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Total Flavonoids Determination and Antioxidant Activity of Ethyl Acetate, Ethanol, and Methanol Extracts from Seluang Belum Root (*Luvunga sarmentosa* (Blume) Kurz.)

Nashrul Wathan[1], M. Ikhwan Rizki[1], Amalia Khairunnisa[1], Hilneser Simamora[1]

[1]Department of Pharmacy Faculty of MIPA, Lambung Mangkurat University, Banjarbaru, Indonesia
Coresspondence Author: nashrul.far@ulm.ac.id

Abstract:

Flavonoids are phenolic compounds from nature that found in almost all parts of the plants. Seluang belum (*Luvunga sarmentosa* (Blume) Kurz.) root is one of the medicinal plants used by Dayak people in Central and South Kalimantan where in previous studies its part was contains flavonoids. This study aims to evaluate the best solvent for extracting flavonoid content and antioxidant activity using ethanol, methanol and ethyl acetate. The antioxidant activity performed by in vitro technique by DPPH assay. Total flavonoids content on the extracts of *L. sarmentosa* root determined using UV-Vis spectrophotometric with aluminium chloride method and calculated as quercetin equivalents (QE). The results showed the highest antioxidant activities confirmed for the methanol extract (IC_{50} = 80.33 ppm). The highest of total flavonoids contents was recorded for the ethanol extract (6.56 ± 0.006% w/w Quercetin Equivalent). The results showed that treatment effect of different solvent extraction was significant (sig <0.05) on total flavonoid and antioxidant activities from *L. sarmentosa* root.

Keywords: *Lavanga,* Saluang bilung; Solvent variation; Dayak tribes; Antioxidant

Introduction

Seluang belum (*Luvunga sarmentosa* (Blume) Kurz.) has long been known by the Dayak people had efficacy as an aphrodisiac that works by increasing stamina, sexual arousal and male fertility. The root of the plant *L. sarmentosa* commonly used by the community to cure back pain, abdominal pain and increase vitality.[1,2] *L. sarmentosa* had central and peripheral analgesic activity.[3] The effects that can be caused by these plants cannot be separated from the performance of the metabolite compounds contained in them. Chemical compounds contained in *L. sarmentosa* based on research by Wathan and Abdullah,[4] showed that the positive root contains steroids and flavonoids.

Flavonoids were one of the natural phenolic compounds found in almost all parts of plants.[5] The properties of flavonoids were known to depend on their chemical structure. In addition, flavonoids were known as potential antioxidants that can prevent the formation of free radicals. The hydroxyl group in flavonoids had an antioxidant effect by counteracting free radicals and/or by chelating metal ions.[6] Flavonoids in their function as aphrodisiacs work by maintaining sperm motility and protecting spermatozoa membranes thereby increasing sperm viability and number.[1] Flavonoids are polar compounds that can be extracted with solvents that have the same properties. The solvent used in this research were methanol and ethanol as the polar solvent and ethyl acetate as a semi-polar solvent where all solvents have a level of polarity that can dissolve flavonoid compounds from the sample.

UV-Vis spectrophotometry was one of the most frequently used techniques in pharmaceutical analysis. It involves measuring the amount of ultraviolet-visible light radiation absorbed by a substance in solution. This instrument was called a UV-Vis spectrophotometer which used to measure the ratio, a ratio function, of the intensity of two light beams in the UV-Vis region.[7] This method was used because flavonoids have a carbonyl system conjugated with an aromatic ring (chromophore and auxochrome groups) the characterization of flavonoids can be carried out by UV-Vis spectrophotometry.[8]

Methods

Sample used in this study were the *L. sarmentosa* root, distilled water, aluminum foil, 5% acetic acid, 10% $AlCl_3$, ethanol 96%, ethyl acetate, ethanol pa, methanol pa, chloroform pa, filter paper, DPPH reagent (Sigma), and standard quercetin (Sigma). *L. sarmentosa* were taken from natural forests in Bukit Batu area, Palangka Raya Regency, Kalimantan Tengah (Kalteng) Province. All parts of the plant taken for determination and the root processed into simplicia.

Five hundred grams of *L. sarmentosa* root was extracted by maceration method using methanol and ethyl acetate as a solvent. Extraction was carried out for 3x24 hours with stirring every 6 hours. The filtrate is then collected and then concentrated using a rotary evaporator and evaporated over a waterbath with a temperature of 50 ºC until a thick extract is obtained to a fixed weight.

Preliminary test for the presence of a class of flavonoid compounds was done with a color reaction using Pb asetat and HCl reagents. After that, the TLC test was carried out to determine the pattern of spot separation of the compounds and to determine whether the sample contained flavonoids and antioxidants using DPPH and $AlCl_3$ reagents and ammonia vapor. Quantitative analysis was performed using UV-Vis spectrophotometry to determine the total flavonoid content and antioxidant activity of the ethanol extract of *L. sarmentosa* root.

Results

L. sarmentosa was carried out to determination at the Herbarium Bogoriense, Botany Division of the Research Center for Biology-Lembaga Ilmu Pengetahuan Indonesia (LIPI) Bogor, Jawa Barat. The purpose of this determination was to ensure the correct identity of the plants used in the study. The result of the determination with letter number 666/IPH.1.01/If.07/III/2019. It was known that the plant used was included in the family Rutaceae with the species name *Luvunga sarmentosa* Kurz.

In extraction the value of the yield obtained from methanol extract is 4.21% and at an ethyl acetate extract of 2.3%. The value of the yield produced by each solvent can be different due to the difference in polarity. Higher polarity on methanol will support its ability to bind all chemical components found in natural ingredients, both non-polar, semi-polar, and polar. Methanol is also an easy-to-fit liquid to enter the cell through the cell wall so that the secondary metabolites contained in the cytoplasm will dissolve in solvents and compounds will be perfectly extracted.[9]

The results of tube test reaction in methanol, ethanol and ethyl acetate extract in the form of light-yellow deposits at the base of the tube. These results indicated extracts were containing flavonoid compounds as mentioned in the literature. The reaction that occurred was caused by a reaction between flavonoids and Pb acetate which formed complex compounds, causing deposits on the basis of the tube reaction.

Determination of quercetin curve was made in 5 serial levels 20, 40, 60, 80, and 100 ppm respectively which from the parent raw solution of 400 ppm. The level of the level series read by the absorbance at a wavelength of 422 nm and operating time in the 26th minute (Figure 1). This step replicated in 3 times. The level of accuracy obtained is 99.88% with 0.12% possible error rate. This was accordance with the literature which states that linearity is good if the linear regression value (r) close to 1.[10]

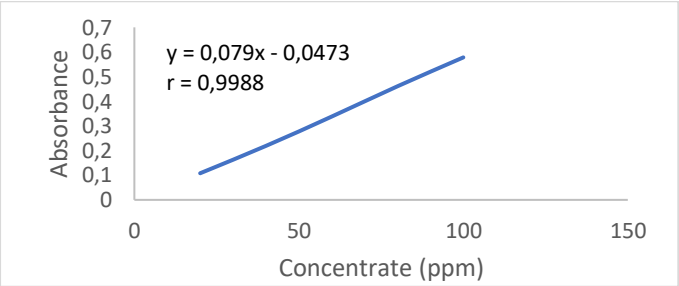

Figure 1. The standard curve of quercetin

The results of the determination of the total flavonoid content of the methanol and ethyl acetate extracts of the roots of *L. sarmentosa* can be seen in Table 1.

Table 1 Calculation of total flavonoid content

Sample	Sample Absorbance	Total Flavonoids (% w/w QE)	Average of Total Flavonoids (% w/w QE) ±SD
Methanol extract	0,029	3,86	
	0,036	4,22	4,10 ± 0,084
	0,036	4,22	
Ethanol extract	0,082	6,57	
	0,081	6,50	6,56 ± 0,006
	0,083	6,61	
Ethyl acetate extract	0,060	5,43	
	0,057	5,28	5,36 ± 0,012
	0,059	5,38	

The results of IC_{50} determination can be seen in Table 2

Table 2. Calculation of IC_{50} value

Sample	Concentrate (ppm)	Inhibition Percentage	IC_{50}
Methanol Extract	50	20,10	80,33 ppm
	60	29,95	
	70	32,69	
	80	51,43	
	90	61,69	
Ethanol Extract	100	9,82	654,85 ppm
	200	16,42	
	300	24,50	
	400	32,49	
	500	38,05	
Ethyl Acetate Extract	100	25,77	152,94 ppm
	125	37,44	
	150	51,33	
	175	57,44	
	200	71,44	

Discussion

Overall, the total flavonoid content of *L. sarmentosa* roots in the ethanol extract had a greatest value when compared to the methanol and ethyl acetate extract. According to Sukmawati,[11] this can happen because ethanol solvent specifically had the ability to dissolve flavonoid compounds greater than other solvents. Determination of the IC_{50} value of the *L. sarmentosa* root ethanol extract was carried out by making a test solution in various series of concentrations.

Based on this case, it can be concluded that the total flavonoid content is not directly proportional to the antioxidant activity. In addition, it can also be caused by the chemical content that has an effect on antioxidants, not only flavonoids, it can also be influenced by the content of other phytochemical compounds such as phenolic groups or tannins.[12]

Conclusions

The highest flavonoid content of *L. sarmentosa* root was 6.56 ± 0.06% w/w Quercetin Equivalent were obtained using ethanol as solvent, and the most active antioxidant activity with IC_{50} value of 80.33 ppm was obtained from extraction with methanol solvent.

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