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Antioxidant Activity Assay of *Eucalyptus pellita* Leaf Extract

Murni Suryani Lumbantoruan[1], Sovia Lenny[2*], Helmina Br. Sembiring[2]

[1]Postgraduate School, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan, 20155, Indonesia
[2]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan, 20155, Indonesia

Abstract. In pharmacy, eucalyptus leaves are used for various pharmacological activities because they contain phenolic compounds. This study aims to detect phytochemical compounds and test the antioxidant activity of *Eucalyptus pellita* leaf extract. The dry powder of *E. pellita* leaves (1900 g) was macerated first with methanol solvent. The concentrated methanol extract was then subjected to partition extraction with n - hexane solvent. The methanol layer was concentrated and then partitioned with ethyl acetate solvent. Methanol extract was obtained as much as 176.75 g (9,30%), n-hexane extract 13.77 g (0.72%), and ethyl acetate extract 27.93g (1.47%). The three extracts were subjected to phytochemical screening, methanol extract and ethyl acetate extract were positive for flavonoids. These three extracts did not contain alkaloids but were positive for tannins and saponins. Antioxidant activity was tested using the DPPH (1,1 diphenyl-2-picrylhydrazyl) method with UV-vis spectrophotometer (max wavelength 517 nm). Sample concentration variations were 12.5; 25; 50; 100; and 200 ppm. Ethyl acetate extract, methanol extract and n - hexane extract have antioxidant activity with IC50 values of 6.811; 17.923; and 31.109 µg/mL, respectively. This shows that *E. pellita* leaf extract has very strong antioxidant activity and ethyl acetate extract has more antioxidant activity.

Keyword: Antioxidant Activity, Leaf Extract, *Eucalyptus pellita,* Phytochemical Screening

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1 Introduction

Antioxidants are substances that stop chain reactions from starting and spreading, thereby inhibiting or delaying a molecule's oxidation. The antioxidant activity of a compound is said to be very strong if its IC50 value is less than 50, strong if between 50 and 100, moderate if between 150 and 200, and very weak if greater than 200. The DPPH method was chosen to evaluate the antioxidant activity of natural materials because it has advantages such as being fast, easy, sensitive, and only requiring a small sample [1]. The usage of synthetic antioxidants that destroy

Corresponding author at: [Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan, Indonesia, 20155]

E-mail address: [*sofia1@usu.ac.id*]

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Type I alveoli and lung endothelial cells, such as butyl hydrocytoluene (BHT) and butyl hydroxyanisole (BHA), has a carcinogenic side effect [2]. Antioxidant substances originating from plants have therefore received a lot of attention and research because they are safe for the body [3].

The flowering plant family Myrtaceae, which includes the genus Eucalyptus, is found worldwide but is most abundant in the tropics. Many species in this genus are considered potential allelopaths. These species are used by indigenous Australians to treat fungal infections and heal wounds [4]. Eucalyptus plantations worldwide are dominated by the "big nine" species (*E. camaldulensis, E. grandis, E. tereticornis, E. globulus, E. nitens, E. urophylla, E. saligna, E. dunnii, and E. pellita*) and their hybrids, which together account for over 90% of eucalyptus plantations[5]. Eucalyptus grows quickly and has advantages in terms of stems, twigs, and leaves. Eucalyptus has been utilized by PT Toba Pulp Lestari Tbk as a raw material for papermaking. The wood and twigs of the eucalyptus plant are still utilized, while the leaves have not undergone maximum processing [5]. Eucalyptus is often used as a medicine because it contains terpenes, porphyrin derivatives, and other phenolic compounds for various pharmacological activities [6]. Leaf extracts from Eucalyptus species have been used traditionally to cure wounds, colds, asthma, cough, diarrhea, dysentery, bleeding, laryngalgia, laryngitis, sore throat, convulsions, trachagia, vermifuge, and fungal infections[7].

Eucalyptus pellita is one of the many species used for afforestation and is considered an attractive biomass due to its environmental resilience and strong resistance to pests and diseases. The rapid growth rate of *E. pellita*, which can grow with a planting rotation of 4-8 years and reach a height of 13-15 m within 3.5 years, also has remarkable [8]. According to [9], *E. pellita* stem bark contains high total phenolate content (TPC) and total flavanol content (TFC), indicating potential antioxidant properties. Based on the description above, *E. pellita* has the potential as an antioxidant as well as one of the plants that contain flavonoid compounds. The utilization of Eucalyptus leaves is still minimal so this study aims to detect phytochemical compounds and test the antioxidant activity of *Eucalyptus pellita* leaf extract.

2 Experimental Section

2.1 Sample Preparation and Partition Extraction of *Eucalyptus pellita* Leaves

Eucalyptus pellita leaf samples were dried in the air, then finely ground until a powder of 1900 grams was obtained. Then macerated with methanol as much as 20 L until all samples are submerged and left for 48 hours,then filtered and concentrated using a rotary evaporator so that a concentrated methanol extract is obtained, then the concentrated methanol extract is repeatedly partitioned using n-hexane until the n-hexane layer is clear. The methanol layer is concentrated until the methanol solvent evaporates. The n-hexane layer was concentrated with a rotary

evaporator to obtain a concentrated n-hexane extract. The methanol extract was then partitioned with an ethyl acetate solvent. The ethyl acetate extract obtained is then concentrated with a rotary evaporator until the ethyl acetate solvent evaporates. Thus, methanol extract, n-hexana extract, and ethyl acetate extract of *Eucalyptus pellita* leaves.

2.2 Phytochemical Screening

Methanol, ethyl acetate, and n-hexane extracts of *Eucalyptus pellita* leaves were each tested for phytochemical screening (alkaloids, flavonoids, tannins, steroids, and triterpenoids and saponins). The same procedure was performed on n-hexane extract and ethyl acetate extract.

Alkaloids: The methanol, ethyl acetate,and n- hexane extracts from *Eucalyptus pellita* leaves were each put in four test tubes. Tube I was tested with Wagner's reagent, tube II with Maeyer's reagent, tube III with Bouchardat's reagent,and tube IV with Dragendorf's reagent. Positive test results are indicated by the presence of a white, brown, or orange precipitate.

Flavonoids: Methanol extract, ethyl acetate, and n - hexane extracts from *E. pellita* leaves were each put in 2 test tubes. Tube I was treated with 10% NaOH, tube II with Mg powder and concentrated HCl. The onset of a yellow, blue, orange, or red color indicates a positive result.

Saponins: Methanol extracts, ethyl acetate and n-hexane extracts from *E.pellita* leaves were each added with 10 ml of distilled water, then shaken vigorously. The formation of stable foam within ± 10 minutes indicates that there are saponin compounds in the sample.

Tannins: Methanol extract, ethyl acetate and n - hexane extracts from *E. pellita* leaves were each put in a test tube, then supplemented with 5% $FeCl_3$. Positive results include the formation of dark blue and greenish-black colors. $FeCl_3$ is used to identify phenol groups; if there are phenol groups in the compound, then there are also tannins, because tannins are polyphenolic compounds.

Triterpenoids and steroids (Liebermann-Burchard test): 1 mg of concentrated methanol extract, ethyl acetate extract and n- hexane extract of *E. pellita* leaves was placed on a drip plate, 6 drops of anhydrous acetic acid were added, then stirred using a spatula until dissolved. Then one drop of concentrated H2SO4 was added. if a purple to orange color is formed in the solution, it indicates that there are triterpenoid compounds, while if it is blue or green, it indicates that there are steroid compounds [10].

2.3 Antioxidant Activity Assay

A concentration variation of the methanol extract solution was made, namely 12.5; 25; 50; 100; and 200 ppm. Furthermore, 0.3 mM DPPH solution was made by dissolving 11.83 mg of DPPH powder (BM 394.32) in methanol p.a in a 100 mL measuring flask covered with aluminum foil, and as much as 1 mL of 0.3 mM DPPH solution was added to 2.5 mL of methanol extract solution in a test tube (which was covered with aluminum foil), then homogenized and incubated for 30 minutes at 37°C. Then the absorbance was measured with a maximum wavelength of 517 nm.

Ascorbic acid was used as a comparator. Antioxidant activity is seen from the IC_{50} value, where the concentration of antioxidants in ppm (µg/mL) is able to inhibit 50% of free radicals. The IC_{50} value can be calculated from the percentage of inhibition with the following formula (1) :

$$\% \text{ Inhibition} = \frac{(\text{Control absorbance} - \text{Sample absorbance})}{(\text{Control absorbance})} \times 100\% \quad (1)$$

The extract can be said to be active if it has an IC50 value of less than 100 ppm.

3. Result And Discussions

3.1 Extraction and Partitioning of *Eucalyptus pellita* Leaves

Maceration using a methanol solvent for the extraction of *Eucalyptus pellita*. The maceration method was chosen because it has numerous benefits, including being simple to use, affordable, and capable of effectively extracting compounds through soaking without heating in order to protect thermolabile substances[11]. Methanol has the ability to draw in analytes such as plant-derived alkaloids, steroids, saponins, and flavonoids. While n-hexane has non-polar characteristics and ethyl acetate is semi-polar, these two solvents are used for partitioning [12]. From the extraction and partitioning stages, three *E. pellita* leaf extracts were obtained, namely methanol extract, n-hexane extract, and ethyl acetate extract.

(a) (b) (c) (a) (b) (c)

Figure 1. (a) Ethyl Acetate Extract; (b) N-hexane Extract; (c) Methanol Extract of *Eucalyptus pellita* Leaf

Due to the difference in polarity between the two solvents, the results of extracts of *E. pellita* in ethyl acetate are better than those in n-hexane. Because the acquisition of compounds is based on the solvent's similarity, this suggests that the compounds in the *E. pellita* extract have a polarity that is near to that of ethyl acetate.Solvent-like compounds will dissolve in other compounds with similar characteristics. Each extract was screened for phytochemicals to determine the presence of secondary metabolite compounds contained in each extract.

Table 1. Phytochemical Screening Results of Methanol Extract, N-hexane Extract, and Ethyl Acetate Extract of E. pellita Leaf

Extract	Mass (g)	Content (%)	Flavonoid	Alkaloid	Steroid/ triterpenoid	Saponin	Tanin
Methanol	176,75	9,30	+	-	+	+	+
N-hexane	13,77	0.72	-	-	+	+	+
Ethyl Acetate	27,93	1.47	+	-	-	+	+

Table 1 shows the results of phytochemical screening of methanol extracts and ethyl acetate extracts of Eucalyptus pellita leaves containing flavonoids, steroids, triterpenoids, and saponins. Methanol and ethyl acetate extracts each contain tannins. The addition of $FeCl_3$ 5% gives a black color, but not because of the presence of tannins in ethyl acetate extract. Because tannins are generally insoluble in ethyl acetate..

3.2 Antioxidant Activity Assay with the 1,1-diphenyl-2-picrylhydrazyl (DPPH) Method

Antioxidant activity was expressed by the IC_{50} value and obtained from the regression line equation, with concentration as the independent variable and percent silencing as the dependent variable. The methanol extract, ethyl acetate extract, and n-hexane extract *of Eucalyptus pellita* leaves were tested for antioxidant activity using DPPH free radical scavenging with UV-visible spectroscopy at a maximum wavelength of 517 nm.

Table 2. Absorbance, Percent Inhibition and Linear Regression Equation of Ascorbic Acid

Concentration (Ppm)	Absorbance	% Inhibition	Linear Regression Equation
3	0,410	53,67	
6	0,298	66,33	y = 3.5669x + 4496
9	0,177	80,00	R^2 = 0.977
12	0,089	89,94	
15	0,041	95,37	

Table 2 shows the Inhibition Concentration 50% (IC_{50}) value of ascorbic acid obtained from the linear regression equation is **1.413 µg/mL.**

The higher the concentration of the sample, the greater the percentage of inhibition, and the lower the concentration of DPPH. This is because DPPH free radicals abstract hydrogen radicals from antioxidant compounds and form DPPH (1,1-diphenyl-2-picrylhydrazine). The reduction of free radicals is also indicated by a change in the color of the solution from purple to pale yellow. The higher the silencing percentage, the stronger the antioxidant activity of the sample. The concentration of an antioxidant substance required to reduce 50% of DPPH free radicals at a certain time (15-30 minutes) is referred to as the IC_{50}[13].

Table 3. Absorbance And Percent Inhibition of Methanol Extract (ME), N-Hexane Extract (NE), And Ethyl Acetate Extract (EE)

Concentration (Ppm)	Absorbance			% Inhibition		
	ME	NE	EE	ME	NE	EE
12,5	0,498	0,513	0,478	43,73	42,03	45,99
25	0,418	0,499	0,398	52,77	43,62	55,03
50	0,344	0,372	0,324	61,13	57,97	63,39
100	0,287	0,207	0,266	67,57	76,61	69,94
200	0,121	0,127	0,102	86,33	85,65	88,47

The regression equation and IC_{50} values of methanol extract, n-hexane extract, ethyl acetate extract, and ascorbic acid as a comparison can be seen in Table 4.

Table 4. Regression Equation and IC_{50} Values of N- hexane Extract, Methanol Extract, Ethyl Acetate Extract, and Ascorbic Acid

Sample	Regression Equation	IC_{50} Values (µg/mL)
N- hexane Extract	Y = 0,2408x + 42,509	31,109
Methanol Extract	Y = 0,2065x + 46,299	17,923
Ethyl Acetate Extract	Y = 0,206x + 48,597	6,811
Ascorbic Acid	Y = 3,5669x + 44,96	1,413

Table 4 shows that the four samples above have activity as DPPH free radical scavengers. Ethyl acetate extract has the highest IC_{50} value of methanol extract and n hexane extract which is 6,811 µg/mL based on the data in Table 3.

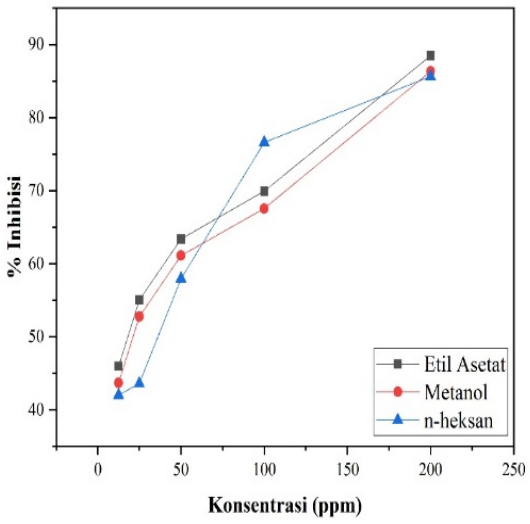

Figure 1. Concentration Relationship Graph of Ethyl Acetate Extract, Methanol Extract,and N-hexane Extract of *Eucalyptus pellita* Leaves

Ethyl acetate extract has strong anti-DPPH antioxidant action. The semi-polar structure of ethyl acetate and the numerous bioactive components that dissolve in it are thought to be related to the

extract's antioxidant efficiency in combating free radicals. Table 4 displays the antioxidant activity test results of the produced extracts.

The IC_{50} value of the leaf extract of *Eucalyptus pellita* in ethyl acetate is less than 50 ppm, as can be observed, and is therefore categorized as a very potent antioxidant. According to phytochemical test results, flavonoids, saponins, and tannins from *E. pellita* leaf extracts may include chemicals that are antioxidants. Because they have a hydroxyl group linked to the aromatic carbon ring, which can absorb free radicals, flavonoids have the potential to act as antioxidants. By contributing one hydrogen atom, this chemical will make peroxy radicals stable [14]

4. Conclusion

1900 g of the dry powder from *E. pellita* leaves were first macerated with methanol solvent. The concentrated methanol extract was next subjected to partition extraction using n-hexane solvent. The methanol layer was concentrated, and the ethyl acetate solvent was used to partition it. Up to 176.75 g of methanol extract (or 9.30%), 13.77 g of n-hexane extract (or 0.72%), and 27.93 g of ethyl acetate extract (or 1.47%) were obtained. Methanol and ethyl acetate extracts tested positive for flavonoids when the three extracts were subjected to phytochemical screening. These three extracts tested positive for tannins and saponins but lacked any alkaloids. Using a UV-vis spectrophotometer and the DPPH (1,1 diphenyl-2-picrylhydrazyl) technique (maximum wavelength 517 nm), antioxidant activity was evaluated. The sample concentrations ranged from 12.5, 25, 50, 100, and 200 ppm. Ethyl acetate extract has the highest IC_{50} value of methanol extract and n hexane extract which is 6,811 µg/mL. The IC_{50} value of the leaf extract of *Eucalyptus pellita* in ethyl acetate is less than 50 ppm, as can be observed, and is therefore categorized as a very potent antioxidant.

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