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Aktsar Roskiana Ahmad ✉; Ishmih Nurul Roudhoh Usman; Rezki Amriati Syarif; ... et. al

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The Identify of Antioxidants Constituents of Cemba Leaves (*Acacia rugata* (Lam.) Fawc. Rendle)

Aktsar Roskiana Ahmad[1, a)], Ishmih Nurul Roudhoh Usman[1], Rezki Amriati Syarif[1], Virsa Handayani[1]

[1]*Faculty of Pharmacy, Universitas Muslim Indonesia, Makassar, Indonesia*

[a)] Corresponden Email: aktsarroskiana@umi.ac.id

Abstract. Cemba (*Acacia rugata* (Lam.) Fawc Rendle), is an endemic plant of Enrekang district and belongs to the Fabaceae family that was empirically used as spicy and could protect from hypertension and diabetic diseases by Enrekang society. Standardization of cemba leaves (Acacia rugata (Lam.) Fawc Rendle) was reported the chemical constituents involved flavonoids, tannins, terpenoids, and alkaloids. This research aims to isolate a compound that has a function as antioxidants. The simplisia of cemba leaves about 300 g were extracted by using maceration and collected 38.751 g crude extract. The isolation of the compound was performed by using vacuum liquid chromatography with a gradient mobile phase (n-hexane: ethyl acetate and ethyl acetate: methanol). The crystal constituent was isolated and identified by using UV-Vis spectrophotometry. From this experiment we conclude that the phenolic compound isolated from methanol extract of cemba leaves *(Acacia rugata* (Lam.) Fawc. Rendle) and it showed an antioxidant activity with IC_{50} 94.039 ug/ml.

INTRODUCTION

An unhealthy lifestyle is one of the causes of various diseases in society. One of the diseases whose number of cases has increased significantly in the last ten years and is the sixth leading cause of death worldwide is diabetes mellitus [1]. The World Health Organization (WHO) states that Indonesia ranks sixth in the world as the country with the highest number of people with diabetes mellitus after India, China, the Soviet Union, Japan, and Brazil [2]. WHO recommends the use of traditional medicines including herbs in the maintenance of public health, prevention and treatment of disease, especially for chronic diseases, degenerative diseases and cancer. WHO also supports efforts to improve the safety and efficacy of traditional medicines [3].

The cemba plant (Acacia pennata) **(Figure 1)** is a typical plant of Enrekang whose leaves are used as a flavor enhancer in food by the people who are often referred to as nasu cemba [4]. Traditionally, Acacia pennata has been used as a medicinal plant to treat coughs, headaches, rheumatism and fever in certain areas of Myanmar. Cemba has a scientific name is *Acacia rugata* (Lam.) Fawc. Rendle) and belongs to fabaceae family. Cemba *(Acaciarugata* (Lam.) Fawc. Rendle) also includes in endemic plants of South Sulawesi, especially in Enrekang [5]. Empirically, cemba leaves used as a spice for traditional culinary of Enrekang community [6]. In which,they believe the cemba could neutralize the fat of meat therefore it doesn't effect to cause hypertension and hyperglycemia. However, previously resecrh reported that the extract of cemba leaves *(Acacia rugata* (Lam.) Fawc. Rendle) contain essential oils, flavonoids, saponins, terpenoids and alkaloids [7].

Antioxidants are compounds that slow down the oxidation process. Lipids and lipid-soluble compounds are easily oxidized components and are found in almost all foodstuffs. These compounds include edible fats (edible fat) [8], edible oils, mono-, di- and tri-glycerides (emulsifiers), sterols, fat-soluble vitamins, phospholipids[9], flavor and aroma components, carotenoids and others[8]. The ideal antioxidant must meet several criteria in order to be used in several food systems.

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FIGURE 1. Cemba leaves [10]

Among these criteria are safe, does not affect color, odor and taste, effective at low concentrations, resistant after processing, stable in the final product, fat soluble and available at low prices[11]. Antioxidants can be broadly divided into 5 groups [12], namely:

- **Primary antioxidants (mostly phenolic compounds)** are a group of compounds that stop the formation of free radicals in lipid oxidation such as tocopherols, Butylated Hydroxytoluene (BHT), Butylated Hydroxyanisole (BHA), Tertiary-Butyl Hydroquinone (TBHQ).
- **Oxygen scavengers** such as vitamin C, ascorbyl palmitate and erythrobic acid.
- **Secondary antioxidants** are compounds that function to decompose lipid hydroperoxides into stable final products; Examples of these antioxidants are diluryl thiopropionic acid and thiodipropionate.
- **Enzymatic antioxidants** such as glucose oxidase, superoxide dismutase, catalase, glutathione peroxidase. These antioxidants function to dissolve oxygen or separate oxidative species from the food system.
- **Chelating agents (chelating agents or sequestrants)** such as citric acid, amino acids, ethylendiaminetetra-acetic acid (EDTA), chelate metal ions that can catalyze lipid oxidation.

According to the chemical constituent of cemba leaves that show the potential to becomes an antioxidants agent [11]. Antioxidants are substances that can neutralize or stabilize the free radicals[13]. Therefore, it is needed to obtain antioxidant compounds of cemba leves by using isolation technic[7, 13,14]. Moreover, this research aims to isolate and identify an antioxidant compound of methanol extract of leaves cemba *(Acacia rugata* (Lam.) Fawc. Rendle) [7].

METHODS

This research was conducted by laboratory analysis of the Faculty of Pharmacy, Indonesian Muslim University, Makassar, Indonesia. The extraction process and preliminary test were carried out before the characterization analysis using UV-Vis was carried out. The research process can be seen in the flow chart **(Figure 2)**:

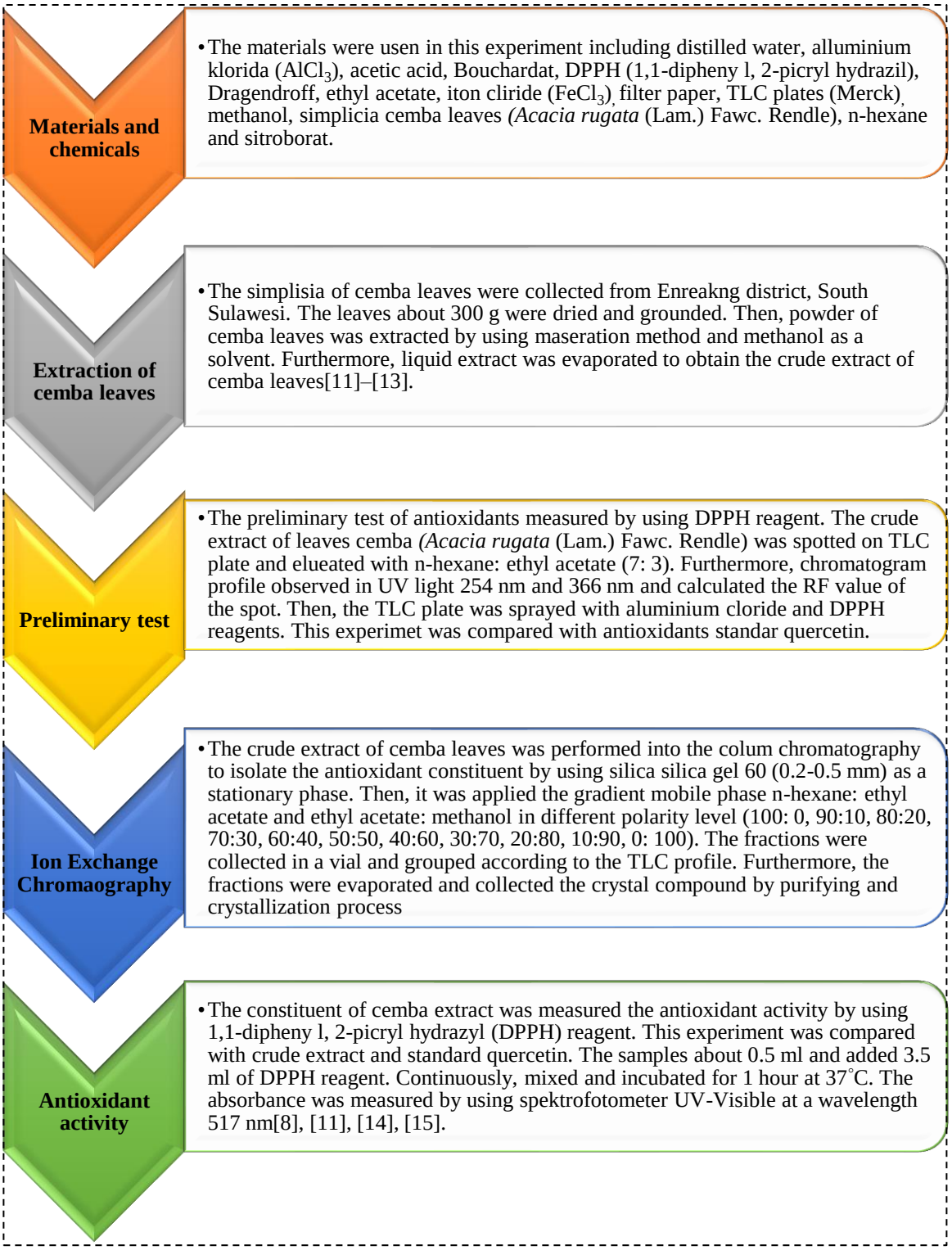

FIGURE 2. The research process of this study

RESULT AND DISCUSSION

This study aims to isolate the antioxidants constituent that contain in the cemba leaves *(Acacia rugata* (Lam.) Fawc. Rendle). The cemba simplisia was collected from Enrekang district Soult Sulawesi and it was determined in Laboratory Pharmacognosy and Phytochemistry Universitas Muslim Indonesia. To extract the chemical constituent of cemba leaves, the maceration method was used. Maceration process is particularly advantageous in the isolation of natural compounds due to cheap, easy, and avoid from the heat which can damage some constituent of the plants/herbs. Then, evaporation of the methanol extract obtained extract as much as 38.751 grams of dry powder weight of 300 grams with a percentage of 12.927%. The percentage of the rendamen percentage calculated to know the ratio between the extract and simplisa **(Table 1)**. According to [3] natural compounds that generally have antioxidant effects are phenols and polyphenols, and the most common are flavonoids (flavonols, isoflavones, flavones, catechins, and flavonones), cinnamic acid derivatives, coumarins, tocopherols, and fatty acids. polyfunctional organic. Phenolic compounds are bioactive components that are widely found in plants. The term phenolic or polyphenol can be defined chemically as a compound having an aromatic ring containing one or more OH substitutions including functional derivatives (esters, metal ethers, glycosides) [8]. Natural antioxidants mainly function as primary antioxidants, namely as free radical acceptors and chain breaker. Volatile phenolic compounds such as eugenol, isoeugenol, thymol and so on have high antioxidant activity but have a strong odor so that their use is limited as food additives. Meanwhile, several other types of natural antioxidants also have some disadvantages [10].

TABLE 1. The percentage of the rendamen extract of cemba leaves

Simplisia (g)	Solvent methanol (mL)	Crude extract (g)	Rendamen extract (%)
300	2500	38.751	12.917

The crude extract of Cemba leaves *(Acacia rugata* (Lam.) Fawc. Rendle) was identified by TLC profiles. TLC method has an several advantages such as versatility, quickly, and high sensitivity. The mobile phase used isocratic n-hexane: ethyl acetate (7: 3). After that, qualitative identification of antioxidant assay by spraying a solution of DPPH. Compounds have antioxidant activity will react with DPPH purple and transformed into a more stable compound to a yellow color[8]. DPPH principle is based on the reaction between the antioxidants with DPPH radical through proton donation. Thus, antioxidants which work by trapping radicals (radical scavenger) can be detect with this method. The principle of this method is the determination of antioxidant activity is based on color changes to yellow from the reaction between DPPH with antioxidant compounds, so DPPH will turn into diphenilpycrilhydrazine non-radical harmless. The increasing number of diphenilpycrilhydrazine will be marked with purple spots discoloration to a yellow color[11, 13, 15].

The methanol extract of leaves purification by performed into the vacuum liquid column chromatography. It was collected about 22 bottles and they were grouped into the 3 fractions based on the TCL profiles **(Table 2)**.

TABLE 2. Fraction cemba leaves *(Acacia rugata* (Lam.) Fawc. Rendle).

Fraction	Weight (g)
I	8.196
II	7, 824
III	10, 216

In the Fraction II was found the crystal. The crystal was purified by washing with methanol and proved the single compound with TLC with gradient mobile phase, TLC two dimension, and detection with specific reagents. After purifying process, we collected the crysal in the amount 2.325 grams (see **Table 3**).

TABLE 3. Results of crystal isolates cemba leaves *(Acacia rugata* (Lam.) Fawc. Rendle)

Isolate	Weight (g)
I	2325

To identify the group of the compound, it was detected by using specific reagent including Dragendorff (alkaloid), sitroborat (flavonoid), $FeCl_3$ (phenolic groups) and DPPH for detecting the potential of antioxidant[6]. The data

showed that the positive result conducted to phenolic groups also interestingly, this compound showed the high antioxidant effect (**Table 4**).

TABLE 4. The identification of compounds

Isolates	Reagent	Description
I	Dragendorf	negative (-)
I	Sitroborat	negative (-)
I	FeCl3	Positive (+)
I	DPPH	Positive (+)

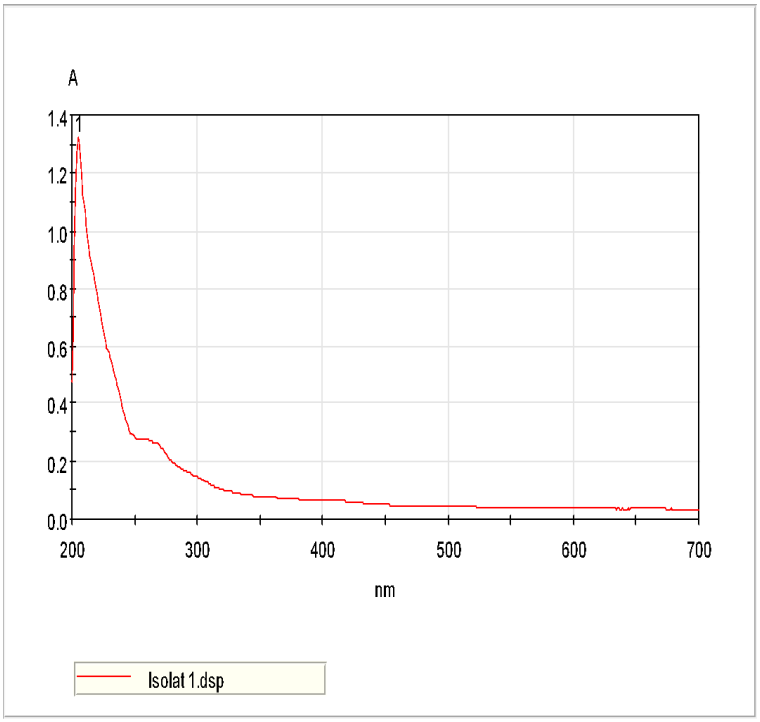

FIGURE 3. Spektra UV-Vis of the compound

The identification of the compound also performed in spectrophotometry UV-Vis to determine the absorption peak. Ultraviolet spectrum of isolates in methanol pa appears at a wavelength of 205 nm with absorbance 1.326 (see **Figure 3**). This data indicated to compound with aromatic ring, which appears at wavelengths longer with great intensity (200-800 nm)[2]. This spectrum showed only one peak that deduced to the purity of the compound. The position of the hydroxy group on the flavonoid core was determined by the addition of shear reagent. Hydroxylation is affected by a bathochromic shift while methylation and glycosylation will cause a shift of the band to a lower wavelength (hypsochromic).

In the other hand, the compound measured for antioxidant activity by using DPPH. The data analized and calculated based on the percentage of the inhibition (**Figure 4**). The results showed that the compound has IC_{50} 94.039 mg/ml/ This data compared with quercetrine with IC_{50} 0.218 mg/ml (**Table 5**). Which quercetrine is strong natural compound as antioxidant. According Phongpaichit et al (2007), a compound categorized to very strong free radical when the value of IC_{50}<10 pg/mL, strong if IC_{50} between 10-50 mg / mL, moderate if IC_{50} ranged from 50-100 mg/mL, undermined when the $ICvalues_{50}$ ranging between 100-250 mg / mL and inactive when the IC_{50} above 250 pg/ ml [9,

11, 13, 16]. Based on these results the possibility compounds have potential as antioxidants in the methanol extract of leaves Cembaisolates*(Acaciarugata* (Lam.) Fawc. Randle) are phenolic compounds.

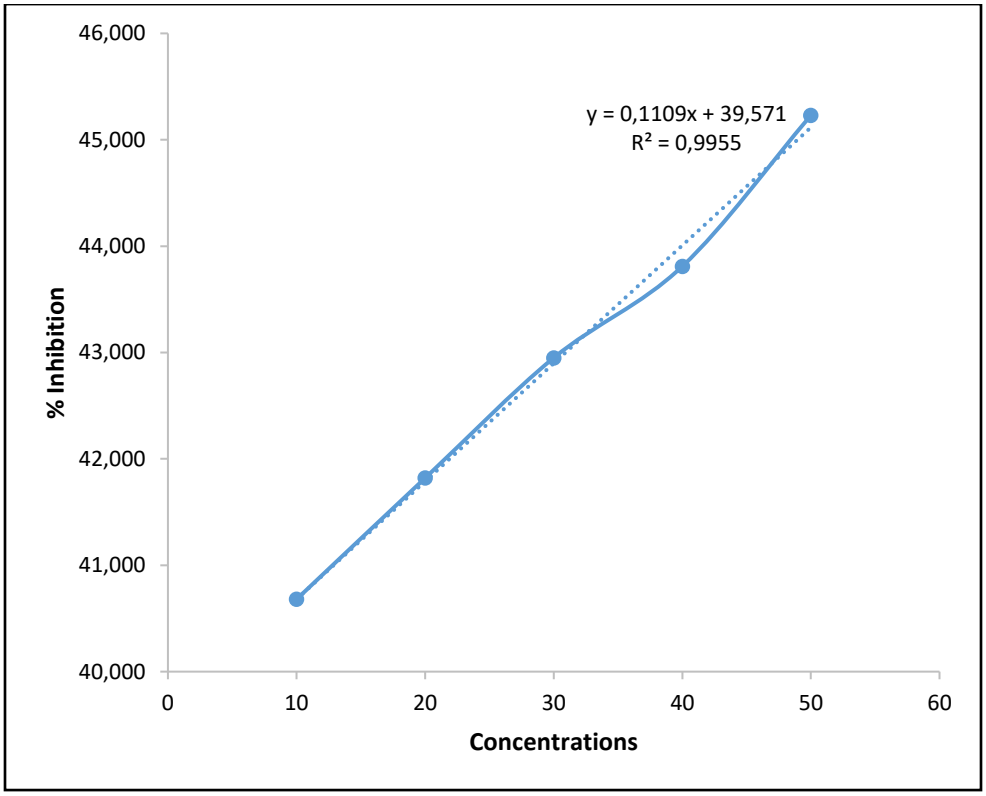

FIGURE 4. The linearity curve of DPPH

The ethyl acetate and hexane extracts did not give a color change which indicated that the extracts did not have antioxidant activity. Besides being able to be seen from the color change (qualitative), antioxidant activity can also be seen from the value of the percentage of inhibition (quantitative). A sample is said to provide antioxidant activity if the concentration of the tested sample is able to inhibit 50 percent of DPPH radicals or what is called IC_{50}. To determine the percentage of resistance, the absorbance of the sample being tested must be known. Below are the absorbance data and the percent resistance obtained from the sample.

TABLE 5. The antioxidant assay of the compound

Sample	Concentration (ppm)	Absorbance	%inhibition	IC_{50} (µg/ml)
Blanko DPPH	25	0,703	-	-
Isolat	10	0.417	40.680	
	20	0.409	41.820	
	30	0.401	42.950	94.039
	40	0.395	43.810	
	50	0.385	45.230	
Quercetrine	0.2	0.351	50.071	
	0.4	0.337	52.062	
	0.6	0.319	54.623	0.218
	0.8	0.300	57.325	
	1	0.280	60.170	

CONCLUSION

The crude extract of Cemba leaves *(Acacia rugata* (Lam.) Fawc. Rendle) was identified by TLC profiles. TLC method has an several advantages such as versatility, quickly, and high sensitivity. The mobile phase used isocratic n-hexane: ethyl acetate (7: 3). After that, qualitative identification of antioxidant assay by spraying a solution of DPPH. Compounds have antioxidant activity will react with DPPH purple and transformed into a more stable compound to a yellow color. Significant color change from purple to yellow. This indicates the presence of antioxidant activity in the sample of 70 percent ethanol with a concentration of 100 ppm. While the ethyl acetate and hexane extracts did not give a color change which indicated that the extracts did not have antioxidant activity. Besides being able to be seen from the color change (qualitative), antioxidant activity can also be seen from the value of the percentage of inhibition (quantitative). From this experiment we conclude that the phenolic compound isolated from methanol extract of cemba leaves *(Acacia rugata* (Lam.) Fawc. Rendle) and it showed an antioxidant activity with IC_{50} 94.039 ug/ml.

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