DETERMINATION OF TOTAL FLAVONOID LEVELS OF KAFFIR LIME (*Citrus hystrix*) LEAF EXTRACT USING SOXHLETATION METHOD USING UV-VIS SPECTROPHOTOMETER

Arlan K. Imran[1)], Vyani Kamba[2)], Zulfiayu Sapiun[3], Sukmawati Damiti[4)], and PriscaSafriani Wicita[5)]

[1,2,3,4,5]Department of Pharmacy, Health Polytechnic of Gorontalo
email: arlanimran25@gmail.com

ABSTRACT

Kaffir lime (*Citrus histrix*) *is one of the plants with abundant flavonoid content in the leaves. The characteristic flavonoids with the two benzene ring groups cause the process of finding an appropriate extraction technique.*

This study aims to determine the total flavonoid levels of kaffir lime leaf extract obtained from the soxhletation extraction method. This research was carried out by extracting simplicia kaffir lime leaves by the soxhletation method using 96% ethanol solvent. Comparison between the simplicia and the solvent used is 1: 5, then the extract obtained was carried out with initial qualitative identification of flavonoids and total flavonoid levels were determined using UV-Vis spectrophotometer.

The results showed that the extract of kaffir lime leaves obtained by soxhletation extraction in qualitative and quantitative tests contained flavonoid compounds with a total content of 5.62%.

Keywords: *Kaffir lime, Flavonoid total, soxhletation, UV-Vis spectrophotometer*

INTRODUCTION

Kaffir lime leaves (Citrus histrix) contain bioactive compounds such as flavonoids, phenolics, tannins, and essential oils. ([1]. Flavonoids are compounds that are described as a row of C6-C3-C6 aromatic rings with 2 main characteristics such as oil that is difficult to dissolve in polar solvents (aglycons) and can also be bound to sugars (glycols) which can be easily dissolved in polar solvents [2]. The characteristics of flavonoids that can be polar and non-polar require proper extraction techniques in searching.The soxhletation method is a method of extraction by utilizing the results of solvent vapors in extracting flavonoid compounds in the sample, compared to other methods in extraction using heat or cold extraction such as reflux and maceration using the soxhletation method is preferred because the sample is preferred not directly exposed to heat which will damage the flavonoid compounds and more effective in increasing the amount of solute by using indirect heat assistance [3].

Research related to the extraction of flavonoids in kaffir lime leaves is still at the stage of the maceration extraction process, so this study aims to measure the total flavonoid levels in kaffir lime leaf extract using the maximum soxhletation heat extraction method in flavonoid extraction as in Jones & Kinghorn 2006.The extract obtained from the extraction

process is then calculated its yield, qualitatively identified and determined quantitatively using UV-Vis spectrophoto meter, its total flavonoid levels.

METHODS

The research conducted is an experimental laboratory study. The materials used in this study were kaffir lime leaves from Karanganyar, Central Java, 96% ethanol (CV Chem-Mix), $FeCl_3$ (CV Chem-Mix), quercetin (CV Chem-Mix), $AlCl_3$ (CV Chem-Mix), potassium acetate (CV Chem-Mix) and Aquadestilata. The research implementation process includes:

Making simplisia kaffie lime leaves (*citrus hystrix*)

500 g Freshly picked kaffir lime leaves are washed clean, wet sorted, chopped in a uniform size, then dried in an oven at 60ºC for 3 days after drying sorting is done to separate the damaged parts and then crushed using a blender to produce Simplicia kaffir lime powder as much as 50 g.

Simplisia Extraction Process of kaffir lime Leaves (*Citrus histrix*) with Soxhletation Method.

Wrap 500 g Simplicia kaffir lime leaves with filter paper tied at both ends, insert the simplicia into a chiffon tube, add 250 mL of 96% ethanol solvent to the round bottom flask, then assemble the soxhlet tool into a series of extraction tools, then heat the round bottom flask which contains solvents at 90^0C until steam forms which will rise again to water and wet the extract, until a clear extract is obtained in the chiffon pipe for 7 cycles. The extract obtained was evaporated on a waterbath to form a thick extract which then the results were calculated, qualitative and quantitative identification of the level of flavonoids.

Qualitative identification of flavonoids extracts of kaffirlime citrus leaves

Qualitative identification begins with weighing the extract as much as 100 mg dissolved in a mixture of 50 mL 96% ethanol and 50 mL of water, then heated over a water bath at 60^0C for 15 minutes. The extract solution in the solvent mixture was put into 3 test tubes then added 0.1 g mg powder and added 2 mL concentrated HCl. Observed the changes that occur, if a yellowish color is formed indicates the presence of flavonoid compounds.

Determination of Total Flavonoid Levels of Kaffir Lime Leaf Extract (*Citrus histrix*) using Spektrofotometer Uv-Vis.
Making of quercetin standard curve.

1000 ppm quercetin mother liquor was made by weighing 25 mg of quercetin dissolved in 25 ml of 96% ethanol, in a measuring flask. each taken as many as 1,250 ml, 0.625 ml, 0.306 ml, 0.156 ml and 0.078 ml from the mother liquor, put in a 25 mL measuring flask sufficient with ethanol 96% to the mark limit. Quercetin levels of 50 ppm, 25 ppm, 12.5 ppm, 6.25 ppm and 3,125 ppm were obtained. As much as 1 ml of solution taken from each concentration was added 0.1 ml of $AlCl_3$, 0.1 ml of potassium acetate, 2.8 ml of aquadest and 1.5 ml of 96% ethanol and allowed to stand for 30 minutes. Its absorbance is read at 415 nm wavelength using UV-Vis spectrophotometer. A standard quartz curve is obtained.

Readings of absorbance sample of kaffir lime leaves extract (*citrus histrix*).

Weighed as much as 10 mg extract of kaffir lime leaves and dissolved in 10 ml of 96% ethanol, so that a 1000 ppm standard solution was obtained. The main solution of ethanol extract of kaffir lime leaves was pipetted 0.5 ml and its volume was sufficient to 10 ml with 96% ethanol obtained a sample solution of 50 ppm concentration. Sample preparation was carried out by taking 1 ml of 50 ppm extract solution, added with 0.1 ml of $AlCl_3$, 0.1 ml of potassium acetate, 2.8 ml of

aquadest, and 1.5 ethanol 96%. After that it is allowed to stand for 30 minutes and the absorbance is measured with a UV-Vis spectrophotometer at a maximum wavelength of 415 nm.

RESULTS AND DISCUSSION

Yield extract result.

The result of the extraction of simplicia of 50 grams of kaffir lime leaves with 96% 250 mL ethanol solvent which was obtained was obtained by thick ethanol extract of kaffir lime leaves as much as 12.59 g, so that the yield of extraction obtained was 25.18%. Extract extraction obtained in this study was more when compared with the maceration method using the same sample, as conducted by Kawiji et al, 2015 which only obtained extract yield of 8.447% [4]. It can be said that the soxhletation extraction method is better at producing extract yields than the cold maceration method.

Results of Qualitative Identification of Extracts.

A positive kaffir lime extract containing flavonoids is characterized by the formation of intense yellow deposits and will fade if a weak acid solution is added. That is caused by the occurrence of conjugations from aromatic groups of the C6-C3-C6 flavonoid structures (Hanani, 2015).

Results of Determination of Total Flavonoids Levels of Extracts of kaffir lime by UV-Vis spectrophotometer.

Standard curvequercetin.

Table 1. Absorbance result of Standard curvequercetin.

Concentration (PPM)	Absorbance
3.12	0.149±0,0002
6.25	0.241±0,0002
12.5	0.431±0,0001
25	0.719±0,0003
50	0.900±0,0002

Measuring the absorbance of the standard quartz as shown in Table 1. Produces a linear regression equation y = 0.01586x + 0.18054 with a value of r = 0.9537. the resulting r value revealed that the standard curve produced has an accuracy of 95.37%, a method is said to be good if the r value produced is close to 1 or in the range 0.95-1 [6]. Furthermore, the linear regression equation is used in determining the total flavonoid levels in extracts of kaffir lime leaves.

Sample Extract kaffir lime leaves

Table2. Absorbance result ofStandard curve quercetin

Sample Conc. (PPM)	Absorbance	Result Conc. (PPM)	% Conc.
50	0.136	2.81	5.62±0,07

The results showed that in 50 PPM the concentration of samples contained only 2.81 PPM flavonoids. This is better than the standard stated in N. F Devy et al, 2010 which states that the flavonoid content of kaffir lime leaves is 1 PPM. It can be interpreted that the method of extracting flavonoids from kaffir lime leaves with the soxhletation extraction method can increase the absorption of flavonoids in kaffir lime leaves.

CONCLUSION

Kaffir lime leaf extract containing flavonoid compounds and total flavonoid levels of kaffir lime leaf extract (Citrus histrix) extracted by soxhletation method was determined by UV-Vis spectrophotometer method of 5.67%.

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