Flavonoid Content Analysis of Ethyl Acetat Fraction of Neem Leaf (Azadiractha indica, A. Juss) by UV-Vis Spectrophotometry

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Submission date: 13-Jul-2021 10:32AM (UTC-0500)

Submission ID: 1619173485

File name: Full_Paper_to_the_3rd_ISNP_Imrawati.docx (32.62K)

Word count: 1949

Character count: 11149

Flavonoid Content Analysis of Ethyl Acetat Fraction of Neem Leaf (*Azadiractha indica*, A. Juss) by UV-Vis Spectrophotometry

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Abstract

Neem leaf is one of the plant containing secondary metabolites. It is unfamiliar to the public and one of the plants used as the traditional medicine. This research aimed to determine the flavonoid content of ethyl acetate fraction of neem leaf (*Azadiractha indica* A Juss). The Neem leaf was extracted by maceration method using ethanol then partitioned by ethyl acetate, where the determination of the flavonoid content by using UV-Vis Spectrophotometry. The result showed that the flavonoid content of athyl acetate fraction of Neem leaf was 7,4520 mgQE/g fraction.

Key word: Neem leaf (Azadiractha indica A.Juss), Flavonoid, UV-Vis Spectrophotometry

Introduction

Indonesian society is now widely use the plant as a traditional medicine to cope with various diseases. Antioxidant utilization can reduce oxidation stress that relate to disease. Research on traditional medicine is necessary to provide scientific evidence of efficacy of medicinal plant in addition can also be used as a source of guidance for the synthesis of compound of new drug compounds. One plant that has long been used as a tradicional remedy is Neem (*Azadiractha indica* A.Juss), whose other name is *Autelaca azadirachta* (Hyine, 1987). In Java, this plant is known as Neem (Hyine, 1987). The title of this plant in the English Language include Neem, Neeim, Margosa, Indian Illac, Bead Tree, pride of Chine, holy tree and Persian Illac (Gruenwald, *et al.*, 1998).

Plant Neem (Azadiractha indica AJuss) including familia Meliaceae. Neem especially in the seeds and leaves contain several components of production of secondary metabolites were very helpful, both in agriculture (pesticides and fertilizers), and pharmaceuticals (drugs). Neem (Azadiractha indica A Juss) is one of the plants that can be used as anti-diarrhea, fever, diabetes and have diverse chemical constituents include triterpenoids, steroids, flavonoids, glycosides (Asrhy, 2009). One class of chemistry that many serves as an active substance that can be used as a treatment is flavonoids. It are compounds reducing good, inhibits many oxidation reactions, either the enzyme or non enzyme and works as an anti-inflammatory. Flavonoids act good container hydroxy and superoxide radicals thereby protecting the membrane lipids against destructive reactions (Robinson, 1995). Quantitative analysis of flavonoids can be done by using UV-Vis Spectrophotometry. Ultraviolet absorption spectra and absorption appears is the single most useful way to identify structures that flavonoids (Markham, 1988). Flavonoids contain aromatic conjugated system and can show strong absorption bands in the UV-Vis(Rohyami, 2007). Based on these description it is necessary to do research on the levels of flavonoids in the leaves of Neem with UV-Vis spectrophotometry method, so that the potential of this plant as a raw material medicine for the prevention and treatment of various disease can be developed to maximum, and it can be used justified by society.

Materials and Methods

Materials used in this study are aluminium chloride 10% (pa. Merck), aquadest sterile, ethanol (pa. Merck), ekstract leave Neem (*Azadiractha indica* A.Juss), ethyl acetate (pa. Merck), sodium acetate (pa. Merck), quarcetine (Pa. Sigma), and Powder magnesium (pa. Merck).

Sampel leaves Neem cleaned of dirt attached to the leaves using water flowing then dried. After the sampel was cut to be small, then ready to extracted with method maceration. Sampel Neem leaves was weighed 300 gram, macerated using ethanol 96% at the temperature room for 3 times 24 hours and put in a countiner sealed and protected from sunlight, occasional done stirring. The result of maceration process was filtered by using a paper filter and evaporated by rotavapor. After that the dried extract was partitioned by liquid-liquid partition method, the ways, 0,5015 gram dried extract was inserted into the funnel separation and partition with ethyl acetate as much as 20 mL, then shaken, occasional cover funnel opened after that allowed to stand for 30 minutes to happen separation between layers of ethyl acetate (fraction of ethyl acetate) and a layer of water (recidues). Residue partitioned again like a way before and done it 3 times. Fraction of ethyl acetate collected and evaporated to obtain the fraction of thick ethyl acetate(Tobo, 2001).

Qualitative analysis of flavonoid was done by way (1) fraction sampel was solved in 5 mL ethanol, whipped, heated, and whipped longer then filtred and added Mg 0,2 gram and 3 drops HCl on each filtrat. The formation of Orange or Red at the layer of ethanol indicate flavonoid (Harbone, 1387), (2) The measurement of the levels flavonoid b ways 3 mL fraction solution then added 0,1 mL AlCl₃ 10% and 0,1 mL sodium acetate. Then allowed to stand for 30 min at room temparture, Then measured absorbance with spectrophotometry UV-Vis the Wavelenght of absorption maximum 445 nm. Done it 3 times replication and calculated levels of flavonoids obtained as mg equivalent quarcetine/gram fraction (Chang, 2002).

Results and Discussion

The plant Mimba (Azadiractha indica A.Juss) including Family meliacae, mimba especially in the seeds and leaves contain several components of production of secondary metabolites were very rewarding, both in agriculture (pesticides and fertilizers), and pharmacy (medicine). Mimba (Azadiractha indica A Juss) is one of the plants that can be used as anti-diarrhea, fever, diabetes and has the content of chemical varied among others triterpenoids, steroids, alkaloids, flavonoids, and glycosides (Asrhy, 2009). In this study, used sample leaves mimba to get a chemical desired used extraction method which is dilution substances efficacious or active substance of the plant parts by using suitable solvent (Ditjen POM, 1986). Where the xetraction precess used is maceration because of this method is a simple method, cheap, and easy. Sampel leaves mimba that has dried 300 gram dissolved in solvent ethanol 96% 1 L. Solvent used in maceration is ethanol 96%, which aims to draw all chemical components in the leaves mimba, as solvent ethanol 96% is a solvent Universal that can be interesting compounds soluble solvent non polar to polar. The extract viscous obtained then partitioned using the solvent ethyl acetate in a way partition liquid-liquid to get a fraction of ethyl acetate. The method pertition liquid-liquid is a method of separation of compounds by using 2 solven different or not mutually mixed, the solvent used the water and ethyl acetate. The result rendamen on the xetraction of 4,1910% and to rendamen fraction of ethyl acetate for 21,1964%. Test qualitative flavonoid to view the content of flavonoids that can be detected by using the powder Mg added a few drops of HCl, the formation of Orange or Red at layer ethanol indicate flavonoids (Harbone, 1987). The identification of show the fraction of ethyl acetate leaves mimba positive flavonoid. On quantitative analysis of flavonoids containing system aromatic that conjugated and can be

shown the ribbon uptake strong in the area UV-Vis on testing spectrophotometry UV-Vis used solution standard quercetin. The first performed running of the wavelength of maximum standard quercetin are area wavelength of 445nm. Solution standard quercetin made in some series concentration of the 2, 4, 6, 8, 10, and 12 ppm, circuits series the concentration, each 3 mL then added 0,1 mL Aluminium Chlorida 10% working to give effect batochrom, so the reaction color formed can be observed and can be measured on spectrophotometry visible. Then added 0,1 mL sodium acetate 1 M, which serves as stabilish, so that the effect of bathroom had happened can be maintained. Then incubation for 30 minutes with aim of order reaction between the solution standard quercetin and reagents can take place perfectly, measured each series the concentration in the wavelength o 445 nm. Reseult absorbance of a series of concentration recorded then generated curve calibration linier quercetine that Y=0.0755X-0.0412 with the velue of the coefficient of determination (R) obtained by 0,9926. So is also on measurement absorbance sample fraction of ethyl acetate leaves mimba made in three replication. The weighed 5 mg fraction of ethyl aptate leaves Mimba then dissolved in 10 mL methanol (solution stock). Took 3 mL then added 0,1 mL aluminium chloride 10% and 0,1 mL of potassium acetate, incubated for 30 minutes, then measured in the wavelength of maximum of 445 nm. Run and earned value absorbance on sample fraction of ethyl acetat leaves Mimba. From the result of this study the content of flavonoids fraction of ethyl acetate leaves Mimba is 7,5420 mgQe/g, which means aech weight fraction of ethyl acetate leave Mimba contain 7,420 mgQe/gram flavonoid equivalent to quercetin.

Table 1. The result rendamen leaves Mimba (Azadicratha indica A. Jusa)

Leaves Mimba	Weight of the end of	Weight of the beginning of	Rendamen (%)
	sample (gram)	sample (gram)	
Extract	12,5731	300	4,1910
Fraction	0,1063	0,5015	21,1964

Table 2. Measuremnat result fraction of ethyl acetate leaves Mimba using spectrophotometry UV-Vis the wavelength of 445 nm

Sample	Replication	Absorbance	Consentration	The content	The average
			of flavonoid	of flavonoid	content of
			early (mg/L)	(mgQE/g)	flavonoid
Fraction of ethyl	I	0,506	7,2476	7,2447	7,5420
Mimba	II	0,515	7,3668	7,3638	mgQE/g fraction
	III	0,544	7,7509	7,7477	

Conclusion

Based on the research conducted can be conclude that the level of flavonoid of fraction of ethyl acetate leaves Mimba is 7,5420 mgQE/g fraction.

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