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Influence of Extraction Method to Total Flavonoid Content of Mareme Leaf Extract (*Glochidion arborescens* Blume)

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Abstract: Leaves mareme (*Glochidion arborescens* Blume) has a very strong antioxidant activity. One potential secondary metabolites as antioxidants are flavonoids. This study aims to determine the effect of the extraction method on total flavonoid levels of mareme leaf extract. Total flavonoid content was determined using UV-Vis spectrophotometry at a wavelength of 430 nm with aluminum chloride reagent uses comparators quercetin so that the results ware calculated as quercetin equivalents (QE). From the research results can be seen ethyl acetate extract resulted in the highest total flavonoid content of 40.3350 mg QE / g using reflux extraction method. Results of analysis using SPSS showed no significant differences in the levels of total flavonoids using extraction methods and there ware significant differences in the levels of total flavonoids of using solvents with increasing polarity.

Keywords: mareme leaves,* Glochidion arborescens *Blume, flavonoid, quercetin, spectrophotometer UV-Vis

I. INTRODUCTION

A methanol extract of mareme leaf has a very strong antioxidant activity [3]. Flavonoids are a group of secondary metabolites produced by plants that are included in a large group of polyphenols and have the ability to capture free radicals and inhibit lipid oxidation [1]. Flavonoids can reduce free radicals by inhibiting enzymes or by blocking metal ions involved in the production of free radicals [9].

The extraction method used affects the total concentration of target compounds for each compound present in the plant have polar properties and stability against heat differently. Some flavonoids that are less polar such as isoflavones, flavanones, flavones, and flavonols methylated can be extracted using a solvent with a low polarity such as chloroform and ether while O-glycoside flavonoids are flavonoids that are easily soluble in polar solvents such as ethanol, methanol, and water [4]. Ethyl acetate is also useful for taking catechins and proanthocyanidins which are included in the class of flavonoid compounds [7]. The total content of the compound flavonoids in the plant mareme leaves are not yet known, but based on research conducted Fajar Budi Sulaksono [10] on the determination of total flavonoids levels in the leaves of Centella Asiatica and Tempuyung leaves using methods Hot and cold extraction, the highest total flavonoids levels in Centella Asiatica leaves ware produced by heat method while the highest total flavonoids levels in Tempuyung leaves ware produced by cold method. From the research, it can be concluded that the stability of the flavonoids compound groups of the temperature can differ in each plant. Total flavonoids levels can be determined using UV-Vis spectrophotometry because flavonoids contain conjugated aromatic systems that can show strong absorption tapes in the UV spectrum and visible spectrum [5]. The study aims to determine the total flavonoids levels of mareme leaf extract using different extraction methods as well as to find out which extraction and solvent methods produce the highest total flavonoids levels of mareme leaves.

II. MATERIAL AND METHOD

A. Materials

The material used in this research includes glassware, steam cups, ash-free filter paper, crus, pliers, distillation tools, reaction tube racks, three feet, analytical scales, reflux tools, macerators, ovens, and UV-Vis spectrophotometry. The materials used in this research are mareme leaf Simplicia (*Glochidion arborescens* Blume), LP chloralhydrate reagent, 70% ethanol, toluene, ammonia, chloroform, HCl 2N, Mayer, dragendorf reagent, aqua dest, Mg metal, amyl alcohol, ether, Lieberman Burchard reagent, FeCl3, 1% gelatin, sulfuric acid vanillin reagent, 70% NaOH ethanol, methanol, ethyl acetate, N-hexane, sodium acetate, 10% aluminum chloride.

B. Sample preparation

The material used in this study was mareme leaves obtained from Cigugur Village, Pangandaran Regency, West Java. Mareme leaves washed with water to remove impurities, then drained and put into an oven at a temperature of 40°C - 50°C, then ground using a grinder to obtain powder bulbs.

C. Simplisia Quality Check

Quality control of Simplicia was carried out to ensure the simplicia used meets the requirements. This examination includes macroscopic and microscopic examination, determination of water-soluble extract, determination of ethanol-soluble extract, determination of insoluble ash content, determination of water-soluble ash, determination of water content, and determination of drying shrinkage.

D. Simplisia Extract of Mareme Leaves Using Maceration Method

Simplisia 500 grams was extracted by multilevel maceration method using n-hexane, ethyl acetate, and methanol. Extraction for each solvent was done for 3 x 24 hours. Each of the resulting filtrates was concentrated by rotary evaporator.

E. Mareme leaf Simplisia extraction using reflux method

Simplisia 300 grams was extracted by multilevel reflux method using n-hexane, ethyl acetate, and methanol as solvent. Each filtrate produced was concentrated with a rotary evaporator.

F. Phytochemical Screening of Simplisia and Mareme Leaf Extracts

Phytochemical screening was carried out to determine the compounds contained in mareme leaf extract. The compounds identified include the examination of alkaloid, flavonoid, quinone, monoterpene and sesquiterpene compounds, steroids and triterpenoids, saponins, tannins, and polyphenols.

G. Determination of Total Flavonoid levels of Mareme using UV-Vis spectrophotometry

1) Preparation of Quercetin Stock and Determination of Maximum Quercetin Waves.
   Quercetin stock solutions were made with a concentration of 1000 ppm, diluted to 60 ppm. 0.3 mL of the mother liquor was put into a 5 mL volumetric flask and methanol p.a was added to the mark, then 0.5 mL pipette was added 0.1 mL 10% AlCl3, 0.1 mL sodium acetate 1 M and 2.8 mL aquadest. Absorbance was measured at a wavelength of 400-800 nm [2].
2) Optimum incubation time determination.
   0.5 mL of standard solution quercetin concentration 60 ppm added 0.1 mL AlCl3 10%, 0.1 mL sodium acetate 1 M and 2.8 mL Aquades, homogenized and incubated at room temperature. Absorption was measured at a wavelength of 430 nm for 1 hour [2].
3) Quercetin Standard Curve Making
   Created series with variations in the concentration of 10, 20, 30, 40, 50, and 60 ppm. Each concentration was added 0.1 mL of 10% AlCl 3, 0.1 mL of sodium acetate 1 M and 2.8 mL of distilled water, then shaken until homogeneous and then left for 30 minutes, absorbance measured at a wavelength of 430 nm [2].
4) Determination of Total Flavonoid Content of Mareme leaf extract
   Mareme leaf extract, each made of 5000 ppm concentration and dissolved in methanol p.a. Then each extract was piped 0.5 mL added 0.1 mL $AlCl_3$ 10%, 0.1 mL sodium acetate 1 M and 2.8 mL distilled water, then shaken until homogeneous and incubated for the optimum time. Absorption was measured at a wavelength of 430 nm [2].

H. Data analysis

The data obtained were analyzed using SPSS with the ANOVA test used for testing two or more samples, while the essence of the test was to find out whether there were significant differences between the average counts of several data groups [8].

III. RESULTS

Macroscopic Examination Results of Mareme Leaves
Macroscopic and microscopic examinations ware in Figures 1 and 2

Figures 1 Mareme Leaf

Figures 2 Simplisia powder

Microscopic Examination Results of Simplisia Mareme Leaves

Identifying fragments contained in mareme leaf can be seen in Figure 3

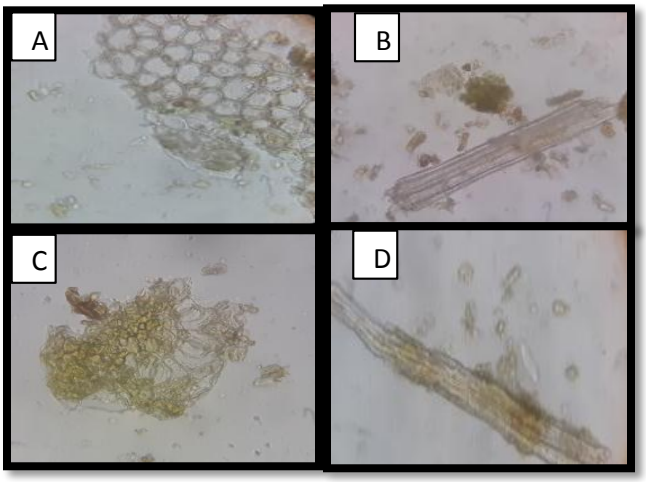

Figure 3 Microscopic powdered Simplisia leaf Mareme enlargement 400x
(A) Epidermis; (B) The Sklerenkim Serabut
(C) diasitic type Stomata; (D) Sklerenkim

Result of quality parameters of Mareme leaf Simplisia

The purpose of determining the parameters of mareme leaf was to ensure the quality of mareme leaf according to established standards The following results of the simplicia parameter check can be seen in Table 1

TABLE 1: RESULTS OF SIMPLICIA MAREME LEAF QUALITY PARAMETER

Parameter	Observation result
Water soluble essence	18,46% ± 0,5484
Ethanol soluble essence	18,48% ± 0,0464
Water content	6,00% ± 0
Dry shrinkage	6,89% ± 0,1096
Total ash content	6,58% ± 0,1285
Acid insoluble ash content	0,73% ± 0,0680
Water soluble ash content	1,82% ± 0,1101

Phytochemical Screening Test of Simplicia and Mareme Leaf Extract

Phytochemical screening was carried out to determine the content of secondary metabolites in simplicia and extracts mareme leaf. The results of phytochemical screening on mareme leaves can be seen in Table 2 below

TABLE 2 : RESULTS OF PHYTOCHEMICAL SCREENING TEST FOR SIMPLISIA AND MAREME LEAF EXTRACTS

Chemical compounds	Simplicia	Extraction Method Refluks			Extraction Method Maserasi		
		Extract N-Heksana	Extract etil asetat	Extract metanol	Extract N- heksana	Extract etil asetat	Extract metanol
Alkaloid	+	+	-	-	+	-	-
Flavonoid	+	+	+	+	+	+	+
Polifenol	+	-	+	+	-	+	+
Tanin	+	-	-	+	-	-	+
Saponin	+	-	-	+	-	-	+
Kuinon	+	-	-	+	-	+	+
Mono-seskuiterpen	+	-	+	-	-	+	-
Steroid	+	+	+	-	+	+	-
Triterpenoid	-	-	-	-	-	-	-

Extraction of Mareme leaves

The extraction of mareme leaves on this study was conducted with two methods namely maceration and reflux and using solvents with increased pollination of N-Hexsan, Ethyl Acetate, and methanol. The yield value was calculated to determine the effectiveness of the solvent used in extracting simplicia leaves. The yield percentage for each extract was in Table 3

TABLE 3: YIELD EXTRACT

Method	% Yield Extract		
	N heksana	Etil asetat	Metanol
Refluksx	3,0 %	3,0%	15,9 %
Maseration	1,2 %	1,5 %	7,0%

The resulting quercetin wavelength was 430 nm and the linear regression equation was obtained sample. Average total flavonoid levels can be seen in Table 4.

TABLE 4 : AVERAGE TOTAL FLAVONOID LEVELS

Sample	Extraction Method Refluks mg QE/g	Extraction Method Maseration mg QE/g
Ekstrac N-Heksana	31,7124 ± 0,49006	32,5654 ± 0,4337
Ekstrac Etil asetat	40,3350 ± 0,4052	33,9028 ± 0,4907
Ekstrac Metanol	10,1953 ± 0,1661	9,4315 ± 0,1248

IV. DISCUSSION

Mareme leaves (*Glochidion arborescens* Blume) ware green, long ovate leaves, pointed leaf tips, flat-leaf edges, leaf bones are pinnate, and the base of the spiky leaves, Besides that the powder of Simplicia mareme leaves was green, has a distinctive odor and bitter taste. Microscopic examination was performed to see the typical fragments contained in mareme leaf.

Based on the test results of extracts, mareme leaf was more absorbed into ethanol solvent than water solvent. From the test results, obtained the value of the water content of mareme leaves 6%. This value has met the standard due to the requirement of simplicia moisture content according to the applicable standard parameters was not more than 10% [11]. The drying shrinkage value was greater than the value of water content (6.89%) that value indicates there was a volatile compound that was lost in the drying process contained in mareme leaves. While the purpose of determining ash content was to provide an overview of internal and external mineral content from the initial process to the formation of simplicia.

Results of Determination of Total Flavonoid Levels of Mareme Leaf Extract Comparative compound used in determining the level of flavonoids is quercetin because it is a strong flavonoid compound of the flavonol group. In addition, most medicinal plants show high quercetin content activity [6]. In determining total flavonoid levels, the sample was reacted first using $AlCl_3$ and Sodium acetate. The addition of $AlCl_3$ has be done to form complex compounds so that there was a visible shift in wavelength $y = 0.0117x + 0.1041$. This equation was used to calculate total flavonoid levels from each mareme leaf extract that can be marked by changes in the sample to a more yellow color. While the addition of Sodium acetate has be done to maintain the wavelength in the visible region [2]. Before the absorbance reading, incubation has be done so that the reaction goes perfectly.

Based on the results of statistical tests using the ANOVA test showed a value of $\alpha < 0.05$ which indicatedthat there ware significant differences in total flavonoid levels by using solvents that have increased polarity. While based on statistical tests using the T-Test the value $\alpha > 0.05$. This value showedthat there was not significant difference between total flavonoid levels using the extraction method. The highest total flavonoid levels ware found in ethyl acetate extract by reflux extraction method.

V. CONCLUSION

Based on research that has been done, it can be concluded that the highest levels of flavonoids in mareme leaves ware produced by the reflux extraction method using ethyl acetate solvent.

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REFERENCES

[1] Banjarnahor, S., & Artanti, N. 2014. *Antioxidant properties of flavonoids*. *Medical Journal of Indonesia* pp. 239–244. doi: 10.20886/jphh.2017.35.3.211-219.

[2] Chang, C.C., Yang., M.H., Wem, H.M., Chern, J.C., 2002. *Estimation of Total Flavonoid Content in Propolis by Two Comlpementary Colorimetric Methods*. *Journal of Food and Drug Analysis*. Vol. 10. No.3. 2002. Pages 178-182.

[3] Dewi, M.K. 2016. Aktivitas Antioksidan Beberapa Ekstrak Daun Mareme (Glochidion borneense (Mull.Arg.) Boerl Terhadap Radikal DPPH (2,2-Diphenyl-1- picrylhydrazil) Menggunakan Spektrofotometri UV-Vis. [Skripsi] Tasikmalaya. Program S-1 Farmasi STIKes BTH Tasikmalaya.

[4] Hanani, Endang. 2016. Analisis Fitokimia. Jakarta: Buku Kedokteran EGC.

[5] Harborne, J.B. 1996. *Metode Fitokimia*. Bandung: ITB Press

[6] Oktaviana, Prima Riska. 2010. Kajian Kadar Kurkuminoid Total Fenol Dan Aktivitas Antioksidan Ekstrak Temulawak Pada Berbagai Teknik Pengeringan Dan Proporsi Pelarutan [Skripsi]. Surakarta: Universitas Sebelas Maret.

[7] Robinson Trevor. 1995. *Kandungan Organik Tumbuhan Tinggi*. Padmawinata K, penerjemah. Bandung: Penerbit ITB. Terjemahan dari: *The Organic Constituents of Higher Plants*.

[8] Santoso S. 2012. *Panduan Lengkap SPSS Versi 20*. Jakarta: Elex Media Komputindo.

[9] Suhartono, E. 2016. Toksisitas Oksigen Reaktif & Antioksidan di Bidang Kedokteran dan Kesehatan. cetakan pertama. Yogyakarta: Goysen Publishing.

[10] Sulaksono, F. B. and AB, S. 2012. Koreksi Kadar Flavonoid Dan Toksisitas Dalam Ekstrak Tempuyung (Sonchus Arvensis) Dan Pegagan (Centella Asiatica 1.

[11] Voight, R. 1984. *Buku Pelajaran Teknologi Farmasi*. Yogyakarta:Gadjah Mada University Press.