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THE EFFECT OF SENGGANI LEAVES (*Melastoma malabathricum* L.) EXTRACTION METHOD ON TOTAL PHENOLIC LEVELS, TOTAL FLAVONOIDS, AND ANTIOXIDANT ACTIVITIES

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ABSTRACT

Extraction method is important in maintaining the stability of the target compound. The purpose of this study was to observe the effect of the extraction method on total phenol levels, Total Flavonoids levels, and antioxidant activity. The extraction process used percolation and soxhletation methods with 96% ethanol as solvent. Preliminary tests of antioxidant activity were carried out by TLC with silica GF_{254} stationary phase and mobile phase using chloroform: methanol: ethyl acetate: water (80: 12: 6: 2). Positive results are characterized by pale yellow spots after being sprayed with 0.2% DPPH. Total phenol levels, Total Flavonoid levels, and antioxidant activity with the percolation method were 255.89±3.16 mg GAE/g sample; 36.92±0.22 mg QE/g sample; 16.00±1.76 µg/mL, respectively. Meanwhile, total phenol levels, Total Flavonoid levels and antioxidant activity with the the soxhletation method were 268.76±2.77 mg GAE/g sample; 41.01±0.35 mg QE/g sample; 15.13±2.67 µg/mL, respectively. In conclusion, extraction method affected the total phenol levels and Total Flavonoids levels (sig p <0.05), but not antioxidant activity (sig p> 0.05).

KEYWORDS: Antioxidant, *Melastoma malabathricum* L., extraction method, DPPH.

INTRODUCTION

Senggani (*Melastoma malabathricum* L.) is a plant that is a wild plant in shrubs. Empirically, senggani leaves are used by the community for various diseases.[1] Research that has been carried out addressing senggani leaves has been shown to have high antioxidant activity.[2] [3] The study reported, ethanol extract of senggani leaves from maceration method obtained Total Phenol levels, Total Flavonoids of 384.33 ± 0.005 mg/g; 85.8 ± 0.009 mg/g followed by IC_{50} values of 11.59 ± 0.84 µmol/L.[2] Total Phenol and Total Flavonoid levels of maceration method were 113.28 ± 0.003 mg GAE/g; 33.08 ± 0.004 mg QE/g.[4] The Reflux method obtained Total Phenol levels of 212.95 ± 4.37 mg GAE/g.[5] The sonicator method shows an IC_{50} value of 16.50 µg/mL in the category of very strong antioxidant activity.[6]

Total Phenol levels, Total Flavonoid levels, and antioxidant activity of plant extracts are influenced by various factors, one of which is the extraction method.[7] The choice of extraction method has an important role in determining an antioxidant activity. Ethanol extract of guava obtained from soxhletation method was better compared to maceration method, resulting an IC_{50} value of 37.67 µg / mL and IC_{50} value of 47.80 µg/mL, respectively.[8] Furthermore, another study shown that soxhletation method produced better result with IC_{50} value of 2.78 µg/mL, compared to maceration method with IC_{50} value of 3.18 µg/mL in ethanol extract of pineapple peels.[9] Ethanol extract of bay leaves of percolation method has better antioxidant activity with IC_{50} value of 49.67 µg/mL, when compared to the soxhletation method with IC_{50} 49.98 µg/mL.[10] Beluntas methanol extract of percolation method generated better Total Phenol and Total Flavonoid compared with maceration method; soxhletation, and reflux. The percolation method obtained a Total Phenol level of 116.96 mg/g; Total Flavonoid levels were 66.75 mg/g, whereas maceration, soxhletation and reflux methods Total Phenol and Total Flavonoid levels were below the level of percolation method.[11]

Based on the descriptions that have been mentioned, the authors are interested in conducting research on the effect of the senggani leaf extraction method on Total Phenolic levels, Total Flavonoids, and antioxidant activity. The purpose of the study was to determine the effect of the extraction method from percolation and soxhletation methods so as to produce Total Phenol levels, Total Flavonoids, and optimum antioxidant activity.

METHODS

Materials

The equipment used in this study were a set of UV-vis spectrophotometer (Shimadzu type 2450), UV lamps 254 and 366 nm (Merck type 1.13203,0001), oven (MODENA), Buther, rotary evaporator (Heodolph), vaporizer cup (pyrex) , desiccator (NORMAX), hot plate (Schott Instrument), fume hood (ESCO model EFH-4A1), micropipette (Raninin E1019705K®), analytical scales (Precise TYP 320-9410-003), glassware (Pyrex), a set of modification percolation tools, a set of soxhletation tools, blenders (Maspion), spray bottles, rubber filter bulb (D and D/standard type), thermometer, alcohol meter, flour sieve, and vortex.

Sample used in this study was senggani leaf (*Melastoma malabathricum* L.) taken in March 2019 on Parit Haji Husen 2 street, Southeast Pontianak, West Kalimantan Indonesia.

The materials used in this study were Ethanol 96%, Distilled Water p.i (Wida WITM Unicap brand), Methanol (pro analysis and technical), 1% $FeCl_3$ solution, $AlCL_3$ solution, 10% Na_2CO_3 solution, $CH_3COOHNa$ solution. 1 M, Folin Ciocalteu reagent, Quercetin, DPPH (2.2 diphenyl-1-picrihydrazyl crystal), Dimethyl Sulfate (DMSO), Silica Plate GF_{254}, Whatman filter paper, Gallic Acid, Chloroform, and Ethyl Acetate.

PROCEDURES

Treatment of Drying Plants

Fresh senggani leaves obtained are washed and drained and then chopped. Drying is carried out at 40-45^{0}C referring to the study Luliana 2016. The results of drying are then sorted dry, grinded, and sieved.

Extraction Process

The extraction process followed Mukhriani, 2014, the chosen method was the percolation method and the soxhletation method with a few modifications.[12]

a) Percolation Method

The percolation extraction process was carried out by modifying the percolator device used. 86.3 grams of zinc powder were put into the percolator. Then, it was flowed with a small pump of 96% ethanol solvent constantly, at the top of the percolator so that the sample was absorbed in the percolator wetted. The extraction process was carried out until the end point specified. The extraction results are then filtered with vacuum buthner and evaporated at 55^{o}C. The remaining solvent from the evaporator is then heated to a thick extract until dry.

b) Soxhletation Method

The process of extracting the soxhletation was carried out by placing the zinc powder as much as 125.07 grams in filter paper and placing it in the soxhlet casings, extraction was carried out 5 times. 96% ethanol solvent and boiling stones are put into the flask then put in a bath. Assembled soxhletation equipment and the extraction process takes place at a temperature of 60-70^{o}C to the specified end point. Determination of the yield of percent yield obtained is calculated by the following formula;

$$\% \text{ Rendemen} = \frac{extract\ weight}{simplisia} x\ 100\%$$

Loss on Drying

Weighed 1-2 grams of senggani leaf ethanol extract, put into a crucible lid that has previously been heated at 105^{o}C for 30 minutes until the weight obtained was constant. The extract in a crucible is then put into the oven and opened the lid, heated at a temperature of 105^{o}C for 30 minutes. After being heated, it is crucial that the extract with the extract is inserted into desiccator for 30 minutes in a closed position. Then it was weighed until the weight remains constant.[13] Drying losses are calculated by the following formula;

$$\% \text{ Loss on Drying} = \frac{Initial\ Weight\ of\ Krusibel - Final\ Weigh\ of\ Krusibel}{Initial\ Weight\ of\ Krusibel} x\ 100\%$$

Preliminary Thin Layer Chromatography Test

The ethanol extract of senggani leaves was spotted on silica plate GF_{254} 2-3 times and allowed to dry. The plate was then eluted in the TLC eluting chamber using the mobile phase of Chloroform: Methanol: Ethyl Acetate: Water (80: 12: 6: 2). The plate was taken out and let to dry. The TLC plate was then observed in UV visible light 254 and 366 nm. To detect the presence of antioxidant activity, the TLC plate was sprayed with a mix of specific reagent of 1% $FeCl_3$, 5% $AlCl_3$ and, 0.2% DPPH solutions.

Determination Test of Total Phenol Levels

Determination of Total Phenol content of senggani leaves was slightly modified from the study Barki, 2017 & Saifudin, 2011). The Total Phenol content unit was determined in (mg GAE/g sample)[14][15]

a) Preparation of Gallic Acid Standard Solution

10 mg Gallic Acid powder was dissolved with 1 mL DMSO and added Distilled Water p.i to volume 100 mL volumetric flask. Then made a concentration series of 15; 25; 35; 45; 55 and; 65 µg / mL. Took 0.5 mL of each concentration, reacted with 0.25 mL Folin Ciocalteau : Distilled Water reagent (1: 1) and 1 mL 1% Na_2CO_3, added Distilled Water p.i to volume in 5 mL volumetric flask, then incubated for 30 minutes. A concentration of 45 µg/mL was used for maximum wavelength. Theoretically, the wavelength for Gallic Acid is around 600-800 nm.[16]

b) Making Senggani Leaf Ethanol Extract Sample

Weighed 18 mg of the ethanol extract of senggani leaves, dissolved with 0.3 mL DMSO and added Distilled Water p.i to volume in 10 mL volumetric flask to obtain 1800 µg / mL. Then, pipetted 1 mL of the solution, diluted with Distilled Water p.i. to volume in a 10 mL flask to produce the concentration of 180 µg/mL. Took 0.5 mL of

180 µg/mL, reacted with 0.25 mL of Folin Ciocalteau Distilled Water reagent (1: 1) and 1 mL 10% Na2CO3, diluted with Distilled Water p.i to volume in 5 mL volumetric flask, then incubated for 30 minutes. It was done for 3 times replication.

c) Incubation Process

The incubation process was carried out by removing the mixture in a reaction tube and heated in warm water at a temperature of 45-50^{o}C for 5 minutes and allowed to stand at room temperature for 10 minutes to measure at a wavelength of 755.4 nm.

Test Determination of Total Flavonoid Levels

Determination of Total Flavonoid levels refers to the Chang method and Saifudin, 2011 with a slight modification. The Total Flavonoid level was determined in (mg QE / g sample)[15]

a) Making Quercetin Standard Solution

Quercetin powder of 11.2 mg was dissolved in methanol p.a to obtain a stock concentration of 112 µg / mL. The series concentration was made for 9; 12; 15; 18; 21; and 24 µg / mL in a 10 mL flask. Each concentration series was then taken 2 mL, reacted with 0.1 mL 10% $AlCl_3$; 0.1 mL CH_3COONa 1M and added to volume with Distilled Water in a 5 mL flask, then incubated for 30 minutes. Concentration of 18 µg / mL was used for maximum wavelength optimization. Theoretically, the wavelength for the determination of Total Flavonoids ranges from 400-600 nm.[16]

b) Making Senggani Leaf Sample Solution

Weighed 24 mg of ethanol extract of senggani leaves, dissolved with methanol p.a, to obtain a stock concentration of 2400 µg / mL. Then the concentration was reduced to 480 µg/mL in a 10 mL flask. Pipetted 2 mL 480 µg/mL solution, reacted with 0.1 mL 10% $AlCl_3$; 0.1 mL CH_3COONa 1M and added to volume with Distilled Water in a 5 mL flask, then incubated for 30 minutes and measured at a wavelength of 433.5 nm Calculation of Total Phenol levels and Total Flavonoids is carried out using the standard curve equation used to determine the concentration of the sample (x). Then enter the following crowd:

$$Phenol\ \&\ Flavonoid\ Content\ Total = \frac{C\ (\mu g/mL)\ x\ F\ x\ V\ (mL) x\ 10^{-3}}{M\ x\ 10^{-3}\ g}$$

Remarks:

C = concentration obtained from y = bx + a [obtained from the standard curve (µg/mL)]

V = initial volume of extract (mL)

M = extract weight (g)

F = dilution factor

Antioxidant Activity Test

Making DPPH Solution

Weighed 25 mg of DPPH crystals, dissolved in 25 mL methanol p.a to obtain 1000 µg / mL. Then taken 0.7 mL from the stock solution 3 times and put in a 50 mL flask, added methanol p.a to volume to produce the concentration of 42 µg/mL. Determination of the maximum wavelength (λmax) DPPH was done by reading the wavelength absorption at 515-520 nm.[16]

Making Quercetin Comparative Solution

Weighed 11.2 mg of quercetin powder, dissolved in methanol p.a in a 100 mL flask to obtain the concentration of 112 µg/mL. A concentration of 50 µg/mL was produced by taking 0.744 mL from the stock solution, diluted with methanol p.a to volume in a 5 mL flask. From the concentration of 50 µg/mL, concentration series were made for 2; 4; 6; 8; and 10 µg / mL with methanol p.a in a 5 mL flask. Each concentration series was taken 1 mL to be reacted with 3 mL DPPH 42 µg/mL in a dark vial. Then, it was incubated for 30 minutes and measured absorption at wavelength of 516.2 nm.

Antioxidant Activity Testing

Weighed 10 mg of senggani leaves ethanol extract, dissolved with methanol p.a to obtain a stock concentration of 1000 µg/mL in a 10 mL flask. Then pipetted 1 mL of stock to obtain 200 µg/mL in a 5 mL flask, diluted with methanol p.a to volume. A series of concentration was made for 3; 7; 12; 18; 24; 30; and 36 µg/mL from 200 µg/mL solution, diluted with methanol p.a to volume in a 5 mL flask. Each series concentration was reacted with 3 mL DPPH 42 µg/mL in a dark vial and incubated for 30 minutes.

Analysis of results

The percent inhibition value obtained was then used to find the IC_{50} value, the linear correlation value between concentration (X) and % inhibition (Y) was then included in the equation y = b.x + a, the value of y as a percent inhibitory 50% and x as the value of IC_{50}. A statistical test was performed with SPSS version 21. to see a significant comparison of the two extraction methods. H0 will be accepted if H0 <0.05 and H1 are rejected if H1> 0.05. Calculation of percent inhibition can be calculated by the following formula:

$$\%\ Inhibition = \frac{(abs.\ DPPH - abs.\ sample)}{abs.\ DPPH}\ x\ 100\%$$

RESULTS AND DISCUSSION

Determination

The sample used in this study was Senggani leaves. Determination confirmed the species *Melastoma malabathricum* L. The purpose of determination was to avoid problems in sampling.

Extraction

The results of the extraction from the percolation and soxhletation methods can be seen in Table 1. The result of the percent rendering method was 23.52% while for the soxhletation method was 21.27%. The extraction method used to attract metabolites in plants has a close relationship with the percent yield obtained. Percentage yield of percolation method was more than the soxhletation method because the percolation method

takes a long time to arrive at the end point compared to soxhletation method which was much faster. Lengthy extraction process makes contact between the solvent and the sample was better, so that the percent yield is obtained more. However, the results obtained can be influenced by several factors such as the solvent used, the condition of the sample, the size of the sample powder, the extraction method used and the length of the extraction process.[17]

Table 1: Percentage of Rendermen and Loss on Drying of Percolation and Soxhletation Methods.

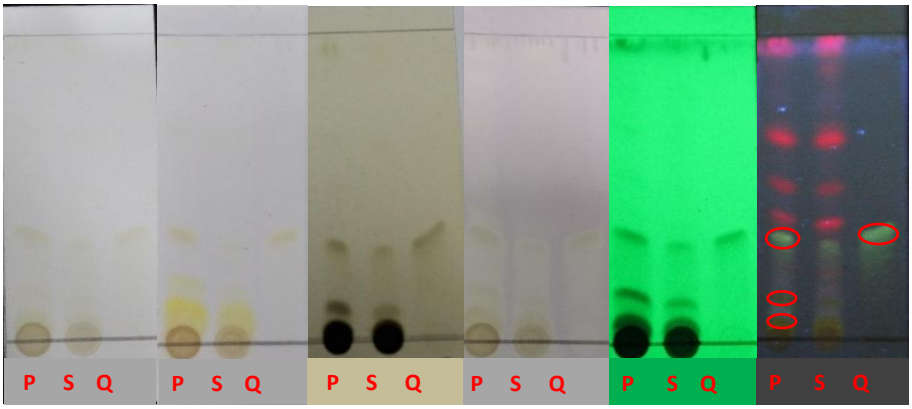
Methods	The Amount of Sample (g)	The Amount of Solvent (L)	% Rendermen	Loss on Drying	The Type of Extract	The Color of Extract
Percolation	86.3	8	23.52	16.15±0,55	Viscous	Dark Black
Soxhletation	125.07	2	21.27	24.77±0,14	Viscous	Dark Black

Determination of Loss on Drying

The result of the determination loss on drying of the percolation method was 16.15 ± 0.55 and the method of soxhletation was 24.77 ± 0.14 as shown in table 1. Loss on drying can deliver the type of extract obtained, which according to Voigt (1994) states the extract can be divided into 3 types namely dry extract, viscous extract, and liquid extract. Loss on drying of the extract <5% was said to be in the dry extract; 5-30% was said to be viscous extract, and <30% is said as a liquid extract.[18] Loss on drying obtained was in the range of 5-30% so that the extracts from the percolation and soxhletation methods are said to be in the viscous extract. The percolation and soxhletation methods produced different drying losses, this is due to the different methods used.[10]

Preliminary Test Results of TLC

TLC results (figure 1) showed that there were phenol compounds, flavonoids, and antioxidant activity which was shown by the discoloration of the spots after being sprayed with specific reagents. $FeCl_3$ to detect the presence of phenol compounds, $AlCl_3$ to detect flavonoids, and DPPH 0.2% to detect the presence of antioxidant activity.



Remarks: (a) The results of observations in visible light, (b) after being sprayed with 5% AlCl3 reagents, (c) after being sprayed with 1% FeCl3 reagents, (d) after being sprayed with a 0.2% DPPH reagent, (e) light UV 254 nm after being sprayed with 1% FeCl3, (f) UV366 nm light after spraying with 5% AlCl3. The percolation method (P), the soxhletation method (S) and Quersetin (Q).

Figure 1. Pattern of Chromatogram TLC Method with Silica GF$_{254}$ and Mobile Phase of Chloroform Methanol: Ethyl Acetate: Water (80: 12: 6: 2)

Determination of Total Phenol Levels

Determination of the Total Phenol content of the percolation and soxhletation methods was obtained from the standard curve of Gallic Acid in Figure 2. The absorbance obtained (y) to determine the initial concentration (x) calculated by the equation y = bx + a. The results of the Total Phenol content of the location method were 255.89 ± 3.16 mg GAE / g sample while the soxhletation method was 268.76 ± 2.77 mg GAE/g sample as shown in Table 2.

T-test results of Total Phenol levels from the percolation and soxhletation methods showed a sig p value <0.05, so it was concluded that there were significant differences. The Soxhletation method produced a greater Total Phenol content than the percolation method, because it uses the optimum heating temperature during the extraction process, so that the phenol content obtained is greater.[19]

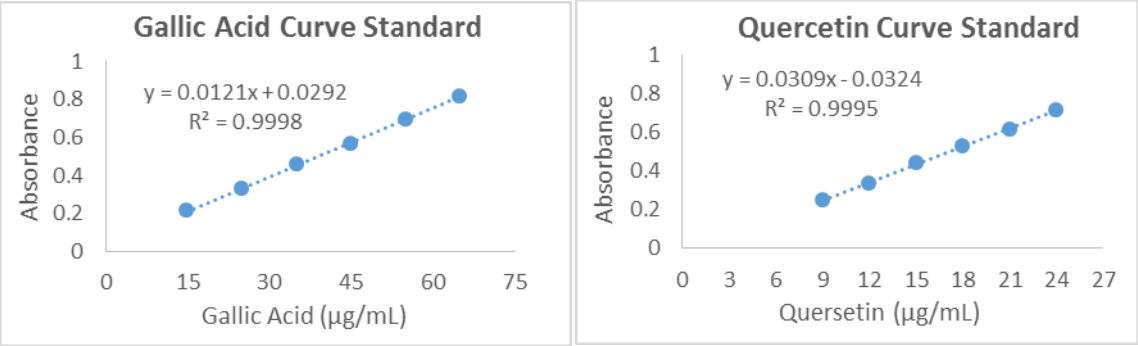


Figure 2: Standard Curve of Gallic Acid and Quercetin.

Designation of Total Flavonoid Levels

Determination of Total Flavonoid levels was carried out by the quercetin equation y = 0.0309 (x) + (-0.324) to determine the concentration of the sample. The result of determination of Total Flavonoid content of percolation method was 36.92 ± 0.22 mg QE / g sample, while the soxhletation method was 41.01 ± 0.35 mg QE / g sample as shown in Table 2.

The t-test results showed that the Total Flavonoid levels of percolation and soxhletation methods were significantly different (sig p <0.05). A significant difference from the method is due to the heating factor used in the extraction process. The study reported that optimum heating will increase Total Flavonoid levels, but more than 120^{o}C can reduce Total Flavonoid levels.[20]

Table 2*:* Phenol and Flavonoid Content Total, of Senggani Leaf Extract.

Methods	Phenol Content Total (mg GAE/g)	Flavonoid Content Total (mg QE/g)	IC_{50} Values (µg/mL)	Activity Category
Percolation	255.89±3.16	36.92±0,22	16.00±1.76	Very Strong
Soxhletation	268.76±2.77	41.01±0,35	15.13±2.67	Very Strong
Quercetin Standard	-	-	4.58±0.012	Very Strong

Antioxidant Activity of Senggani Leaf Extract Percolation and Soxhletation Methods

The results of quantitative antioxidant activity tests can be seen in Table 2. The IC_{50} value of the soxhletation method was stronger than the percolation method, this is presumably due to the heating factor which is carried out during the extraction process. Optimum heating can increase antioxidant activity.[21] However, heating more than 120^{o}C can deteriorate and reduce antioxidant activity.[22]

T-test results, IC_{50} values of percolation and soxhletation methods showed sig p value> 0.05. Thus, it was concluded that there was no significant difference. Table 2 also explains the strength of the antioxidant activity of the percolation and soxhletation methods with quercetin comparison. The aim is to see the effect of the extraction method shown by the IC_{50} value. The results showed the percolation and soxhletation methods have IC_{50} values greater than IC_{50} quercetin. But even so, both methods have very strong antioxidant activity.

The percolation and soxhletation methods have three times lower antioxidant activity than the quercetin standard because the sample levels used are still extracts. Thus, it is suspected that antioxidant activity that should be optimally inhibited by other metabolites. While the standard compound used is a pure compound that produces optimum antioxidant activity.

CONCLUSIONS

The results of research conducted can be concluded as follows:

1. Total Phenol levels and Total Flavonoid levels of the percolation method were 255.89 ± 3.16 mg GAE / g sample; 36.92 ± 0.22 mg QE/g sample. Whereas the soxhletation method was 268.76 ± 2.77 mg GAE / g sample; 41.01 ± 0.35 mg QE / g of the sample. T-test results of the two methods showed significant differences (sig p <0.05). it was concluded that the extraction method affected Total Phenol levels and Total Flavonoid levels.
2. IC_{50} value The percolation method was 16.00 ± 1.70 µg / mL while the soxhletation method was 15.14 ± 2.70 µg/mL. The IC_{50} value obtained indicates that the soxhletation method is better than the percolation method, although the results of the t-test show no significant difference (sig p> 0.05).

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