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Antioxidant Activity Test of Ethanol Extract of Kesum Leaves *(Polygonum Minus Huds)* Using the DPPH Method

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Kesum leaves (*Polygonum minus* Huds.) are herbal plants with potential as natural sources of antioxidants. This study aimed to evaluate the antioxidant activity of the ethanol extract of kesum leaves using the DPPH method and to identify the types of secondary metabolites present. Extraction was performed by maceration using 70% ethanol. Phytochemical screening was conducted qualitatively, while antioxidant activity was determined based on IC_{50} values measured using a UV–Vis spectrophotometer at a wavelength of 517 nm, with vitamin C used as a reference standard. The results showed that the ethanol extract of kesum leaves contained alkaloids, flavonoids, tannins, triterpenoids, and steroids. The antioxidant activity test revealed that the ethanol extract had an IC_{50} value of 7.9241 µg/mL, which was classified as having very strong antioxidant activity. In comparison, vitamin C exhibited a lower IC_{50} value of 3.2886 µg/mL, indicating stronger antioxidant activity. These findings indicate that although the antioxidant activity of the ethanol extract of kesum leaves is lower than that of vitamin C, the extract still falls into the category of very strong antioxidants and has potential as a natural antioxidant source.
Keywords: Antioxidant Activity Test, Ethanol Extract, Kesum Leaves, DPPH Method

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1. Introduction

Alternative medicine using herbal plants has attracted significant public attention and has developed rapidly. Herbal medicinal plants are widely distributed across almost all regions of Indonesia. People utilize these plants to overcome both physical and mental health disorders. Indonesia has various plants that are used in herbal medicine, one of which is kesum leaves (Polygonum minus Huds.) [1].

Kesum, scientifically known as Polygonum minus Huds., is an endemic plant found in West Kalimantan. In Kalimantan, this plant is widely recognized by the local community as an important biological resource. Empirically, kesum leaves have been used as herbal medicine. Kesum leaves are considered a potential source of natural antioxidants due to their high content of flavonoid and phenolic compounds [2].

Several previous studies have reported that kesum leaf extract exhibits strong antioxidant activity, as indicated by its low IC_{50} value, which reflects an excellent ability to scavenge free radicals [3]. Most earlier investigations on the antioxidant potential of kesum leaves mainly used methanol as the extraction solvent and consistently demonstrated high antioxidant activity [3]. However, the type of solvent used can significantly influence extraction outcomes and antioxidant capacity, since solvent polarity affects the amount of active compounds extracted. Studies evaluating the antioxidant activity of kesum leaves using ethanol, particularly 70% ethanol, remain limited compared to those using methanol. In addition, differences in solvent concentration and extraction conditions may lead to variations in IC_{50} values reported across

studies. Therefore, further research is necessary to evaluate the antioxidant activity of kesum leaf ethanol extract using the DPPH method as an effort to develop natural antioxidant sources.

One commonly used qualitative phytochemical screening method is the color reaction test using specific reagents. In this method, natural samples are tested with certain reagents that produce characteristic color changes when specific secondary metabolites are present. For instance, particular reagents can be used to detect the presence of flavonoids, alkaloids, tannins, saponins, and other compounds. The observed color changes provide preliminary indications of the presence of these compounds in the sample [4].

Antioxidants are substances that play an important role in protecting and maintaining cellular stability from damage caused by free radicals generated through oxidative stress processes. Antioxidants function by neutralizing free radicals, converting them into more stable metabolic products that can later be eliminated from the body. The intake of antioxidant-rich foods, such as fruits and vegetables, is known to help reduce the risk of diseases associated with oxidative stress. Adequate consumption of antioxidants is also necessary to prevent an increase in free radicals in the body [5].

Furthermore, oxidative stress can trigger various cellular processes such as vasodilation, signal transduction, cell differentiation, development, and the onset of degenerative diseases. Antioxidants can prevent tissue and cellular damage caused by free radicals. Commonly used antioxidants include synthetic antioxidants; however, the use of synthetic antioxidants may cause several health problems, such as rheumatoid arthritis, cancer, and premature aging. Therefore, it is necessary to replace synthetic antioxidants with natural antioxidants derived from natural sources [6].

The DPPH (1,1-Diphenyl-2-Picrylhydrazyl) method is one of the testing techniques used to evaluate the effectiveness of antioxidant compounds in scavenging free radicals. This measurement approach is characterized by its simplicity, rapid procedure, and limited use of chemicals. In this method, DPPH radicals react with antioxidant compounds, resulting in the formation of DPPH-H and new antioxidant radicals. Antioxidant compounds have the ability to donate hydrogen atoms to DPPH radicals, filling the electron deficiency, and ultimately forming stable or non-radical antioxidant products. The antioxidant activity test using the DPPH method can be conducted in vitro [7].

Research on kesum plants (Polygonum minus Huds.) has a significant impact and contributes to the utilization of kesum leaves as a basic ingredient for traditional medicine. Therefore, this study aims to determine the antioxidant activity of kesum leaf extract using the DPPH method with ethanol as the solvent.

2. Method

The research used an experimental method, which included the collection and identification of plant material, preparation of simple preparations, characterization of simple preparations, preparation of extracts, and testing the antioxidant activity of kesum (Polygonum minus Huds.) leaf extract. This research was conducted at the Prima Indonesia University Laboratory. The study population was kesum (Polygonum minus Huds.) leaves obtained from Deli Serdang Regency, while the sample used was the ethanol extract of kesum leaves.

The data collection method in this study utilized various tools and materials. The tools used for extraction included a maceration kit, including a beaker, measuring cylinder, volumetric flask, glass funnel, filter paper, stirring rod, dark-colored bottle, blender, electric scale, and water bath. To determine antioxidant activity, a set of glassware, test tubes, measuring pipettes, a stopwatch, a micropipette, and a UV-VIS spectrophotometer were used. For chemical reaction tests, glassware, test tubes, measuring cylinders, dropper pipettes, and a water bath were used.

The materials used in this study were dried kesum leaves *(Polygonum minus Huds.)* obtained from the Medan Krio area, Deli Serdang Regency, along with 70% ethanol, methanol, DPPH powder, ethanol, and vitamin C.

Procedure

Raw Material Preparation

Collected kesum leaves *(Polygonum minus Huds.)* were wet sorted by washing to remove any remaining impurities. Dry sorting was then performed. The samples were then dried in a drying cabinet at ± 50°C. They were then ground into powder using a blender until smooth and ready for extraction [8].

Preparation of Kesum Leaf Extract

A 300-gram sample of the dried kesum leaves was weighed and placed in a maceration vessel for maceration. Three liters of solvent were then added until the leaves were completely submerged. Extraction was carried out by sequentially soaking the samples in 70% ethanol. Then, it was re-macerated for 3 days with occasional stirring, then filtered using filter paper to separate the pulp and filtrate. The liquid ethanol extracts were then concentrated using a rotary vacuum evaporator at 50°C to obtain a 70% ethanol extract [8].

Simplicia Formula Characterization Examination

Simplicia formula characterization examination included determining water content, water-soluble extract content, ethanol-soluble extract content, total ash content, and acid-insoluble ash content.

Water Content Determination

a. Toluene Saturation
   200 ml of toluene was placed in a round-bottom flask, 2 ml of distilled water was added, and the apparatus was then set up and distillation was carried out for 2 hours. The distillation was stopped and allowed to cool for approximately 30 minutes. The volume of water in the receiving tube was then read to the nearest 0.1 ml.
b. Determining the Water Content of the Simplex
   5 g of accurately weighed simplex powder was added to the toluene flask and heated gently for 15 minutes. After the toluene boiled, the toluene rate was adjusted to 2 drops per second until most of the water had distilled off. The rate was then increased to 4 drops per second. After all the water had distilled off, the inside of the condenser was rinsed with toluene. The distillation was continued for 5 minutes, and the receiving tube was allowed to cool to room temperature. After the water and toluene had completely separated, the water volume was read to the nearest 0.1 ml. The difference between the two water volumes corresponded to the water content of the material being tested. The water content was calculated as a percentage.

Moisture Content Formula:
Moisture Content (%) = (Final volume of distilled water obtained / Weight of the weighed simplicia sample) × 100

Determination of Water-Soluble Extract Content

Five grams of powdered medicinal plants were placed in a stoppered flask and macerated with 100 ml of water-chloroform (2.5 ml of chloroform in 1000 ml of water) for 24 hours, with occasional shaking for the first 6 hours, then allowed to stand for 18 hours. After filtering, 20 ml of the filtrate was evaporated to dryness in a shallow, flat-bottomed, tared dish. The remainder was heated at 105°C until a constant weight was achieved. The water-soluble extract content was calculated based on the dried material.

Water-Soluble Extract Formula:
Water-soluble extract = (weight of water-soluble extract (g))/(weight of the drug (g)) x 100/20 x 100%

Determination of Ethanol-Soluble Extract

5 g of powdered drug was placed in a stoppered flask, macerated with 100 ml of 96% ethanol for 24 hours, with occasional shaking for the first 6 hours, and then left for 18 hours. The filtrate was filtered, and 20 ml of the filtrate was evaporated to dryness in a shallow, flat-bottomed, tared dish. The remainder was heated at 105°C until a constant weight was reached. The ethanol-soluble extract was calculated based on the dried material.

Ethanol-Soluble Extract Formula:

Ethanol-soluble extract = (weight of ethanol-soluble extract (g)) / (weight of the drug (g)) x 100/20 x 100%

Determination of Total Ash Content

A 2 g of accurately weighed powder is placed in a tared porcelain crucible, then leveled. The crucible is heated until a constant weight is achieved. The ash content is calculated for the air-dried material.
Ash Content Formula:

Total ash content = (weight of ash (g)) / (weight of initial sample (g)) x 100%

Determination of Acid-Insoluble Ash Content

The ash obtained in the total ash content determination is boiled in 25 ml of 2N hydrochloric acid for 5 minutes. The insoluble portion is collected, filtered through ash-free filter paper, and washed with hot water. The residue and filter paper are heated until a constant weight is reached, then cooled and weighed. The acid-insoluble ash content is calculated based on the dried material.
Acid-insoluble ash content formula:

Acid-insoluble ash content = (ash weight (g))/(raw material weight (g)) x 100%

Phytochemical Screening Identification

a. Alkaloid Test
   Performed using Dragendorff's reagent. 1 ml of extract is placed in a test tube, 0.5 mL of 2% HCl is added, and the mixture is shaken until homogeneous. Then, 2–3 drops of Dragendorff's reagent are added to the tube. If a brown precipitate forms in the tube, the sample contains alkaloids [9].
b. Flavonoid Test
   Performed by placing 1 mL of extract in a test tube and adding sufficient hot water. 5 mL of the filtrate is taken, and 2 cm of Mg band and 1 mL of concentrated HCl are added, then shaken. A red, yellow, or orange color is formed, indicating the presence of flavonoids [9].
c. Saponin Test
   Performed by the Forth method on the extract. 1 mL of extract is placed in a test tube, followed by 2 mL of hot water. The sample will foam, then 1 mL of 2% HCl is added. If the foam persists for 30 seconds, the extract is positive for saponins [9].
d. The Tannin Test
   On the extract is performed by adding a few drops of 5% iron (III) chloride solution to 1 mL of the extract. If a dark blue or greenish-black precipitate forms, the extract is positive for tannins [9].
e. The Triterpenoid And Steroid Tests
   On the extract sample are preceded by mixing 1 mL of the extract with 2 mL of 98% chloroform in a test tube and then stirring. Afterward, the chloroform layer formed is taken and dropped onto a dropper plate and allowed to dry. Then, 5 drops of 98% anhydrous acetic acid and 3 drops of 98%

H_2SO_4 are added. If a red, orange, or yellow color forms, the sample contains triterpenoids, and if a green color forms, the sample contains steroids [9].

Antioxidant Activity Testing with the DPPH Method

1. Preparation of DPPH Solution
   A ppm DPPH solution was prepared by weighing 5 mg of DPPH powder and dissolving it in 25 mL of methanol in a volumetric flask to produce a concentration of 200 ppm. A 50 ppm concentration was prepared by pipetting 6.25 mL from a 200 ppm DPPH stock solution, then making up to 25 mL and measuring it using a UV-Vis spectrophotometer [10].
2. Preparation of Vitamin C Standard Solution
   The 1000 ppm vitamin C standard solution was prepared by weighing 10 mg of vitamin C and dissolving it in methanol using a 10 mL volumetric flask. The solution was then diluted to obtain a 100 ppm concentration by pipetting 1 mL of the stock solution and adding methanol up to a final volume of 10 mL. Furthermore, several concentration variations were prepared, namely 4, 6, 8, 10, and 12 ppm. These solutions were prepared by pipetting 0.2 mL, 0.3 mL, 0.4 mL, 0.5 mL, and 0.6 mL of the 100 ppm solution, respectively, and diluting each to 5 mL with methanol in a 5 mL volumetric flask. The absorbance of these solutions was then measured using a UV-Vis spectrophotometer [10].
3. Preparation of Kesum Leaf Ethanol Extract Sample Solution
   A 2000 ppm stock solution was prepared by weighing 20 mg of kesum leaf ethanol extract and dissolving it in the solution, homogenizing it, and making up to 10 mL. Then, from the 2000 ppm stock solution, dilutions were made at five concentrations: 50, 75, 100, 125, and 200 ppm by pipetting 0.125 mL, 0.187 mL, 0.312 mL, 0.25 mL, and 0.5 mL, adding methanol to a 5 mL volumetric flask, and measuring the absorbance using a UV-Vis spectrophotometer [10].
4. Determination of the Maximum Wavelength of DPPH
   The test was performed by measuring a 50 ppm DPPH solution that had been incubated for 30 minutes at room temperature and measured at a wavelength range of 400-800 nm. The wavelength obtained was 517 nm [10].
5. Determination of Antioxidant Activity of Vitamin C Reference Standard
   The test was conducted by pipetting 1 mL of vitamin C reference solution of five concentrations, then adding 3 mL of 50 ppm DPPH to each. The mixture was then homogenized by vortexing and incubated at room temperature for 30 minutes. The absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm [10].
6. Measuring the Antioxidant Activity of Ethanol Extract of Kesum Leaves
   The test was conducted by pipetting 1 mL of sample solutions of various concentrations, then adding 3 mL of 50 ppm DPPH to each. The mixture was then homogenized by vortexing and incubated at room temperature for 30 minutes. The absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm [10]

Data Analysis

Determination of Percent Inhibition

Antioxidant activity was expressed as percent inhibition using the formula.

Percent Inhibition Formula:

% Inhibition = [(Blank absorbance – Sample absorbance) / Blank absorbance] × 100%

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Determining IC50

The IC50 value is obtained by calculating the concentration required to inhibit free radicals by 50% based on the linear regression equation using the formula:

y = ax + b

Where:

y = 50

x = Concentration of the test solution

3. Result and Discussion

Ethanol Extract of Kesum Leaves

The extraction yielded 72.54 grams of thick ethanol extract of kesum leaves. Using 300 grams of the herb, the yield was 24.18%. Percentage yield is the ratio of the amount of metabolites obtained after extraction to the weight of the dried sample/herb. A yield is considered good if the yield exceeds 10%. Therefore, the ethanol extract of kesum leaves obtained in this study is considered good, as the yield is >10%.

Table 1. Results

Simplicia	Weight of simplicia	Weight of extract	%Rendement
Kesum Leaves *(Polygonum Minus Huds.)*	300 gram	72,54 gram	24,18%

Rendemen Extract of Kesum Leaves

Characteristics of Herb

Table 2. Results of the examination of the characteristics of Kesum Leaves Herb

NO	Parameters	Result
1.	Moisture Content	9,31%
2.	Water-Soluble Extract Content	13,40%
3.	Ethanol-Soluble Extract Content	17,68%
4.	Total Ash Content	9,90%
5.	Acid-Insoluble Ash Content	3,41%

Water Content

Determining water content is a parameter that indicates the minimum amount of water contained in a medicinal herb. The distillation process is carried out using the toluene method, where the water volume is read after the toluene and water have completely separated. After the volume is measured, the water content is calculated [11]. The resulting water content was 9.31%, which serves as the maximum water content limit in the medicinal herb.

Water-Soluble Extract Content

Determining the water-soluble extract content was carried out to understand the number of components in the medicinal herb that can dissolve in the solvent, namely water. The analysis results showed that the water-soluble extract content reached 13.40%. When compared to the ethanol-soluble extract content, the figure was lower. This indicates that the dominant substance in the kesum leaf medicinal herb is semi-polar, or that there are fewer water-soluble components [11].

Ethanol-Soluble Extract Content

Determining the concentration of the ethanol-soluble extract was carried out to determine the amount of substance that can be extracted from the medicinal herb using ethanol as a solvent. The percentage of ethanol-soluble extract was then calculated. The calculation results showed that the concentration of ethanol-soluble extract reached 17,68 %. Compared to the concentration of soluble extract obtained from water, the figure was higher. This indicates that the dominant compounds in the Kesum leaf simplex are semipolar, meaning that more compounds can dissolve in ethanol [11]..

Total Ash Content

Determining the total ash content aims to identify the minerals contained in the sample. High ash content indicates the presence of inorganic contamination. The process of determining total ash content is related to the level of purity and the presence of contamination [11]. The total ash content of the Kesum leaves obtained was 9.90%.

Acid-Insoluble Ash Content

Determining the acid-insoluble ash level aims to determine the amount of ash originating from external impurities that are insoluble in acid solutions. The acid-insoluble ash content of the Kesum leaf simplex obtained was 3.41%. Higher ash content indicates the presence of minerals and silicates originating from soil or sand [11].

Table 3. Results of Identification of Secondary Metabolite Content in Condensed Ethanol Extract of Kesum Leaves

Phytochemical Screening	The results	Description
alkaloids	Orange Sediment	+
flavanoids	Reddish brown sediment	+
saponins	Unstable Foam	-
tannins	Dark blue	+
Steroids	greenish	+
Terpenoids	Reddish	+

Phytochemical Screening

Phytochemical testing was conducted on the ethanol extract of kesum leaves to identify the secondary metabolites present. The test results, as shown in the table, indicate that the extract contains alkaloids, flavonoids, tannins, triterpenoids, and steroids. However, the saponin test did not detect the presence of saponins in the kesum leaf extract [9].

Alkaloid testing using Dragendorff's reagent will produce an orange precipitate if the sample contains alkaloids. The appearance of this colored precipitate is caused by the interaction between the reagent and the structure of the alkaloid compound. This precipitation process occurs in alkaloid compounds containing nitrogen. The nitrogen in these compounds has a lone pair of electrons, allowing it to form covalent bonds with metal ions. Specifically, nitrogen will react with potassium ions, which then form a potassium-alkaloid complex [12].

For flavonoid testing, magnesium (Mg) powder and hydrochloric acid (HCl) are used. A positive result is indicated by the appearance of a reddish-brown color, indicating a reduction reaction. Flavonoid compounds tend to be polar due to their hydroxyl groups. The same holds true for tannins, which are also

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polar due to the presence of hydroxyl groups. Tannin identification is performed by adding iron(III) chloride (FeCl3), and the sample shows a blue-black color change, indicating hydrolysis of the tannin compound [9].

Saponins are compounds with hydrophilic groups, which tend to bind to water, while their hydrophobic groups bind to air. In a micelle structure, the polar groups face outward, while the non-polar groups face inward. This condition allows for foam formation. However, in this analysis, the sample did not contain saponins because they were unable to form a stable foam. In general, if the test result is positive, the addition of hydrochloric acid (HCl) aims to increase the polarity, allowing the hydrophilic groups to bind more stably and the resulting foam to last longer [13].

Testing for triterpenoids and steroids is based on the compound's ability to produce a specific color when reacting with concentrated sulfuric acid (H2SO4) in an aqueous acetic acid solvent. The principle of this reaction involves the release of water molecules (H2O) and interaction with carbocations. Research findings indicate that the kesum leaf extract contains triterpenoids, indicated by the appearance of a red color, and also positive for steroids, indicated by the appearance of a green color [9].

Table 4. Results of calculating the % inhibition and IC50 of kesum leaf extract and Vitamin C at 517 nm using the DPPH method.

Test Solution	Concentration(ppm)	Sample Absorbance	%Inhibition	Linear Regression	IC50 (ppm)
Vitamin C	4	0,6467	53,36	y= 15,854x +	3,2866
	6	0,5643	59,30	31,136	(Very
	8	0,4997	63,97	R^2=0.9978	Strong)
	10	0,4530	67,33		
	12	0,4027	70,96		
Kesum Leaf	50	0,645	53,51	y= 17,309x +	7,9241
Extract	75	0,553	60,14	14,172	(Very
	100	0,474	65,84	R^2=0.9979	Strong)
	125	0,417	69,92		
	200	0,317	77,16		

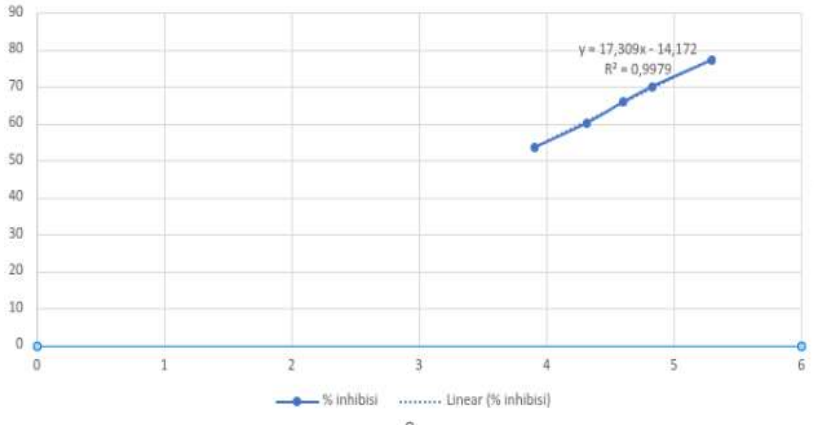

Figure 1. Calibration curve of Kesum leaves

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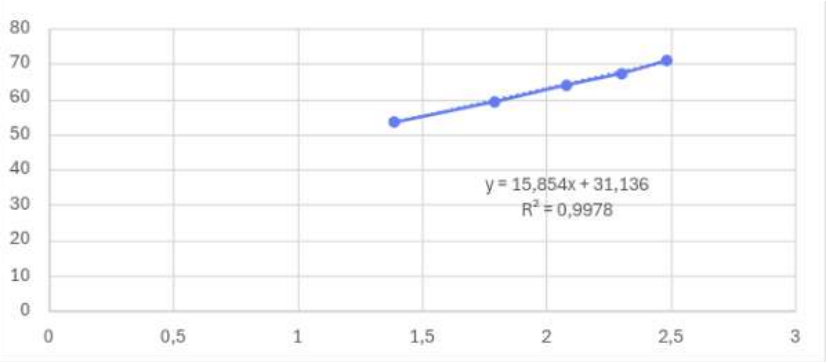

Figure 2. Vitamin C calibration curve

Antioxidant Activity Testing

The antioxidant activity of kesum leaf extract and vitamin C was tested using the DPPH method and UV-Vis spectrophotometry at a wavelength of 517 nm. The level of antioxidant activity was determined based on the IC_{50} value, which indicates the amount of sample solution required to inhibit 50% of DPPH free radicals. To determine antioxidant activity, the IC_{50} value was calculated using a regression equation obtained from a graph showing the relationship between concentration and percentage of inhibition, where the x-axis represents the sample concentration (ppm) and the y-axis represents the percentage inhibition value [14].

Sibua *et al.* stated that an antioxidant compound is categorized as very strong if the IC_{50} value is less than 50 mg/L, strong if the IC_{50} value ranges from 50 to 100 mg/L, moderate if the IC_{50} value ranges from 100 to 150 mg/L, weak if the IC_{50} value ranges from 150 to 200 mg/L, and very weak if the IC_{50} value is more than 200 mg/L [15].

The antioxidant activity test of the ethanol extract of kesum leaves was carried out at five different concentration levels, namely 50 ppm, 75 ppm, 100 ppm, 125 ppm, and 200 ppm. The purpose of using various concentrations was to determine the IC_{50} value using a mathematical formula based on the relationship between inhibition percentage and extract concentration. The results showed that the IC_{50} value of the ethanol extract of kesum leaves was 7.9241 µg/mL, indicating very high antioxidant activity.

Vitamin C solution was used as a reference standard in the antioxidant activity test of the ethanol extract of kesum leaves at concentrations of 4 ppm, 6 ppm, 8 ppm, 10 ppm, and 12 ppm. Vitamin C was used as a benchmark to evaluate the antioxidant potential contained in the ethanol extract of kesum leaves. Referring to Table 4, the IC_{50} value of vitamin C was found to be 3.2866 µg/mL, indicating that its antioxidant activity was very strong compared to the ethanol extract of kesum leaves.

A lower IC_{50} value indicates a higher ability of a compound to scavenge free radicals. Vitamin C belongs to the category of secondary antioxidants, which are capable of neutralizing various types of free radicals outside the cells. This property is due to the presence of free hydroxyl groups in the structure of vitamin C, which function as free radical scavenging agents. The presence of several hydroxyl groups in vitamin C also enhances its antioxidant effectiveness [16].

Based on the results presented in Table 4 for kesum leaf extract data, it can be observed that there was an increase in the percentage of inhibition from the lowest concentration of 50 ppm, which was 53.80%, to the highest concentration of 200 ppm, reaching 76.42%. Furthermore, Table 4 also shows an increase in the percentage of inhibition for vitamin C, starting from the lowest concentration of 4 ppm (53.36%) to the highest concentration of 12 ppm (71.42%). This increase indicates that higher extract concentrations result

in higher inhibition percentages. Damanis *et al.* reported similar findings, stating that the percentage of free radical inhibition increases as the concentration increases [17].

4. Conclusion

Based on the results of this study, it can be concluded that the ethanol extract of kesum leaves obtained through the maceration method using 70% ethanol as the solvent produced a yield of 24.18%. This value indicates that the extraction process was carried out effectively and efficiently. The yield obtained was greater than 10%, thus meeting the standard for a good extract yield. In addition, these results demonstrate that 70% ethanol is able to extract various metabolite compounds effectively from kesum leaves.

The characterization analysis of the simplicia showed that kesum leaves had good quality, based on parameters such as moisture content, water-soluble extract content, ethanol-soluble extract content, total ash content, and acid-insoluble ash content. The moisture content obtained was still within a safe limit, which can reduce the possibility of microbial growth and prevent degradation of active compounds. Furthermore, the ethanol-soluble extract content was higher than the water-soluble extract content, indicating that the main active compounds in kesum leaves are semi-polar in nature, making them more easily extracted using ethanol as the solvent.

The qualitative phytochemical screening results indicated that the ethanol extract of kesum leaves contained various secondary metabolite compounds, such as alkaloids, flavonoids, tannins, triterpenoids, and steroids. However, saponins were not detected in the extract. The presence of these compounds plays an important role in the biological activity of the extract, particularly in its antioxidant activity. Flavonoids and tannins, which contain hydroxyl groups, are known to have an important role in scavenging free radicals by donating hydrogen atoms, thereby inhibiting oxidation processes.

The antioxidant activity test using the DPPH method showed that the ethanol extract of kesum leaves was able to neutralize free radicals very effectively, with an IC_{50} value of 7.9241 µg/mL. This value falls into the category of very strong antioxidants based on the classification criteria of antioxidant activity. From this experiment, it can be observed that the lower the IC_{50} value, the higher the ability of the extract to inhibit free radicals. In comparison, vitamin C showed a lower IC_{50} value of 3.2866 µg/mL, indicating that vitamin C has stronger antioxidant activity than the ethanol extract of kesum leaves.

Although there is a difference in IC_{50} values, this does not diminish the potential of the ethanol extract of kesum leaves as a natural antioxidant source. The kesum leaf extract still demonstrated excellent antioxidant activity and has the potential to be developed as an alternative natural antioxidant derived from natural resources. Therefore, kesum leaves *(Polygonum minus Huds.)* have strong potential to be utilized in the development of pharmaceutical or health products as antioxidants and may serve as a basis for further research, particularly in formulation studies and other biological activity evaluations.

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