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Effect of Surfactants on Total Phenol Content from Sonication Extraction of Moringa (*Moringa oleifera Lamk*) Leaves in Vegetable Oil

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Abstract

This research aimed to determine the effect of variations in surfactant addition and sonication extraction time on the total phenolic content of Moringa leaves in vegetable oils, namely Virgin Coconut Oil (VCO) and Extra Virgin Olive Oil (EVOO). Moringa leaf extract in VCO and EVOO at varying volume additions of tween 80 of 0 mL; 0.1 mL; 0.2 mL; 0.3 mL; and 0.4 mL, as well as variations in extraction time, namely 0, 10, 20, and 30 minutes. The solution mixture was sonicated at room temperature with a frequency of 42 kHz. The extraction results were tested for total phenol content using the total phenol measurement method (Folin-Ciocalteu), whose absorption was measured using a UV-Vis Spectrophotometer. The best variation was the addition of tween 80 volume of 0.4 mL and extraction time of 30 minutes; sonicated Moringa leaf extract in VCO was 39.28 ± 1.75 mg GAE/g and EVOO was 57.80 ± 2.89 mg GAE/g. Furthermore, the typical functional groups of phenolic compounds in the best Moringa leaf herbal oil extract in VCO and EVOO were identified using an FTIR spectrophotometer.

Keywords: *EVOO, Moringa leaves, total phenol content, VCO*

Introduction

Moringa (*Moringa oleifera* Lamk.) is a plant that contains 8 mg/mL of phenolic compounds in its leaves and produces a good source of natural phytochemicals (Toppo *et al*., 2015). Moringa leaves contain phenolic compounds in the form of flavonoids as active compounds, namely isoflavones, flavanones, flavone alcohols, and flavonols) which are non-polar (Rifkia & Prabowo, 2020). VCO and EVOO are two different vegetable oils that provide health benefits to skin tissue (Aulia *et al*., 2014). The VCO's main composition is lauric acid (saturated fatty acid), which contains Medium Chain Triglycerides (MCT) and the phenolic compound α-tocopherol, which is very good for controlling skin softness because it is biodegradable (Ja'afar et al., 2019). EVOO contains several vitamins and monounsaturated fatty acids (MUFA) in the form of oleic acid in olives (Kusuma *et al*., 2022). Oleic acid as a topical treatment helps reduce the volume of skin lesions by 75% and resolves all lesions in 83% of patients (Gustafsson *et al*., 2004). Using the hot maceration extraction method, Moringa leaf extract with VCO and EVOO positively contains phenolic compounds, alkaloids, tannins, flavonoids, terpenoids, and steroids. Moringa leaves extract VCO and EVOO contain total phenol levels, namely 7.473% GAE and 44.83% GAE at a concentration of 40%, better than without the addition of Moringa leaves, namely 5.982% GAE and 9.44% GAE (Mahmudah, Yulianti, *et al*., 2023). Curcumin levels in turmeric-extract

virgin coconut oil using the ultrasonic method have a more excellent value than the maceration extraction method (Mahmudah, Nada, *et al*., 2023).

Vegetable oils function as alternative solvents because they have high solubility and selectivity, do not evaporate quickly, are easily regenerated, and are economically viable (Yara-Varón *et al*., 2017). Herbal oil can be obtained using the sonication method, which utilizes ultrasonic waves to break down the cell walls and release the cell contents into the extraction medium easily, requiring a shorter extraction time (Rifkia & Prabowo, 2020). Variations in extraction time were carried out to determine which extraction process ran optimally without any active compounds being damaged. Meanwhile, variations in the addition of Tween 80 were carried out to find out which Moringa leaves produce the maximum phenolic compounds, where Tween 80 is one of the surfactant groups with an excellent ability to dissolve phenolic compounds and has a high HLB (Hyrophylic-Lipophylic Balance) value of 15, which functions as a stable oil-in-water emulsifier so that it can maximize the solubility of the active compound in oil solvents (Farah Diba *et al*., 2014). The curcumin content in turmeric virgin coconut oil extract with the addition of Tween 80 has a more excellent value than without Tween 80 (Mahmudah, Nada, *et al*., 2023).

This research aimed to determine the effect of variations in surfactant addition and sonication extraction time on the total phenolic content of Moringa leaves in VCO and EVOO. The addition of Tween 80 to the extraction is expected to attract more phenolic compounds from Moringa leaves to obtain higher total phenol levels. The compounds in moringa and vegetable oils have good benefits for the skin, so you can take advantage of these two ingredients in herbal oil. The Moringa vegetable oil extract results can be an antioxidant product for skin health.

Methods

Sonication Extraction Method with Variations in Surfactant Addition and Extraction Time in VCO and EVOO

A sample of 4 grams of Moringa powder was added to 10 mL each of VCO and EVOO. Then, variations in the volume of tween 80 0 mL; 0.1 mL; 0.2 mL; 0.3 mL; and 0.4 mL. Next, sonication was extracted using a water bath sonicator with a frequency of 42 kHz with time variations of 0, 10, 20, and 30 minutes. The thick extract, the result of the herbal oil mixture, is then filtered using a cheesecloth to take the filtrate and stored in a dark glass bottle. Then, calculate the yield (Mahmudah, Nada, *et al*., 2023):

$$\% \; yield = \frac{extract\ weight}{sample\ weight} \times 100\% \qquad (1)$$

Preparation of Gallic Acid Standard Solution

The gallic acid standard was weighed at 0.5 mg, put into a 5 mL volumetric flask, and dissolved in distilled water to the mark so that the standard concentration of gallic acid was 100 ppm. Next, dilute the gallic acid stock solution by pipetting to 0.15; 0.3; 0.6; 0.9; 1.2; and 1.5 mL is then put into a 5 mL measuring flask and distilled water is added up to the mark so that the final concentration of the standard gallic acid solution is 3, 6, 12, 18, 24, and 30 ppm (Barki *et al*., 2017).

Determination of Total Phenol Content

Determination of total phenol content was carried out by weighing 5 mg of herbal oil and then placing it in a 5 mL volumetric flask. Then ethanol was added up to the limit mark to obtain a concentration of 1000 ppm. Then 0.5 mL of the herbal oil test solution was pipetted, and the standard solution was added with 2.5 mL of Folin-Ciocalteu reagent. Then, 2 mL of Na_2CO_3 was added. Then vortexed for 3 minutes until homogeneous and incubated for 45 minutes. Then, the absorbance was measured at a maximum wavelength of 759.9 nm (Barki *et al*., 2017).

Calculation of Total Phenol Content

The concentration of the oil solution is entered into the regression equation for the

gallic acid standard solution, and the formula y = ax + b is used where y is the absorbance value of the sample. After obtaining the x value (phenol concentration), the total phenol content is calculated through calculations using the formula below (Barki *et al*., 2017):

$$□□□ = \frac{C.V.DF}{w} \qquad (2)$$

Identification of Functional Groups in Liquid and Solid Samples using FTIR

Samples of VCO, EVOO, tween 80, Moringa leaves with VCO extract, Moringa leaves with EVOO extract, Moringa leaves with VCO extract with variations in surfactant volume and the best extraction time, and EVOO extract Moringa leaves with variations in surfactant volume and best extraction time were taken in several drops. The pellet-making plate includes one part of potassium bromide pellets (KBr). Then, it was pressed at a pressure of 80 torr to obtain pellet plates. Then, smear a small amount of sample on the pellet. Pellet plates were identified at wave numbers 4000-500 cm^{-1} using FTIR.

Moringa leaf powder was crushed with potassium bromide (KBr) using an agate mortar. After being crushed, the sample is placed in a pellet-making plate. Then, it is pressed at a pressure of 80 torr (8 tons per unit area) to obtain pellet plates. Pellet plates were identified at wave numbers 4000-500 cm^{-1} using FTIR (Batool *et al*., 2017).

Result and Discussion

Sonication Extraction

The resulting extract is a mixture of Moringa leaf extract with a dark green color. This indicates that the secondary metabolite compounds in Moringa leaf powder have been extracted in VCO and EVOO. The color change of the filtrate resulting from sonication becomes a thick, cloudy green extract, which is assumed to contain active compounds from Moringa leaves, namely phenolic compounds dissolved in VCO and EVOO. The yield values obtained after the filtrate filtering process from Moringa leaf herbal oil extract in VCO and EVOO are shown in Table 1 and Table 2.

Table 1. Results of the yield of Moringa leaf herbal oil extract in VCO

Volume Tween 80	yield (%)			
	Extraction Time (minute)			
	0	**10**	**20**	**30**
0 mL	23.82	23.42	20.60	18.15
0.1 mL	23.70	23.64	21.21	21.12
0.2 mL	25.40	24.55	21.39	21.17
0.3 mL	25.48	25.40	23.02	22.93
0.4 mL	26.78	26.15	25.84	23.94

Table 2. Results of the yield of Moringa leaf herbal oil extract in EVOO

Volume Tween 80	yield (%) Extraction Time (minute)			
	0	10	20	30
0 mL	25.10	25,00	22.22	21.52
0.1 mL	25.98	25.29	22.56	22.32
0.2 mL	26.55	26.27	23.75	22.83
0.3 mL	27.51	27.45	24.07	24.04
0.4 mL	29.11	28.51	25.66	24.83

The higher the added volume of surfactant and the shorter the extraction time, the higher the yield will be. This can happen because surfactants can stabilize the emulsion of particles in solution by preventing clumping between particles so that breaking down active compounds will be more effective (Suptijah *et al.*, 2011). A high yield value was obtained with a shorter extraction time, and this is thought to be because the oil was not absorbed into the Moringa powder optimally, where there has not been much reaction between the active phenolic compounds in the oil and Moringa leaves. However, filtering was done, so the yield was high with a low total phenol content. The highest yield of Moringa leaf herbal oil in VCO and EVOO was obtained by adding a surfactant volume of 0.4 mL and an extraction time of 0 minutes, respectively, at 26.7824% and 29.1151%.

Tables 3 and 4 show the results of the total phenolic content of herbal oil from sonicated Moringa leaf extract in VCO and EVOO.

Table 3. Total phenol content of herbal oil from sonicated Moringa leaf extract in VCO

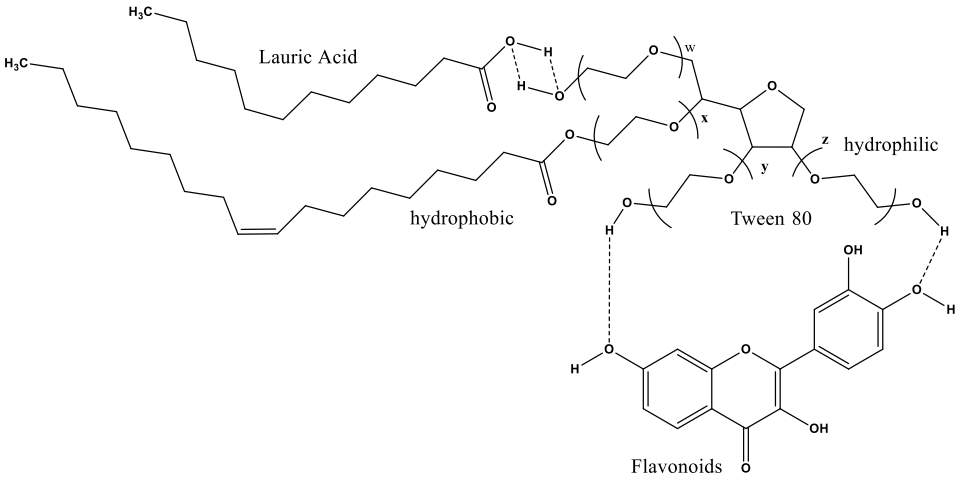
Volume Tween 80	Mean and Std. Dev. Total Phenol Content Moringa Extract in VCO (mg GAE/g) Extraction Time (minutes)			
	0	10	20	30
0 mL	1,22±0,40^{ij*}	5,94±1,45^{gh}	14,41±1,84^{cd}	16,36±2,12^{c}
0.1 mL	1,23±0,70^{j}	7,11±0,40^{fgh}	14,98±0,80^{cd}	17,06±1,06^{c}
0.2 mL	3,17±1,75^{hij}	10,81±1,45^{def}	31,64±1,45^{b}	32,34±1,75^{b}
0.3 mL	5,49±1,84^{ghi}	13,13±1,84^{cde}	30,95±1,45^{b}	34,42±1,06^{b}
0.4 mL	8,73±2,00^{efg}	15,44±1,45^{c}	35,16±1,45^{ab}	39,28±1,75^{a}

Table 4. Total phenol content of herbal oil from sonicated Moringa leaf extract in EVOO

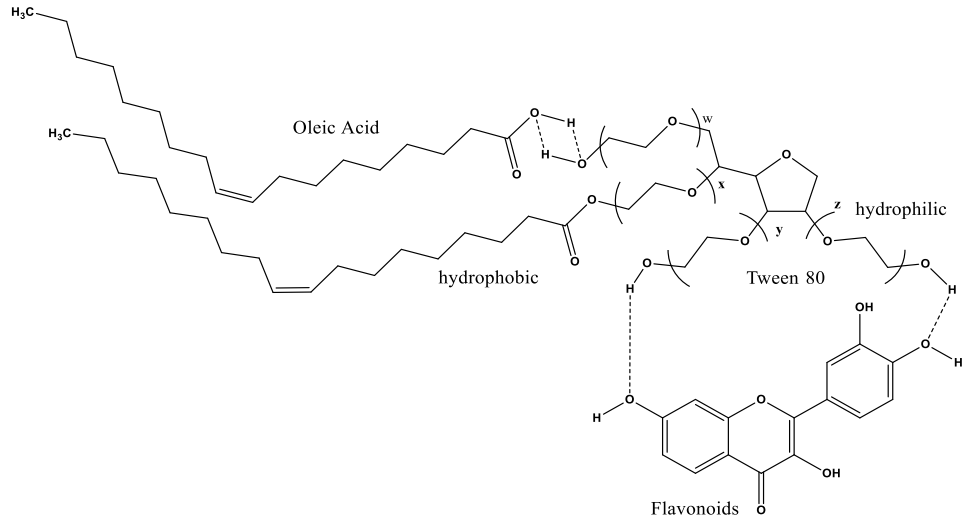
Volume Tween 80	Mean and Std. Dev. Total Phenol Content Moringa Extract in EVOO (mg GAE/g)			
	Extraction Time (minute)			
	0	10	20	30
0 mL	0,95±0,45^{h}	8,73±1,45^{efg}	14,75±1,06^{ef}	15,21±1,39^{ef}
0.1 mL	8,03±1,45^{g}	13,13±1,84^{ef}	15,67±0,40^{ef}	15,67±2,00^{ef}
0.2 mL	8,73±1,75^{g}	15,44±2,23^{ef}	32,11±2,00^{c}	33,11±2,12^{c}
0.3 mL	10,58±2,12^{fg}	22,38±2,23^{d}	35,35±1,39^{c}	52,48±1,75^{a}
0.4 mL	16,60±2,50^{e}	23,54±2,10^{d}	41,83±1,75^{b}	57,80±2,89^{a}

*Different subset notations on each row indicate significant differences ($p < 0.05$)

Table 3 and Table 4 show that the highest total phenol content is directly proportional to the increase in surfactant volume addition and extraction time. The total phenol content increased with increasing surfactant volume and extraction time in the sample. This indicates that the ability of Tween 80 surfactant as an emulsifying agent can stably reduce the surface tension of oil in water so that it can optimally increase the solubility process of hydrophilic phenolic compounds in sonicated Moringa leaf herbal oil extract samples in VCO and EVOO. The presence of the hydrophilic -OH group in the surfactant causes interaction between the H atom and the O atom of the secondary metabolite compound to form a hydrogen bond, which can attract secondary metabolite compounds that have the -OH group. Meanwhile, van der Waals forces are formed due to the interaction between hydrophobic groups and oil. Increasing the surfactant concentration can reduce the surface tension between the organic phase and the water phase, where at a smaller particle size, the surface area of the sample to be extracted becomes more significant and requires more surfactant to fill that surface area (Isabella *et al.*, 2022). The following is the alleged interaction between surfactants and phenolic compounds in Figure 1.



(a)



(b)

Figure 1. Interactions of (a) tween 80 with lauric acid (VCO) and flavonoids, (b) tween 80 with oleic acid (EVOO) and flavonoids

The total phenol content in EVOO of Moringa leaf herbal oil is higher than that of VCO. This can happen because the Tween 80 and EVOO carbon chains in the unsaturated fatty acid (oleic acid) have a double C bond at number 9, where overlapping π-π interactions occur. The robust van der Waals forces between the two compounds can increase the total phenol content. This differs from VCO, which does not have a double C bond in its saturated fatty acid (lauric acid), where the interaction between Tween 80 and VCO is weaker, resulting in lower total phenol levels.

In Table 3 and Table 4, the highest total phenol content was obtained from variations in the addition of tween 80 volumes of 0.4 mL and extraction time of 30 minutes in the sonicated extract of Moringa leaves in VCO reaching 39.28 ± 1.75 mg GAE/ g and EVOO reached 57.80±2.89 mg GAE/g. Compared with Mahmudah *et al.* (2023), who tested the total phenol content in Moringa leaf extract using the maceration method and found 25.90 mg GAE/g at the highest level of VCO and 30.35 mg GAE/g at the highest level of EVOO (Mahmudah, Yulianti, *et al.*, 2023)This shows that variations in the addition of surfactant volume and the extraction time significantly influenced the total phenol content in the sonication results of Moringa leaf extract in VCO and EVOO.

Identification of Functional Groups using an FTIR Spectrophotometer

The highest total phenol content value was then identified using an FTIR (Fourier Transform Infrared) Spectrophotometer to identify the functional groups of phenolic compounds found in herbal oil samples. Therefore, functional groups of phenolic compounds in Moringa leaf powder, VCO, were also identified. EVOO, and tween 80 as a spectral comparison. The following are the results of functional group identification obtained in Figure 2.

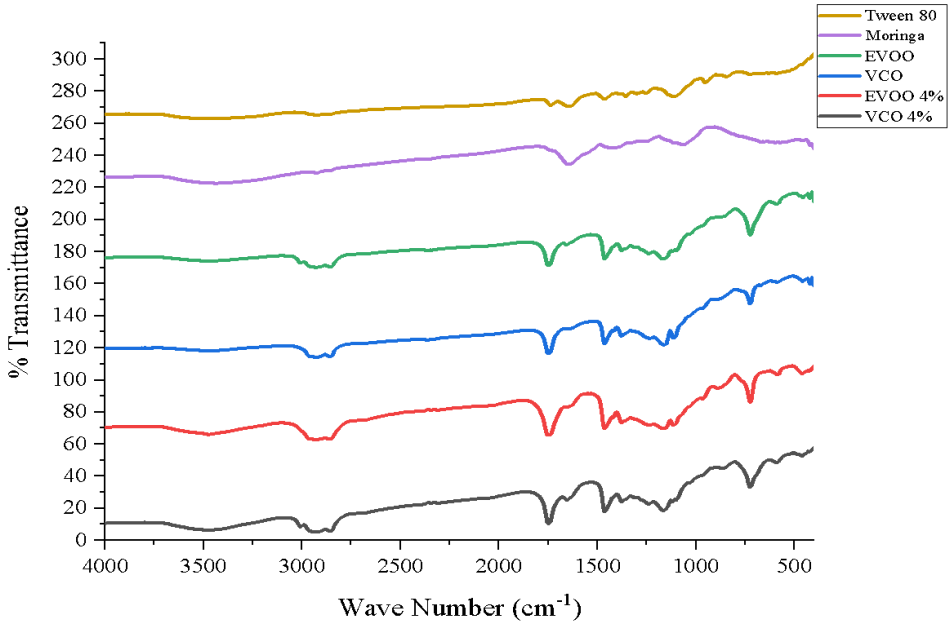


Figure 2. FTIR Spectra tween 80, Moringa leaves, VCO, EVOO, extract *herbal oil* VCO, extract *herbal oil* EVOO

The following FTIR interpretation results are shown in Table 5.

Table 5. Functional group interpretation of Moringa leaf herbal oil extract in VCO and EVOO with adding 0.4 mL of tween 80, VCO, EVOO, Moringa leaves, and tween 80.

Wave Number (cm^{-1})							
***Herbal Oils* (VCO)**	***Herbal Oils (*EVOO)**	**VCO**	**EVOO**	**Moringa Leaves**	**Tween 80**	**Literature (Socrates, 1994)**	**Functional groups**
3472	3476	3470	3470	3435	3448	3550-3250	O-H *stretching*
-	3005	-	3004	-	-	3100-3000	C-H sp^2
2924	2922	2925	2926	2924	2924	3000-2800	$-CH_3$ *stretching*
1747	1745	1746	1746	1741	1735	1740-1720	C=O aldehid
1650	1654	-	1654	1645	1645	1660-1580	C=C *stretching*
1231	1239	1231	1238	1264	1251	1260-1180	C-O-C *stretching*
722	723	723	723	667	723	900-650	CH Bending aromatic

The FTIR interpretation of the best VCO herbal oil sample shows an absorption band at wave number 3472 $cm^{-1,}$ indicating OH (hydroxyl) stretching vibrations. Then, at wave number 2924 cm-1, there is the $-CH_3$ stretching vibration. At wave numbers 1747

cm^{-1} and 1650 cm^{-1}, respectively, are C=O aldehyde and C=C vibrations. Wave number 1231 cm^{-1} indicates C-O-C stretching vibrations, while wave number 722 cm^{-1} indicates aromatic C-H vibrations. The FTIR interpretation of the best EVOO herbal oil sample shows an absorption band at wavenumber 3476 $cm^{-1,}$ indicating OH (hydroxyl) stretching vibrations. Then, wave numbers 3005 cm^{-1} and 2922 cm^{-1} are CH sp^2 and -CH_3 stretching vibrations. At wave numbers 1745 cm^{-1} and 1654 cm^{-1}, respectively, are C=O aldehyde and C=C vibrations. The wave number 1239 cm^{-1} indicates COC stretching vibrations, while the wave number 723 cm^{-1} indicates aromatic CH vibrations.

The results of the FTIR interpretation of VCO samples, the best VCO herbal oil, Moringa leaves, and tween 80 show a difference with the results of the interpretation of EVOO samples and the best EVOO herbal oil, namely that there is no absorption pattern at wave numbers 3004 cm^{-1} and 3005 cm^{-1}. which shows the functional group of C-H sp^2. This is because, in the best EVOO and EVOO herbal oil samples, there is a CH sp^2 group in the oleic acid carbon chain from the unsaturated fatty acid compound of olive oil.

Conclusion

Variations in surfactant addition and sonication extraction time on the total phenolic content of Moringa leaves in VCO and EVOO had a significant influence, with the highest total phenol content, respectively being 39.28 ± 1 .75 mg GAE/g and 57.80±2.89 mg GAE/g. Furthermore, identification using FTIR on the best total phenol content value from Moringa leaves VCO extract produces typical functional groups of phenolic compounds, namely O-H, -CH_3, C=O aldehyde, C=C, C-O-C, and aromatic C-H at a wave number of 3472 cm^{-1}, 2924 cm^{-1}, 1747 cm^{-1}, 1650 cm^{-1}, 1231 cm^{-1}, and 722 cm^{-1}. Meanwhile, the best total phenol content in Moringa leaves EVOO extract was at wave numbers 3476 cm^{-1}, 2922 cm^{-1}, 1745 cm^{-1}, 1654 cm^{-1}, 1239 cm^{-1}, and 723 cm^{-1}, as well as the CH sp^2 group at wave numbers 3005 cm^{-1}.

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