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The Effect of Different Ethanol Concentrations as The Extraction Solvent on Total Flavonoid Content of White Mulberry Leaves Extracts *(Morus alba L.)*

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ABSTRACT

White mulberry leaves (Morus alba L.) contain flavonoid which empirically have many health benefits, such as reducing fever, relieving headaches and throats, coughs, dysentery, digestive disorders, flu, and according to research it has antioxidant activity. Many factors effect the total flavonoid content, one of which is the concentration of the extraction solvent. The purpose of the research was to determine the effect of different ethanol concentrations as the extraction solvent on total flavonoid content of white mulberry (Morus alba L.) leaves extract. White mulberry leaves (Morus alba L.) were extracted using the maceration method, the extract was tested for phytochemical screening and thin layer chromatography using chloroform: ethyl acetate (6: 4) as the eluent. The resulting spots were observed visually and with λ 254 nm and λ 366 nm UV light. Determination of flavonoid content by UV-Vis spectrophotometry using the AlCl3 colorimetric method at a maximum wavelength of 415 nm. The research results showed that the yield of 50% and 96% ethanol extract of white mulberry leaves (Morus alba L.) was 17.42 % and 11.04 %. Phytochemical screening showed that both extracts positively contained flavonoids. The presence of flavonoids was confirmed using a thin layer chromatography test, resulting in an average Rf value for 50% and 96% ethanol extracts are 0.71 and 0.70, close to quercetin Rf standard is 0,73 as comparison. The total flavonoid content obtained from the 50% ethanol extract was 36.0902 mgQE/g and the 96% ethanol extract was 56.4699 mgQE/g. The conclusions are the concentration of the extraction solvent effect the yield and total flavonoid content.

Keywords: White mulberry leaves (Morus alba L.), Flavonoid, Ethanol, UV-Vis Spectrophotometry

I. INTRODUCTION

Indonesia has abundant biodiversity so that it is rich in medicinal plants whose presence has the potential to be utilized and explored optimally. Medicinal plants are easy to obtain, relatively do not have a bad effect on health, the costs are cheaper than chemical drugs, and can be easly reached by people. [1],[2]

One of the interesting plants to study is the white mulberry leaves (Morus alba L.). Empirically, people utillize white mulberry leaves (Morus alba L.) to treat headaches and throats, fever, coughs, dysentery, digestive disorders, malaria, increased and breastfeeding. In addition, white mulberry leaves (Morus alba L.) have antioxidant activitiy.[3], [4]

Based on previous research, methanol extract of white mulberry leaves (Morus alba L.) using the DPPH method showed higher antioxidant activity with an IC50 of 837.4 μg mg-1 compared to white mulberry branches extract with an IC50 of 158.3 μg mg-1.4 White mulberry leaves (Morus alba L.) have antioxidant activity because they contain flavonoid compounds which have antioxidant activity are quercetin, isoquercetin, routine, and quercetin 3-O- β- D- glucosyl - (1-6)- β- Dglucopyrosinase.[5],[6]

To extract flavonoid compounds, an extraction process can be carried out in which differences in solvent concentration affect the extraction results obtained.[7] The extraction solvent is used ethanol with concentrations of 50% and 96% which has a polarity similar to flavonoid compounds such as quercetin, which is one of the flavonoids in the flavonol group.[8] Quercetin is a compound that has low solubility in water, more soluble in alcohol compounds and organic solvents. Ethanol is a polar solvent that is often used in various extraction methods and has a hydroxyl group that can form a hydrogen bond with the hydroxyl group of flavonoid compounds so that it can increase the solubility of flavonoid compounds in ethanol.[9]

The concentration of extraction solvent affects total flavonoid content. Previous research has been carried out regarding the determination of the total flavonoid content of white mulberry leaf extract (Morus alba L.) which was extracted with 70% ethanol. It was found that the total flavonoid content of white mulberry leaves was 33.303 mg RE/g higher than white mulberry fruit was 0.899 mg RE/g.[10] Furthermore white mulberry (Morus alba L.) leaf extract using 80% ethanol resulted in total flavonoid content of 19.64 mg RE/g higher than black mulberry (Morus nigra L.) leaves of 18.40 mg RE/g.[11]

Based on what was mentioned above, this study to determine the effect of differences in extraction solvent concentrations using 50% and 96% ethanol on the total flavonoid content of white mulberry (Morus alba L.) leaves extract.

II. METHODS AND MATERIAL

Source of plant material

The samples used in this study were the leaves of white mulberry (Morus alba L.) obtained from Tapak Sungkai gardens of local farmer in Bogor, Indonesia. The plant parts were then inspected at the Laboratory of the Biological Research Center, LIPI, Bogor Indonesia.

Source of equipments

Oven (Memmert®), analytical balance (Ohaus®), UV-Vis spectrophotometer (Analytic Jena Specord 200 plus), water bath (Alab Tech®), cuvette, buchner flask, buchner funnel, vacump pump (Abn aspira®).

Source of materials

The materials used in this research were white mulberry leaf (Morus alba L.), technical 96% ethanol, ethanol p.a (Smartlab®), quercetin (sigma®), AlCl3 p.a (Merck®), CH3COOH p.a (Merck®), silica gel GF254 thin layer chromatography plate (Merck®), HCl p.a (Merck®).

Extraction of white mulberry (Morus alba L.)

500 grams of mulberry leaves powder soaked for 3 x 24 hours while occasionally stirring in 2 L of 96% and 50% ethanol solvent. The dregs were remacerated for 2 x 24 hours with 1 L of the same solvent. After 2 days, the sample was filtered, filtrate and dregs were obtained. After that concentrated using a water bath at a temperature of 40 oC to obtain extract.[12],[13]

Phytochemical screening of white mulberry (Morus alba L.)

Flavonoid identification was carried out by weighing 0.5 g of the extract added with 1N HCl and 0.2 g of magnesium powder. A sample is positive for flavonoids if it forms a yellow or orange color.[14]

Qualitative Identification of Flavonoids Using Thin Layer Chromatography

Saturate the container for the thin layer chromatography process which contains filter paper using 20 ml of chloroform and ethyl acetate (6:4) as mobile phase. Prepare an F254 silica gel TLC plate measuring 10 x 8 cm. Identify spots visually and using 366 nm UV light.[15],[16]

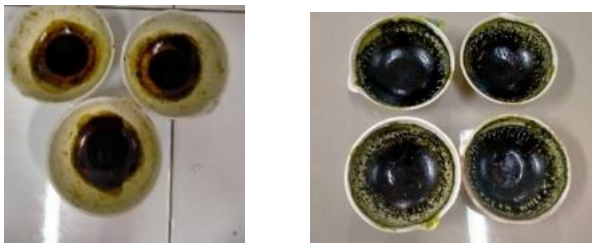
Determination of Total Flavonoid Content using UV-Vis Spectrophotometry

A standard solution of quercetin was prepared at a concentration of 1000 ppm into a 50 ml volumetric flask. A standard solution of 100 ppm quercetin was made to determine the maximum wavelength. Pipet 1 ml of 100 ppm quercetin standard solution added with 1 ml of 10% AlCl3 and 8 ml of 5% acetic acid, put into a test tube. The maximum absorption was measured at a wavelength of 350-500 nm. Making of 60 ppm; 80 ppm; 100 ppm; 120 ppm; and 140 ppm series standard solutions, put into a 50 ml volumetric flask, add ethanol p.a. Ethanol extracts of 50% and 96% white mulberry leaves were made 2000 ppm concentration, the extract was put into a 10 ml volumetric flask and added ethanol p.a. 1 ml of extract solution and standard solution were pipetted into a test tube and 1 ml of 10% AlCl3 and 8 ml of 5% acetic acid were added.[17–20]

III.RESULTS AND DISCUSSION

Table 1 Organoleptic Results of White Mulberry Leaves Extract *(Morus alba L.)*

Identification	50% Ethanol extract	96% Ethanol extract
Smell	Specific	Specific
Color	Dark brown	Blackish green
Form	Thick	Thick



(a) (b)

Figure 1. Results of White Mulberry Leaves Extract

Notes: a) Ethanol 50% extract and (b) Ethanol 96% extract.

Organoleptic observations on the 50% and 96% ethanol extract of white mulberry leaves *(Morus alba L.)* are a thick liquid, have a distinctive smell. However the 50% ethanol extract is dark brown color and the 96% ethanol extract is blackish green.

Table. 2 Results of The Ethanol Extract off White Mulberry Leaves

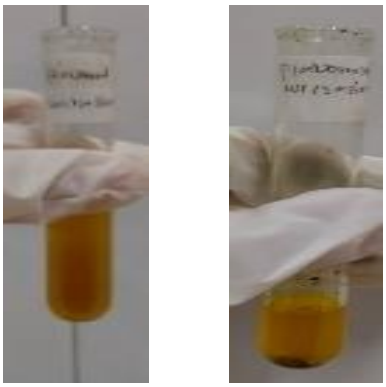
Samples	Powder (gram)	Thick Extract (gram)	Yield Extract
50% Ethanol	500	87,1	17,42%
96% Ethanol	500	55,2	11,04%

The yields of 50% and 96% ethanol extracts of white mulberry (*Morus alba* L.) leaves were 17.42% and 11.04%.

Phytochemical screening of white mulberry *(Morus alba L.)*

Phytochemical screening was carried out on 50% ethanol extract and 96% ethanol extract of white mulberry leaves *(Morus alba L.)* as a preliminary test using a color reagent to determine the metabolites contained in the extract.

Figure 2. Phytochemical Screening Results of 50% and 96% Ethanol Extract of White Mulberry Leaves *(Morus alba L.).*



(a) (b)

Notes: a) 50% ethanol extract, b) 96% ethanol extract

Tabel 3 Phytochemical Screening Results of 96% Ethanol Extract of White Mulberry Leaves *(Morus alba L.)*

Secondary Metabolites	50% Ethanol Extract	96% Ethanol Extract
Flavonoid	+++	+++

The results of the study show that the 50% and 96% ethanol extracts of white mulberry leaves *(Morus alba L.)* are positive for containing secondary metabolites of flavonoid. This is in line with previous study, extract positive for containing secondary metabolites of flavonoids.[21]

Qualitative Identification of Flavonoids Using Thin Layer Chromatography

The resulting spots were observed using UV light λ 366 nm. White mulberry leaves extract *(Morus alba L.)* contains flavonoids if the Rf value of the extracts are the same or close to the standard Rf value of quercetin. The identification results are shown in table 4 and figures 4.

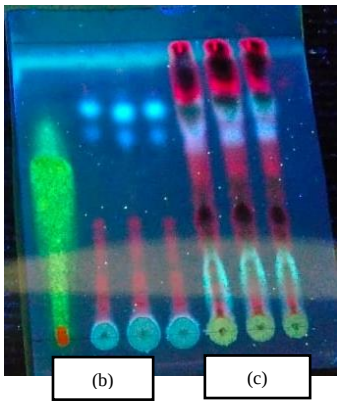


Figure 4. Thin Layer Chromatography of 50% Ethanol Extract of White Mulberry Leaves *(Morus alba L.)* Observation in UV Light 366 nm.

Notes: (a) quercetin, (b) 50% ethanol extract, (c) 96% ethanol extract

Table 4. Table of TLC Test Results

Samples	R*f* ± SD
Quercetin	0,73
50% ethanol extract	0,71 ± 0,005
96% ethanol extract	0,70 ± 0,005

Note: SD (Standar Deviation)

Table 4 show that the 50% and 96% ethanol extracts of white mulberry leaves *(Morus alba L.)* positive flavonoid. The Rf value of quercetin was 0.73, 50% ethanol extract was 0.71 and 96% ethanol extract was 0.70. The Rf value of both extracts is close to the Rf value of the standard comparator. This is in line with

previous research, in Sky Mustard extract, the Rf value of flavonoids is 0.71.[22]

Determination of Total Flavonoid Content Using UV-Vis Spectrophotometry

Based on the results of the study using quercetin used as a standard at a concentration of 100 ppm. The results show that the maximum wavelength of quercetin standard is at a wavelength of 415 nm. Determination of the standard curve of quercetin was carried out at 60-140 ppm, obtained the average absorbance of 0.3535; 0.4664; 0.6087; 0.7138; and 0.8395, respectively (Figure 6). The resulting measurements show that the higher the concentration value, the higher the absorbance obtained.

The results of the linear regression test are y = 0.0061x - 0.0133 with a correlation coefficient value obtained R^2 of 0.9984 and R of 0.9992. The value of R which is close to one indicates that the standard curve obtained is linear so that the concentration of quercetin solution with absorbance is interrelated.[23]

Total flavonoid content in the sample is expressed as mg Equivalents Quercetin (EQ)/gram extract.

Table 5. Total Flavonoid Content of White Mulberry Leaf Extract

Extract Samples	Mean Absorbance± SD	Total Flavonoid Content (mg QE/Gram Extract) ± SD
50% Ethanol	0,4270 ± 0,002	36,0902 ± 0,166
96% Ethanol	0,6756 ± 0,002	56,4699 ± 0,155

Based on the results of the study, the average total flavonoid content of 50% and 96% ethanol extracts of white mulberry leaves (*Morus alba* L.) was 36.0902 mgQE/g and 56.4699 mgQE/g, respectively (Table 5).

These results show that the total flavonoid content extracted with 96% ethanol is higher than that with 50% ethanol. It can be said that the concentration of ethanol solvent affects the total flavonoid content obtained.[24] This is because the 96% ethanol solvent concentration has a level of solubility equivalent to flavonoid compounds, so it is more effective in dissolving flavonoid compounds in white mulberry leaf extract (*Morus alba* L.) as the like dissolve like principle.[25]

The total flavonoid content of white mulberry leaf extract (*Morus alba* L.) extracted with 96% ethanol in this study is higher when compared to previously conducted studies using 70%, ethanol and 80% ethanol obtained total flavonoid content of 33.303 µg RE/mg and 19.64 µg RE/mg, respectively.

IV. CONCLUSION

From the results of study that has been conducted on 50% and 96% ethanol extracts of white mulberry leaves (*Morus alba* L), the yield of 50% ethanol extract is 17,42% higher than 96% ethanol extract of 11,04%.

The 50% and 96% ethanol extracts are positive for flavonoid compounds with an average Rf value of 50% ethanol extract of 0,71 and 96% ethanol extract of 0,70 which is close to the Rf value of quercetin standard comparison of 0,73.

The concentration of extraction solvent affects the total flavonoid content as seen from the higher total flavonoid content of 96% ethanol extract at 56,4699 mgQE/g compared to the total flavonoid content of 50% ethanol extract at 36,0902 mgQE/g.

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