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Original Article

ANTIOXIDANT ACTIVITY OF DECHLOROPHYLLATED CEMBA (*Acacia rugata* (Lam.) Fawc. Rendle) LEAF EXTRACT

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Abstract

Cemba *(Acacia rugata (Lam.) Fawc. Rendle)* belongs to the genus acacia. The use of cemba leaves as a spice for traditional dishes in Enrekang Regency has many health benefits. This study aims to dechlorophyllated cemba leaf extract and screen for antioxidant activity testing using several methods. Determination of antioxidant activity using free radical scavenging 1,1-diphenyl picryl hydrazine (DPPH), measurement of phenolic content, and measurement of flavonoid content. The results showed that the free radical scavenger in quercetin was IC_{50} 6.787 µg/mL, a free radical scavenger in dechlorophyllated cemba leaf extract of IC_{50} 349.65. µg/mL total phenolic content of 611.9 mg GAE/g extract and total flavonoid content of 74.8 mgQE/g extract. Based on the IC_{50} value, the dechlorophyllated Cemba *(Acacia rugata (Lam.) Fawc. Rendle)* leaf extract has a fragile antioxidant intensity, whereas quercetin as a comparator has a very strong antioxidant intensity. Therefore, dechlorophyllated cemba leaf extract has antioxidant activity.

Keywords: Cemba (*Acacia rugata)*; dechlorophyllated; antioxidant; phenol ;flavonoid

Introduction

Antioxidants are compounds that can reduce, restrain and prevent the oxidation process by donating one or more electrons to free radicals so that these free radicals can be suppressed (Gulcin, 2020). The accumulation of free radicals in the body can cause oxidative stress. This condition can affect the physiological and biochemical processes of the body, resulting in disruption of cell function metabolism and can end in cell death. Cell damage by free radicals seems to be the main cause of premature aging and degenerative diseases such as anti-diabetic cancer, heart disease, cataracts, decreased immune system, and brain dysfunction (Ahmed et all, 2017; Santos et all, 2019). The use of antioxidants will help prevent or slow down oxidation. Two types of antioxidants have been widely used, namely synthetic antioxidants and natural antioxidants. Synthetic antioxidants such as butylated hydroxy anisole, butylated hydroxytoluene, and tertiary butylhydroquinone. Natural antioxidants found in most plants are phenolic groups such as tocopherols, flavonoids, lignins, and phenolic acids. (Dolatabadi, 2014; Dontha, 2016). This potential is also found in cemba leaves so it has the potential to be developed as a candidate for traditional medicine. Our research in 2014 regarding the standardization of cemba leaf extract (*Acacia rugata* (Lam.) Fawc. Rendle) which refers to the Indonesian Herbal Pharmacopeia (FHI) stated that this plant meets the requirements as a traditional medicinal ingredient. The antioxidant potential of chemical compounds has different mechanisms, so fractionation is one way of exploring potential natural chemical compounds. Therefore, it is necessary to screen the antioxidant activity analysis method for a type of

sample (Haida, Hakiman, 2019; Asmawati, 2021). Based on the description of the potential and chemical content of Cemba leaf extract, it is necessary to carry out basic research on screening for antioxidant activity, namely scavenging the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, measuring the capacity of antioxidant compounds for phenolic and total flavonoid content in extracts and extract fractions from cemba leaves (*Acacia rugata* (Lam.) Fawc. Rendle).

Research Procedure

a. Sampling

The research sample was cemba leaves (*Acacia rugata*) obtained from Enrekang Regency. The herbarium of the sample was deposited at Pharmacognosy laboratory, Universitas Muslim Indonesia Makassar.

b. Extraction

Cemba powder was extracted by maceration method using methanol solvent. As much as 1200 mg of powder was placed in the maceration vessel, then 2000 ml of methanol solvent was added. The extraction process was carried out for 3 days with occasional stirring. The liquid extract was filtered and collected, followed by rotary evaporation at a temperature of 50°C and a rotation of 60 rpm to separate the extract from the solvent to obtain a thick extract. To obtain a dry extract, the thick extract was placed in a desiccator for 3 days.

c. Dechlorophyllation of the extract with n-Hexane

The crude extract obtained was dechlorophyllated by the liquid partition method using hexane solvent. The extract was dissolved with distilled water and put into a separatory funnel, then added with 50 mL of n-hexane, was separated between the water layer 1 phase and the n-hexane layer. The water fraction was then dried and continued for antioxidant testing.

d. Antioxidant Activity Test

- **DPPH Radical Scavenging Method**

The extract was prepared by weighing 10 mg and dissolved in 10 ml of methanol pa. DPPH solution was prepared by dissolving 5 mg of solid DPPH in 100 ml of methanol pa. Measurement of extracts by placing a test solution of 2 ml and 2 ml of DPPH solution respectively. Then, incubated for 30 minutes at 27°C in a dark room. Then the absorbance was measured using a Uv-vis spectrophotometer at a wavelength of 516 nm. The test results were measured against the quercetin standard using equation:

$$Inhibition\ (\%) = \frac{Sample\ absorbance - Blank\ absorbance}{Blank\ absorbance} \text{x} 100\%$$

- **Total Phenolic Compound**

Measurement of total phenolic content in extracts and fractions was carried out using the Folin-Ciocalteu reagent. Gallic acid was used to create a calibration curve with various concentrations of 10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml, and 100 µg/ml. Cemba leaf extract and fractions at a concentration of 1,000 µg/ml were then taken 1.0 ml, put into a 10.0 ml volumetric flask, added 500 µl of Folin Ciocalteu reagent, then shaken until homogeneous for 1 minute. Before the eighth minute, 4.0 ml of Na2CO3 7.5% w/v was added and shaken for 1 minute and distilled water was added and shaken until homogeneous. Furthermore, measurements were made with a spectrophotometer at a wavelength of 745 nm, where the wavelength was obtained when the wavelength screening was carried out. The results of these measurements are expressed as the equivalent weight of gallic acid per sample weight based on a linear curve.

- **Total Flavonoid Compound**

Measurement of the total flavonoid content of the extract and fractions of cemba leaves was carried out using aluminum chloride reagent. To prepare the calibration curve, quercetin standards were used with various concentrations of 2 µg/ml, 4 µg/ml, 6 µg/ml, 8 µg/ml, and 10 µg/ml. Cemba leaf extract and fractions at a

concentration of 1000 µg/mL were added to 1.5 ml of 96% ethanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M sodium acetate, and 2.8 ml of distilled water. After being incubated at room temperature for 30 minutes, the absorbance was measured at a wavelength of 431 nm with a UV-VIS spectrophotometer. The results of these measurements are expressed as a weight equivalent to quercetin for each sample weight based on a linear curve.

- **Data Analysis**

The IC_{50} of extracts and fractions was determined using a linear regression equation from the data on the relationship between concentration and percentage of radical absorption/absorbance. The determination of IC_{50} was analyzed using GraphPad Prism software the latest version GraphPad Software, La Jolla, California, USA.

Result and Discussion

Cemba leaves are endemic plants from Enrekang Regency. According to the identification and determination results (LIPI), cemba has the scientific name *Acacia rugata* (Lam.) Fawc. Rendle is a member of the Fabaceae family. Cemba leaf extract standardization testing shows that this plant is needed as a raw material for traditional medicine (Ahmad et al, 2015).

The purpose of this study was to test the antioxidant activity of dechlorophyllated cemba (*Acacia rugata* (Lam.) Fawc. Rendle) leaf extract which has antioxidant activity using several antioxidant testing methods of dechlorophyllated cemba (*Acacia rugata* (Lam.) Fawc. Rendle) leaf extract.

Antioxidant activity test used three methods, namely determination of total phenolic content, determination of total flavonoid content, and DPPH radical scavenging. The phenol content was determined using the Folin-Ciocalteu method (Nugroho, 2012, Ahmad et al & Malik et al 2015). In this experiment, gallic acid was used as a standard which has 3 phenolic hydroxy groups, and gallic acid has high purity, is stable, and is affordable (Mongkolsip et al, 2004). Gallic acid when reacted between the Folin-Ciocalteu reagent and phenolic compounds will change color to yellow which indicates the presence of phenolic compounds. Phenolic compounds react with Folin-Ciocalteu in alkaline conditions (Aspari et al, 2011), therefore Na2CO3 is added, this addition also shows a shift in bathochromic phenolic compounds (Harbone, 1987) and produces a blue color. The reaction that occurs is an oxidation-reduction reaction, phenolic compounds reduce phosphomolybdate phosphotungstate in Folin-Ciocalteu to form blue molybdenum. Absorbance was measured at a wavelength of 745 nm using UV-Vis spectrometry.
The blue complex formed between the reaction of the phenolic hydroxyl groups in the extract with the Folin Ciocalteu reagent can be measured by visible spectrophotometry, the maximum absorption of the chromophore depends on the alkaline solution and the concentration of phenolic compounds (Blainski et al, 2013), which were previously incubated for 2 hours until a reaction was formed. perfect (Eghdami et al, 2010). The total phenolic content in plants is expressed in GAE (Gallic Acid Equivalent) which is the equivalent number of milligrams of gallic acid in 1 gram of sample. The results showed that the phenolic content of the methanol extract of cemba leaves (*Acacia rugata* (Lam.) Fawc. Rendle) from Enrekang Regency, South Sulawesi was 611.9 mg GAE/g extract with a total phenolic content percentage (w/w) of 61.19. %

Table 1. Results of Absorbance Measurement of Gallic Acid Standard Solution at a Wavelength of 745 nm

Concentration (ppm)	Absorbance
30	0,207
40	0,281
50	0,347

Table 2. The results of determining the total phenolic content of methanol extract of Cemba (*Acacia rugata* (Lam.) Fawc. Rendle) Leaves

Replication	Absorbance (y)	Initial Phenolic Content	Total Phenolic Content (mgGAE/g extract)	Average Phenolic Content (mgGAE/g ekstrak)	% Phenolic Content (b/b)
1	0.42	59.76	597,6		
2	0.44	62.61	626,1	611,9	61,19
3	0.43	61.19	611,9		

In determining the levels of total flavonoid compounds in the sample, quercetin was used as a standard solution. In determining the total flavonoid content, the addition of methanol pa which functions as a solvent, $AlCl_3$ to form a complex, resulting in a shift in wavelength towards visible (visible) which is marked by the solution producing a more yellow color. The addition of potassium acetate is to maintain the wavelength in the visible (Chang et al., 2002). The results showed that the flavonoid content of the methanol extract of cemba leaves (*Acacia rugata* (Lam.) Fawc. Rendle) from Enrekang Regency, South Sulawesi was 74.8 mgQE/g extract with a percentage of flavonoid content (w/w) of 7.48. %

Tabel 3. Results of Absorbance Measurement of Quercetin Standard Solution at a Wavelength of 431 nm

Concentration (ppm)	Absorbance
5	0,59
10	0,644
15	0,712
20	0,766
25	0,828

Tabel 4. The results of determining the Total Flavonoid content of methanol extract of Cemba (*Acacia rugata* (Lam.) Fawc. Rendle) Leaves

Replication	Absorbance (y)	Initial flavonoid content	Total Content extract)	Flavonoid (mgQE/g	Average content ekstrak)	Flavonoid (mgQE/g	% Flavonoid content (b/b)
1	0.631	8.53	85.3				
2	0.611	6.87	68.7		74.8		7.48
3	0.613	7.03	70.3				

Quantitative test, the extract was dissolved using methanol pa solvent with a reaction time (incubation) of 30 minutes at 37oC (Pokorny et al, 2001 & Molyneux, 2004), measured using a UV-Vis spectrophotometer at a maximum wavelength of 516 nm. Determination of antioxidant activity refers to the procedure Brand-William (1997) and Ahmad et.al (2012) and uses quercetin as a standard at various concentrations of 2,4,6,8,10 g/mL

with IC50 6,787g/mL. Cemba leaf extract was weighed as much as 10 mg dissolved in 100 ml of methanol pa, diluted with various concentrations of 20,40,60,80 g/mL. The results of the antioxidant activity of the methanol extract of cemba leaves were 349.650 g/mL.

Tabel 5. The results of determining the Radical Scavenging activity of methanol extract of Cemba (*Acacia rugata* (Lam.) Fawc. Rendle) Leaves

Sample	Concentration (µg/mL)		% Inhibition	y=bx+a	IC_{50} (µg/mL)
Quercetin	2	0,591	24,80916031	y=4,9109x + 16,667	6,787554216
	4	0,472	39,94910941		
	6	0,441	43,89312977		
	8	0,344	56,23409669		
Cemba (*Acacia rugata* (Lam.) Fawc. Rendle)	20	0,685	20,16317016	y=0,0915x + 18,007	349,6502732
	40	0,677	21,0955711		
	60	0,655	23,65967366		
	80	0,64	25,40792541		

The antioxidant activity of dechlorophyllated methanol extract of cemba leaves is included in the weak category because cemba extract contains compounds that can reduce free radicals such as polyphenols and flavonoids (Ahmad, 2014). Bimlesh et al (2011) reported that flavonoids are strong antioxidants against free radicals. Its activity is based on the ability to donate protons from the phenol group of flavonoids so that free radicals can be delocalized.

Conclusion

Based on the data obtained, we conclude that the dechlorophyllated cemba methanol extract has antioxidants with a phenolic content of 611.9 mg GAE/g, a flavonoid content of 74.8 mgQE/g and a DPPH radical scavenger of 349.6502732 g/mL.

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