

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

Wahyu Priyo Legowo¹, Irma Erika Herawati^{1*}, Lisna Dewi²

¹Sekolah Tinggi Farmasi Indonesia, Jl. Soekarno Hatta No.354, Bandung, Indonesia

²Jurusan Farmasi, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas al-Ghifari, Jl. Cisaranten Kulon No.140, Bandung, Indonesia

*Email Corresponding: irmaerika@stfi.ac.id

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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 IC₅₀ 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 frutescens* 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 frutescens* 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%, FeCl₃, gelatin 1%, HCl, magnesium powder (Mg), gallic acid, quercetin, Folin-Ciocalteu reagents, AlCl₃ powder, sodium carbonate (Na₂CO₃), 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 H₂SO₄ 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:

$$\% \text{ Inhibition DPPH} = \left[\frac{(Ab - Aa)}{Ab} \right] \times 100$$

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 IC₅₀ 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

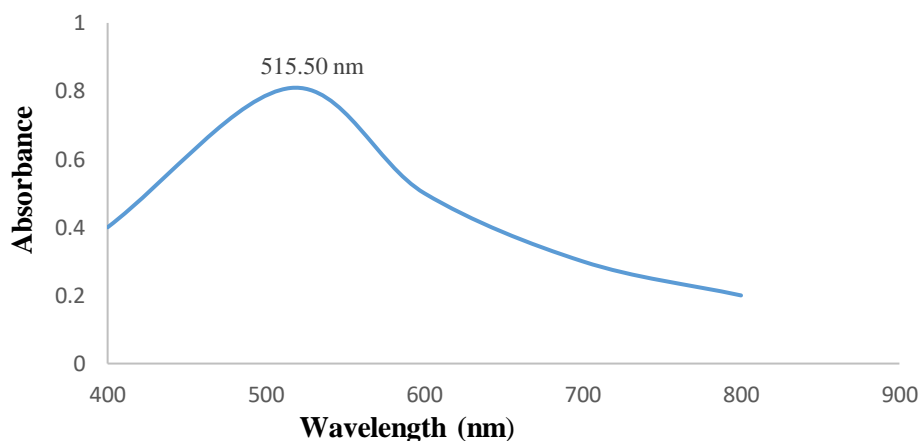


Figure 2. Maximum Wavelength of DPPH Solution in 96% Ethanol

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	$y=1.6119x+ 53.152$	63.99
	60	45.49		
	65	52.78		
	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 activity ($IC_{50} = 12.62$ 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).

Determination 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.

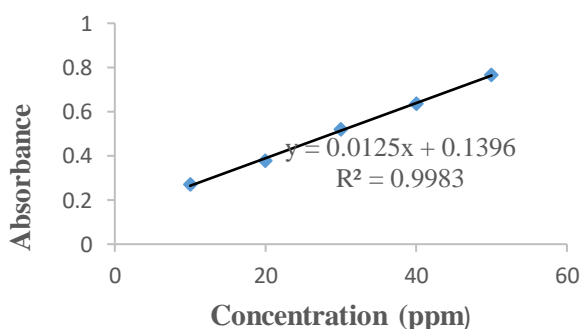


Figure 3. Calibration Curve of Gallic Acid Standard the Measurement was Performed in Triplicates (n=3)

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 Extract

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.

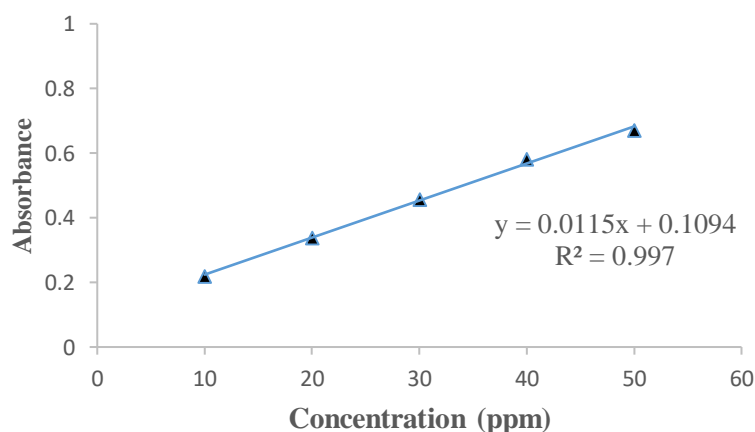


Figure 4. Calibration Curve of Quercetin Standard The Measurement was Performed in Triplicates (n=3)

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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