Pharmacogn J. 2020; 12(2): 356-360
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Research Article

Determination of Total Flavonoid Levels of Ethanol Extract Sesewanua Leaf (*Clerodendrum Fragrans* Wild) With Maceration Method Using UV-Vis Spectrofotometry

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ABSTRACT

Introduction: Sesewanuwa (*Clerodendrum fragrans* Wild) 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. **Objective:** This study aims to determine the total flavonoid levels of ethanol extract of sesewanuwa leaves obtained from maceration extraction methods. **Method:** This research was carried out by extracting the simplicia of sesewanuwa leaves by maceration method using 96% ethanol solvent. Comparison between the simplicia and the solvent used is 1:7, then the extract obtained was carried out with initial qualitative identification of flavonoids with simple reagents and the total flavonoid levels were determined using UV-Vis spectrophotometry. **Results:** The results showed that the ethanol extract of sesewanuwa leaves obtained by maceration extraction method in qualitative and quantitative tests contained flavonoids with quercetin standard with a total content of 13.47%.This research was carried out by extracting the simplicia of sesewanuwa leaves by maceration method using 96% ethanol solvent. Comparison between the simplicia and the solvent used is 1:7, then the extract obtained was carried out with initial qualitative identification of flavonoids with simple reagents and the total flavonoid levels were determined using UV-Vis spectrophotometry. **Conclusion:** The results showed that the ethanol extract of sesewanuwa leaves obtained by maceration extraction method in qualitative and quantitative tests contained flavonoids with quercetin standard with a total content of 13.47%.

Key words: Sesewanuwa, Total Flavonoid, Quercetin, Maceration, UV-Vis spectrophotometry.

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History
- Submission Date: 28-11-2019;
- Review completed: 15-12-2019;
- Accepted Date: 10-01-2020.

DOI : 10.5530/pj.2020.12.56

Article Available online
http://www.phcogj.com/v12/i2

INTRODUCTION

Sesewanua leaves (*Clerodendrum fragrans* Wild) contain bioactive compounds such as flavonoids, phenolics, tannins, alkaloids and saponins.[1] The classification of Sesewanua was king: Plantae, class : Equisetopsida, Ordo: Lamiales, Family : Lamiaceae, Genus: Clerodendrum, Species: *Clerodendrum fragrans* Wild. Sinonim: *Clerodendrum chinense* (Osbect), *Volkameria fragrans* Vent. Local name: Sesewanua, Bulahu.[2-4] Chemical ingredients of Sesewanua were Tannin (++), Saponin (++), Flavonoid (++), dan Alkaloid (++),[1] while[5] only showed possitive in alkaloid and flavonoid. Acteoside, leucosceptoside A, isoacteoside, ester metil dan etil dari asam kafeat, jnoside dan kaempferol,[2] Uncinatone, Prunasin, Acacetin-7-O-metylgluconate, Clerosterol, Neolignan I, II, dan III, Serratagenic acid, dan Scutellarin.[6]

Clerondrum genus have Larvicidal and Pupicidal activity.[7,8] In empirical studies, sesewanua used as antitumor, antiinflamation, antipyretic, spa,antioxidant, antiinflamation and antihematome.[9-16] Sesewanua leaves had been studied for it's antipyretic activity[1,10] Antioxidant test at etil acetate, etanol and n-heksan extract showed that Sesewanua had a strong antioxidant.[12] It cytotoxic effect to T47D cell found at moderate level.[11]

Flavonoid is a compound which is 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 (aglycone) and can also be bound to sugars (glycons) which can be easily dissolved in polar solvents.[17] The characteristic of flavonoids that can be polar and non-polar require proper extraction techniques in carrying out the search.

Macerated extraction method is a simple method of extraction with the principle of immersion and stirring of the sample in a suitable solvent in extracting flavonoid compounds in the sample, compared to other methods in maceration extraction having the advantage of using more solvents than other extraction methods. Optimization that can be done on the maceration method is to vary the ratio of the number of samples and solvents used. Factors that can affect the amount of yield and total levels of extracted compounds include the type of solvent and the amount of solvent used.[18]

Research related to the search for secondary metabolism of sesewanuwa leaves is still in the process of maceration extraction using different solvents and comparison of solvents, namely using ethyl acetate solvent at a ratio of 1: 4[12] and ethanol solvents in a ratio of 1: 3,[19] so this study aims to measure the total flavonoid levels in sesewanuwa leaf extract using maceration extraction methods with

Cite this article: Sapiun Z, Pangalo P, Imran AK, Wicita PS, Daud RPA. Determination of Total Flavonoid Levels of Ethanol Extract Sesewanua Leaf (*Clerodendrum Fragrans* Wild) With Maceration Method Using UV-VIS Spectrofotometry. Pharmacog J. 2020;12(2):356-60.

a higher sample and solvent ratio of 1: 7 which more leverage in the search process.[18]

The extract obtained from the extraction process is then calculated its yield, qualitatively identified and determined quantitatively using UV-Vis spectrophotometry its total flavonoid levels.

METHODS

The research conducted was an experimental laboratory study. The materials used in this study were leaves of sesewanuwa from Tilango District, Gorontalo Indonesia, ethanol 96% (CV Chem-Mix), FeCl3 (CV Chem-Mix), quarsetin (CV Chem-Mix), AlCl3 (CV Chem-Mix), potassium acetate (CV Chem-Mix) and Aquadestilata. The research implementation process includes:

Making simplisia sesewanua leaves (*Clerodendrum fragrans* Wild)

One Kg fresh leaves were collected from healthy sesewanuwa at Tilango District, Gorontalo Province, Indoensia during April 2019. The plant sample was identified to be *Clerodendum fragrans* Wild. by Indonesian Institute of Sciences – Research Center for Plant Conservation and Botanic Garden, Bogor, Indoensia (Reference no.B-418/IPH.3/KS/II/2019). This leaves are washed washed thoroughly with running water to remove the dirt from these samples.[20] These samples were blotted dry with tissue papers and carried out chopped with a uniform size, then carried out drying in the oven at 60°C for 3 days after drying dry sorting is done to separate the damaged parts and then mashed using a blender to produce simplicia 500 grams of sesewanuwa leaf powder.[12]

The simplisia extraction process of sesewanua leaves (*Clerodendrum fragrans* Willd) with the maseration method

Weigh as much as 500 g of simplicia leaves of an animal and then add 96% ethanol[21] 3.5 L until the simplicia is completely submerged. Extraction was carried out for 3x24 hours protected from light and stirred every 24 hours. On days 2 and 3 the solvent was filtered for remaseration to obtain 3 filtrates for each solvent. The filtrate was then evaporated with a rotary evaporator to obtain a thick ethanol extract from the leaves of an animal.[22]

Qualitative identification of flavonoid ethanol extract of sesewanua 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°C for 15 minutes. The extract solution in the solvent mixture was put into 6 test tubes each of 3 tubes tested using 10% NaOH as much as 5 ml and the other 3 tubes added with 5 ml concentrated acetate Pb shaken strongly. Observed the changes that occur, if formed yellow, red, brown to test with NaOH and precipitate formed to test with positive Pb acetate samples containing flavonoids.

Determination of total flavonoid content of ethanol extract of sesewanua leaf (*Clerodendrum fragrans* Wild) using UV-Vis spectrophotometry

Making of quercetin raw curve

1000 ppm quarsetin mother liquor was made by weighing 25 mg of quercetin dissolved in 25 ml of 96% ethanol, in a measuring flask. each taken 3 ml, 4 ml, 5 ml, 6 ml, 7 mL, 8 mL and 9 ml from the mother liquor, put in a 10 mL measuring flask sufficient with ethanol 96% to the mark limit. Quercetin levels of 60 ppm, 80 ppm, 100 ppm, 120 ppm, 140 ppm, 160 ppm and 180 ppm are 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 spectrophotometry. A standard quartz curve is obtained.

Readings of absorbance samples of sesewanua leaf ethanol extract (Clerodendrum fragrans Wild)

Weighed as much as 10 mg of ethanol extract from sesewanuwa leaves and dissolved in 10 ml of 96% ethanol, so a 1000 ppm sample solution was obtained. Sample preparation was carried out by taking 1 ml of 1000 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%.[23] After that it was allowed to stand for 30 minutes and the absorbance was measured with a UV-Vis spectrophotometer at a maximum wavelength of 415 nm.

RESULTS AND DISCUSSION

Extract yield extract

The result of the extraction of simplicia Sesewanua leaves 500 grams with 96% 3.5 L ethanol solvent that has been done was obtained as much as 38 g of ethanol extract of sesewanuwa leaves, so that the yield of extraction obtained is equal to 7.6%. The results obtained are still better than the number of research ramen[12] using the same extraction method but different solvents and solvent comparisons, each with 1: 4 ethyl acetate and n hexane solvents. The yield obtained in the research[12] was 5% and 2% respectively. The increase in the amount of yield obtained from this study was caused by 2 things namely the type of solvent and the ratio of samples and solvents used (Figure 1).

Ethanol solvent has a high polarity value compared to ethyl acetate and n-hexane. The abundant secondary metabolite compounds in the leaves of sesewanuwa are polar compounds so that the use of more sample and solvent comparisons of 1: 7 causes more substances to be found in the ethanol solvent than other solvents.[12,24]

Results of qualitative identification of extracts

Ethanol extract of positive sesewanua leaves containing flavonoids both tested using NaOH and Pb acetate with each marked an intense yellow color and a precipitate formed.

Testing with the addition of NaOH into the solution will cause a reaction between Na + ions and H + ions will bind to OH- in NaOH to form H_2O compounds in the extract, whereas Na + ions will bind to O- ions in the cyclic groups to form sodium phenoxide compounds which will cause the solution to turn yellow.[25,26]

Testing using Pb Acetate will produce a white precipitate at the bottom of the tube which is caused by the reaction between the Pb^+ ion and the hydroxyl group on the cyclic structure of the flavonoids, because Pb is one of the heavy metals whose properties will always produce a precipitate when reacted (Figure 2).[25,26]

Result of determination of total flavonoid levels of ethanol extract of sesewanua leaves by UV-Vis spectrophotometry

Quercetin raw curve

Measurement of quercetin standard absorbance as shown in Table 1. produces a linear regression equation y = 0.0043x + 0.0196 with a value of r = 0.9878. the resulting r value indicates that the standard curve produced has an accuracy of 98.78%, a method is said to be good if the r value produced is close to 1 or in the range 0.95-1. Furthermore, the linear regression equation is used in determining total flavonoid levels in ethanol extracts of sesewanuwa leaves (Table 2).

Figure 1: Sesewanua (*Clerodendrum fragrans* Wild.).

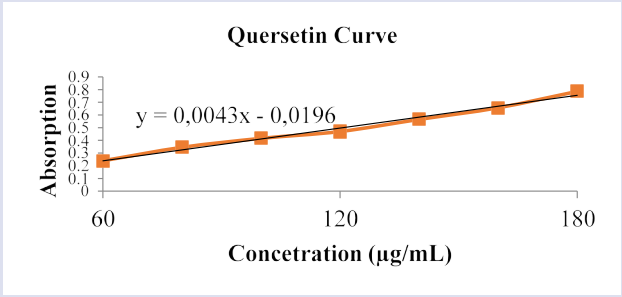

Figure 2: Quantitative test flavonoid ethanol extract of Sesewanua leaf (*Clerodendrum fragrans* Wild).

Table 1: Results of absorbance of quercetin raw curve.

Concentration (PPM)	Absorbance
60	0.240 ± 0,0006
80	0.346 ± 0,0012
100	0.415 ± 0,0010
120	0.470 ± 0,0010
140	0.565 ± 0,0006
160	0.657 ± 0,0010
180	0.786 ± 0,0015

Table 2: Results of absorbance of quercetin raw curve.

Absorbance	Sample Content (PPM)	Level of Results (PPM)	% Content
0.560	1000	134.79	13.47 ± 0,07

Samples of sesewanuwa leaf ethanol extract

The results showed that in 1000 PPM the sample concentrations contained 134.79 PPM flavonoids. The results obtained are higher in total flavonoid levels compared to the total number of total flavonoids from leaves of plants that are optimized for the drying temperature of their simplicity.[19] Hohakay et al showed that fresh leaves, dried leaves, dried at 40°C and 60°C respectively had total flavonoid levels of 1.2%, 0.72%, 0.78% and 0.62%. The increase in the total amount of flavonoids obtained from this study was due to the comparison of samples and solvents used in the extraction process by maceration method. The factor that can affect the extraction process is the amount of solvent used.[18] The ratio of samples and solvents used in this study is 1: 7 whereas in research Hohakay et al. only 1: 3.[19]

CONCLUSION

Ethanol extract of sesewanuwa leaves contains flavonoid compounds and total flavonoid levels of ethanol extract of sesewanuwa leaves extracted by maceration method with a 1: 7 solvent ratio determined by UV-Vis spectrophotometry method of 13.47%.

ACKNOWLEDGEMENT

The authors thanks to Health Ministry of Indonesia for financial support in this research.

CONFLICTS OF INTEREST

None.

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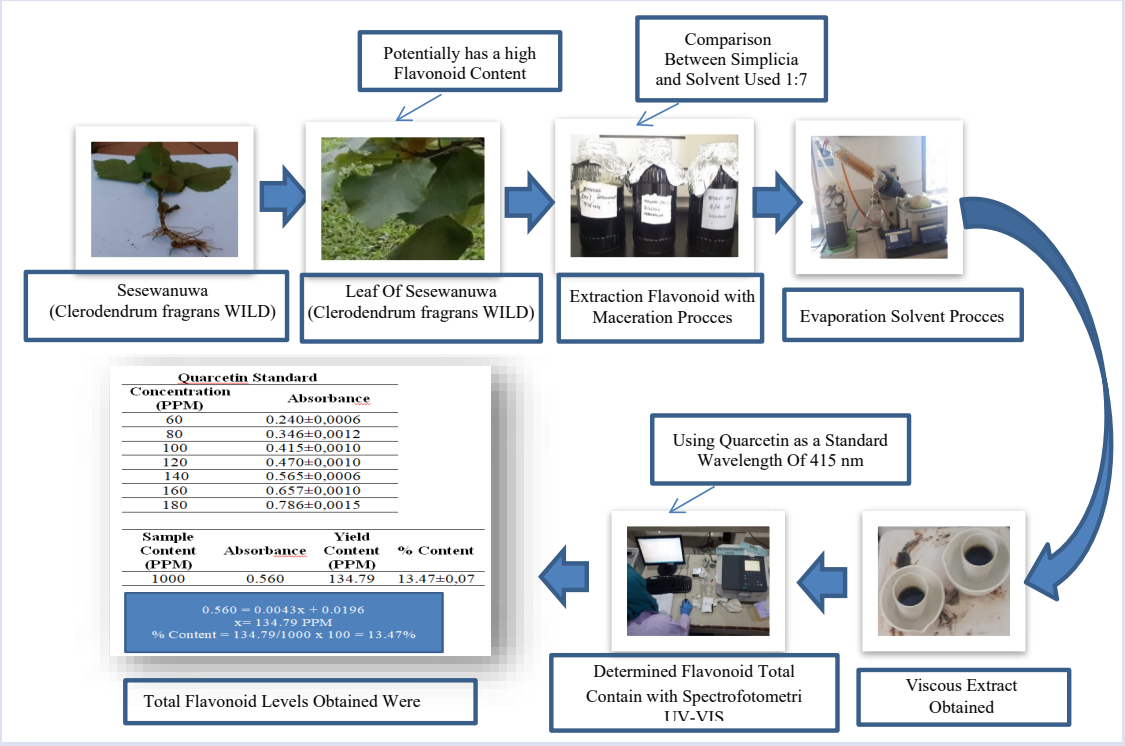
GRAPHICAL ABSTRACT



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Cite this article: Sapiun Z, Pangalo P, Imran AK, Wicita PS, Daud RPA. Determination of Total Flavonoid Levels of Ethanol Extract Sesewanua Leaf (*Clerodendrum Fragrans* Wild) With Maceration Method Using UV-VIS Spectrofotometry. Pharmacog J. 2020;12(2):356-60.