° which shows a strong positive correlation,

meaning the higher the flavonoid content, the better the antioxidant activity. Flavonoids 70% ethanol and ethyl acetate correlated with the antioxidant activity of the DPPH method.

4. Conclusions and Suggestions

This study showed that the highest total flavonoid content was 70% ethanol-solvent kersen leaf extract of 3.374 ± 0.082 mg EK/mL, the highest total phenolic content of ethyl acetate solvent kersen leaf extract of 262.78 ± 6.27 mg EAG/mL, and the highest antioxidant activity result of the DPPH method was ethyl acetate solvent kersen leaf extract of 262.78 6.27 mg EAG/mL, and the highest antioxidant activity result of the DPPH method was ethyl acetate solvent kersen leaf extract of 262.78 6.27 mg EAG/mL. 34.45 ± 1.23 µg/ml with a very strong antioxidant category. Loading plot analysis on chemometrics showed a correlation between 70% ethanol flavonoids and ethyl acetate with the antioxidant activity of the DPPH method. Based on this study, it is recommended that the formulation of kersen leaf extract preparations be carried out, and further research in vivo on animals.

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