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Solvent optimization of flavonoid extraction from *Moringa oleifera* L. using simplex lattice design

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ABSTRACT

Background: Flavonoids found mainly as pharmacologically active natural ingredient of moringa (*Moringa oleifera* L.). Therefore, optimizing the solvent to maximize the levels of flavonoid components is required.

Objective: This study aims to determine the optimum solvent composition for extracting flavonoid compounds from moringa leaves using the simplex lattice design (SLD).

Methods: Moringa leaves were extracted using the sonication method with five combinations of 96% ethanol and water as the solvent. The solvent composition was optimized using Design-Expert version 11 software. Moreover, UV-Vis spectrophotometry was used to screen and identify flavonoid content.

Results: Flavonoids were detected in moringa extract. Using the F test, a valid SLD equation, y= 440.243(A) + 142.983(B) – 47.324(A)(B), was obtained. This equation showed that the solvent ethanol 96% : water (100:0) was the best solvent and produced the maximum amount of moringa flavonoid content (440 mg QE/g extract).

Conclusion: The flavonoid content was directly proportional to the high composition of 96% ethanol.

Keywords: flavonoid, Moringa oleifera, solvent optimization, simplex lattice design

Introduction

Moringa (*Moringa oleifera* L.) is a plant with numerous benefits, one of which is an alternative food for combating malnutrition [1–3]. One of the metabolites in Moringa leaves that are beneficial as therapeutic compounds and offer health advantages is flavonoids [4]. Flavonoids are polyphenolic compounds that are widely distributed in plants in the form of sugar-binding glycosides [5]. Flavonoids are known to act as an antianemia agent by stimulating the synthesis of erythropoietin (EPO) during red blood cell formation [6,7]. Typically, flavonoid compounds are extracted with polar solvents including methanol, acetone, ethanol, water, and isopropyl alcohol [8].

Several studies have determined the optimal solvent percentage for the extraction of Moringa leaves to yield the maximum total flavonoid content. Compared to 50% and 70% ethanol solvents, Moringa leaves extracted with 96% ethanol produced the greatest flavonoid content of 13,15 mg QE/g of extract [9]. In contrast to 60% and 70% ethanol solvents, 80% ethanol solvent in sonicated moringa leaf extract produced the greatest flavonoid concentration of 50 mg QE/g extract [10]. The higher the concentration of ethanol solvent, the higher the produced of flavonoid compounds. In the absence of solvent optimization, however, the optimal ethanol solvent concentration for the synthesis of flavonoid compounds yields varying outcomes.

To achieve the highest flavonoid content based on the optimal solvent composition, further research into extraction solvent optimization is still required. Simplex lattice design (SLD) is a method used to optimize material components with varying amounts of material composition so that the total amount is equalized. This method can determine the optimal material components, such as solvents. In addition, the

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SLD method is practical and fast because it eliminates the need for trial-and-error determination of the solvent composition.

Previous studies found that different the best concentrations of ethanol solvents to flavonoids [9,10]. This is because there was no solvent optimization performed. Therefore, we optimized the solvent in flavonoid extraction from moringa leaves using simplex lattice design. This study seeks to establish the optimal composition of 96% ethanol and water for extracting flavonoid components from moringa leaves using SLD, as well as the flavonoid content of Moringa leaf extract based on the optimal composition.

Methods

Producing of simplisia

A total of 4.2 kg of moringa leaves were harvested at the Indonesian village of Apitaik, Pringgabaya District, East Lombok Regency, West Nusa Tenggara. The samples were initially sorted and cleaned with clean water three times. The sample was then covered with a black cloth and sun-dried. The dried samples were sorted, mashed using a blender, and sieved with a 70-mesh sieve before to the extraction procedure [11].

Sample extraction

Fifty grams of samples were placed in a glass jar and extracted with 500 mL of 96% ethanol and water per sample. There were five variations of the 96% ethanol : water ratio, namely 100:0, 75:25, 50:50, 25:75, and 0:100. The extraction process was carried out using a sonicator (Elmasonic) for 3x30 minutes. The filtrate was concentrated with a rotary evaporator (Heidolph) to obtain a thick extract. Furthermore, the percent yield of each extract was calculated [12]. The calculation of the yield of Moringa leaf extract refers to Equation 1.

$$\% \text{ yield} = \frac{\text{thick extract weight (g)}}{\text{initial simplicia weightl (g)}} \text{ x } 100\% \quad \text{.....1)}$$

Flavonoids qualitative test

A total of 2 mL of moringa leaf extract was added with 1 mL of concentrated HCl (Merck) and 0.1 g of magnesium powder (Merck). Flavonoids positive result was represented by the formation of a yellow, orange to red color [13].

Determination of operating time and maximum wavelength

Determination of total flavonoid content was carried out using UV-Vis spectrophotometry (Analytik Jena Specord 200 Plus). Quercetin 100 ppm master stock was made by weighed 10 mg of quercetin then dissolved in p.a ethanol (Merck) in a 100 mL volumetric flask (Iwaki).

The operating time was determined by measure the standard solution at a theoretical wavelength of 428 nm. The absorbance was read every 5 to 60 minutes until it reached a stable absorbance. The standard solution contains quercetin was produced by adding 0.5 mL of 50 ppm quercetin, 1.5 mL of ethanol p.a. (Merck), 0.1 mL of 10% aluminum chloride (Merck), 0.1 mL of 1 M potassium acetate (Merck), and 2.8 ml of aquabidest (Merck). The mixture of the solution was vortexed until homogenous. The maximum wavelength (λ max) was determined by measuring the quercetin standard at a wavelength between 400 and 750 nm, using the same preparation described above.

Standard curve determination

Standard solutions were made with concentrations of 30, 40, 50, 60, and 70 ppm, and each was pipetted using a micropipette (Labnet) as much as 0.5 mL. After that, 1.5 mL of ethanol p.a (Merck), 0.1 mL of 10% aluminum chloride (Merck), 0.1 mL of 1 M potassium acetate (Merck), and 2.8 mL of aquabidest (Merck) were added. The mixture of the solution was vortexed until homogenous. After incubating the solution for the duration of the operating time, the absorbance at the maximum wavelength was measured using UV-Vis spectrophotometry (Analytik Jena Specord 200 Plus).

Total flavonoid content

The sample solution of moringa leaf extract was prepared at a concentration of 2500 ppm: 0.0125 g of extract was weighed before being diluted in 5 mL of ethanol p.a (Merck). The sample was added with 1.5 mL of ethanol p.a (Merck), 0.1 mL of 10% aluminum chloride (Merck), 0.1 mL of 1 M potassium acetate (Merck), and 2.8 mL aquabidest (Merck). The mixture of the solution was vortexed until homogenous.

The solution was incubated for the duration of the operating time. Absorbance was measured using UV-Vis spectrophotometry (Analytik Jena Specord 200 Plus) at the maximum wavelength. The test was conducted three times (triplo). The total flavonoid content was

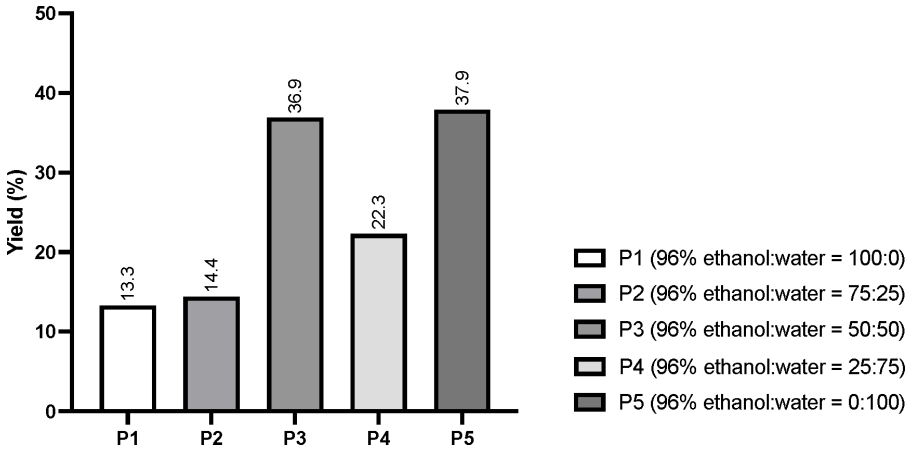

Figure 1. The yield extract with variation percentage solvent composition

Table 1. Moringa leaf extract flavonoid qualitative test

Sample	Organoleptic test			
	Taste	Color	Scent	Form
P1 (ethanol 96%: water = 100:0)	Bitter	Green-black	Aromatic	Very thick
P2 (ethanol 96%: water = 75:25)	Bitter	Black-brown	Aromatic	Thick
P3 (ethanol 96%: water = 50:50)	Bitter	Black-brown	Aromatic	Thick
P4 (ethanol 96%: water = 25:75)	Bitter	Black-brown	Aromatic	Thick
P5 (ethanol 96%: water = 0:100)	Bitter	Black-brown	Aromatic	Thick

calculated using Equation 2, in which TFC = total flavonoid content (mgQE/g extract), V = sample volume (mL), Fp = dilution factor, and m = sample weight (g).

$$TFC = \frac{C \times V \times Fp}{m} \quad \text{.....2)}$$

Extraction solvent optimization using simplex lattice design (SLD)

The flavonoid content of extract were then analyzed using the simplex lattice design (SLD) method to calculate the coefficients a, b, and ab to obtain Equation 3, where A = proportion of 96% ethanol, B = proportion of water, Y = response (total flavonoid contents).

$$Y = a(A)+b(B)+ab(A)(B) \quad \text{.....3)}$$

On the basis of Equation 3, a profile representing the total flavonoid content produced by the ethanol and water mixture was constructed. The profile data were utilized to calculate the optimal fluid composition. Each equation derived from each formula is confirmed using a statistical technique, specifically the F test, with a degree of confidence of 95% [11].

Data analysis

SPSS version 25 was used to carry out a parametric statistical analysis. The analysis consists of a test for normality, a test for homogeneity, and a test for the null hypothesis using one-way ANOVA and the Tukey test. This statistical analysis was conducted to see whether there was a significant difference in the study's results if p 0.05 was obtained.

Results

Sample extraction and flavonoid qualitative test

A total of 4.2 kg of moringa leaves that have been sorted wet were then cleaned and drained. Moringa leaf samples were then dried and sorted dry until it reach a weight of 1 kg. The dried samples were powdered using a blender to obtain 900 g of simplicia powder. Extract yield in each sample obtained the lowest value in sample P1, while the highest value in sample P5, indicates that the high ethanol concentration used produced a less but thicker extract (Figure 1, Table 1). In the organoleptic test, moringa leaf extract with P1 solvent composition was greenish-black, but the other four solvent composition variations were brownish-black.

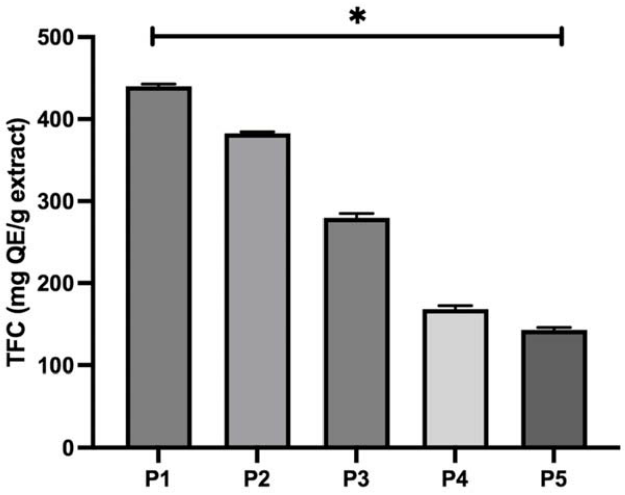

Figure 2. Total flavonoid content (TFC) of Moringa leaf extract. *significantly different among sample groups. $p < 0.05$

Determination of total flavonoid content of moringa leaf extract

The maximum wavelength used for the measurement of quercetin absorbance was 434 nm, and the operating time was 60 minutes. Based on the determination of the total flavonoid content, the P1 composition (ethanol:water = 100:0) has the highest average total flavonoid content, with 440 mg QE/g extract, while the P5 composition (ethanol:water = 0:100) has the lowest average total flavonoid content, with 142.9 mg QE/g extract. The results of the total flavonoid levels obtained in the five samples of moringa leaf extract also showed significant differences between the sample groups in the total flavonoid levels produced (Figure 2).

Optimization of flavonoid compound extraction solvent composition

Based on the calculation, the simplex lattice design (SLD) equation was obtained, which refers to the equation: Y = 440.243 [A] + 142.983 [B] – 47.243 [A][B]. For the validation results of the SLD equation, the F test value for the total flavonoid content obtained is F count 27.34, while the F table is 4.67, so it is concluded valid.

Discussion

This research aimed to find the optimal solvent components for flavonoid extraction from moringa leaves. The components of this study are composed of 96% ethanol and water. Polarity-dependent solvent selection, such as ethanol and water, can determine the extracted substance type. Composition P1 with 100 portions of 96% ethanol as solvent yielded a greenish-black and very thick extract, suggesting that the solvent attracts more chlorophyll than other solvent components. This result is consistent with the previous report that the chlorophyll content extracted increases with increasing ethanol solvent concentration [14,15].

The high concentration of ethanol in the solvent evaporated, results in the decrease of the extract yielded. However, the extract yield is not directly proportional to the total flavonoid content of moringa leaf extract. The yield of 30%, 50%, 70%, and 96% ethanol extracts were 2.60%, 1.88%, 1.88%, and 1.92%, respectively, while the flavonoid content was 12.3, 8.2, 18.0, and 44.7 mg QE/g extract [16]. These results indicate that the yield is not positively correlated with the total flavonoid content obtained; therefore, further investigation is required to ascertain the total flavonoid content of moringa leaf extracts.

The presence of flavonoid compounds was determined by the formation of a yellow orange or red color during the Shinoda test [17]. The results of flavonoid screening in five samples obtained solutions with different colors, indicates that the types of flavonoids extracted from moringa leaves are also different. The flavonoid group can be distinguished based on the reaction of flavonoid discoloration. In the qualitative test, P1 produced red when the extract was added with HCl and magnesium, indicating the presence of flavanone, flavonol, and flavanonol. In sample P2, the orange color shift indicates the presence of a flavone group, whereas in samples P3, P4, and P5, the yellow color change shows the presence of an isoflavone group.

The total flavonoid concentration was determined using the colorimetric method. This method is based

on the complex synthesis of aluminum chloride with a ketone group attached to the C-4 atom and a hydroxy group attached to the C-3 or C-5 atom [18]. This study utilized quercetin as a standard for evaluating total flavonoid contents because quercetin is the flavonoid with the widest distribution in plants [18].

The composition of 96% ethanol and water as solvent was optimized using the SLD method to extract moringa leaves. The obtained SLD equation is valid because the F count value was 28.189 > F table 4.670. The optimum solvent composition based on SLD for extracting flavonoid compounds from moringa leaves was 100 portions of ethanol and 0 portions of water (P1 composition). The optimal solvent produced the highest flavonoid concentration of 440 mg/QE g extract. The results coincided with a previous report on optimizing the filtered fluid for extracting flavonoid compounds in fragrant pandan leaves, namely 96% ethanol solvent [19]. The 96% ethanol solvent yielded the highest levels of flavonoid compounds compared to other solvent concentration variations, including 30%, 50%, and 70% ethanol [16]. This is due to the ability and nature of the solvent to dissolve flavonoids varied, depending on the level of the polarity of the solvent and the extracted component.

Conclusion

The optimum solvent composition for the extraction of flavonoid compounds from Moringa leaves using the SLD method is 100 portions of 96% ethanol and 0 portions of water with a total flavonoid content of 440 mg QE/g extract.

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Author contributions

WH, NIH design the study; SMO contribute to data acquisition; SMO, WH write the first draft; WH, NIH performed analytical statistic; SMO, WH, NIH finalize and revise final manuscript; all authors agreed to the final version of the manuscript.

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