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

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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

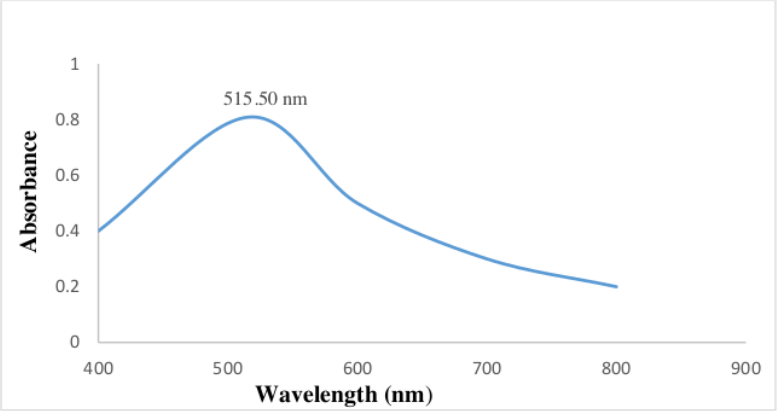

Figure 1. Maximum Wave Length of DPPH

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**.

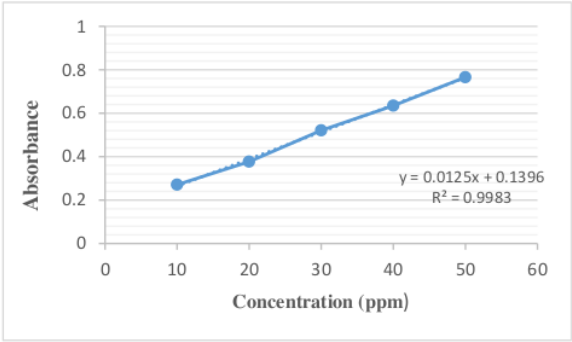

Figure 2. Calibration Curve of Gallic Acid (n=3)

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.

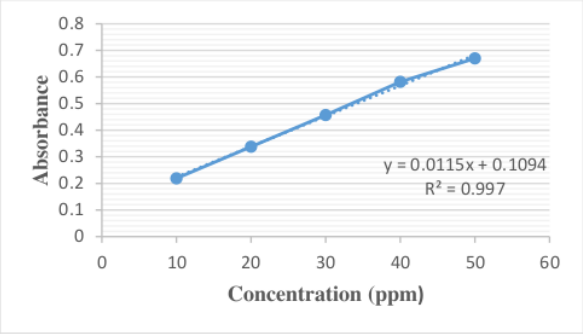

Figure 3. Calibration Curve of Quercetin (n=3)

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.

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