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***Abstract*—The use of Moringa leaves is increasing because it contains β carotene, phenolic compounds and flavonoids which are efficacious as antioxidants. The solvent concentration is a factor that affects the active content in the extract. This study aims to determine the effect of ethanol concentration on β carotene levels, total phenolic and total flavonoids in ethanol extract of Moringa leaves. In this study the variation of ethanol solvent concentration was 50%, 70% and 96% for extracting Moringa leaves by maceration method at a ratio of 1:40. The extract obtained was determined as β carotene levels by using HPLC, total phenolic content and total flavonoids contend by spectrophotometer. The results of each test were analyzed by using one-way ANOVA to see a significant difference between solvent concentrations for each test parameter. The test results showed that in each ethanol extract 50%, 70%, 96% had β carotene levels of 0.24 ± 0.01; 0.40 ± 0.00; 5.33 ± 0.15; total phenolic 122.26 ± 1.49; 132.54 ± 2.56; 84.62 ± 2.44; total flavonoids 2.51 ± 0.06; 5.03 ± 0.08; 13.15 ± 0.47, respectively. The highest solvent containing active ingredients is 96% ethanol.**

***Keywords*—moringa oleifera, β carotene, total phenolic, total flavonoid, solvent**

I. INTRODUCTION

Free radical is mediator of some diseases because it has unpair electron. To overcome this, antioxidant can protect our cells from negative effect of free radical. However using the synthetic antioxidant can make a side effect. Accordingly many studies have been contucted by using plant which is have many coumpound that can protect from free radical.

The results showed that Moringa leaves contained phenolic compounds, flavonoids and carotenes [1], [2]. Based on the content of active ingredients, especially compounds of Vitamin C, flavonoids, phenolic and carotene, Moringa leaves have properties as antioxidants, antimicrobials, aterio-sclerosis, anti-inflammatory and antiaging due to the influence of sunlight [2]-[5]. The content of phenol causes Moringa leaf extract can protect collagen [6]. Antioxidant activity is also caused by the active ingredient β-carotene [3], which functions to fight free radicals in the body that cause of aging [7]. The β-carotene can increase the amount of collagen, which is very closely related to the appearance of wrinkles [6]. Therefore β-carotene is also one of the agents in plants that functions as anti-aging [8]. In addition, Moringa leaves also contain quercetin and kaempferol compounds which are useful as anti-tyrosinase agents [9].

Moringa leaf extract activity was also seen from research on its formulation in several dosage forms. Moringa leaf extract applied in topical [14] can be used for prevention and treatment of diseases of oxidative stress and anti-aging [9]. Previous research showed that a concentration of 3% extract in cream was able to reduce the occurrence of evenness [10]. The formulation of 70% ethanol extract in lotions and gels with a concentration of 5% has activity as a strong sunscreen [11]. The formulation of petroleum ether extract of Moringa leaves purified with hexane provides 8.02% Vitamin E levels. Increasing the concentration of the purified extract in the emulgel causes an increase in viscosity and a decrease in dispersion [12].

The activity of Moringa leaf extract is strongly influenced by the content of the active ingredients in the extract. Based on the concept of like dissolve like, polar compounds will dissolve in polar solvents and non-polar compounds will dissolve in non-polar solvents [13]. To optimize the process of extracting the content of active compounds in Moringa leaf extract such as β-carotene, phenolic and flavonoids, we performed a variety of different concentrations of ethanol 50%, 70% and 96%. Concentration variations were carried out to determine the most optimal concentration of solvents in pursuing the levels of active substances in Moringa leaves.

II. METHODOLOGY OF RESEARCH

A. Materials

This study used Moringa leaves that collected from Bringharjo market, aquadest, methanol absolute (*Merck*), ethanol p.a (*Merck*), natrium carbonat (Na2CO3) (*Merck*),

aluminum chloride (AlCL3) (*Merck*), Folin-ciocalteu (*Merck*), 1,1 difenil-2-pikrihidrazil (DPPH) (*Aldrich*), askorbit acid (*Sigma*), quercetin (*Aldrich*), octil methoxycinamat (*Sigma*), galic acid (*Aldrich*). The tools are High Pressure Liquid Chromatography (*Shimadzu*), Spectrophotometer (Shima-dzu), water bath (memmerth).

B. Procedure of Research

1. Making extracts

Dry powder samples of Moringa leaves were dried in room temperature and smashed manualy. After that it was weighed of 10 grams and then extracted using ethanol 50%, 70% and 96% as much as 400 mL. The weighed sample powder was soaked in solvent and mixture for 72 hours at room temperature. During the maceration process stirring was also carried out. After that it was filtered with filter paper and vacuum pump. The extract was placed in an air-conditioned chamber until the solvent evaporates and a thick extract was obtained [1].

2. Determination of β carotene levels

B carotene levels were determined by the High Performance Liquid Chromatography method. The HPLC system configuration used Phenomenenx Luna 5µm C18 coloumn. The mobile phase was mixture of acetonitril: metanol:diklorometan (37:10:53v/v) at a flow rate 11ml/min. The total run time was 12 min.

Validation of the method for determining carotene β levels:

a. Determination of specificity

Specificity was determined based on the resolution value between the 2 peaks produced by the chromatogram.

b. Determination of linearity

Linearity was determined based on the r value of the regression equation the relationship between the concentration of the active substance and the area of the chromatogram.

c. Determination of precision

Precision was determined by the value of CV generated from 6 sample levels at a concentration of 0.6 µg/ml.

d. Determination of accuracy

Accuracy was determined by the recovery value of the data in the 3 concentration variations. Each of concentration was replicated 3 times. The solution with three known of standart concentration were added to 100 µg Moringa oleifera extract samples. The recovery of each standart concentration was calculated.

e. Determination of detection limits

The detection limit of the analysis method is determined based on the Limit of Detection and Limit of Quantification values.

Determination of β carotene levels:

In the determination of β carotene content the mobile phase of the mixture between acetonitrile:methanol: dichloromethan (37:10:53 v/v) was used. The extract was diluted in mobile phase.The 20 µl of sample was injected to HPLC.

3. Determination of total phenolic levels

Total phenolic levels are determined by Folin-Ciocalteau reagents. Absorbance of the extract solution was measured by UV-Vis spectrophotometer at the maximum absorbance wavelength with replication 3 times [14]. In this study the standard used in the form of gallic acid. Sample was diluted in mixture of ethanol:aquadest (1:1). The amount of total phenolic (mg GAE/g) was measured by using spectrophotometer with operating time at 60 minute and wave length at 761 nm.

4. Determination of total levels of flavonoids

Determination of total flavonoid content was carried out based on the colorimetric method using aluminum chloride (AlCl3) [15]. The sample absorbance of three replications was measured by a UV-Vis spectrophotometer at the maximum wavelength wavelength [16]. In this study, quercetin was used as a standard for total flavonoids. The amount of total flavonoid (%) was measured by using spectrophotometer with operating time at 43 minute and wave length at 422 nm.

III. Result and Discussion

A. Validation of Determination of β Carotene Levels with HPLC

The purpose of the validation of the level determination method is to prove that the level determination method used can provide precise and precise results. The parameters used include selectivity, linearity, LOD / LOQ determination, accuracy and recovery. In the test of carotene β content determination using the level determination method with HPLC.

1. Specificity

Based on the calculation results it is known that the resolution value between the two peaks is 6.08. (table I). This shows that the separation between two adjacent peaks has shown a fairly good separation that is fulfilling the requirements of the value Rs s 1.5 [17].

TABLE I. Retention Time Data and Adjacent Peak Pedestal Width

	Peak before β caroten	Peak β caroten
Retention time (minute)	2.92	5.05
Width of peak (cm)	0.1	0.6

2. Linearity

The test results show that the standard curve equation obtained is linear because it has r = 0.999. This r value is greater than r table (0.707) so that this linear equation can be used to calculate carotene β levels based on the area obtained using the HPLC method. The test results are presented in Fig 1.

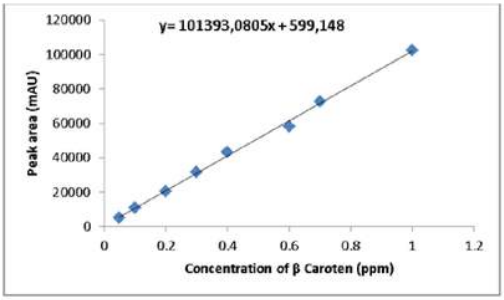

Fig. 1. The relationship curve between β carotene levels and peak area.

3. *Accuracy*

In this study the accuracy was measured by placebo method. Based on the data in table III, the recovery data for each concentration is 99.92%; 100.05%; 99.98%. Accuracy in the analysis method has an average recovery criteria for each replication of 50% -150% [18]. Based on the results of the recovery, the accuracy requirements are met.

TABLE II. ACCURACY DETERMINATION DATA

Conc. before analysis (µg/ml)	Conc. after analysis (µg/ml)	Recovery (%)	average (%)
0.2	0.198	99	99.92
	0.201	100.5	
	0.200	100	
	0.199	99.5	
	0.193	96.5	
	0.208	104	
0.6	0.603	100.5	100.05
	0.591	98.5	
	0.588	98	
	0.597	99.5	
	0.615	102.5	
	0.608	101.3	
1	1.038	103.8	99.98
	1.049	104.9	
	0.928	92.8	
	1.030	103	
	0.997	99.7	
	0.957	95.7	

4. *Precision*

Careful analytical methods will provide measurement results that are fixed at all times from the same sample. In table II, it can be seen that the CV value is less than 2%, the precision requirements are fulfilled [19].

TABLE III. PRECISION DETERMINATION RESULTS

Conc β Karoten (µg/ml)	Conc after analysis (µg/ml)	recovery (%)	SD	CV (%)
0.6	0.6003	100.05	1.71	1.70

5. *LOD and LOQ Detection Limits*

Based on the regression line equation, the detection limit value is 0.16 µg / ml and the quantification limit is 0.05 µg / ml as presented in table IV.

TABLE IV. CALCULATION OF LOD AND LOQ

concentration (µg/ml) (X)	Area (mAU) (Y)	Yi = bX+a	$(Y-Yi)^2$
0.05	5171	5668.802	247806/831
0.1	10931	10738.456	37073.192
0.2	20460	20877.764	174526.760
0.3	31586	31017.072	323679.070
0.4	43215	41156.380	4237916.304
0.6	58268	61434.996	10029863.664
0.7	72465	71574.304	793339.364
1	102364	101992.229	138213.676
Y = 101393.0805x +599.148 r = 0.999			Σ = 15982418.9
Slope (b) = 101393.0805			
Standar Deviasi Residual (SD) = 1632.0957			
LOQ = 0.05 µg/ml		LOD = 0.16 µg/ml	

B. *Calculation of β Carotene Levels on Ethanol Extract of Kelor Leaves*

The results of the study show data on carotene β levels in samples of Moringa leaf ethanol extract (EEDK) as shown in Table V.

TABLE V. AVERAGE Β CAROTENE LEVELS IN THE EXTRACT WITH VARIATION CONCENTRATION OF SOLVENT

Sample	Conc (µg/ml)	CV
EEDK 50%*	0.24 ± 0.01	4.17
EEDK 70%*	0.4 ± 0	1.70
EEDK 96%*	5.33 ± 0.15	2.86

EEDK = ethanol extract of Moringa leaf
* Value p <0.05, which means significantly different

The highest β carotene content was obtained from ethanol extract 96% of Moringa leaf compared to 50% ethanol extract and 70% Moringa leaf due to differences in solvent polarity. Where according to the concept of like dissolve like, where polar compounds will dissolve in polar solvents and non-polar compounds will dissolve in non-polar solvents [20]. From the experiments the levels of non-polar β carotene will be higher in the 96% ethanol solvent which is non polar, this is in accordance with the concept of like dissolve like. Statistical test results showed that there were significant differences between extracts with different variations in solvent concentration ($p < 0.05$). This proves that the use of ethanol solvent concentrations on the extraction of different Moringa leaves causes differences in the levels of β carotene in the ethanol extract of Moringa leaves.

C. Determination of Total Phenolic Levels

The results of the phenolic assay are shown in Figure 2 which shows that the total phenolic content of 70% ethanol extract of Moringa leaves is the highest compared to 50% ethanol extract and 96% Moringa leaf. Ethanol has polar and non-polar groups. The hydroxyl group (-OH) is a highly polar group because of the high electronegativity of oxygen. On the other hand, ethanol also has non-polar carbon (C2H5-) so it can dissolve non-polar compounds [21]. The presence of 4% water may help the process of diffusion of polar leaves of Moringa compounds. The increase in water content in the solvent so that 70% ethanol was obtained caused an increase in the acquisition of Total Phenolic Content (TPC) from 122.26 mg GAE per gram of 50% ethanol extract to 132.54 mg GAE per gram of 70% ethanol extract. This shows that with the greater composition of the water in the solvent, more polar compounds in the Moringa leaf can diffuse into the solvent, although this can also reduce the possibility of extracted non-polar compounds into the solvent. The greater increase in water content, namely in the use of 50% ethanol, gives lower TPC gain of 122.26 mg GAE.

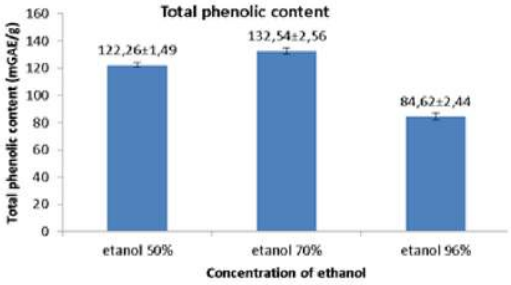

Fig. 2. The amount of total phenolic content in ethanol extract with variation of concentration.

D. Determination of Total Flavonoid Levels

Based on the results of testing the total flavonoid content in Figure 3 it is known that the total flavonoid content in the ethanol extract 96% of Moringa leaves is higher, compared to 50% ethanol extract and 70% Moringa leaf. This is due to the ability and the nature of the solvent to dissolve the flavonoids differently, depending on the level of polarity of the solvent and the extracted compound. According to the principle of polarization, a compound will dissolve in a solvent that has the same polarity [25]. Flavonoid compounds are polar compounds because they have a number of sugars that are bound, therefore flavonoids are more likely to dissolve in polar solvents. Flavonoid compounds are divided into several types, each type of flavonoids has different polarity depending on the number and position of the hydroxyl groups of each type of flavonoids so that it will affect the solubility of flavonoids in solvents [26]. The highest total flavonoids in ethanol extract 96% of Moringa leaves explained that the characteristics of flavonoids in ethanol extract 96% Moringa leaves have the same polarity with 96% ethanol, so Moringa leaf extract with ethanol 96% solvent produces the highest content of flavonoids. Statistical test results showed that there were significant differences between extracts with different variations in solvent concentration ($p < 0.05$). This proves that the use of ethanol solvent concentrations on the extraction of different Moringa leaves causes differences in the total content of flavonoids in the ethanol extract of Moringa leaves.

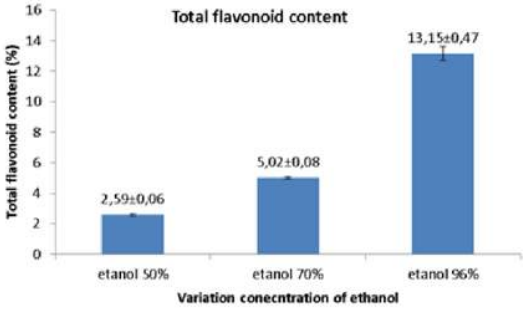

Fig. 3. Total flavonoid content in ethanol extract with variation of concentration.

IV. Conclusion

The variation concentration of ethanol influenced the amount of β carotene, total phenolic and total flavonoid. The optimum concentration of solvent was 96% of ethanol.

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