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

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ABSTRACT

Introduction: Sesewanua (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 sesewanua leaves obtained from maceration extraction methods. Method: This research was carried out by extracting the simplicia of sesewanua 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 sesewanua 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 sesewanua 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 sesewanua 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: Sesewanua, Total Flavonoid, Quercetin, Maceration, UV-Vis spectrophotometry.

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 only showed positive in alkaloid and flavonoid. Acteoside, leucosceptoside A, isoaacteose, ester metil dan etil dari asam kafeat, jnoside dan kaempferol;1 Uncinatone, Prunasin, Acacetin-7-O- methylgluconate, Clerosterol, Neolignan I, II, dan III, Serratagenic acid, dan Scutellarin.5 Clerondrum genus have Larvicidal and Pupicidal activity.7,8 In empirical studies, sesewanua used as antitumor, antiinflamation, antipyretic, spa,antioxidant, antiinflammation and antihematome.5-10 Sesewanua leaves had been studied for it's antipyretic activity.11 Antioxidant test at etil acetate, etanol and n-heksan extract showed that Sesewanua had a strong antioxidant.11 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.12 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.13

Research related to the search for secondary metabolism of sesewanua 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 1:412 and ethanol solvents in a ratio of 1:3,14 so this study aims to measure the total flavonoid levels in sesewanua leaf extract using maceration extraction methods with

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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 Province, Indonesia during April 2019. The plant sample was identified to be Clerodendrum fragrans Wild, by Indonesian Institute of Sciences – Research Center for Plant Conservation and Botanic Garden, Bogor, Indonesia (Reference no.B-418/IPH.3/KS/II/2019). This leaves are 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

Making simpisia sesewanu leaves (Clerodendrum fragrans Wild)

One Kg fresh leaves were collected from healthy sesewanuwa at Tilango District, Gorontalo Province, Indonesia during April 2019. The plant sample was identified to be Clerodendrum fragrans Wild, by Indonesian Institute of Sciences – Research Center for Plant Conservation and Botanic Garden, Bogor, Indonesia (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 simpisia extraction process of sesewanu leaves (Clerodendrum fragrans Wild) with the maceration method

Weigh as much as 500 g of simpicia leaves of an animal and then add 96% ethanol3.5 L. until the simpicia 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 sesewanu 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 sesewanu leaf (Clerodendrum fragrans Wild) using UV-Vis spectrophotometry

Making of quercetin raw curve

1000 ppm quaretin 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 AlCl₃, 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.

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).
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CONFLICTS OF INTEREST
None.

REFERENCE

1. Nonke E, Kaunang S, Samuel MY. Botanical and phytochemical constituents of several medicinal plants from mount Klatab north Minahasa. J Med Plants Stud. 2017;5(2):29-35.

2. Uno WZ. Isolation and Identification Antibacterial Activity of liem leaves Extract (Clerodendrum minahassae Tejiam dan Binn). Hassanuddin University; 2015.

3. WHO. Medicinal plants in Viet Nam (Institute of Materia Medica - HANOI - WHOHNPJPR, 1990, 444 p.). Part II: Medicinal plants. 55. Clerodendrum squamatum Vahl. Institute of Materia Medica, Hanoi. 1990. (cited 2019 Jan 18), p. 444. Available from: http://www.nzdl.org/gsdlmod?e=d-00000-00---off-Zz-8-00&a=d&c=whoedm&cl=CL1.1&d=HASH16e13d8f984a47e857913.55

4. Franca F, Atkins S. Neotropical Verbenaceae - Neotropikey from Kew. Kew, Royal Botani Garden; 2018.

5. Shrivastava, N. Patel T. Clerodendrum and Healthcare: An Overview. Med Aromat Plant Sci Biotechnol. 2007;1(1):42.

6. Muthukumaran P, Saraswathy N, Aswitha V, Balan R, Gokhul VB, Indumathi P, Balan R, Gokhul VB, Indumathi P, et al. Assessment of total phenolic, flavonoid, tannin content and phytochemical screening of leaf and flowers extracts from Peltophorum pterocarpum (DC.) Backer ex K.Heyne: A comparative study. Pharmacogn J. 2016;8(2):140-3.

7. Mihai CM, Al L, Bobi D, Dezmirian D. Estimation of Flavonoid Content in Propolis by Two Different Colorimetric Methods. 2010;43(1):407-10.

8. Hamidu L, Ahmad AR, Najib A. Qualitative and quantitative test of total flavonoid buni fruit (Antidesma bunius (L.) Spreng) with UV-Vis spectrophotometry method. Pharmacogn J. 2018;10(1):60-3.

9. Suryanto. Phytochemical Antioxidant. Surabaya, Indonesia: Putra Media Nusantara; 2012.

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

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 |

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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 amount of flavonoids from leaves of plants that are optimized for the drying temperature of their simplicity. Hohakay et al. showed that fresh leaves, dried leaves, dried at 40°C and 60°C respectively had total flavonoid levels of 1.2%, 0.78% and 0.62%. The increase in the total amount of flavonoids dried at 40°C and 60°C respectively had total flavonoid levels of 1.2%, 0.78%, and 0.62%. The UV-Vis spectrophotometry method of 13.47%.

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 |

Figure 1: Sesewanua (Clerodendrum fragrans Wild).

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

CONCLUSION

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