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Jurnal : Medical Sains: Jurnal Ilmiah Kefarmasian (Sinta 4)

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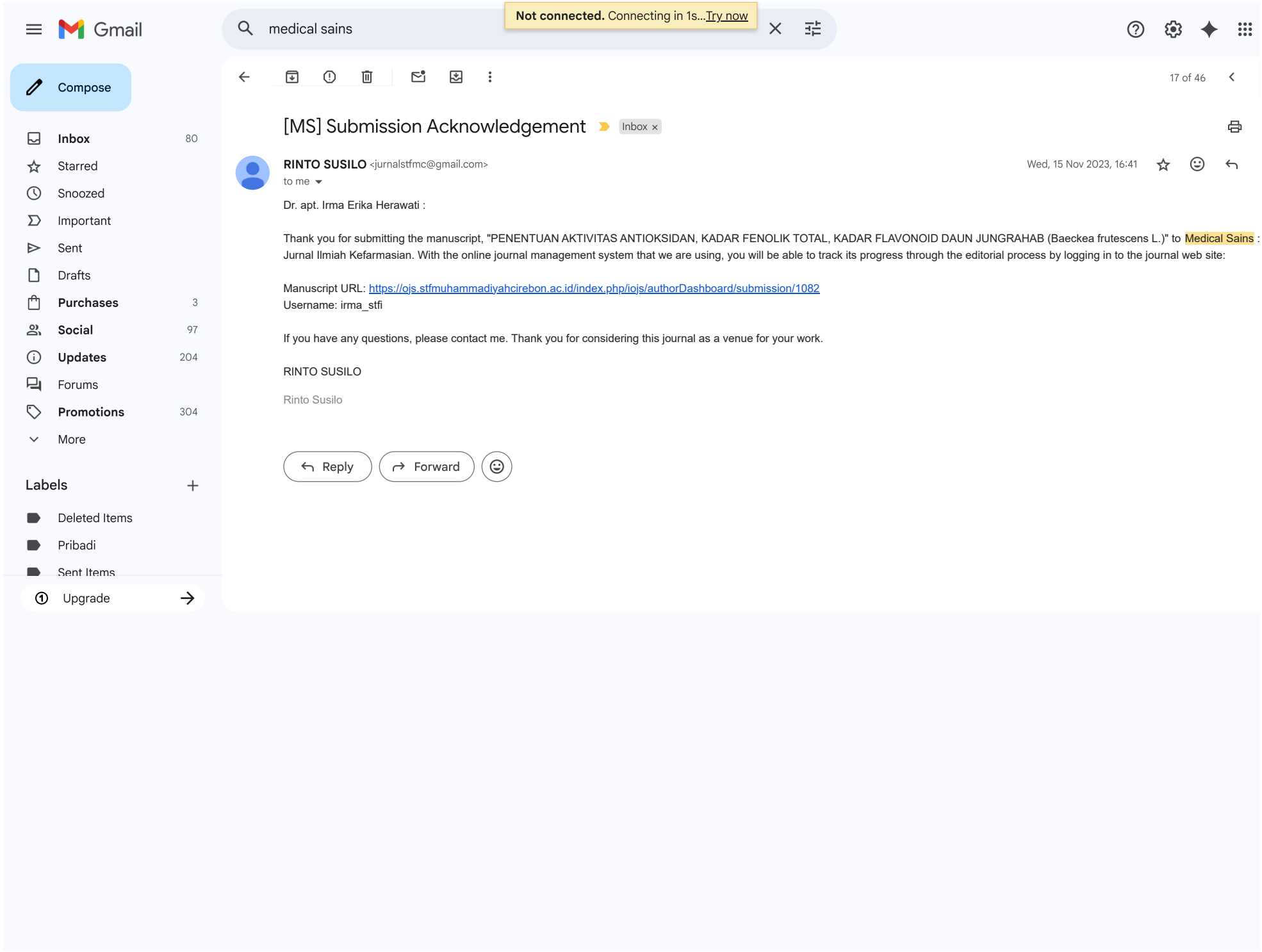
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DETERMINATION ANTIOXIDANT ACTIVITY, TOTAL PHENOLICS, FLAVONOIDS CONTENT, OF JUNGRAHAB LEAVES (*Baeckea frutescens* L.)

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ABSTRACT

Medicinal plants are currently widely used to treat various diseases, and one of the reasons is the safety of medicinal plants. The active compounds in medicinal plants include phenolics and flavonoids, which are widely known to have antioxidant activity. Antioxidants played an important role in the body's defense against various diseases because antioxidant compounds were able to prevent the bad effects caused by free radicals. Jungrahab (*Baeckea frutescens* L.) was a medicinal plant that contained phenolics and flavonoids. The aim of this research was to determine antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, total phenolic content, and flavonoid content from jungrahab leaf extract. The results show the antioxidant activity of the extract, water fraction, ethyl acetate, and n-hexane of jungrahab leaves with IC_{50} values of 12.62, 61.74, 60.66, and 63.99 ppm, respectively. Meanwhile, jungrahab extract has a total phenolic content of 52.40 mg GAE/g and a flavonoid content of 56.72 mg QE/g. Jungrahab extract is the strongest antioxidant category compared to its fractions.

Keywords: phenolics, flavonoids, antioxidant, jungrahab

INTRODUCTION

Medicinal plants have been utilized to treat a variety of diseases in traditional herbal methods since ancient times. Despite recent advances in contemporary drug systems, herbal medicine continues to play an important role in health care. its lengthy history in traditional medicine, as well as its potential benefits to human health, caught the interest of many people, particularly in nations that are developing. It is now well recognized that medicines produced from plants are safer than synthetic versions (Phuyal et al, 2020).

Plants contain an abundance in phytochemicals such as phenolics, flavonoids, alkaloids, glycosides, lignins, and tannins. The most prevalent phytoconstituents of many fruits, vegetables, medicinal, and aromatic plants that are responsible for antioxidant activity are phenols and flavonoids. Natural antioxidants, such as phenol and flavonoid chemicals derived from plants, are gaining benefit due to the potential toxicological consequences of synthetic antioxidants. An antioxidant is a chemical that prevents or delays oxidative damage to organism cells by scavenging free radicals such as peroxide or hydroperoxide, hence lowering the risk of degenerative diseases. Cancer, Alzheimer's disease, heart, kidney, and liver diseases, fibrosis, atherosclerosis, arthritis, and neurological disorders can all be caused by abnormal free radical production (Phuyal et al, 2020). Several medicinal plants have been investigated for antioxidant and other biological properties.

Jungrahab (*Baeckea frustescens* L.) is an Australian plant of the Myrtaceae family. The shrimp plant Jungrahab has curled branches, linear leaves, and white flower petals. Jungrahab leaves have been used for the medical treatment of headaches, rheumatism, and fever. Secondary metabolites found in Jungrahab leaves include flavonoids, sesquiterpenes, triterpenoids, and essential oils. (Huong et al., 2023). The purpose of this study was to examine the antioxidant activity of extracts and fractions of jungrahab leaves, as well as the total phenolic and flavonoid contents of the extract.

RESEARCH METHODS

Equipment and Materials

The instruments used in this study are the UV-Vis spectrophotometer (Shizuma), the rotary vaporator (Buchi), the 500 mL round cloves, the 100 mL measurement clove, the 10 mL scales, the droplets, the 1 mL volume pipette, the 10-mL volume pipette, cylinder paper, scales, and other instruments commonly used in the laboratory.

Jungrahab leaves were obtained from Balai Penelitian Tanaman Rempah dan Obat, Kebun Percobaan Manoko, Cikahuripan Kecamatan Lembang Jawa Barat. The plant was identified in the Plant Taxonomy Laboratory of the Department of Biology, Faculty of Mathematics and Natural Sciences, University of Padjajaran, Jatinangor, with the letter number No. 20/HB/06/2022 stating that the plant was used correctly (*Baeckea frutescens* L.).

The chemicals used are ethanol 70%, $FeCl_3$, gelatin 1%, HCl, magnesium powder (Mg), gallic acid, quercetin, Folin-Ciocalteu reagents, $AlCl_3$ powder, sodium carbonate (Na_2CO_3), and DPPH. (Sigma Aldrich). All the chemicals used are analytical solvents (Merck, Jerman).

Research Procedure

1. **Extraction of Jungrahab Leaves**

   The leaves were extracted with 70% ethanol by the maseration method for three days, with a solvent replacement every 24 hours. The liquid extract is collected and applied with a rotary vaporator at a temperature of 50℃ at a speed of 100 rpm, and then the extract yield is calculated (Yuliana et al., 2023).

2. **Fractination of Jungrahab Extract**

   10 grams of extracts are dissolved in aquadest that have been heated to 60˚ and then liquidly extracted using n-hexane and ethyl acetate three times for each solvent. The entire fraction is collected and applied, and then the fraction yield is calculated (Herawati & Hanifah, 2018).

3. **Phytochemical Screening**

   Phytochemical screening was performed against simplisia, extracts, and fractions of jungrahab leaves using the Harbone method (2007), which included secondary metabolites of alkaloids, flavonoids, tannins, phenolics, saponins, steroids, triterpenoids, and glycosides.

4. **Antioxidant Activity Extract and Fraction Jungrabah Leaves using 2,2-diphenyl-1-picrylhydrazyl (DPPH)**

   Dissolve 4 mg DPPH (2,2-diphenyl-1-picrylhydrazil) with 96% ethanol in a 100 mL (40 g/mL) volumetric flask. Dissolves 5 mg of vitamin C and 50 mg of sample (extract) with 96% ethanol, respectively, in 100 mL of volumetric flask, then dilutes to obtain concentrations of 1; 2; 3; 4; and 5 ppm for vitamin C and 5; 10; 25; 20; and 25 ppm for extract, while for fractions the concentrations are 55; 60; 65; 70; and 75 ppm. A total of 2 mL of vitamin C, extract, and fraction, inserted respectively in the tube, were added to 3 mL of 40 g/mL DPPH. The mixture is diluted and incubated in

a dark space for 20 minutes, then its absorption is measured at 515 nm using a spectroscopic photometer. The blank used is 96% ethanol.

The percentage of antioxidant activity is calculated using the formula:

$$\% \text{ inhibition DPPH} = [(Ab-Aa)/Ab] \times 100$$

Where Aa and Ab are the respective sample and blanko absorption values, A percentage of the inhibition curve versus the plasma concentration and sample concentration required for 50% inhibition is determined and expressed as an IC_{50} value (Herawati & Hanifah, 2018).

5. Determination Total Phenolics Content of Jungrahab Leaves Extract

The determination of total phenolic levels was done using the Folin-Ciocalteu method according to Chun et al. (2003), with modifications. The sample was produced at a concentration of 2500 ppm with a 70% ethanol solvent. A maximum of 0.5 mL of sample is added with 5 mL of the Folin-Ciocalteu reaction (which has been diluted with aquades at a ratio of 1:10) and 4 mL of 1M sodium carbonate. The mixture is incubated for 15 minutes, and then the absorption is measured at the maximum wavelength. Total phenols are calculated using the linear regression equation of the acid calibration curve.

6. Determination Flavonoids Content of Jungrahab Leaves Extract

The determination of flavonoid levels was done using the Chang et al., 2020 method with modifications. The sample was produced at a concentration of 5000 ppm using 70 percent ethanol. A total of 0.5 mL of the sample was added with 1.5 mL of 70 percent ethanol, then added with 0.1 mL of $AlCl_3$, 10%, 0.1 mL of 1 M sodium acetate, and 2.8 mL of aquades. The mixture is incubated for 30 minutes, and the solution absorption of the sample is measured with UV-Vis spectroscophy at maximum wavelengths. Total flavonoids are calculated using the linear regression equation of the quersetin calibration curve.

RESULTS AND DISCUSSION

1. Extraction of Jungrahab Leaves

In this study, the extraction method used was maseration with 70% ethanol solvent. Maseration was chosen because it was a simple method and suitable for secondary metabolite compounds that are soluble (not resistant to heat). Maseration has the advantage that there is no heating at the time of the secondary metabolite withdrawal process, so it does not damage the compounds present in the simplisia. (Widiastuti et al., 2023). The result of maseration on the thick extracts of jungrahab leaves is brown-green, with an extract yield of 18.78%.

2. Fractination of Jungrahab Leaves

Fractionation is a technique for separating and grouping the chemical contents of extracts based on polarization. In the process of fractioning, two solvents are used that are not mixed. The liquid-liquid extraction method is the method chosen in this study. The purpose of fractionation is to separate compounds according to their polarity, so that the number and type of the compound are a different fraction. (Saptarini & Herawati, 2017). The yield results from the fraxination of the extracts of jungrahab leaves can be seen in **Table 1.**

Table 1. Results Fraction Yield of Jungrahab

Fraction	Yield (%)
Water	39.6
n-hexane	23.5
Ethyl Acetate	15.6

From **Table 1** above, it can be seen that the secondary metabolites present in the jungrahab leaves are more polar, followed by the non-polar secondary, and the latter is the semi polar secondary.

3. Phytochemical Screening

Phytochemical screening is performed qualitatively by observing the color or changes formed after a reaction with a particular reaction. The purpose of this phytochemical screening is to identify the contents of the secondary metabolites present in the simplicia, extracts, and fractions of the leaf. Results of phytochemical screening can be seen in **Table 2.**

Table 2. Results of Phytochemical Screening for Jungrab Leaves

No	Compound	Simplicia	Extract	Fraction		
				Water	n-hexsan	Ethyl Acetate
1	Alkaloids	+	+	+	+	+
2	Phenolics	+	+	+	+	+
3	Flavonoids	+	+	+	+	-
4	Tannins	+	+	+	+	-
5	Saponins	+	+	+	-	-
6	Steroids and Terpenoids	+	+	-	+	-
7	Glycosides	+	-	-	-	-

Notes:
(+): detected
(-): not detected

According to the results of phytochemical screening performed on simplicia, extracts, and fractions of leaves, leaves have potential as antioxidants due to the presence of secondary phenolic metabolites and flavonoids (Saptarini & Herawati, 2017).

4. Antioxidant Activity Extract and Fraction of Jugrahab Leaves using DPPH

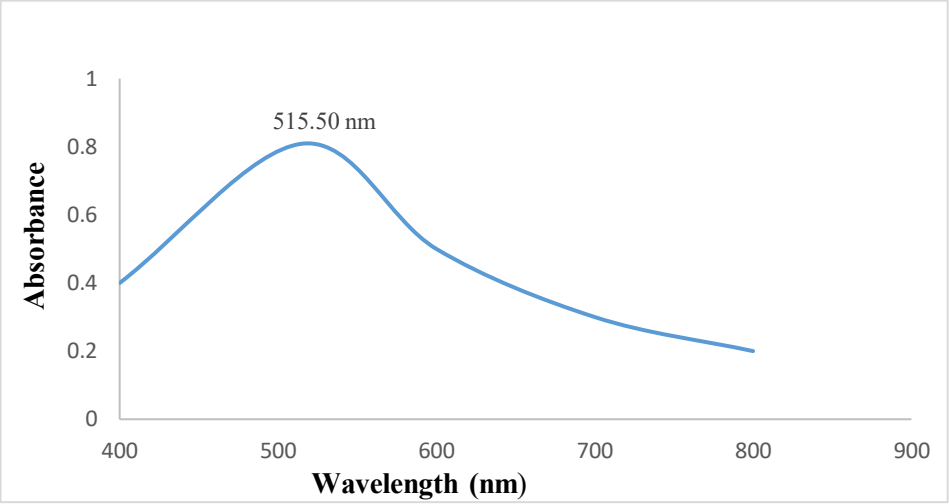

Figure 1. Maximum Wave Length of DPPH

Table 3. Results of Antioxidant Activity on Jungrahab Leaves Extract and Fraction

Sample	Concentration (ppm)	Absorbance	Inhibition (%)	Linear Regression	IC_{50} (ppm)
Vitamin C (standard)	1	0.620 ± 0.013	23.36	y=7.058x + 16.477	4.75
	2	0.562 ± 0.028	30.53		
	3	0.492 ± 0.017	39.18		
	4	0.465 ± 0.011	42.52		
	5	0.383 ± 0.016	52.66		
Extract	5	0.462 ± 0.034	42.89	y=0.9914x+ 37.49	12.62
	10	0.435 ± 0.015	46.23		
	15	0.375 ± 0.014	53.65		
	20	0.352 ± 0.006	56.49		
	25	0.303 ± 0.027	65.55		
Water Fraction	55	0.478 ± 0.025	40.91	y=1.4512x+ 39.592	61.74
	60	0.433 ± 0.002	46.48		
	65	0.361 ± 0.005	55.38		
	70	0.316 ± 0.005	60.94		
	75	0.243 ± 0.035	69.96		
Ethyl Acetate Fraction	55	0.490 ± 0.006	39.43	y=1.7182x+ 54.229	60.66
	60	0.414 ± 0.006	48.83		
	65	0.334 ± 0.012	58.71		
	70	0.267 ± 0.015	66.99		
	75	0.216 ± 0.005	73.30		
n-hexane Fraction	55	0.538 ± 0.037	33.50	y=1.6119x+ 53.152	63.99
	60	0.441 ± 0.013	45.49		
	65	0.382 ± 0.021	52.78		
	70	0.327 ± 0.006	59.58		
	75	0.269 ± 0.014	66.75		

The DPPH (2,2-diphenyl-1-picrylhydrazil) technique is used for determining antioxidant activity. The DPPH approach was chosen because DPPH is a stable free radical that absorbs at 515 nm. The antioxidant activity of pure phenolic compounds or plant extracts is commonly determined using this approach (Shalaby & Shanab, 2013). Since vitamin C can neutralize free radicals through electron donation and transfer mechanisms, it is employed as a standard in antioxidant activity testing (Carita et al, 2020).

The wavelength of DPPH is 500–520 nm; in this study, the wave length was obtained at 515,5 nm, as shown in **Figure 1**, which is in line with the literature (Saptarini et al, 2019).

The results showed that ethanol extract has the highest antioxidant activity with an IC_{50} of 12.62 ppm, whereas n-hexane extract is the lowest antioxidant with an IC_{50} value of 63.99 ppm, as shown in **Table 3**. (Houghton & Raman, 1998) categorized antioxidant activity into four categories: strong (IC_{50}: 50–100 ppm), moderate (IC_{50}: 100–150 ppm), weak (IC_{50}: 150–200 ppm), and very weak (IC_{50}: >200 ppm). So extracts and all fractions of jungrahab leaves belong to the category of strong antioxidants.

Plant-derived antioxidants, with or without side effects, can protect the human body from disease caused by free radicals. The mechanism of antioxidant action is to prevent oxidative chain reactions that would otherwise cause damage to the organism (Saptarini et al, 2019).

5. Detemination Total Phenolics Content of Jungrahab Leaves Extract

Phenolic compounds are important secondary metabolites in plants that have antioxidant activity. The total phenolics level of jungrahab leaves extract was measured using the Folin-Ciocalteu method (Aryal et al., 2019). From the results of the study, we obtained the calibration curve for acid gallic as shown in **Figure 2**.

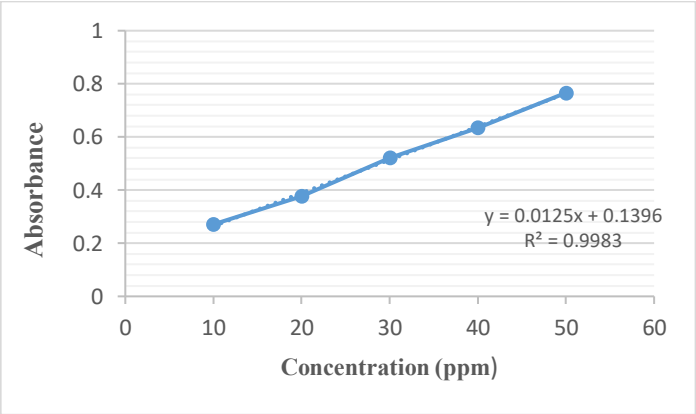

Figure 2. Calibration Curve of Gallic Acid (n=3)

Table 4. Results of Total Phenolics Content on Jungrahab Leaves Extract

Sample	Replication	Concentration (ppm)	Absorbance	Total Phenolics Content (mg GAE/g)	Average of Total Phenolics Content (mg GAE/g)
Extract	1	2500	0.793	52.27	52.40±0.011
	2		0.795	52.43	
	3		0.796	52.51	

From the gallic acid calibration curve in **Figure 2**, a linear regression equation is obtained to determine the total phenolic content extract. Gallic acid is chosen as a comparator because it is one of the phenolic compounds with a simple structure, has stable properties, and is available in pure condition. (Senet et al., 2018). Gallic acid has an aromatic-OH group, reactioned in a basal atmosphere with Folin-Ciocalteau, will produce a blue-colored molybdenum-tungsten and measurable absorption. The

absorption measurement of gallic acid is performed at maximum wavelength of 751 nm. The higher the concentration of phenolic compounds, the more phenolic ions will be reduced to molybdenum-tungsten complexes, and the resulting color will become more concentrated (Husain et al., 2023). The results of the study showed that the total phenolic content of jungrahab leaves extract was 52.40 mg GAE/g, as shown in **Table 4.**

6. Determination Flavonoids Content of Jungrahab Leaves Extraxt

Flavonoids are a secondary metabolite known to have antioxidant activity because of their ability to fight free radicals that play a role in the development of degenerative diseases, which can damage the body's immune system and also oxidize proteins and lipids (Husain et al., 2023). The group that has antioxidants in flavonoids is the hydroxy (-OH) group (Sholikhah et al., 2023). The method used to determine content of flavonoids is Chang et al., 2020 method. The principle of this method is a reaction between $AlCl_3$ and the flavonoid, which will form a stable complex compound with C-4 keto groups as well as C-3 or C-5 hydroxyl groups of flavons and flavonols.

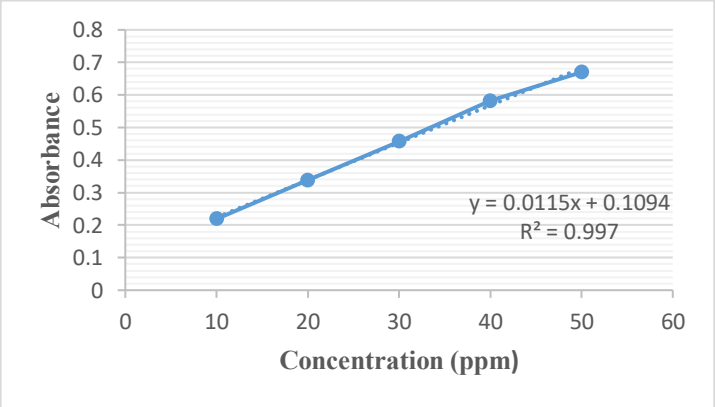

Figure 3. Calibration Curve of Quercetin (n=3)

Table 5. Results of Flavonoids Content on Jungrahab Leaves Extract

Sample	Replication	Concentration (ppm)	Absorbance	Flavonoids content (mg QE/g)	Average Flavonoids content (mg QE/g)
Extract	1	5000	0.762	56.75	56.72±0.002
	2		0.760	56.58	
	3		0.763	56.83	

The standard used for determining the content of flavonoids is quercetin, because quersetin is a flavonoid of the flavonol group that has keto groups in the C-4 atom and also hydroxyl groups in neighboring C-3 and C-5 atoms (Azizah et al., 2014). As seen in **Figure 3**, the linear regression obtained on the quersetin calibration curve is used to determine the content of flavonoids extract from jungrahab leaves. Quercetin absorption measurements were performed at a maximum wavelength of 435 nm. The result of the determination of the flavonoids content of the leaf extract is 56.72 mg QE/g, as shown in **Table 5**.

CONCLUSION

Jungrahab leaves have high levels of phenolics and flavonoids, so they can be categorized as having strong antioxidant potential in both extracts and fractions.

ACKNOWLEDGMENT

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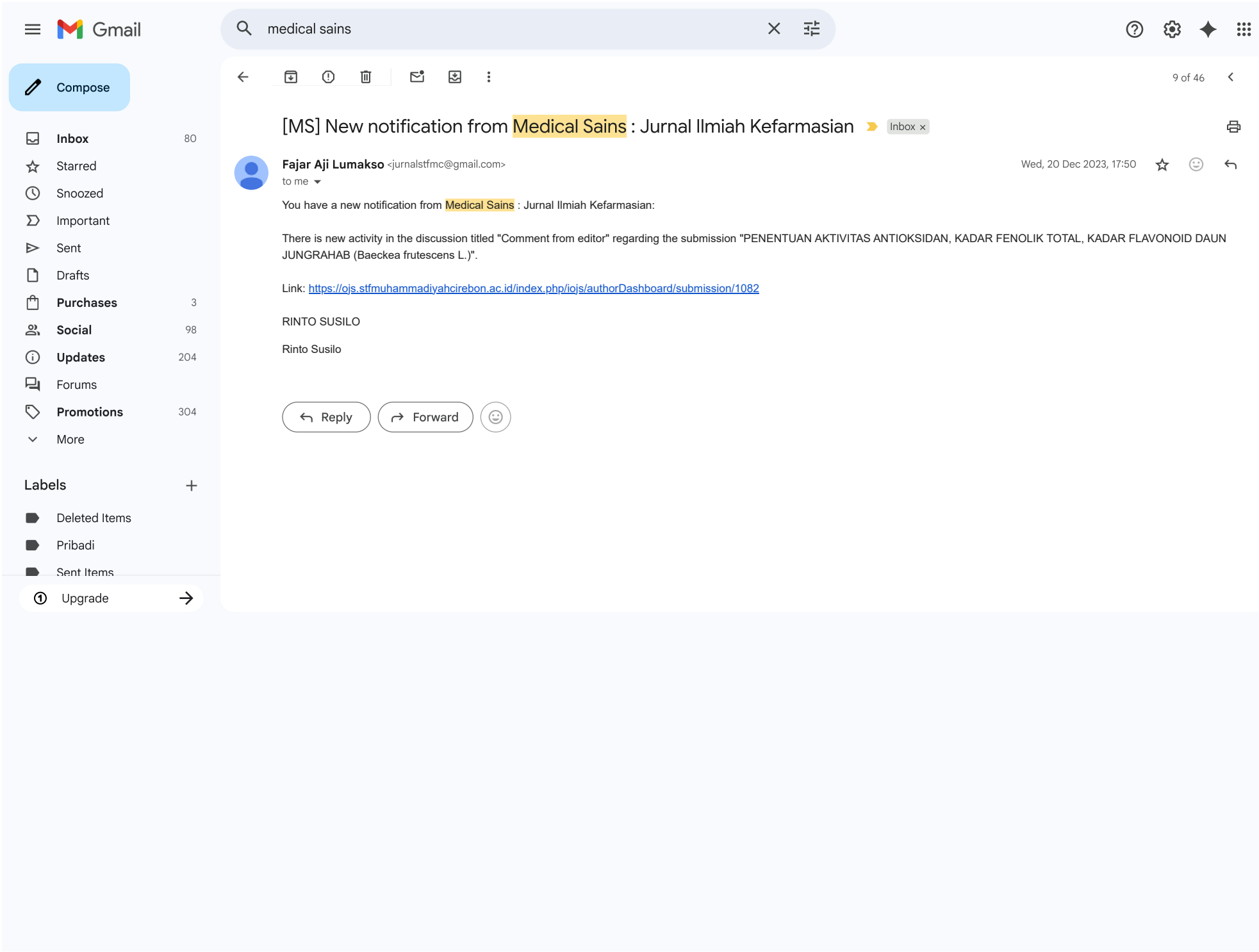
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Our decision is: Revisions Required

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DETERMINATION OF ANTIOXIDANT ACTIVITY, TOTAL PHENOLICS, FLAVONOIDS CONTENT, OF JUNGRAHAB LEAVES (*Baeckea frutescens* L.)

Submitted : December 2, 2023 Revised :..... Accepted:.....

ABSTRACT

Medicinal plants are currently widely used to treat various diseases, and one of the reasons is the safety of medicinal plants. The active compounds in medicinal plants include phenolics and flavonoids, which are widely known to have antioxidant activity. Antioxidants played an important role in the body's defense against various diseases because antioxidant compounds were able to prevent the bad effects caused by free radicals. Jungrahab (*Baeckea frutescens* L.) was a medicinal plant that contained phenolics and flavonoids. The aim of this research was to determine antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, total phenolic content, and flavonoid content from jungrahab leaf extract. The results show the antioxidant activity of the extract, water fraction, ethyl acetate, and n-hexane of jungrahab leaves with IC_{50} values of 12.62, 61.74, 60.66, and 63.99 ppm, respectively. Meanwhile, jungrahab extract has a total phenolic content of 52.40 mg GAE/g and a flavonoid content of 56.72 mg QE/g. Jungrahab extract is the strongest antioxidant category compared to its fractions.

Keywords: phenolics, flavonoids, antioxidant, jungrahab

INTRODUCTION

Medicinal plants have been utilized to treat a variety of diseases in traditional herbal methods since ancient times. Despite recent advances in contemporary drug systems, herbal medicine continues to play an important role in health care. its lengthy history in traditional medicine, as well as its potential benefits to human health, caught the interest of many people, particularly in nations that are developing. It is now well recognized that medicines produced from plants are safer than synthetic versions (Phuyal *et al.*, 2020).

Plants contain an abundance in phytochemicals such as phenolics, flavonoids, alkaloids, glycosides, lignins, and tannins. The most prevalent phytoconstituents of many fruits, vegetables, medicinal, and aromatic plants that are responsible for antioxidant activity are phenols and flavonoids. Natural antioxidants, such as phenol and flavonoid chemicals derived from plants, are gaining benefit due to the potential toxicological consequences of synthetic antioxidants. An antioxidant is a chemical that prevents or delays oxidative damage to organism cells by scavenging free radicals such as peroxide or hydroperoxide, hence lowering the risk of degenerative diseases. Cancer, Alzheimer's disease, heart, kidney, and liver diseases, fibrosis, atherosclerosis, arthritis, and neurological disorders can all be caused by abnormal free radical production (Phuyal *et al.*, 2020). Several medicinal plants have been investigated for antioxidant and other biological properties.

Jungrahab (*Baeckea frustescens* L.) as shown in **Figure 1**, is an Australian plant of the Myrtaceae family. The shrimp plant Jungrahab has curled branches, linear leaves, and white flower petals. Jungrahab leaves have been used for the medical treatment of headaches, rheumatism, and fever. Secondary metabolites found in Jungrahab leaves include flavonoids, sesquiterpenes, triterpenoids, and essential oils (Huong, Duc and Son, 2023). The purpose of

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Commented [TA9]: ~~It is now well recognized that medicines produced from plants are safer than synthetic versions~~ = It is now well-recognized that plant medicines are safer than synthetic ones

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Commented [TA13]: Jungrahab (*Baeckea frustescens* L.), as shown in **Figure 1**

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this study was to examine the antioxidant activity of extracts and fractions of jungrahab leaves, as well as the total phenolic and flavonoid contents of the extract.

Figure 1. Jungrahab (*Baeckea frustescens* L.)
Source: https://id.wikipedia.org/wiki/Ujung_atap

RESEARCH METHODS

Equipment and Materials

The instruments used in this study are the UV-Vis spectrophotometer (Shizuma), the rotary vaporator (Buchi), 500 mL round flask, 100 mL volumetric flask, 10 mL volumetric flask, dropper pippete, 1 mL volume pipette, 10 mL volume pipette, filter paper, funnel, and other instruments commonly used in the laboratory.

Jungrahab leaves were obtained from Balai Penelitian Tanaman Rempah dan Obat, Kebun Percobaan Manoko, Cikahuripan Kecamatan Lembang Jawa Barat. The plant was identified in the Plant Taxonomy Laboratory of the Department of Biology, Faculty of Mathematics and Natural Sciences, University of Padjajaran, Jatinangor, with the letter number No. 20/HB/06/2022 stating that the plant was used correctly (*Baeckea frutescens* L.).

The chemicals used are ethanol 70%, $FeCl_3$, gelatin 1%, HCl, magnesium powder (Mg), gallic acid, quercetin, Folin-Ciocalteu reagents, $AlCl_3$ powder, sodium carbonate (Na_2CO_3), and DPPH. (Sigma Aldrich). All the chemicals used are analytical solvents (Merck, Jerman).

Research Procedure

1. **Extraction of Jungrahab Leaves**

   The leaves were extracted with 70% ethanol by the maseration method for three days, with a solvent replacement every 24 hours. The liquid extract is collected and applied with a rotary vaporator at a temperature of 50℃ at a speed of 100 rpm, and then the extract yield is calculated (Yuliana *et al.*, 2023).

2. **Fractination of Jungrahab Extract**

   10 grams of extracts are dissolved in aquadest that have been heated to 60˚ and then liquidly extracted using n-hexane and ethyl acetate three times for each solvent. The entire fraction is collected and applied, and then the fraction yield is calculated (Herawati and Hanifah, 2018).

3. **Phytochemical Screening**

   Phytochemical screening was performed against simplisia, extracts, and fractions of jungrahab leaves using the Harborne. 2007 method, which included

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secondary metabolites of alkaloids, flavonoids, tannins, phenolics, saponins, steroids, triterpenoids, and glycosides.

Commented [TA22]: 2007 method included secondary metabolites of alkaloids, flavonoids, tannins, phenolics, saponins, steroids, triterpenoids, and glycosides.

4. **Antioxidant Activity Extract and Fraction Jungrabah Leaves using 2,2-diphenyl-1-picrylhydrazyl (DPPH)**

Dissolve 4 mg DPPH (2,2-diphenyl-1-picrylhydrazil) with 96% ethanol in a 100 mL (40 g/mL) volumetric flask. Dissolves 5 mg of vitamin C and 50 mg of sample (extract) with 96% ethanol, respectively, in 100 mL of volumetric flask, then dilutes to obtain concentrations of 1; 2; 3; 4; and 5 ppm for vitamin C and 5; 10; 25; 20; and 25 ppm for extract, while for fractions the concentrations are 55; 60; 65; 70; and 75 ppm. A total of 2 mL of vitamin C, extract, and fraction, inserted respectively in the tube, were added to 3 mL of 40 g/mL DPPH. The mixture is diluted and incubated in a dark space for 20 minutes, then its absorption is measured at 515 nm using a spectroscopic photometer. The blank used is 96% ethanol.

The percentage of antioxidant activity is calculated using the formula:

$$\% \text{ inhibition DPPH} = [(Ab-Aa)/Ab] \times 100$$

Where Aa and Ab are the respective sample and blanko absorption values, A percentage of the inhibition curve versus the plasma concentration and sample concentration required for 50% inhibition is determined and expressed as an IC_{50} value (Herawati and Hanifah, 2018).

5. **Determination Total Phenolics Content of Jungrahab Leaves Extract**

Commented [TA23]: Determination OF Total Phenolics

The determination of total phenolic levels was done using the Folin-Ciocalteu method according to Chun, Kim and Lee, 2003, with modifications. The sample was produced at a concentration of 2500 ppm with a 70% ethanol solvent. A maximum of 0.5 mL of sample is added with 5 mL of the Folin-Ciocalteu reaction (which has been diluted with aquades at a ratio of 1:10) and 4 mL of 1M sodium carbonate. The mixture is incubated for 15 minutes, and then the absorption is measured at the maximum wavelength. Total phenols are calculated using the linear regression equation of the acid calibration curve.

Commented [TA24]: Determining total phenolic levels was done using the Folin-Ciocalteu method according to Chun, Kim, and Lee, 2003, with modifications

6. **Determination Flavonoids Content of Jungrahab Leaves Extract**

Commented [TA25]: 1.Determination of Flavonoids

The determination of flavonoid levels was done using Chang *et al*., 2020 method with modifications. The sample was produced at a concentration of 5000 ppm using 70 percent ethanol. A total of 0.5 mL of the sample was added with 1.5 mL of 70 percent ethanol, then added with 0.1 mL of $AlCl_3$, 10%, 0.1 mL of 1 M sodium acetate, and 2.8 mL of aquades. The mixture is incubated for 30 minutes, and the solution absorption of the sample is measured with UV-Vis spectroscophy at maximum wavelengths. Total flavonoids are calculated using the linear regression equation of the quersetin calibration curve.

Commented [TA26]: Flavonoid levels were determined using the Chang *et al*. 2020 method with modifications

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RESULTS AND DISCUSSION

1. **Extraction of Jungrahab Leaves**

In this study, the extraction method used was maseration with 70% ethanol solvent. Maseration was chosen because it was a simple method and suitable for secondary metabolite compounds that are soluble (not resistant to heat). Maseration has the advantage that there is no heating at the time of the secondary metabolite

Commented [TA29]: *Maceration was chosen because it was a simple method and suitable for soluble secondary metabolite compounds (not resistant to heat).*

withdrawal process, so it does not damage the compounds present in the simplisia. (Widiastuti *et al.*, 2023). The result of maseration on the thick extracts of jungrahab leaves is brown-green, with an extract yield of 18.78%.

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2. Fractination of Jungrahab Leaves

Fractionation is a technique for separating and grouping the chemical contents of extracts based on polarization. In the process of fractioning, two solvents are used that are not mixed. The liquid-liquid extraction method is the method chosen in this study. The purpose of fractionation is to separate compounds according to their polarity, so that the number and type of the compound are a different fraction. (Saptarini and Herawati, 2017). The yield results from the fraxination of the extracts of jungrahab leaves can be seen in **Table 1.**

Commented [TA32]: In the process of fractioning, two solvents that are not mixed are used

Commented [TA33]: The purpose of fractionation is to separate compounds according to their polarity so that the number and type of the compound are different fractions

Table 1. Results Fraction Yield of Jungrahab

Fraction	Yield (%)
Water	39.6
n-hexane	23.5
Ethyl Acetate	15.6

From **Table 1** above, it can be seen that the secondary metabolites present in the jungrahab leaves are more polar, followed by the non-polar secondary, and the latter is the semi polar secondary.

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3. Phytochemical Screening

Phytochemical screening is performed qualitatively by observing the color or changes formed after a reaction with a particular reaction. The purpose of this phytochemical screening is to identify the contents of the secondary metabolites present in the simplicia, extracts, and fractions of the leaf. Results of phytochemical screening can be seen in **Table 2.**

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Commented [TA36]: This phytochemical screening aims to identify the contents of the secondary metabolites present in the Simplicia extracts and fractions of the leaf

Table 2. Results of Phytochemical Screening for Jungrab Leaves

No	Compound	Simplicia	Extract	Fraction		
				Water	n-hexsan	Ethyl Acetate
1	Alkaloids	+	+	+	+	+
2	Phenolics	+	+	+	+	+
3	Flavonoids	+	+	+	+	-
4	Tannins	+	+	+	+	-
5	Saponins	+	+	+	-	-
6	Steroids and Terpenoids	+	+	-	+	-
7	Glycosides	+	-	-	-	-

Notes:
(+): detected
(-): not detected

According to the results of phytochemical screening performed on simplicia, extracts, and fractions of leaves, leaves have potential as antioxidants due to the presence of secondary phenolic metabolites and flavonoids (Saptarini and Herawati, 2017).

4. Antioxidant Activity Extract and Fraction of Jugrahab Leaves using DPPH

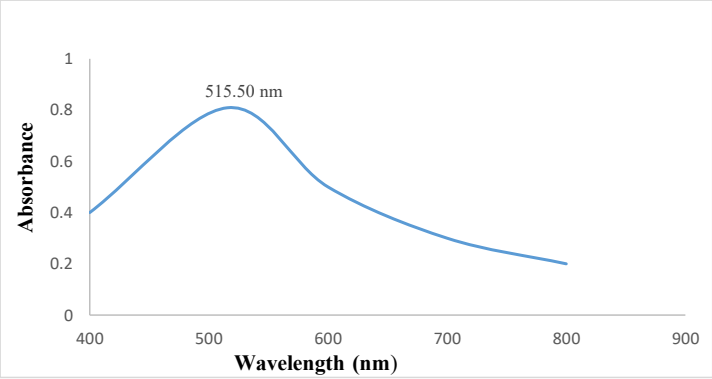

Figure 2. Maximum Wave Length of DPPH

Table 3. Results of Antioxidant Activity on Jungrahab Leaves Extract and Fraction

Sample	Concentration (ppm)	Absorbance	Inhibition (%)	Linear Regression	IC_{50} (ppm)
Vitamin C (standard)	1	0.620 ± 0.013	23.36	y=7.058x + 16.477	4.75
	2	0.562 ± 0.028	30.53		
	3	0.492 ± 0.017	39.18		
	4	0.465 ± 0.011	42.52		
	5	0.383 ± 0.016	52.66		
Extract	5	0.462 ± 0.034	42.89	y=0.9914x+ 37.49	12.62
	10	0.435 ± 0.015	46.23		
	15	0.375 ± 0.014	53.65		
	20	0.352 ± 0.006	56.49		
	25	0.303 ± 0.027	65.55		
Water Fraction	55	0.478 ± 0.025	40.91	y=1.4512x+ 39.592	61.74
	60	0.433 ± 0.002	46.48		
	65	0.361 ± 0.005	55.38		
	70	0.316 ± 0.005	60.94		
	75	0.243 ± 0.035	69.96		
Ethyl Acetate Fraction	55	0.490 ± 0.006	39.43	y=1.7182x+ 54.229	60.66
	60	0.414 ± 0.006	48.83		
	65	0.334 ± 0.012	58.71		
	70	0.267 ± 0.015	66.99		
	75	0.216 ± 0.005	73.30		
n-hexane Fraction	55	0.538 ± 0.037	33.50	y=1.6119x+ 53.152	63.99
	60	0.441 ± 0.013	45.49		
	65	0.382 ± 0.021	52.78		
	70	0.327 ± 0.006	59.58		
	75	0.269 ± 0.014	66.75		

The DPPH (2,2-diphenyl-1-picrylhydrazil) technique is used for determining antioxidant activity. The DPPH approach was chosen because DPPH is a stable free radical that absorbs at 515 nm. The antioxidant activity of pure phenolic compounds or plant extracts is commonly determined using this approach (Shalaby and Shanab, 2013). Since vitamin C can neutralize free radicals through electron donation and transfer mechanisms, it is employed as a standard in antioxidant activity testing (Caritá *et al.*, 2020).

Commented [TA37]: Please showed the functional groups on Vitamin C can attack free radicals ?

The wavelength of DPPH is 500–520 nm; in this study, the wave length was obtained at 515,5 nm, as shown in **Figure 2**, which is in line with the literature (Saptarini, Rahayu and Herawati, 2019).

The results showed that ethanol extract has the highest antioxidant activity with an IC_{50} of 12.62 ppm, whereas n-hexane extract is the lowest antioxidant with an IC_{50} value of 63.99 ppm, as shown in **Table 3**. (Houghton and Raman, 1998) categorized antioxidant activity into four categories: strong (IC_{50}: 50–100 ppm), moderate (IC_{50}: 100–150 ppm), weak (IC_{50}: 150–200 ppm), and very weak (IC_{50}: >200 ppm). So, extracts and all fractions of jungrahab leaves belongs to the category of strong antioxidants.

Plant-derived antioxidants, with or without side effects, can protect the human body from disease caused by free radicals. The mechanism of antioxidant action is to prevent oxidative chain reactions that would otherwise cause damage to the organism (Saptarini, Rahayu and Herawati, 2019).

5. Detemination Total Phenolics Content of Jungrahab Leaves Extract

Phenolic compounds are important secondary metabolites in plants that have antioxidant activity. The total phenolics level of jungrahab leaves extract was measured using the Folin-Ciocalteu method (Aryal *et al.*, 2019). From the results of the study, we obtained the calibration curve for acid gallic as shown in **Figure 3**.

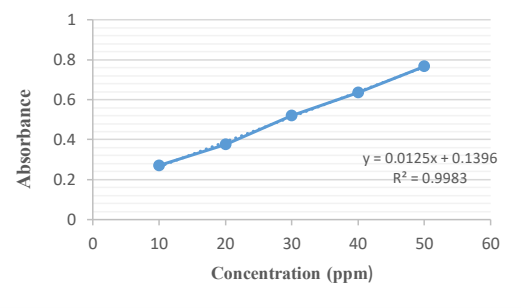

Figure 3. Calibration Curve of Gallic Acid (n=3)

Table 4. Results of Total Phenolics Content on Jungrahab Leaves Extract

Sample	Replication	Concentration (ppm)	Absorbance	Total Phenolics Content (mg GAE/g)	Average of Total Phenolics Content (mg GAE/g)
Extract	1	2500	0.793	52.27	52.40±0.011
	2		0.795	52.43	
	3		0.796	52.51	

From the gallic acid calibration curve in **Figure 2**, a linear regression equation is obtained to determine the total phenolic content extract. Gallic acid is chosen as a comparator because it is one of the phenolic compounds with a simple structure, has stable properties, and is available in pure condition (Senet *et al.*, 2018). Gallic acid has an aromatic-OH group, reactioned in a basal atmosphere with Folin-Ciocalteau, will produce a blue-colored molybdenum-tungsten and measurable absorption. The

absorption measurement of gallic acid is performed at maximum wavelength of 751 nm. The higher the concentration of phenolic compounds, the more phenolic ions will be reduced to molybdenum-tungsten complexes, and the resulting color will become more concentrated (Husain, Yunus and Basri, 2023). The results of the study showed that the total phenolic content of jungrahab leaves extract was 52.40 mg GAE/g, as shown in **Table 4.**

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6. Determination Flavonoids Content of Jungrahab Leaves Extraxt

Flavonoids are a secondary metabolite known to have antioxidant activity because of their ability to fight free radicals that play a role in the development of degenerative diseases, which can damage the body's immune system and also oxidize proteins and lipids (Husain, Yunus and Basri, 2023). The group that has antioxidants in flavonoids is the hydroxy (-OH) group (Sholikhah, Riyanti and Wahyono, 2023). The method used to determine content of flavonoids is Chang *et al*., 2020 method. The principle of this method is a reaction between $AlCl_3$ and the flavonoid, which will form a stable complex compound with C-4 keto groups as well as C-3 or C-5 hydroxyl groups of flavons and flavonols.

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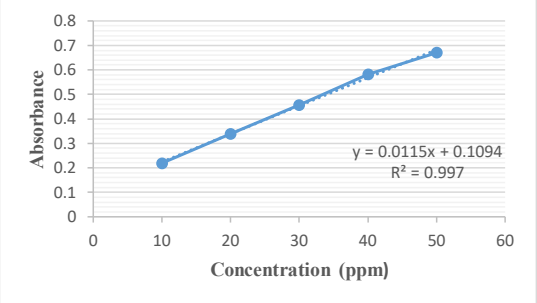

Figure 4. Calibration Curve of Quercetin (n=3)

Table 5. Results of Flavonoids Content on Jungrahab Leaves Extract

Sample	Replication	Concentration (ppm)	Absorbance	Flavonoids content (mg QE/g)	Average Flavonoids content (mg QE/g)
Extract	1	5000	0.762	56.75	56.72±0.002
	2		0.760	56.58	
	3		0.763	56.83	

The standard used for determining the content of flavonoids is quercetin, because quersetin is a flavonoid of the flavonol group that has keto groups in the C-4 atom and also hydroxyl groups in neighboring C-3 and C-5 atoms (Azizah, Kumolowati and Faramayuda, 2014). As seen in **Figure 4**, the linear regression obtained on the quersetin calibration curve is used to determine the content of flavonoids extract from jungrahab leaves. Quercetin absorption measurements were performed at a maximum wavelength of 435 nm. The result of the determination of the flavonoids content of the leaf extract is 56.72 mg QE/g, as shown in **Table 5**.

CONCLUSION

Jungrahab leaves have high levels of phenolics and flavonoids, so they can be categorized as having strong antioxidant potential in both extracts and fractions.

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ACKNOWLEDGMENT

Authors thanked Septia Anjani Suherman and Pegi Mugi Putri Wijaya for their technical assistance in this research.

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3. Bukti perbaikan manuskrip-1 (30 Desember 2023)

DETERMINATION ANTIOXIDANT ACTIVITY, TOTAL PHENOLICS, FLAVONOIDS CONTENT, OF JUNGRAHAB LEAVES (*Baeckea frutescens* L.)

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ABSTRACT

Medicinal plants are currently widely used to treat various diseases, and one of the reasons is the safety of medicinal plants. The active compounds in medicinal plants include phenolics and flavonoids, which are widely known to have antioxidant activity. Antioxidants played an essential role in the body's defense against various diseases because antioxidant compounds prevent the bad effects caused by free radicals. Jungrahab (*Baeckea frutescens* L.) was a medicinal plant that contained phenolics and flavonoids. The aim of this research was to determine antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, total phenolic content, and flavonoid content from jungrahab leaf extract. The results show the antioxidant activity of the extract, water fraction, ethyl acetate, and n-hexane of jungrahab leaves with IC_{50} values of 12.62, 61.74, 60.66, and 63.99 ppm, respectively. Meanwhile, jungrahab extract has a total phenolic content of 52.40 mg GAE/g and a flavonoid content of 56.72 mg QE/g. Jungrahab extract is the strongest antioxidant category compared to its fractions.

Keywords: phenolics, flavonoids, antioxidant, jungrahab

INTRODUCTION

Medicinal plants have been utilized to treat a variety of diseases in traditional herbal methods since ancient times. Despite recent advances in contemporary drug systems, herbal medicine continues is essential in health care. Its lengthy history in conventional medicine and its potential benefits to human health caught the interest of many people, particularly in developing nations. It is now well-recognized that plant medicines are safer than synthetic ones (Phuyal *et al.*, 2020).

Plants contain an abundant in phytochemicals such as phenolics, flavonoids, alkaloids, glycosides, lignins, and tannins. The most prevalent phytoconstituents responsible for the antioxidant activity of many fruits, vegetables, and medicinal and aromatic plants are phenols and flavonoids. Natural antioxidants, such as phenol and flavonoid chemicals derived from plants, are gaining benefits due to the potential toxicological consequences of synthetic antioxidants. An antioxidant is a chemical that prevents or delays oxidative damage to organism cells by scavenging free radicals such as peroxide or hydroperoxide, hence lowering the risk of degenerative diseases. Cancer, Alzheimer's disease, heart, kidney, and liver diseases, fibrosis, atherosclerosis, arthritis, and neurological disorders can all be caused by abnormal free radical production (Phuyal *et al.*, 2020). Several medicinal plants have been investigated for antioxidant and other biological properties.

Jungrahab (*Baeckea frustescens* L.), as shown in **Figure 1**, is an Australian plant of the Myrtaceae family. The shrimp plant Jungrahab has curled branches, linear leaves, and white flower petals. Jungrahab leaves have been used to treat headaches, rheumatism, and fever. Secondary metabolites found in Jungrahab leaves include flavonoids, sesquiterpenes, triterpenoids, and essential oils (Huong, Duc and Son, 2023). This study aimed to examine the antioxidant activity of extracts and fractions of jungrahab leaves and the extract's total phenolic and flavonoid contents.

Figure 1. Jungrahab (*Baeckea frustescens* L.)
Source: https://id.wikipedia.org/wiki/Ujung_atap

RESEARCH METHODS

Equipment and Materials

The instruments used in this study are the UV-Vis spectrophotometer (Shizuma), the rotary evaporator (Buchi), 500 mL round flask, 100 mL volumetric flask, 10 mL volumetric flask, dropper pipettes, 1 mL volume pipettes, 10 mL volume pipettes, filter paper, funnel, and other instruments commonly used in the laboratory.

Jungrahab leaves were obtained from Balai Penelitian Tanaman Rempah dan Obat, Kebun Percobaan Manoko, and Cikahuripan Kecamatan Lembang Jawa Barat. The plant was identified in the Plant Taxonomy Laboratory of the Department of Biology, Faculty of Mathematics and Natural Sciences, University of Padjajaran, Jatinangor, with the letter number No. 20/HB/06/2022 stating that the plant was used correctly (*Baeckea frutescens* L.).

The chemicals used are ethanol 70%, $FeCl_3$, gelatin 1%, HCl, magnesium powder (Mg), gallic acid, quercetin, Folin-Ciocalteu reagents, $AlCl_3$ powder, sodium carbonate (Na_2CO_3), and DPPH. (Sigma Aldrich). All the chemicals used are analytical solvents (Merck, Jerman).

Research Procedure

1. **Extraction of Jungrahab Leaves**

   The leaves were extracted with 70% ethanol by the maceration method for three days, with a solvent replacement every 24 hours. The liquid extract is collected and applied with a rotary vaporator at a temperature of 50℃ at a speed of 100 rpm, and then the extract yield is calculated (Yuliana *et al.*, 2023).

2. **Fractionation of Jungrahab Extract**

   Ten grams of extracts are dissolved in aquadest that have been heated to 60˚ and then liquidly extracted using n-hexane and ethyl acetate three times for each solvent. The entire fraction is collected and applied, and then the fraction yield is calculated (Herawati and Hanifah, 2018).

3. **Phytochemical Screening**

Phytochemical screening was performed against simplisia, extracts, and fractions of jungrahab leaves using the Harborne. 2007 method, included secondary metabolites of alkaloids, flavonoids, tannins, phenolics, saponins, steroids, triterpenoids, and glycosides.

4. **Antioxidant Activity Extract and Fraction Jungrabah Leaves using 2,2-diphenyl-1-picrylhydrazyl (DPPH)**

Dissolve 4 mg DPPH (2,2-diphenyl-1-picrylhydrazil) with 96% ethanol in a 100 mL (40 g/mL) volumetric flask. Dissolves 5 mg of vitamin C and 50 mg of sample (extract) with 96% ethanol, respectively, in 100 mL of volumetric flask, then dilutes to obtain concentrations of 1; 2; 3; 4; and 5 ppm for vitamin C and 5; 10; 25; 20; and 25 ppm for extract, while for fractions the concentrations are 55; 60; 65; 70; and 75 ppm. A total of 2 mL of vitamin C, extract, and fraction, inserted respectively in the tube, were added to 3 mL of 40 g/mL DPPH. The mixture is diluted and incubated in a dark space for 20 minutes, then its absorption is measured at 515 nm using a spectroscopic photometer. The blank used is 96% ethanol.

The percentage of antioxidant activity is calculated using the formula:

$$\% \text{ inhibition DPPH} = [(Ab-Aa)/Ab] \times 100$$

Where Aa and Ab are the respective sample and blanko absorption values, A percentage of the inhibition curve versus the plasma concentration and sample concentration required for 50% inhibition is determined and expressed as an IC_{50} value (Herawati and Hanifah, 2018).

5. **Determination of Total Phenolics Content from Jungrahab Leaves Extract**

Determining total phenolic levels was done using the Folin-Ciocalteu method according to Chun, Kim and Lee, 2003, with modifications. The sample was produced at a concentration of 2500 ppm with a 70% ethanol solvent. A maximum of 0.5 mL of sample is added with 5 mL of the Folin-Ciocalteu reaction (which has been diluted with aquades at a ratio of 1:10) and 4 mL of 1M sodium carbonate. The mixture is incubated for 15 minutes, and then the absorption is measured at the maximum wavelength. Total phenols are calculated using the linear regression equation of the acid calibration curve.

6. **Determination of Flavonoids Content from Jungrahab Leaves Extract**

Flavonoid levels were determined using Chang *et al.*, 2020 method with modifications. The sample was produced at a concentration of 5000 ppm using 70 percent ethanol. A total of 0.5 mL of the sample was added with 1.5 mL of 70 percent ethanol, then with 0.1 mL of $AlCl_3$, 10%, 0.1 mL of 1 M sodium acetate, and 2.8 mL of aquadest. The mixture is incubated for 30 minutes, and the solution absorption of the sample is measured with UV-Vis spectroscopy at maximum wavelengths. Total flavonoids are calculated using the linear regression equation of the quersetin calibration curve.

RESULTS AND DISCUSSION

1. **Extraction of Jungrahab Leaves**

In this study, the extraction method used was maceration with 70% ethanol solvent. Maceration was chosen because it was a simple method and suitable for soluble secondary metabolite compounds (not resistant to heat). Maceration has the

advantage of no heating during the secondary metabolite withdrawal process, so it does not damage the compounds in the simplicia. (Widiastuti *et al.*, 2023). The result of maceration on the thick extracts of jungrahab leaves is brown-green, with an extract yield of 18.78%.

2. Fractionation of Jungrahab Leaves

Fractionation is a technique for separating and grouping the chemical contents of extracts based on polarization. In the process of fractioning, two solvents that are not mixed are used. The liquid-liquid extraction method is the method chosen in this study. The purpose of fractionation is to separate compounds according to their polarity so that the number and type of the compound are different fractions. (Saptarini and Herawati, 2017). The yield results from the fractionation of the extracts of jungrahab leaves can be seen in **Table 1.**

Table 1. Results Fraction Yield of Jungrahab

Fraction	Yield (%)
Water	39.6
n-hexane	23.5
Ethyl Acetate	15.6

From **Table 1** above, it can be seen that the secondary metabolites present in the jungrahab leaves are more polar, followed by the non-polar secondary, and the latter is the semi-polar secondary.

3. Phytochemical Screening

Phytochemical screening is performed qualitatively by observing the color or changes formed after a reaction with a particular response. This phytochemical screening aims to identify the contents of the secondary metabolites present in the Simplicia extracts and fractions of the leaf. Results of phytochemical screening can be seen in **Table 2.**

Table 2. Results of Phytochemical Screening for Jungrab Leaves

No	Compound	Simplicia	Extract	Fraction		
				Water	n-hexsan	Ethyl Acetate
1	Alkaloids	+	+	+	+	+
2	Phenolics	+	+	+	+	+
3	Flavonoids	+	+	+	+	-
4	Tannins	+	+	+	+	-
5	Saponins	+	+	+	-	-
6	Steroids and Terpenoids	+	+	-	+	-
7	Glycosides	+	-	-	-	-

Notes:
(+): detected
(-): not detected

According to the results of phytochemical screening performed on simplicia, extracts, and fractions of leaves, leaves have potential as antioxidants due to the presence of secondary phenolic metabolites and flavonoids (Saptarini and Herawati, 2017).

4. Antioxidant Activity Extract and Fraction of Jugrahab Leaves using DPPH

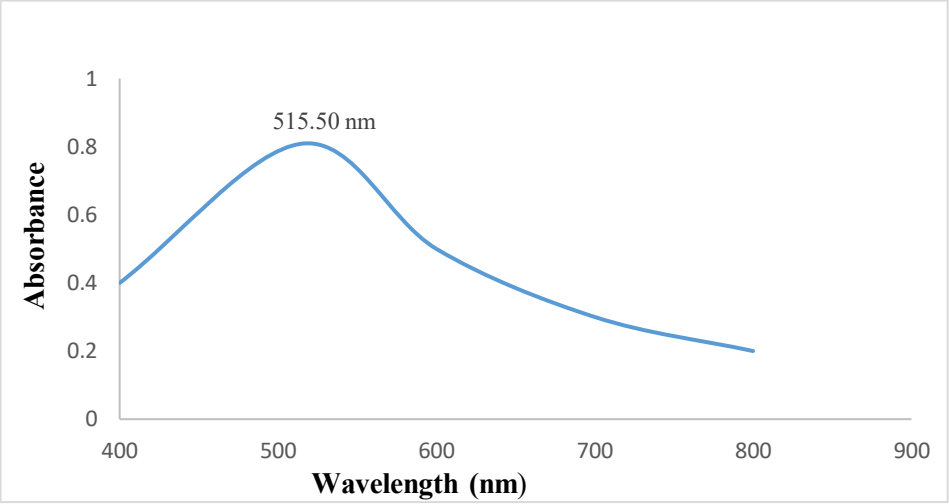

Figure 2. Maximum Wave Length of DPPH

Table 3. Results of Antioxidant Activity on Jungrahab Leaves Extract and Fraction

Sample	Concentration (ppm)	Absorbance	Inhibition (%)	Linear Regression	IC_{50} (ppm)
Vitamin C (standard)	1	0.620 ± 0.013	23.36	y=7.058x + 16.477	4.75
	2	0.562 ± 0.028	30.53		
	3	0.492 ± 0.017	39.18		
	4	0.465 ± 0.011	42.52		
	5	0.383 ± 0.016	52.66		
Extract	5	0.462 ± 0.034	42.89	y=0.9914x+ 37.49	12.62
	10	0.435 ± 0.015	46.23		
	15	0.375 ± 0.014	53.65		
	20	0.352 ± 0.006	56.49		
	25	0.303 ± 0.027	65.55		
Water Fraction	55	0.478 ± 0.025	40.91	y=1.4512x+ 39.592	61.74
	60	0.433 ± 0.002	46.48		
	65	0.361 ± 0.005	55.38		
	70	0.316 ± 0.005	60.94		
	75	0.243 ± 0.035	69.96		
Ethyl Acetate Fraction	55	0.490 ± 0.006	39.43	y=1.7182x+ 54.229	60.66
	60	0.414 ± 0.006	48.83		
	65	0.334 ± 0.012	58.71		
	70	0.267 ± 0.015	66.99		
	75	0.216 ± 0.005	73.30		
n-hexane Fraction	55	0.538 ± 0.037	33.50	y=1.6119x+ 53.152	63.99
	60	0.441 ± 0.013	45.49		
	65	0.382 ± 0.021	52.78		
	70	0.327 ± 0.006	59.58		
	75	0.269 ± 0.014	66.75		

The DPPH (2,2-diphenyl-1-picrylhydrazil) technique is used for determining antioxidant activity. The DPPH approach was chosen because DPPH is a stable free radical that absorbs at 515 nm. The antioxidant activity of pure phenolic compounds or plant extracts is commonly determined using this approach (Shalaby and Shanab, 2013). Since vitamin C can neutralize free radicals through electron donation and transfer mechanisms, it is employed as a standard in antioxidant activity testing (Caritá

et al., 2020). Vitamin C is a six-carbon lactone ring structure with 2,3-enediol moiety. The antioxidant activity of vitamin C comes from 2,3-enediol (Akbari *et al.*, 2016).

The wavelength of DPPH is 500–520 nm; in this study, the wave length was obtained at 515,5 nm, as shown in **Figure 2**, which is in line with the literature (Saptarini, Rahayu and Herawati, 2019).

The results showed that ethanol extract has the highest antioxidant activity with an IC_{50} of 12.62 ppm, whereas n-hexane extract is the lowest antioxidant with an IC_{50} value of 63.99 ppm, as shown in **Table 3**. (Houghton and Raman, 1998) categorized antioxidant activity into four categories: strong (IC_{50}: 50–100 ppm), moderate (IC_{50}: 100–150 ppm), weak (IC_{50}: 150–200 ppm), and very weak (IC_{50}: >200 ppm). So, extracts and all fractions of jungrahab leaves belong to the category of strong antioxidants.

Plant-derived antioxidants, with or without side effects, can protect the human body from disease caused by free radicals. The mechanism of antioxidant action is to prevent oxidative chain reactions that would otherwise cause damage to the organism (Saptarini, Rahayu and Herawati, 2019).

5. Detemination Total Phenolics Content of Jungrahab Leaves Extract

Phenolic compounds are important secondary metabolites in plants that have antioxidant activity. The total phenolics level of jungrahab leaves extract was measured using the Folin-Ciocalteu method (Aryal *et al.*, 2019). From the results of the study, we obtained the calibration curve for acid gallic as shown in **Figure 3**.

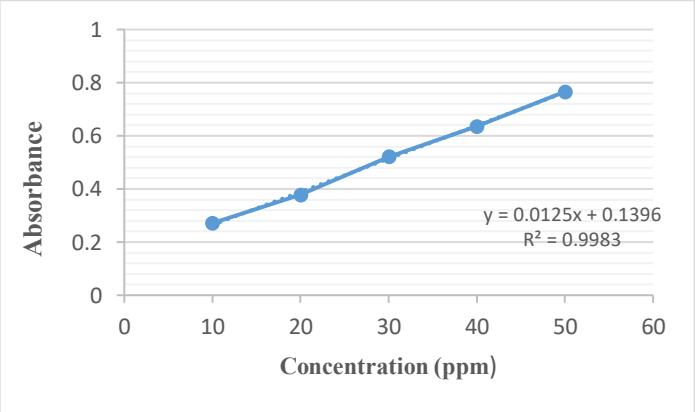

Figure 3. Calibration Curve of Gallic Acid (n=3)

Table 4. Results of Total Phenolics Content on Jungrahab Leaves Extract

Sample	Replication	Concentration (ppm)	Absorbance	Total Phenolics Content (mg GAE/g)	Average of Total Phenolics Content (mg GAE/g)
Extract	1	2500	0.793	52.27	52.40±0.011
	2		0.795	52.43	
	3		0.796	52.51	

From the gallic acid calibration curve in **Figure 3**, a linear regression equation is obtained to determine the total phenolic content extract. Gallic acid is chosen as a comparator because it is one of the phenolic compounds with a simple structure, has stable properties, and is available in pure condition (Senet *et al.*, 2018). Gallic acid

has an aromatic-OH group, reactioned in a basal atmosphere with Folin-Ciocalteau, will produce a blue-colored molybdenum-tungsten and measurable absorption. The absorption measurement of gallic acid is performed at maximum wavelength of 751 nm. The higher of the concentration of phenolic compounds, the more phenolic ions will be reduced to molybdenum-tungsten complexes, and the resulting color will become more concentrated (Husain, Yunus and Basri, 2023). The results of the study showed that the total phenolic content of jungrahab leaves extract was 52.40 mg GAE/g, as shown in **Table 4.**

6. Determination Flavonoids Content of Jungrahab Leaves Extraxt

Flavonoids are a secondary metabolite known to have antioxidant activity because of their ability to fight free radicals that play a role in the development of degenerative diseases, which can damage the body's immune system and also oxidize proteins and lipids (Husain, Yunus and Basri, 2023). The group that has antioxidants in flavonoids is the hydroxy (-OH) group (Sholikhah, Riyanti and Wahyono, 2023). Because flavonoids have a high redox potential, they may serve as reducing agents, hydrogen donors, and singlet oxygen quenchers (Zehiroglu and Sarikaya, 2019). The method used to determine content of flavonoids is Chang *et al*., 2020 method. The principle of this method is a reaction between $AlCl_3$ and the flavonoid, which will form a stable complex compound with C-4 keto groups as well as C-3 or C-5 hydroxyl groups of flavons and flavonols.

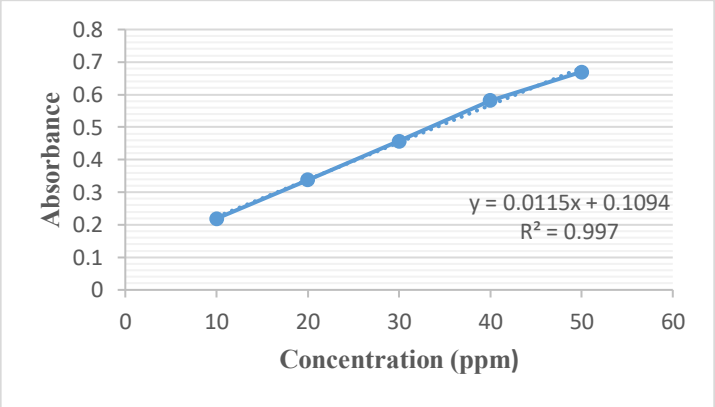

Figure 4. Calibration Curve of Quercetin (n=3)

Table 5. Results of Flavonoids Content on Jungrahab Leaves Extract

Sample	Replication	Concentration (ppm)	Absorbance	Flavonoids content (mg QE/g)	Average Flavonoids content (mg QE/g)
Extract	1	5000	0.762	56.75	56.72±0.002
	2		0.760	56.58	
	3		0.763	56.83	

The standard used for determining the content of flavonoids is quercetin, because quersetin is a flavonoid of the flavonol group that has keto groups in the C-4 atom and also hydroxyl groups in neighboring C-3 and C-5 atoms (Azizah, Kumolowati and Faramayuda, 2014). As seen in **Figure 4**, the linear regression obtained on the quersetin calibration curve is used to determine the content of flavonoids extract from jungrahab leaves. Quercetin

absorption measurements were performed at a maximum wavelength of 435 nm. The result of the determination of the flavonoids content of the leaf extract is 56.72 mg QE/g, as shown in **Table 5**.

CONCLUSION

Jungrahab leaves have high levels of phenolics (52.40 mg GAE/g) and flavonoids (56.72 mg QE/g), so they can be categorized as having strong antioxidant potential in both extracts (12.62 ppm) and fractions.

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4. Bukti revisi manuskrip (reviewer-2) (18 Januari 2024)

DETERMINATION ANTIOXIDANT ACTIVITY, TOTAL PHENOLICS, FLAVONOIDS CONTENT, OF JUNGRAHAB LEAVES (*Baeckea frutescens* L.)

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Submitted :December 2, 2023 Revised :..... Accepted:.....

ABSTRACT

Medicinal plants are currently widely used to treat various diseases, and one of the reasons is the safety of medicinal plants. The active compounds in medicinal plants include phenolics and flavonoids, which are widely known to have antioxidant activity. Antioxidants played an essential role in the body's defense against various diseases because antioxidant compounds prevent the bad effects caused by free radicals. Jungrahab (*Baeckea frutescens* L.) was a medicinal plant that contained phenolics and flavonoids. The aim of this research was to determine antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, total phenolic content, and flavonoid content from jungrahab leaf extract. The results show the antioxidant activity of the extract, water fraction, ethyl acetate, and n-hexane of jungrahab leaves with IC_{50} values of 12.62, 61.74, 60.66, and 63.99 ppm, respectively. Meanwhile, jungrahab extract has a total phenolic content of 52.40 mg GAE(gallic acid equivalent)/g and a flavonoid content of 56.72 mg QE(quercetin equivalent)/g. Jungrahab extract is the strongest antioxidant category compared to its fractions.

Keywords: phenolics content, flavonoids content, antioxidant activity, DPPH (2, 2-Diphenyl-1- picrylhydrazyl), jungrahab

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INTRODUCTION

Medicinal plants have been utilized to treat a variety of diseases in traditional herbal methods since ancient times. Despite recent advances in contemporary drug systems, herbal medicine continues is essential in health care. Its lengthy history in conventional medicine and its potential benefits to human health caught the interest of many people, particularly in developing nations. It is now well-recognized that plant medicines are safer than synthetic ones (Phuyal *et al.*, 2020).

Plants contain abundant in phytochemicals such as phenolics, flavonoids, alkaloids, glycosides, lignins, and tannins. The most prevalent phytoconstituents responsible for the antioxidant activity of many fruits, vegetables, and medicinal and aromatic plants are phenols and flavonoids. Natural antioxidants, such as phenol and flavonoid chemicals derived from plants, are gaining benefits due to the potential toxicological consequences of synthetic antioxidants. An antioxidant is a chemical that prevents or delays oxidative damage to organism cells by scavenging free radicals such as peroxide or hydroperoxide, hence lowering the risk of degenerative diseases. Cancer, Alzheimer's disease, heart, kidney, and liver diseases, fibrosis, atherosclerosis, arthritis, and neurological disorders can all be caused by

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Jungrahab (*Baeckea frustescens* L.), as shown in **Figure 1**, is an Australian plant of the Myrtaceae family. The shrimp plant Jungrahab has curled branches, linear leaves, and white flower petals. Jungrahab leaves have been used to treat headaches, rheumatism, and fever. Secondary metabolites found in Jungrahab leaves include flavonoids, sesquiterpenes, triterpenoids, and essential oils (Huong, Duc and Son, 2023). This study aimed to examine the antioxidant activity of extracts and fractions of jungrahab leaves and the extract's total phenolic and flavonoid contents.

Figure 1. Jungrahab (*Baeckea frustescens* L.)
Source: https://id.wikipedia.org/wiki/Ujung_atap

RESEARCH METHODS

Equipment and Materials

The instruments used in this study are the UV-Vis spectrophotometer (Shimadzu), the rotary evaporator (Buchi), 500 mL round flask, 100 mL volumetric flask, 10 mL volumetric flask, dropper pipettes, 1 mL volume pipettes, 10 mL volume pipettes, filter paper, funnel, and other instruments commonly used in the laboratory.

Jungrahab leaves were obtained from Balai Penelitian Tanaman Rempah dan Obat, Kebun Percobaan Manoko, and Cikahuripan Kecamatan Lembang Jawa Barat. The plant was identified in the Plant Taxonomy Laboratory of the Department of Biology, Faculty of Mathematics and Natural Sciences, University of Padjajaran, Jatinangor, with the letter number No. 20/HB/06/2022 stating that the plant was used correctly (*Baeckea frutescens* L.).

The chemicals used are ethanol 70%, $FeCl_3$, gelatin 1%, HCl, magnesium powder (Mg), gallic acid, quercetin, Folin-Ciocalteu reagents, $AlCl_3$ powder, sodium carbonate (Na_2CO_3), and DPPH. (Sigma Aldrich). All the chemicals used are analytical solvents (Merck, Jerman).

Research Procedure

1. **Extraction of Jungrahab Leaves**

   The leaves were extracted with 70% ethanol by the maceration method for three days, with a solvent replacement every 24 hours. The liquid extract is collected and applied with a rotary vaporator at a temperature of 50℃ at a speed of 100 rpm, and then the extract yield is calculated (Yuliana *et al.*, 2023).

2. **Fractionation of Jungrahab Extract**

   Ten grams of extracts are dissolved in aquadest that have been heated to 60˚ and then done liquid-liquid extraction (LLC) with n-hexane and ethyl acetate, three

times for each solvent. The entire fraction is collected and applied, and then the fraction yield is calculated (Herawati and Hanifah, 2018).

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3. **Phytochemical Screening**

Phytochemical screening was performed against simplisia, extracts, and fractions of jungrahab leaves using the Harborne. 2007 method, of following secondary metabolites.

Alkaloids Test
Approximately 500 mg of sample was stirred with a few 2N HCl and 9 mL of aquadest, then heat on the water for 2 minutes. The mixtures were then cooled and filtered. The filtrate was used to perform a test with reagen Dragendorff and Mayer.

Flavonoids Test
Approximately 3 mL of sample was treated with 1 mL of 10% NaOH solution. The formation of the intense yellow color showed an indication of the presence of flavonoids.

Phenolics Test
Sample (50 mg) is dissolved in 5 mL of disstiled water. To this few drops of neutral 5% ferric chloride solution are added. A dark green color indicates the presence of phenolic compound.

Tannins Test
Sample (50 mg) is dissolved in 5 mL of distilled water and 2 mL of 1% solution of gelatin containing 10% NaCl is added to it. White precipitate indicates the presence of tannins.

Saponins Test
Approximately 3 mL of sample were added to 3 mL of distilled water and shaken vigorously. The formation of a stable, persistent froth was taken as a positive test for saponins.

Steroids/Triterpenoids
Approximately 2 mL of chloroform and concentrated H_2SO_4 were added to 5 mL of the prepared plant extracts. A layer of red color indicated the presence of steroids in the lower chloroform.

Glycosides Test
For 50 mg sample is hydrolysed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate is subjected to the Borntrager's test. Which to 2 mL of filtered hydrolysate, 3 mL of chloroform is added and shaken, chloroform layer is separated and 10% ammonia solution is added to it. Pink color indicates presence of glycosides.

4. **Antioxidant Activity Extract and Fraction Jungrabah Leaves using 2,2-diphenyl-1-picrylhydrazyl (DPPH)**

Dissolve 4 mg DPPH (2,2-diphenyl-1-picrylhydrazil) with 96% ethanol in a 100 mL (40 g/mL) volumetric flask. Dissolves 5 mg of vitamin C and 50 mg of sample (extract) with 96% ethanol, respectively, in 100 mL of volumetric flask, then dilutes to obtain concentrations of 1; 2; 3; 4; and 5 ppm for vitamin C and 5; 10; 25; 20; and 25 ppm for extract, while for fractions the concentrations are 55; 60; 65; 70; and 75 ppm. A total of 2 mL of vitamin C, extract, and fraction, inserted respectively in the tube, were added to 3 mL of 40 g/mL DPPH. The mixture is diluted and incubated in

a dark space for 20 minutes, then its absorption is measured at 515 nm using a spectroscopic photometer. The blank used is 96% ethanol.

The percentage of antioxidant activity is calculated using the formula:

$$\% \text{ inhibition DPPH} = [(Ab-Aa)/Ab] \times 100$$

Where Aa and Ab are the respective sample and blanko absorption values, A percentage of the inhibition curve versus the plasma concentration and sample concentration required for 50% inhibition is determined and expressed as an IC_{50} value (Herawati and Hanifah, 2018).

5. Determination of Total Phenolics Content from Jungrahab Leaves Extract

Determining total phenolic levels was done using the Folin-Ciocalteu method according to Chun, Kim and Lee, 2003, with modifications. The sample was produced at a concentration of 2500 ppm with a 70% ethanol solvent. A maximum of 0.5 mL of sample is added with 5 mL of the Folin-Ciocalteu reaction (which has been diluted with aquades at a ratio of 1:10) and 4 mL of 1M sodium carbonate. The mixture is incubated for 15 minutes, and then the absorption is measured at the maximum wavelength. Total phenols are calculated using the linear regression equation of the acid calibration curve.

6. Determination of Flavonoids Content from Jungrahab Leaves Extract

Flavonoid levels were determined using Chang *et al*., 2020 method with modifications. The sample was produced at a concentration of 5000 ppm using 70 percent ethanol. A total of 0.5 mL of the sample was added with 1.5 mL of 70 percent ethanol, then with 0.1 mL of $AlCl_3$, 10%, 0.1 mL of 1 M sodium acetate, and 2.8 mL of aquadest. The mixture is incubated for 30 minutes, and the solution absorption of the sample is measured with UV-Vis spectroscopy at maximum wavelengths. Total flavonoids are calculated using the linear regression equation of the quersetin calibration curve.

RESULTS AND DISCUSSION

1. Extraction of Jungrahab Leaves

In this study, the extraction method used was maceration with 70% ethanol solvent. Maceration was chosen because it was a simple method and suitable for soluble secondary metabolite compounds (not resistant to heat). Maceration has the advantage of no heating during the secondary metabolite withdrawal process, so it does not damage the compounds in the simplicia. (Widiastuti *et al.*, 2023). The result of maceration on the thick extracts of jungrahab leaves is brown-green, with an extract yield of 18.78%.

2. Fractionation of Jungrahab Leaves

Fractionation is a technique for separating and grouping the chemical contents of extracts based on polarization. In the process of fractioning, two solvents that are not mixed are used. The liquid-liquid extraction method is the method chosen in this study. The purpose of fractionation is to separate compounds according to their polarity so that the number and type of the compound are different fractions. (Saptarini and Herawati, 2017). The yield results from the fractionation of the extracts of jungrahab leaves can be seen in **Table 1.**

Table 1. Results of jungrahab fractination

Fraction	Yield (%)
Water	39.6
n-hexane	23.5
Ethyl Acetate	15.6

From **Table 1** above, it can be concluded that jungrahab leaf extract has a secondary metabolite that is mostly attracted to polar solvents, then to non-polar solvents, and the latter to the secondary metabolite that is attracted to semi-polar solvents~~. it can be seen that the secondary metabolites present in the jungrahab leaves are more polar, followed by the non-polar compounds and the latter is the semi-polar secondary.~~

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3. Phytochemical Screening

Phytochemical screening is performed qualitatively by observing the color or changes formed after a reaction with a particular response. This phytochemical screening aim was for identification the contents of the secondary metabolites present in the Simplicia extracts and fractions of the leaf. Results of phytochemical screening can be seen in **Table 2.**

Table 2. Results of Phytochemical Screening for Jungrab Leaves

No	Compound	Crude material	Extract	Fraction		
				Water	n-hexane	Ethyl acetate
1	Alkaloids	+	+	+	+	+
2	Phenolics	+	+	+	+	+
3	Flavonoids	+	+	+	+	-
4	Tannins	+	+	+	+	-
5	Saponins	+	+	+	-	-
6	Steroids and terpenoids	+	+	-	+	-
7	Glycosides	+	-	-	-	-

Notes:
(+): detected
(-): not detected

According to the results of phytochemical screening performed on crude material, extracts, and fractions of leaves, leaves have potential as antioxidants due to the presence of secondary phenolic metabolites and flavonoids (Saptarini and Herawati, 2017).

4. Antioxidant Activity Extract and Fraction of Jugrahab Leaves using DPPH

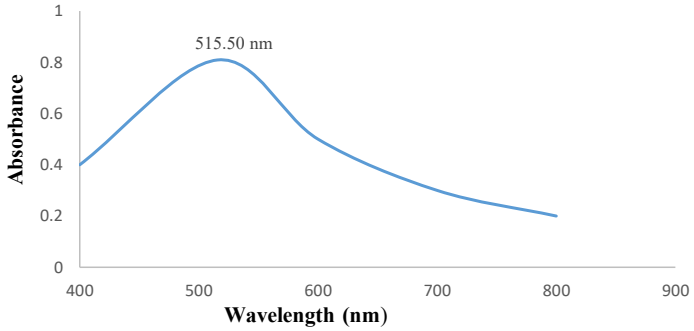

Figure 2. Maximum wavelength of DPPH solution in 96% ethanol

Table 3. Results of antioxidant activity on jungrahab leaves extract and fraction

Sample	Concentration (ppm)	Inhibition (%)	Linear Regression	IC_{50} (ppm)
Vitamin C (standard)	1	23.36		
	2	30.53		
	3	39.18	y=7.058x + 16.477	4.75
	4	42.52		
	5	52.66		
Extract	5	42.89		
	10	46.23		
	15	53.65	y=0.9914x+ 37.49	12.62
	20	56.49		
	25	65.55		
Water fraction	55	40.91		
	60	46.48		
	65	55.38	y=1.4512x+ 39.592	61.74
	70	60.94		
	75	69.96		
Ethyl acetate fraction	55	39.43		
	60	48.83		
	65	58.71	y=1.7182x+ 54.229	60.66
	70	66.99		
	75	73.30		
n-hexane fraction	55	33.50		
	60	45.49		
	65	52.78	y=1.6119x+ 53.152	63.99
	70	59.58		
	75	66.75		

The DPPH (2,2-diphenyl-1-picrylhydrazil) technique is used for determining antioxidant activity. The DPPH approach was chosen because DPPH is a stable free radical that absorbs at 515 nm. The antioxidant activity of pure phenolic compounds or plant extracts is commonly determined using this approach (Shalaby and Shanab, 2013). Since vitamin C can neutralize free radicals through electron donation and transfer mechanisms, it is employed as a standard in antioxidant activity testing (Caritá *et al.*, 2020). Vitamin C is a six-carbon lactone ring structure with 2,3-enediol moiety. The antioxidant activity of vitamin C comes from 2,3-enediol (Akbari *et al.*, 2016).

The wavelength of DPPH is 500–520 nm; in this study, the wave length was obtained at 515,5 nm, as shown in **Figure 2**, which is in line with the literature (Saptarini, Rahayu and Herawati, 2019).

The results showed that ethanol extract has the highest antioxidant activity with an IC_{50} of 12.62 ppm, whereas n-hexane extract is the lowest antioxidant with an IC_{50} value of 63.99 ppm, as shown in **Table 3**. (Houghton and Raman, 1998) categorized antioxidant activity into four categories: strong (IC_{50}: 50–100 ppm), moderate (IC_{50}: 100–150 ppm), weak (IC_{50}: 150–200 ppm), and very weak (IC_{50}: >200 ppm). So, extracts and all fractions of jungrahab leaves belong to the category of strong antioxidants.

Plant-derived antioxidants, with or without side effects, can protect the human body from disease caused by free radicals. The mechanism of antioxidant action is to prevent oxidative chain reactions that would otherwise cause damage to the organism (Saptarini, Rahayu and Herawati, 2019).

5. Detemination Total Phenolics Content of Jungrahab Leaves Extract

Phenolic compounds are important secondary metabolites in plants that have antioxidant activity. The total phenolics level of jungrahab leaves extract was measured using the Folin-Ciocalteu method (Aryal *et al.*, 2019). From the results of the study, we obtained the calibration curve for acid gallic as shown in **Figure 3**.

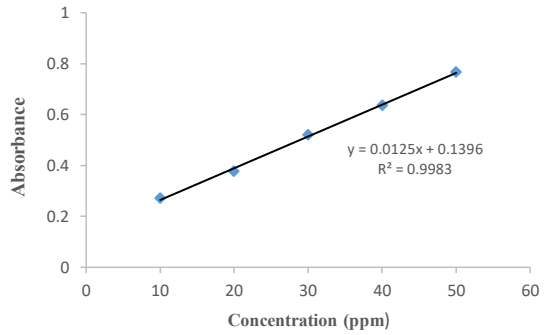

Figure 3. Calibration curve of gallic acid standard. The measurement was performed in triplicates (n=3)

Table 4. Results of total phenolics content on jungrahab leaves extract

Sample	Replicates	Concentration (ppm)	Total phenolics content (mg GAE/g)	Average of total phenolics content (mg GAE/g)
Extract	1	2500	52.27	52.40±0.011
	2		52.43	
	3		52.51	

From the gallic acid calibration curve in **Figure 3**, a linear regression equation is obtained to determine the total phenolic content extract. Gallic acid is chosen as a comparator because it is one of the phenolic compounds with a simple structure, has stable properties, and is available in pure condition (Senet *et al.*, 2018). Gallic acid has an aromatic-OH group, reactioned in a basal atmosphere with Folin-Ciocalteau,

will produce a blue-colored molybdenum-tungsten and measurable absorption. The absorption measurement of gallic acid is performed at maximum wavelength of 751 nm. The higher the concentration of phenolic compounds, the more phenolic ions will be reduced to molybdenum-tungsten complexes, and the resulting color will become more concentrated (Husain, Yunus and Basri, 2023). The results of the study showed that the total phenolic content of jungrahab leaves extract was 52.40 mg GAE/g, as shown in **Table 4.**

6. Determination Flavonoids Content of Jungrahab Leaves Extraxt

Flavonoids are a secondary metabolite known to have antioxidant activity because of their ability to fight free radicals that play a role in the development of degenerative diseases, which can damage the body's immune system and also oxidize proteins and lipids (Husain, Yunus and Basri, 2023). The group that has antioxidants in flavonoids is the hydroxy (-OH) group (Sholikhah, Riyanti and Wahyono, 2023). Because flavonoids have a high redox potential, they may serve as reducing agents, hydrogen donors, and singlet oxygen quenchers (Zehiroglu and Sarikaya, 2019). The method used to determine content of flavonoids is Chang *et al*., 2020 method. The principle of this method is a reaction between $AlCl_3$ and the flavonoid, which will form a stable complex compound with C-4 keto groups as well as C-3 or C-5 hydroxyl groups of flavons and flavonols.

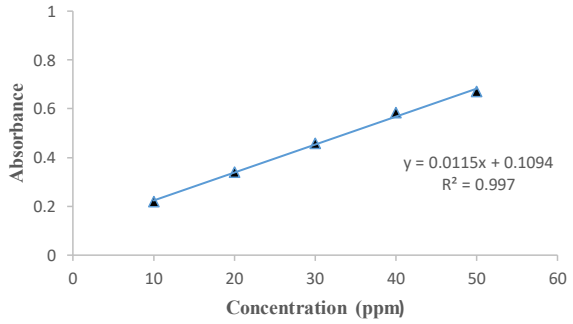

Figure 4. Calibration curve of quercetin standard. The measurement was performed in triplicates (n=3)

Table 5. Results of Flavonoids Content on Jungrahab Leaves Extract

Sample	Replication	Concentration (ppm)	Flavonoids content (mg QE/g)	Average flavonoids content (mg QE/g)
Extract	1	5000	56.75	56.72±0.002
	2		56.58	
	3		56.83	

The standard used for determining the content of flavonoids is quercetin, because quersetin is a flavonoid of the flavonol group that has keto groups in the C-4 atom and also hydroxyl groups in neighboring C-3 and C-5 atoms (Azizah, Kumolowati and Faramayuda, 2014). As seen in **Figure 4**, the linear regression obtained on the quersetin calibration curve is

used to determine the content of flavonoids extract from jungrahab leaves. Quercetin absorption measurements were performed at a maximum wavelength of 435 nm. The result of the determination of the flavonoids content of the leaf extract is 56.72 mg QE/g, as shown in **Table 5**.

CONCLUSION

Jungrahab leaves have high levels of phenolics (52.40 mg GAE/g) and flavonoids (56.72 mg QE/g), so they can be categorized as having strong antioxidant potential in both extracts (12.62 ppm) and fractions.

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5. Bukti perbaikan manuskrip-2 (18 Januari 2024)

DETERMINATION ANTIOXIDANT ACTIVITY, TOTAL PHENOLICS, FLAVONOIDS CONTENT, OF JUNGRAHAB LEAVES (*Baeckea frutescens* L.)

Wahyu Priyo Legowo[1], Irma Erika Herawati[1*], Lisna Dewi[2]
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[2] *Jurusan Farmasi, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas al-Ghifari, Jl. Cisaranten Kulon No.140, Bandung, Indonesia*
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Submitted :December 2, 2023 Revised :..... Accepted:.....

ABSTRACT

Medicinal plants are currently widely used to treat various diseases, and one of the reasons is the safety of medicinal plants. The active compounds in medicinal plants include phenolics and flavonoids, which are widely known to have antioxidant activity. Antioxidants played an essential role in the body's defense against various diseases because antioxidant compounds prevent the bad effects caused by free radicals. Jungrahab (*Baeckea frutescens* L.) was a medicinal plant that contained phenolics and flavonoids. The aim of this research was to determine antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, total phenolic content, and flavonoid content from jungrahab leaf extract. The results show the antioxidant activity of the extract, water fraction, ethyl acetate, and n-hexane of jungrahab leaves with IC_{50} values of 12.62, 61.74, 60.66, and 63.99 ppm, respectively. Meanwhile, jungrahab extract has a total phenolic content of 52.40 mg GAE(gallic acid equivalent)/g and a flavonoid content of 56.72 mg QE(quercetin equivalent)/g. Jungrahab extract is the strongest antioxidant category compared to its fractions.

Keywords: phenolics content, flavonoids content, antioxidant, DPPH (2, 2-Diphenyl-1-picrylhydrazyl), jungrahab

INTRODUCTION

Medicinal plants have been utilized to treat a variety of diseases in traditional herbal methods since ancient times. Despite recent advances in contemporary drug systems, herbal medicine continues is essential in health care. Its lengthy history in conventional medicine and its potential benefits to human health caught the interest of many people, particularly in developing nations. It is now well-recognized that plant medicines are safer than synthetic ones (Phuyal *et al.*, 2020).

Plants contain abundant in phytochemicals such as phenolics, flavonoids, alkaloids, glycosides, lignins, and tannins. The most prevalent phytoconstituents responsible for the antioxidant activity of many fruits, vegetables, and medicinal and aromatic plants are phenols and flavonoids. Natural antioxidants, such as phenol and flavonoid chemicals derived from plants, are gaining benefits due to the potential toxicological consequences of synthetic antioxidants. An antioxidant is a chemical that prevents or delays oxidative damage to organism cells by scavenging free radicals such as peroxide or hydroperoxide, hence lowering the risk of degenerative diseases. Cancer, Alzheimer's disease, heart, kidney, and liver diseases, fibrosis, atherosclerosis, arthritis, and neurological disorders can all be caused by

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abnormal free radical production (Phuyal *et al.*, 2020). Several medicinal plants have been investigated for antioxidant and other biological properties.

Jungrahab (*Baeckea frustescens* L.), as shown in **Figure 1**, is an Australian plant of the Myrtaceae family. The shrimp plant Jungrahab has curled branches, linear leaves, and white flower petals. Jungrahab leaves have been used to treat headaches, rheumatism, and fever. Secondary metabolites found in Jungrahab leaves include flavonoids, sesquiterpenes, triterpenoids, and essential oils (Huong, Duc and Son, 2023). This study aimed to examine the antioxidant activity of extracts and fractions of jungrahab leaves and the extract's total phenolic and flavonoid contents.

Figure 1. Jungrahab (*Baeckea frustescens* L.)
Source: https://id.wikipedia.org/wiki/Ujung_atap

RESEARCH METHODS

Equipment and Materials

The instruments used in this study are the UV-Vis spectrophotometer (Shimadzu), the rotary evaporator (Buchi), 500 mL round flask, 100 mL volumetric flask, 10 mL volumetric flask, dropper pipettes, 1 mL volume pipettes, 10 mL volume pipettes, filter paper, funnel, and other instruments commonly used in the laboratory.

Jungrahab leaves were obtained from Balai Penelitian Tanaman Rempah dan Obat, Kebun Percobaan Manoko, and Cikahuripan Kecamatan Lembang Jawa Barat. The plant was identified in the Plant Taxonomy Laboratory of the Department of Biology, Faculty of Mathematics and Natural Sciences, University of Padjajaran, Jatinangor, with the letter number No. 20/HB/06/2022 stating that the plant was used correctly (*Baeckea frutescens* L.).

The chemicals used are ethanol 70%, $FeCl_3$, gelatin 1%, HCl, magnesium powder (Mg), gallic acid, quercetin, Folin-Ciocalteu reagents, $AlCl_3$ powder, sodium carbonate (Na_2CO_3), and DPPH. (Sigma Aldrich). All the chemicals used are analytical solvents (Merck, Jerman).

Research Procedure

1. **Extraction of Jungrahab Leaves**

   The leaves were extracted with 70% ethanol by the maceration method for three days, with a solvent replacement every 24 hours. The liquid extract is collected and applied with a rotary vaporator at a temperature of 50℃ at a speed of 100 rpm, and then the extract yield is calculated (Yuliana *et al.*, 2023).

2. **Fractionation of Jungrahab Extract**

   Ten grams of extracts are dissolved in aquadest that have been heated to 60˚ and then liquidly extracted using n-hexane and ethyl acetate three times for each

solvent. The entire fraction is collected and applied, and then the fraction yield is calculated (Herawati and Hanifah, 2018).

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3. **Phytochemical Screening**

Phytochemical screening was performed against simplisia, extracts, and fractions of jungrahab leaves using the Harborne. 2007 method, included secondary metabolites of alkaloids, flavonoids, tannins, phenolics, saponins, steroids, triterpenoids, and glycosides.

4. **Antioxidant Activity Extract and Fraction Jungrabah Leaves using 2,2-diphenyl-1-picrylhydrazyl (DPPH)**

Dissolve 4 mg DPPH (2,2-diphenyl-1-picrylhydrazil) with 96% ethanol in a 100 mL (40 g/mL) volumetric flask. Dissolves 5 mg of vitamin C and 50 mg of sample (extract) with 96% ethanol, respectively, in 100 mL of volumetric flask, then dilutes to obtain concentrations of 1; 2; 3; 4; and 5 ppm for vitamin C and 5; 10; 25; 20; and 25 ppm for extract, while for fractions the concentrations are 55; 60; 65; 70; and 75 ppm. A total of 2 mL of vitamin C, extract, and fraction, inserted respectively in the tube, were added to 3 mL of 40 g/mL DPPH. The mixture is diluted and incubated in a dark space for 20 minutes, then its absorption is measured at 515 nm using a spectroscopic photometer. The blank used is 96% ethanol.

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$$\% \text{ inhibition DPPH} = [(Ab-Aa)/Ab] \times 100$$

Where Aa and Ab are the respective sample and blanko absorption values, A percentage of the inhibition curve versus the plasma concentration and sample concentration required for 50% inhibition is determined and expressed as an IC_{50} value (Herawati and Hanifah, 2018).

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Determining total phenolic levels was done using the Folin-Ciocalteu method according to Chun, Kim and Lee, 2003, with modifications. The sample was produced at a concentration of 2500 ppm with a 70% ethanol solvent. A maximum of 0.5 mL of sample is added with 5 mL of the Folin-Ciocalteu reaction (which has been diluted with aquades at a ratio of 1:10) and 4 mL of 1M sodium carbonate. The mixture is incubated for 15 minutes, and then the absorption is measured at the maximum wavelength. Total phenols are calculated using the linear regression equation of the acid calibration curve.

6. **Determination of Flavonoids Content from Jungrahab Leaves Extract**

Flavonoid levels were determined using Chang *et al*., 2020 method with modifications. The sample was produced at a concentration of 5000 ppm using 70 percent ethanol. A total of 0.5 mL of the sample was added with 1.5 mL of 70 percent ethanol, then with 0.1 mL of $AlCl_3$, 10%, 0.1 mL of 1 M sodium acetate, and 2.8 mL of aquadest. The mixture is incubated for 30 minutes, and the solution absorption of the sample is measured with UV-Vis spectroscopy at maximum wavelengths. Total flavonoids are calculated using the linear regression equation of the quersetin calibration curve.

RESULTS AND DISCUSSION

1. **Extraction of Jungrahab Leaves**

In this study, the extraction method used was maceration with 70% ethanol solvent. Maceration was chosen because it was a simple method and suitable for

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From **Table 1** above, it can be seen that the secondary metabolites present in the jungrahab leaves are more polar, followed by the non-polar compounds and the latter is the semi-polar secondary.

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3. Phytochemical Screening

Phytochemical screening is performed qualitatively by observing the color or changes formed after a reaction with a particular response. This phytochemical screening aim was for identification the contents of the secondary metabolites present in the Simplicia extracts and fractions of the leaf. Results of phytochemical screening can be seen in **Table 2.**

Table 2. Results of Phytochemical Screening for Jungrab Leaves

No	Compound	Crude material	Extract	Fraction		
				Water	n-hexane	Ethyl acetate
1	Alkaloids	+	+	+	+	+
2	Phenolics	+	+	+	+	+
3	Flavonoids	+	+	+	+	-
4	Tannins	+	+	+	+	-
5	Saponins	+	+	+	-	-
6	Steroids and terpenoids	+	+	-	+	-
7	Glycosides	+	-	-	-	-

Notes:
(+): detected
(-): not detected

According to the results of phytochemical screening performed on crude material, extracts, and fractions of leaves, leaves have potential as antioxidants due to the presence of secondary phenolic metabolites and flavonoids (Saptarini and Herawati, 2017).

4. Antioxidant Activity Extract and Fraction of Jugrahab Leaves using DPPH

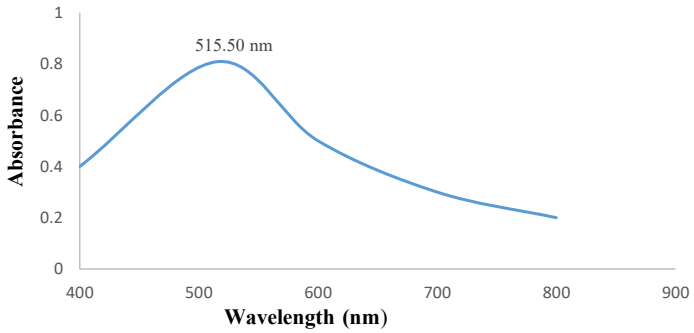

Figure 2. Maximum wavelength of DPPH solution in 96% ethanol

Table 3. Results of antioxidant activity on jungrahab leaves extract and fraction

Sample	Concentration (ppm)	Inhibition (%)	Linear Regression	IC_{50} (ppm)
Vitamin C (standard)	1	23.36		
	2	30.53		
	3	39.18	y=7.058x + 16.477	4.75
	4	42.52		
	5	52.66		
Extract	5	42.89		
	10	46.23		
	15	53.65	y=0.9914x+ 37.49	12.62
	20	56.49		
	25	65.55		
Water fraction	55	40.91		
	60	46.48		
	65	55.38	y=1.4512x+ 39.592	61.74
	70	60.94		
	75	69.96		
Ethyl acetate fraction	55	39.43		
	60	48.83		
	65	58.71	y=1.7182x+ 54.229	60.66
	70	66.99		
	75	73.30		
n-hexane fraction	55	33.50		
	60	45.49		
	65	52.78	y=1.6119x+ 53.152	63.99
	70	59.58		
	75	66.75		

The DPPH (2,2-diphenyl-1-picrylhydrazil) technique is used for determining antioxidant activity. The DPPH approach was chosen because DPPH is a stable free radical that absorbs at 515 nm. The antioxidant activity of pure phenolic compounds or plant extracts is commonly determined using this approach (Shalaby and Shanab, 2013). Since vitamin C can neutralize free radicals through electron donation and transfer mechanisms, it is employed as a standard in antioxidant activity testing (Caritá

et al., 2020). Vitamin C is a six-carbon lactone ring structure with 2,3-enediol moiety. The antioxidant activity of vitamin C comes from 2,3-enediol (Akbari *et al.*, 2016).

The wavelength of DPPH is 500–520 nm; in this study, the wave length was obtained at 515,5 nm, as shown in **Figure 2**, which is in line with the literature (Saptarini, Rahayu and Herawati, 2019).

The results showed that ethanol extract has the highest antioxidant activity with an IC_{50} of 12.62 ppm, whereas n-hexane extract is the lowest antioxidant with an IC_{50} value of 63.99 ppm, as shown in **Table 3**. (Houghton and Raman, 1998) categorized antioxidant activity into four categories: strong (IC_{50}: 50–100 ppm), moderate (IC_{50}: 100–150 ppm), weak (IC_{50}: 150–200 ppm), and very weak (IC_{50}: >200 ppm). So, extracts and all fractions of jungrahab leaves belong to the category of strong antioxidants.

Plant-derived antioxidants, with or without side effects, can protect the human body from disease caused by free radicals. The mechanism of antioxidant action is to prevent oxidative chain reactions that would otherwise cause damage to the organism (Saptarini, Rahayu and Herawati, 2019).

5. Detemination Total Phenolics Content of Jungrahab Leaves Extract

Phenolic compounds are important secondary metabolites in plants that have antioxidant activity. The total phenolics level of jungrahab leaves extract was measured using the Folin-Ciocalteu method (Aryal *et al.*, 2019). From the results of the study, we obtained the calibration curve for acid gallic as shown in **Figure 3**.

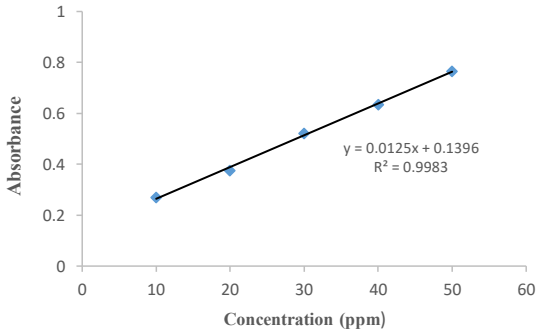

Figure 3. Calibration curve of gallic acid standard. The measurement was performed in triplicates (n=3)

Table 4. Results of total phenolics content on jungrahab leaves extract

Sample	Replicates	Concentration (ppm)	Total phenolics content (mg GAE/g)	Average of total phenolics content (mg GAE/g)
Extract	1	2500	52.27	52.40±0.011
	2		52.43	
	3		52.51	

From the gallic acid calibration curve in **Figure 3**, a linear regression equation is obtained to determine the total phenolic content extract. Gallic acid is chosen as a comparator because it is one of the phenolic compounds with a simple structure, has

stable properties, and is available in pure condition (Senet *et al.*, 2018). Gallic acid has an aromatic-OH group, reactioned in a basal atmosphere with Folin-Ciocalteau, will produce a blue-colored molybdenum-tungsten and measurable absorption. The absorption measurement of gallic acid is performed at maximum wavelength of 751 nm. The higher the concentration of phenolic compounds, the more phenolic ions will be reduced to molybdenum-tungsten complexes, and the resulting color will become more concentrated (Husain, Yunus and Basri, 2023). The results of the study showed that the total phenolic content of jungrahab leaves extract was 52.40 mg GAE/g, as shown in **Table 4.**

6. Determination Flavonoids Content of Jungrahab Leaves Extraxt

Flavonoids are a secondary metabolite known to have antioxidant activity because of their ability to fight free radicals that play a role in the development of degenerative diseases, which can damage the body's immune system and also oxidize proteins and lipids (Husain, Yunus and Basri, 2023). The group that has antioxidants in flavonoids is the hydroxy (-OH) group (Sholikhah, Riyanti and Wahyono, 2023). Because flavonoids have a high redox potential, they may serve as reducing agents, hydrogen donors, and singlet oxygen quenchers (Zehiroglu and Sarikaya, 2019). The method used to determine content of flavonoids is Chang *et al*., 2020 method. The principle of this method is a reaction between $AlCl_3$ and the flavonoid, which will form a stable complex compound with C-4 keto groups as well as C-3 or C-5 hydroxyl groups of flavons and flavonols.

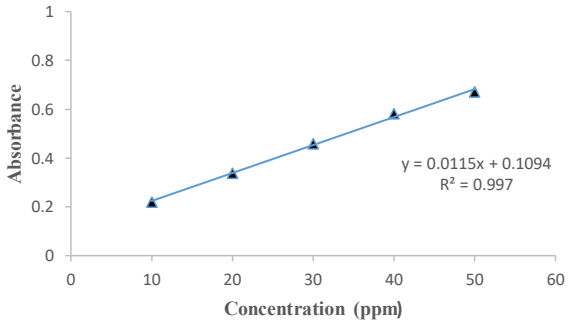

Figure 4. Calibration curve of quercetin standard. The measurement was performed in triplicates (n=3)

Table 5. Results of Flavonoids Content on Jungrahab Leaves Extract

Sample	Replication	Concentration (ppm)	Flavonoids content (mg QE/g)	Average flavonoids content (mg QE/g)
Extract	1	5000	56.75	56.72±0.002
	2		56.58	
	3		56.83	

The standard used for determining the content of flavonoids is quercetin, because quersetin is a flavonoid of the flavonol group that has keto groups in the C-4 atom and also

hydroxyl groups in neighboring C-3 and C-5 atoms (Azizah, Kumolowati and Faramayuda, 2014). As seen in **Figure 4**, the linear regression obtained on the quersetin calibration curve is used to determine the content of flavonoids extract from jungrahab leaves. Quercetin absorption measurements were performed at a maximum wavelength of 435 nm. The result of the determination of the flavonoids content of the leaf extract is 56.72 mg QE/g, as shown in **Table 5**.

CONCLUSION

Jungrahab leaves have high levels of phenolics (52.40 mg GAE/g) and flavonoids (56.72 mg QE/g), so they can be categorized as having strong antioxidant potential in both extracts (12.62 ppm) and fractions.

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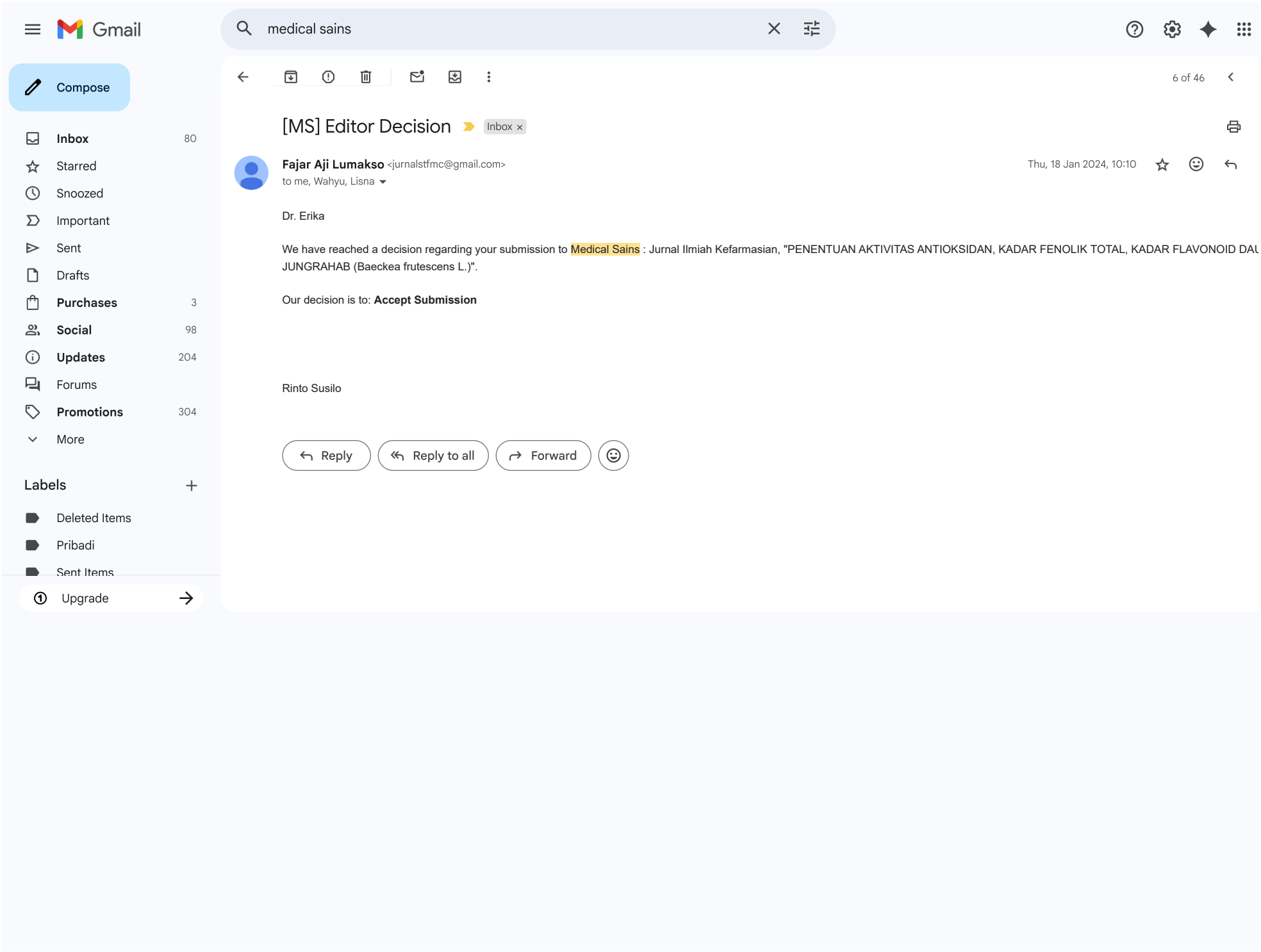
6. Bukti manuskrip diterima (18 Januari 2024)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DETERMINATION ANTIOXIDANT ACTIVITY, TOTAL PHENOLICS, FLAVONOIDS CONTENT, OF JUNGRAHAB LEAVES (*Baeckea frutescens* L.)

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ABSTRACT

Medicinal plants are widely used to treat various diseases, and one of the reasons for this is the safety of medicinal plants. The active compounds in medicinal plants include phenolics and flavonoids, which are known to have antioxidant activity. Antioxidants play an essential role in the body's defense against various diseases because they prevent the negative effects of free radicals. Jungrahab (*Baeckea frutescens* L.) is a medicinal plant containing phenolics and flavonoids. This study aimed to determine the antioxidant activity of jungrahab leaf extracts using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method. The results showed the antioxidant activity of the extract, water fraction, ethyl acetate, and n-hexane of jungrahab leaves with IC_{50} values of 12.62, 61.74, 60.66, and 63.99 ppm, respectively. Meanwhile, jungrahab extract has a total phenolic content of 52.40 mg GAE (Gallic Acid Equivalent)/g and a flavonoid content of 56.72 mg QE (Quercetin Equivalent)/g. Jungrahab extract showed strongest antioxidant category compared to its fractions.

Keywords: phenolics content, flavonoids content, antioxidant activity, DPPH, jungrahab

INTRODUCTION

Medicinal plants have been used to treat a variety of diseases using traditional herbal methods since ancient times. Despite recent advances in contemporary drug systems, herbal medicine continues to be essential for healthcare. Its lengthy history in conventional medicine and potential benefits to human health have attracted the interest of many people, particularly in developing nations. It is now well recognized that plant medicines are safer than synthetic medicines (Phuyal et al., 2020).

Plants contain abundant phytochemicals, such as phenolics, flavonoids, alkaloids, glycosides, lignins, and tannins. Phenols and flavonoids are the most prevalent phytoconstituents responsible for the antioxidant activity of many fruits, vegetables, and medicinal and aromatic plants. Natural antioxidants such as phenol and flavonoid chemicals derived from plants are gaining benefits because of the potential toxicological consequences of synthetic antioxidants. An antioxidant is a chemical that prevents or delays oxidative damage to cells by scavenging free radicals such as peroxide or hydroperoxide, thereby lowering the risk of degenerative diseases. Cancer; Alzheimer's disease; heart, kidney, and liver diseases; fibrosis; atherosclerosis; arthritis; and neurological disorders can all be caused by abnormal free radical production (Phuyal et al., 2020). Several medicinal plants have been investigated for their antioxidant and biological properties.

Jungrahab (*Baeckea frustescens* L.), shown in Figure 1, is an Australian plant belonging to the Myrtaceae family. The shrimp plant Jungrahab has curled branches, linear leaves, and white flower petals. Jungrahab leaves have been used to treat headache, rheumatism, and fever. Secondary metabolites found in jungrahab leaves include flavonoids,

sesquiterpenes, triterpenoids, and essential oils (Huong et al., 2023). This study aimed to examine the antioxidant activity of jungrahab leaf extracts and fractions and the total phenolic and flavonoid contents of the extract.

Figure 1. Jungrahab (*Baeckea frustescens* L.)

RESEARCH METHODS

Equipment and Materials

The instruments used in this study were a UV-Vis spectrophotometer (Shimadzu), rotary evaporator (Buchi), 500 mL round flask, 100 mL volumetric flask, 10 mL volumetric flask, dropper pipettes, 1 mL volume pipettes, 10 mL volume pipettes, filter paper, funnel, and other instruments commonly used in the laboratory.

Jungrahab leaves were obtained from Balai Penelitian Tanaman Rempah dan Obat, Kebun Percobaan Manoko, and Cikahuripan Kecamatan Lembang Jawa Barat. The plant was identified in the Plant Taxonomy Laboratory of the Department of Biology, Faculty of Mathematics and Natural Sciences, University of Padjajaran, Jatinangor, with letter number No. 20/HB/06/2022, indicating that the plant was correctly used (*Baeckea frutescens* L.).

The chemicals used were ethanol 70%, $FeCl_3$, gelatin 1%, HCl, magnesium powder (Mg), gallic acid, quercetin, Folin-Ciocalteu reagents, $AlCl_3$ powder, sodium carbonate (Na_2CO_3), and DPPH. (Sigma Aldrich). All the chemicals used were analytical solvents (Merck, Jerman).

Research Procedure

1. **Extraction of Jungrahab Leaves**

   The leaves were extracted with 70% ethanol by the maceration method for three days, with a solvent replacement every 24 hours. The liquid extract was collected and applied with a rotary vaporator at a temperature of 50 °C and a speed of 100 rpm, and then the extract yield was calculated (Yuliana et al., 2023).

2. **Fractionation of Jungrahab Extract**

   Ten grams of extracts were dissolved in an aquadest that had been heated to 60 °C and then subjected to liquid-liquid extraction (LLC) with n-hexane and ethyl acetate three times for each solvent. The entire fraction was collected and applied, and the fraction yield was calculated (Herawati and Hanifah, 2018).

3. **Phytochemical Screening**

   Phytochemical screening was performed against simplisia, extracts, and fractions of jungrahab leaves using the Harborne, (2007) method, of following secondary metabolites.

Alkaloids Test

Approximately 500 mg of the sample was stirred with a few 2N HCl and 9 mL of aquadest, and then heated in water for 2 minutes. The mixture was then cooled and filtered. The filtrate was used to perform a test with Dragendorff and Mayer reagents.

Flavonoids Test

Approximately 3 mL of the sample was treated with 1 mL of 10% NaOH solution. The formation of an intense yellow color indicates the presence of flavonoids.

Phenolics Test

The sample (50 mg) was dissolved in 5 mL distilled water. To this, a few drops of a neutral 5% ferric chloride solution were added. The dark green color indicated the presence of a phenolic compound.

Tannins Test

The sample (50 mg) was dissolved in 5 mL of distilled water and 2 mL of a 1% solution of gelatin containing 10% NaCl was added. The white precipitate indicated the presence of tannins.

Saponins Test

Approximately 3 mL of each sample was added to 3 mL of distilled water and shaken vigorously. The formation of a stable, persistent froth was considered a positive result for saponins.

Steroids/Triterpenoids

Approximately 2 mL of chloroform and concentrated H_2SO_4 was added to 5 mL of the prepared plant extracts. A layer of red color indicates the presence of steroids in the lower chloroform.

Glycosides Test

The 50 mg sample was hydrolyzed with concentrated hydrochloric acid for 2 hours in a water bath, filtered and the hydrolysate is subjected to Borntrager's test. To 2 mL of filtered hydrolysate, 3 mL of chloroform was added and shaken, the chloroform layer was separated, and 10% ammonia solution was added. Pink color indicates the presence of glycosides.

4. Antioxidant Activity Extract and Fraction Jungrabah Leaves using 2,2-diphenyl-1-picrylhydrazyl (DPPH)

Dissolve 4 mg DPPH (2,2-diphenyl-1-picrylhydrazil) in 96% ethanol in a 100 mL (40 g/mL) volumetric flask. Dissolves 5 mg of vitamin C and 50 mg of sample (extract) were mixed with 96% ethanol in a 100 mL volumetric flask, and then diluted to obtain concentrations of 1, 2, 3, 4, and 5 ppm for vitamin C and 5, 10, 25, 20, and 25 ppm for extract, while for fractions the concentrations were 55, 60, 65, 70, and 75 ppm. A total of 2 mL of vitamin C, extract, and fraction, inserted respectively in the tube, were added to 3 mL of 40 g/mL DPPH. The mixture was diluted and incubated in the dark for 20 minutes, and its absorption was measured at 515 nm using a spectroscopic photometer. The blank was 96% ethanol.

The percentage antioxidant activity was calculated using the following formula:

$$\%\ Inhibition\ DPPH = \left[\frac{(Ab - Aa)}{Ab}\right] x100$$

Where Aa and Ab are the sample and blanko absorption values, respectively, and the percentage of the inhibition curve versus the plasma concentration and sample concentration required for 50% inhibition was determined and expressed as an IC_{50} value (Herawati & Hanifah, 2018).

5. Determination of Total Phenolics Content from Jungrahab Leaves Extract

Total phenolic levels were determined using the Folin-Ciocalteu method, as described by Chun et al., (2003), with modifications. The sample was produced at a concentration of 2500 ppm using 70% ethanol. The sample (0.5 mL) was added to 5 mL

of the Folin-Ciocalteu reaction (diluted with aquades at a ratio of 1:10) and 4 mL of 1M sodium carbonate. The mixture was incubated for 15 minutes, and absorption was measured at the maximum wavelength. The total phenol content was calculated using a linear regression equation of the acid calibration curve.

6. Determination of Flavonoids Content from Jungrahab Leaves Extract

Flavonoid levels were determined using the method described by Chang et al. (2020), with modifications. The sample was produced at a concentration of 5000 ppm using 70 percent ethanol. A total of 0.5 mL of the sample was added with 1.5 mL of 70 percent ethanol, then with 0.1 mL of $AlCl_3$, 10%, 0.1 mL of 1 M sodium acetate, and 2.8 mL of aquadest. The mixture was incubated for 30 minutes, and the solution absorption of the sample was measured using UV-Vis spectroscopy at the maximum wavelengths. The total flavonoid content was calculated using the linear regression equation of the quercetin calibration curve.

RESULTS AND DISCUSSION

Extraction of Jungrahab Leaves

In this study, maceration with 70% ethanol was used as the extraction method. Maceration was chosen because it is a simple method suitable for soluble secondary metabolite compounds (not resistant to heat). Maceration has the advantage of no heating during the secondary metabolite withdrawal process; therefore, it does not damage the compounds in the simplicial process (Widiastuti et al., 2023). The result of maceration on the thick extracts of jungrahab leaves was brown-green with an extract yield of 18.78%.

Fractionation of Jungrahab Leaves

Fractionation is a technique for separating and grouping the chemical content of extracts based on polarization. In the fractioning process, two solvents that were not mixed were used. The liquid-liquid extraction method was chosen in this study. The purpose of fractionation is to separate compounds according to their polarity so that the number and type of compounds are different fractions (Saptarini et al., 2019). The yield results from the fractionation of the extracts of jungrahab leaves can be seen in Table I.

Table I. Results of Jungrahab Fractination

Fraction	Yield (%)
Water	39.6
n-hexane	23.5
Ethyl Acetate	15.6

From Table I, it can be concluded that jungrahab leaf extract had a secondary metabolite that was mostly attracted to polar solvents, then to non-polar solvents, and the latter to the secondary metabolite that was attracted to semi-polar solvents.

Phytochemical Screening

Phytochemical screening is performed qualitatively by observing the color or changes formed after a reaction with a particular response. This phytochemical screening aimed to identify the contents of secondary metabolites present in the Simplicia extracts and fractions of the leaf. The results of phytochemical screening are shown in Table II.

Table II. Results of Phytochemical Screening for Jungrahab Leaves

No.	Compound	Crude material	Extract	Fraction		
				Water	n-hexane	Ethyl acetate
1.	Alkaloids	+	+	+	+	+
2.	Phenolics	+	+	+	+	+

3.	Flavonoids	+	+	+	+	-
4.	Tannins	+	+	+	+	-
5.	Saponins	+	+	+	-	-
6.	Steroids and terpenoids	+	+	-	+	-
7.	Glycosides	+	-	-	-	-

Notes:
(+): detected
(-): not detected

According to the results of phytochemical screening performed on crude material, extracts, and fractions of leaves, leaves have potential as antioxidants due to the presence of secondary phenolic metabolites and flavonoids (Saptarini et al., 2019).

Antioxidant Activity Extract and Fraction of Jugrahab Leaves using DPPH

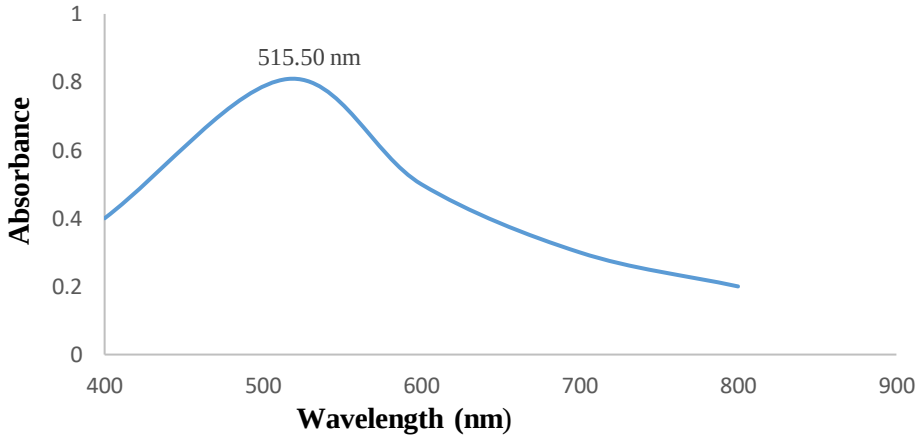

Figure 2. Maximum Wavelength of DPPH Solution in 96% Ethanol

Table III. Results of Antioxidant Activity on Jungrahab Leaves Extract and Fraction

Sample	Concentration (ppm)	Inhibition (%)	Linear Regression	IC_{50} (ppm)
Vitamin C (standard)	1	23.36	y=7.058x + 16.477	4.75
	2	30.53		
	3	39.18		
	4	42.52		
	5	52.66		
Extract	5	42.89	y=0.9914x+ 37.49	12.62
	10	46.23		
	15	53.65		
	20	56.49		
	25	65.55		
Water fraction	55	40.91	y=1.4512x+ 39.592	61.74
	60	46.48		
	65	55.38		
	70	60.94		
	75	69.96		
Ethyl	55	39.43	y=1.7182x+ 54.229	60.66

acetate fraction	60	48.83		
	65	58.71		
	70	66.99		
	75	73.30		
n-hexane fraction	55	33.50		
	60	45.49		
	65	52.78	y=1.6119x+ 53.152	63.99
	70	59.58		
	75	66.75		

The DPPH (2,2-diphenyl-1-picrylhydrazil) technique was used to determine antioxidant activity. The DPPH approach was chosen because DPPH is a stable free radical that absorbs at 515 nm. The antioxidant activity of pure phenolic compounds or plant extracts is commonly determined using this approach (Shalaby and Shanab, 2013). Since vitamin C can neutralize free radicals through electron donation and transfer mechanisms, it has been employed as a standard in antioxidant activity testing (Caritá et al., 2020). Vitamin C has a six-carbon lactone ring structure with a 2,3-enediol moiety. The antioxidant activity of vitamin C comes from 2,3-enediol (Akbari et al., 2016).

The wavelength of DPPH was 500–520 nm; in this study, the wavelength was obtained at 515,5 nm, as shown in Figure 2, which is in line with the literature (Saptarini et al., 2019).

The results showed that the ethanol extract had the highest antioxidant activith (IC_{50} = 12.62 ppm, whereas the n-hexane extract had the lowest antioxidant activity (IC_{50} value of 63.99 ppm, as shown (Table III). Houghton and Raman (1998) categorized antioxidant activity into four categories: strong (IC_{50}:50–100 ppm), moderate (IC_{50}:100–150 ppm), weak (IC_{50}:150–200 ppm), and very weak (IC_{50} >200 ppm). Therefore, the extracts and all fractions of jungrahab leaves belong to the category of strong antioxidants.

Plant-derived antioxidants with or without side effects can protect the human body from diseases caused by free radicals. The mechanism of antioxidant action is to prevent oxidative chain reactions that would otherwise cause damage to the organism (Saptarini et al., 2019).

Detemination Total Phenolics Content of Jungrahab Leaves Extract

Phenolic compounds are important secondary metabolites in plants that exhibit antioxidant activities. The total phenolic content of the jungrahab leaf extract was measured using the Folin-Ciocalteu method (Aryal et al., 2019). From the results of this study, we obtained a calibration curve for gallic acid, as shown in Figure 3.

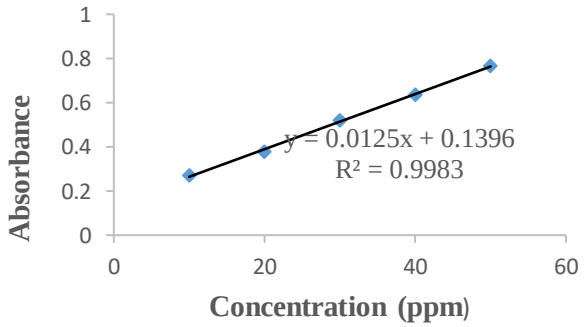

Figure 3. Calibration Curve of Gallic Acid Standard the Measurement was Performed in Triplicates (n=3)

Table IV. Results of Total Phenolics Content on Jungrahab Leaves Extract

Sample	Replicates	Concentration (ppm)	Total phenolics content (mg GAE/g)	Average of total phenolics content (mg GAE/g)
Extract	1	2500	52.27	52.40±0.011
	2		52.43	
	3		52.51	

From the gallic acid calibration curve shown in Figure 3, a linear regression equation was obtained to determine the total of the extract. Gallic acid was chosen as a comparator because it is a phenolic compound with a simple structure, has stable properties, and is available under pure conditions (Senet et al., 2018). Gallic acid has an aromatic OH group and reacts in a basal atmosphere with Folin-Ciocalteu reagent, which produces blue-colored molybdenum-tungsten and measurable absorption. The absorption of gallic acid was measured at a maximum wavelength of 751 nm. The higher the concentration of phenolic compounds, the more phenolic ions are reduced to molybdenum-tungsten complexes, and the resulting color becomes more concentrated (Husain et al., 2023). The results of the study showed that the total phenolic content of jungrahab leaves extract was 52.40 mg GAE/g, as shown in Table IV.

Determination Flavonoids Content of Jungrahab Leaves Extraxt

Flavonoids are secondary metabolites known to have antioxidant activity because of their ability to fight free radicals that play a role in the development of degenerative diseases, which can damage the immune system and oxidize proteins and lipids (Husain et al., 2023). The hydroxyl (-OH) group contains antioxidants in flavonoids (Sholikhah et al., 2023). As flavonoids have a high redox potential, they may serve as reducing agents, hydrogen donors, and singlet oxygen quenchers (Zehiroglu & Sarikaya, 2019). Flavonoid content was determined using the method described by Chang et al., (2020). The principle of this method is a reaction between $AlCl_3$ and the flavonoid, which forms a stable complex with C-4 keto groups, as well as the C-3 or C-5 hydroxyl groups of flavons and flavonols.

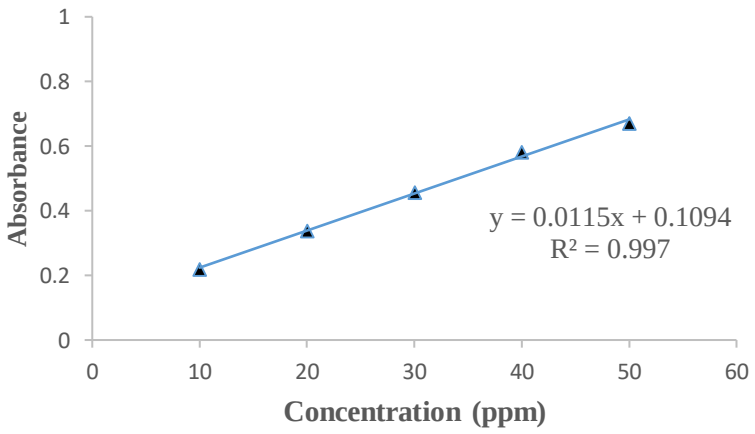

Figure 4. Calibration Curve of Quercetin Standard The Measurement was Performed in Triplicates (n=3)

Table V. Results of Flavonoids Content on Jungrahab Leaves Extract

Sample	Replication	Concentration (ppm)	Flavonoids content (mg QE/g)	Average flavonoids content (mg QE/g)
Extract	1	5000	56.75	56.72±0.002
	2		56.58	
	3		56.83	

The standard used for determining the flavonoid content is quercetin, because quercetin is a flavonoid of the flavonol group that has keto groups in the C-4 atom and hydroxyl groups in neighboring C-3 and C-5 atoms (Azizah et al., 2014). As shown in Figure 4, the linear regression obtained from the quercetin calibration curve was used to determine the content of flavonoid extracts from jungrahab leaves. Quercetin absorption was measured at a maximum wavelength of 435 nm. The result of the determination of the flavonoids content of the leaf extract is 56.72 mg QE/g, as shown in Table V.

CONCLUSION

Jungrahab leaves have high levels of phenolics (52.40 mg GAE/g) and flavonoids (56.72 mg QE/g), so they can be categorized as having strong antioxidant potential in both extracts (12.62 ppm) and fractions.

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