Effect of variation of amount of extracting solutions on total flavonoid currency of ethanol extract of kelor leaves (*Moringa oleifera* L.)

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Abstract. Flavonoids in *Moringa oleifera* leaves have the potential as antioxidants and are utilized in various health products. So information is needed about optimizing the right solvent ratio during the extraction process to obtain effective and optimal results. This study was conducted to determine the effect of ethanol solvent ratio on total flavonoid content and yield. The ratio used was 1:10, 1:20, 1:40, and 1:60. Preparation of *Moringa oleifera* leaves ethanol extract using maceration method with 50% ethanol solvent. Extract standardization test includes organoleptic test and drying shrinkage test. The amount of yield was statistically analyzed using Linear Regression using the SPSS application. Determination of total flavonoid content using UV-Vis spectrophotometer instrument with $AlCl_3$ reagent. Total flavonoid levels obtained were then statistically analyzed using the SPSS application One Way Anova method. Based on the results of the standardization test, *Moringa oleifera* leaves ethanol extract has met the test requirements. The yield of *Moringa oleifera* leaves ethanol extract from 1:10, 1:20, 1:40, and 1:60 ratios were 29.17%; 30.97%; 33.15%; and 37.15%, respectively. The optimal point of total flavonoid content was reached at a ratio of 1:20. The addition of more solvent than 1:20 ratio is no longer effective in increasing the total flavonoid content. The results of this study can then be used as a reference in the formulation of *Moringa oleifera* leaves extract cream and lotion.

1 Introduction

Flavonoids are secondary metabolite compounds that can be found in *Moringa oleifera* leaves and have good bioactive functions [1]. One of the plants that contain many flavonoid compounds is *Moringa oleifera*. In previous studies, *Moringa oleifera* leaves has phenolic content, flavonoids which are known to have good antioxidant activity [2]. To obtain the benefits of flavonoids in *Moringa oleifera* leaves, it is necessary to extract them first. Flavonoid compounds in *Moringa oleifera* leaves can be extracted using maceration method. The extraction method is a type of extraction that is easy because the process is done by soaking the simplisia in the liquid [3].

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The highest flavonoid content in *Moringa oleifera* plants is reported to be found in the leaves with a level of 4.44 ± 0.0045 mg/ml extract [4]. In a study conducted by 2, it was explained that the ethanol extract of *Moringa oleifera* leaves had a flavonoid content of 3.03 ± 0.20 g IQE/100 g. Based on other sources, it is explained that the IC50 value of *Moringa oleifera* leaves is 3.03 ± 0.20 g IQE/100 g [2]. Based on other sources explain that the IC50 value of *Moringa oleifera* leaves ethanol extract is 143.99 [12].

The ratio of materials and solvents during the extraction process can affect the yield of a particular compound. In *Moringa oleifera* leaves extraction research that has been done, there are two types of ratios between *Moringa oleifera* leaves powder and solvents that are commonly used, namely 1:6 and 1:40. The study explained that the greater the solvent ratio, the greater the yield obtained [5]. A similar study that conducted experiments with solvent ratios of 1:10; 1:5; and 1:2.5 obtained results that an increase in solvent ratio will slightly increase total flavonoid content [6].

So, in this study, variations in the ratio of the number of extracting solvents will be carried out to see the parameters of total flavonoid content and yield. The ratio variations used in the experiment will be more and with a wider interval, namely 1:10, 1:20, 1:40, and 1:60. The selection of 50% ethanol as a solvent is because, according to the highest flavonoid content shown in the extract with 50% ethanol solvent [7]. In addition, it is also because flavonoids that are polar will dissolve more easily in polar solvents such as 50% ethanol.

2 Material and Methods

2.1 Tools and materials

The tools used in this research are oven, blender, 20/40 mesh sieve, analytical balance (Brookfield), Halogen Moisture Analyzer, water bath (Memmert), vacuum rotary evaporator (Heidolph), Buchner funnel, vacuum suction, UV-Vis spectrophotometer (1800 Shimadzu), quartz cuvette, volumetric flask (Iwaki), micro pipette, volume pipette (Iwaki), watch glass (Iwaki), porcelain cup, test tube (Iwaki), measuring cup (Iwaki), beaker (Iwaki), dropper, funnel, filter paper. The materials used in this study are *Moringa oleifera* leaves, distilled water, 96% ethanol (technical), ethanol p.a (Merck), Mg powder (Merck), HCl (Merck), aluminum (III) chloride (Merck), quercetin (Aldrich), n-Butanol (Merck), glacial acetic acid (Merck), NaOH 10%, NH_3 (ammonia).

2.2 Simplisia Preparation

Moringa oleifera leaves were dried using an oven at 50°C for 24 hours. The dried *Moringa oleifera* leaves were powdered and sieved with a 20/40 mesh sieve.

2.3 Extraction of *Moringa oleifera* Leaves Simplisia

The extraction process was carried out by maceration method with a powder and solvent ratio of 1:10, 1:20, 1:40, and 1:60. The extract was then evaporated with a rotary evaporator at 40°C and concentrated again with the help of a water bath [2].

2.4 Organoleptic Testing of Extract

Organoleptic testing of extracts is done by observing the extracts that have been obtained using the five senses. Some aspects observed were the extract's color, shape, smell, and taste [8].

2.5 Determination of Drying Shrinkage of Extract

Determination of drying shrinkage of extracts instrument Halogen Moisture Analyzer. The extracted sample obtained weighed approximately 0.5 grams, was placed in a container made of aluminium foil, and then tared. The sample is incandescent at 105°C for 30 minutes or until constant weight [9].

2.6 Determination of Total Flavonoid Content

The method for determining total flavonoid content refers to several modifications sourced from several studies [8,10]. The method modification is adjusted to the research conditions.

2.7 Data Analysis

The data obtained in the form of yield and total flavonoid content in the extract will then be analysed statistically with SPSS at a confidence level of 95%.

3 Results and Discussion

Making simplisia begins with collecting materials, namely *Moringa oleifera* leaves obtained from Yogyakarta. The leaves taken were all leaves, both young and old leaves on each stalk. Drying was carried out at a temperature of 50°C. The dried leaves were then pollinated. The powder was sieved using a 20/40 mesh sieve.

The extraction process was carried out by the maceration method. The maceration method was chosen because the leaves have a soft texture, and the extraction process does not use heating, where heating can reduce flavonoid levels [11]. The sifted powder was then weighed as much as 50 grams and dissolved with 50% ethanol in accordance with the predetermined ratio, namely 1:10, 1:20, 1:40, and 1:60. The extract obtained by varying the ratio of the number of solvents was calculated the yield obtained as presented in Figure 1. The results of statistical tests show that there is an effect of differences in the ratio of the number of solvents to the yield produced. The more solvent causes, the greater the contact of the material with the solvent. If the extracted compounds are more and more, the resulting yield is higher [12]. The highest yield was obtained in the ratio of powder and solvent 1: 60.

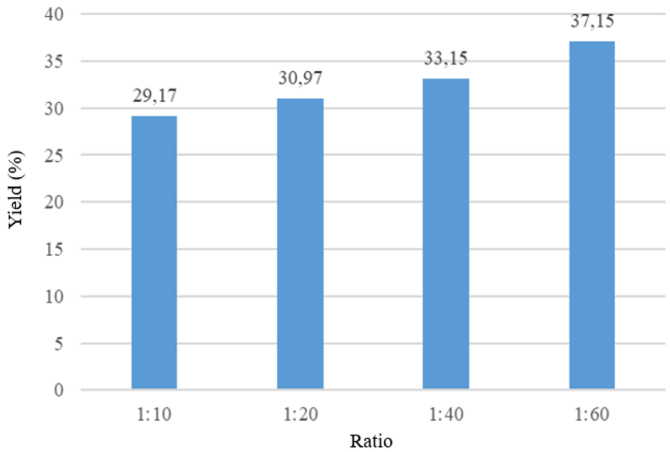

Fig. 1. Diagram of Yield Results on Extracts with Variations in the Ratio of the Number of Solvents

Extract standardization tests carried out include organoleptic tests and drying shrinkage tests. The extract obtained was then identified by observing several organoleptic parameters such as shape, color, odor, and taste. This test is carried out to obtain an initial description of the extract produced. Based on some literature that has been read, *Moringa oleifera* leaves extract has not been listed in the official publication of the Indonesian Ministry of Health either Materia Medika Indonesia or Monograph of Medicinal Plant Extracts. So that the organoleptic comparison data for *Moringa oleifera* leaves extract is obtained from previous research [13]. Organoleptic testing can be seen in Table 1.

Table 1. Organoleptic Test of Extract

Test Parameters	Result	comparator
Shape	Viscous	Viscous
Color	Dark green	Dark green
Smell	Characteristic Moringa odor	Characteristic odor
taste	Bitter	Bitter

Furthermore, the extract was tested for drying shrinkage. The maximum limit of drying shrinkage of the extract is 11%. The goal is to provide an overview of the maximum limit on the amount of compounds, water content, and solvents lost during the heating process [8]. Based on the test results in Table 2, all samples of *Moringa oleifera* leaves ethanol extracts were found to have drying shrinkage results below 11%, which means that all extracts with various solvent ratios meet the stipulated conditions. In special cases (if it does not contain volatile oils and the rest of the organic solvent evaporates) is identical to the water content, which is the water content due to being in the outside air environment [8]. If the value is below the requirements, the possibility of growing fungi and molds can be minimized. So that the quality and quality of *Moringa oleifera* leaves ethanol extract can be better maintained [14]. The results of the drying shrinkage determination are presented in Table 2.

Table 2. Results of Determination of Drying Shrinkage of Extract

Ratio	Replication	Weight (gram)	Drying shrinkage (%)	Average (%)	SD	CV	$\bar{x} \pm Le$
1:10	1	0.503	6.24	6.29	0.17	2.70%	6.29 ± 0.42
	2	0.505	6.48				
	3	0.502	6.15				
1:20	1	0.505	5.88	5.61	0.39	6.95%	5.61 ± 0.97
	2	0.505	5.79				
	3	0.502	5.16				
1:40	1	0.501	5.89	5.97	0.11	1.84%	5.97 ± 0.27
	2	0.505	6.08				
	3	0.503	5.94				
1:60	1	0.504	4.67	4.6	0.25	5.4%	4.6 ± 0.62
	2	0.502	4.32				
	3	0.505	4.81				

Determination of total flavonoid content in *Moringa oleifera* leaves ethanol extract samples refers to previous research [15]. In this method, 2% aluminium chloride is added, and there will be a color change from pale yellow to a more intense yellow. This color change is due to the complex formation reaction between flavonoids and aluminium chloride reagents. The presence of chromophore groups in the flavonoid structure can absorb monochromatic light, making it analysable using a visible spectrophotometric instrument. The results of the total flavonoid content determination test are presented in Table 3.

Table 3. Total Flavonoid Content in Extract Samples

Ratio	Average total flavonoids (mg EQ/g extract)	*Limit of Error (L.e)*	SD	CV
1:10	5.947	0.035	0.022	0.377 %
1:20	8.620	0.231	0.145	1.683 %
1:40	5.471	0.285	0.179	3.276 %
1:60	5.455	0.170	0.107	1.962 %

Data on total flavonoid content in ethanol extracts of *Moringa oleifera* leaves with four variations in the ratio of the number of solvents are presented in Table 3. If the CV value produced is less than 5%, then the method is declared to have good accuracy [16]. In addition, according to the Association of Official Analytical Chemistry, if the CV value is less than 5.3%, the method is considered to have good accuracy [17]. In the four variations of the ratio, the CV value obtained is less than 5%, so the proposed requirements have been met.

The content data was then statistically analysed to determine the effect of powder and solvent ratio. The statistical test results are presented in Table 4. Based on Table 4, between the ratio groups 1:40 and 1:60, there is no significant difference or in other words is the same. Meanwhile, there are significant differences between other groups. When viewed from Figure 2, the 1:20 ratio has the highest average level compared to other groups. There was an increase in the average total flavonoid content from the 1:10 ratio to the 1:20 ratio. This can occur because the more solvent used in the process, the more flavonoids can dissolve. The existence of an increasing amount of solvent can optimize the diffusion of flavonoids in the plant cell membrane into the solvent due to the pressure difference between inside and outside the cell, which is quite high [18].

Table 4. Analysis of Flavonoid Levels in Extract Samples with Tukey HSD Method

Solvent amount ratio		Significance	Conclusion
1:10	1:20	0.000	Significant difference
	1:40	0.001	Significant difference
	1:60	0.001	Significant difference
1:20	1:10	0.000	Significant difference
	1:40	0.000	Significant difference
	1:60	0.000	Significant difference
1:40	1:10	0.001	Significant difference
	1:20	0.000	Significant difference
	1:60	0.998	No different (same)
1:60	1:10	0.001	Significant difference
	1:20	0.000	Significant difference
	1:40	0.998	No different (same)

However, there was a further decrease in the average total flavonoid content at a ratio of 1:40 and 1:60. The optimal point of obtaining the average total flavonoid content was reached at a simplistic and solvent ratio of 1:20. The presence of a solvent volume that is too large can reduce the turbulence (rotation of the solution) that exists in the search process [19]. This can be attributed to the selection of containers used during maceration. The volume of solvent entered is a maximum of 2/3 of the total volume capacity of the container. Because, when the volume of solvent entered exceeds the requirements, it can also reduce the turbulence in the process of extracting during stirring, resulting in a smaller number of flavonoids extracted. Therefore, the addition of solvent more than the ratio of 1:20 is no longer effective in increasing the total flavonoid content.

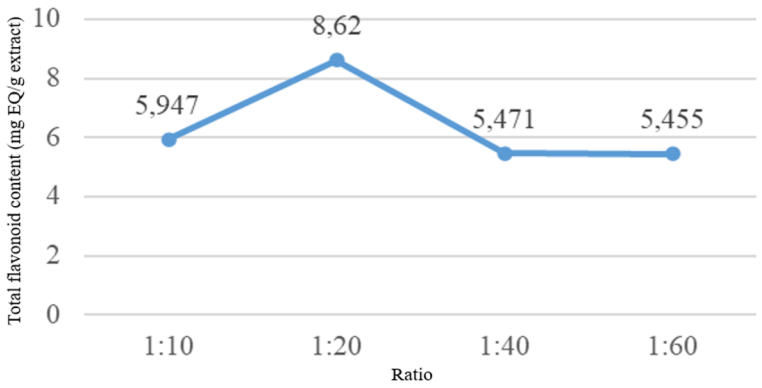

Fig. 2. Total flavonoid content curve at each ratio

In previous studies, the ratio variation carried out was 1:10 to 1:35, and the highest flavonoid content was obtained at a ratio of 1:25 [19]. In addition, in other studies, there was also an increase in total flavonoid levels at a simplistic-solvent ratio of 1:20 to 1:60, but then a decrease when the amount of solvent exceeded the ratio of 1:60. This is possible because the appropriate amount of solvent volume can dissolve flavonoids optimally from the simplisia. If the ratio exceeds 1:60, more impurities will dissolve, which can then block the dissolution process of flavonoids [20].

4 Conclusion

The yield of *Moringa oleifera* leaves ethanol extract from the ratio of 1:10; 1:20; 1:40; and 1:60 is 29.17%; 30.97%; 33.15%; and 37.15%, respectively. The more the amount of solvent, the more the weight of the extract or yield obtained will be. The total flavonoid content of *Moringa oleifera* leaves ethanol extract in the ratio of 1:10; 1:20; 1:40; 1:60 was (5.947 ± 0.035) mg EQ/g extract, (8.620 ± 0.231) mg EQ/g extract, (5.471 ± 0.295) mg EQ/g extract, and (5.455 ± 0.170) mg EQ/g extract, respectively. There was an increase in total flavonoid levels from a ratio of 1:10 to 1:20, but the addition of solvents beyond a ratio of 1:20 was no longer effective for increasing flavonoid levels because there would be a decrease in total flavonoid levels.

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