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Abstract. Edible oil, as an environmentally friendly solvent, supports the extraction of secondary metabolites based on green technology. Secondary metabolite compounds function as natural antioxidant agents found in moringa leaves (Moringa oleifera L.), also edible oils such as extra virgin olive oil (EVOO) and virgin coconut oil (VCO). Furthermore, Moringa leaves extract in edible oil has been identified total phenolic compounds as relatively high. This research focuses on extracting phenolic compounds specifically from a formulation of moringa leaves powder, either in VCO or EVOO, by sonication methodology using variability in the weight of moringa leaves (0 to 4 grams) and the length of the extraction process (0 to 30 minutes). The best formula herbal oil uses 4 grams of moringa leaves and an extraction time of 20 minutes, with a total phenol content of 41.13 ± 1.06 mg GAE/g in moringa extract EVOO and 33.26 ± 1.84 mg GAE/g in moringa extract VCO. Further identification of functional groups using Fourier Transform Infrared Spectroscopy (FTIR) has demonstrated that our formulation products have phenolic compound spectra, namely O-H, C-H stretching and bending, C=O, C=C aromatic, and groups from C-O.

Keyword : edible oils, moringa leaves, phenolics, sonication

1. Introduction

Indonesia has a lot of natural resources that serve as potential sources of bioactive compounds, including Moringa oleifera L., which is indigenous and commercially available as a traditional medicine and functional food for treating wounds, pain, ulcers, liver and heart disorders, and inflammation [1]. The plant is well-known as ''kelor'' by the Indonesians; the plant characteristics are easy to grow in drought-prone areas, for instance, Central and East Java, and West and East Nusa Tenggara [2]. This current study aims to understand how to develop the best formulation for regular dose intake of antioxidant substances from moringa leaves. A recent study shows that Moringa leaves have the highest flavonoid content among various leaves commonly used as a source of nutrition, as well as having the potential as a renewable biomass resource that supports sustainable use. It was for about 327.2 mg GAE within 100 g dried weight, five times higher than the lowest flavonoid quantity in other sources [3]. Another recent study found that total phenolic content (TPC) in moringa leaves was the greatest, 170 mg Gallic acid equivalent (GAE)/100 g, out of flower (114 mg GAE/100 g) and seed (22 mg GAE/100 g)[4]. Furthermore, the compounds of flavonoids that have been identified to have antioxidant activity in several studies were rutin,

kaempferol, quercetin, o-coumaric, myricetin, and isorhamnetin. Moreover, the phenolic compounds, such as ellagic acid, ferulic acid, caffeic acid, sinapic acid, gallic acid, and syringic acid, have been tested as antioxidant, antimicrobial, and anti-inflammatory agents.

The use of edible oil as a solvent in the extraction process of secondary metabolite compounds from natural materials is one form of green technology application, because it not only utilizes solvents that are safe and can be consumed, but also supports the principle of sustainability through the use of environmentally friendly materials, which are easy to obtain, and have the potential to reduce dependence on organic solvents that are toxic and harmful to the environment [5]. Moringa leaf extract in vegetable oil is a herbal oil rich in phenolic compounds, antioxidants that have antibacterial, anti-inflammatory, anti-acne, and antioxidant properties [6][7]. Herbal oil is a traditional medication derived from nourishing plants and extracted using vegetable oil solvents [8]. The VCO comprises MCT (Medium Chain Triglycerides), with lauric acid being the major fatty acid at 48.24%, and phenolic substances, α-tocopherols as natural antioxidants [9]. EVOO includes oleuropein, the primary polyphenol in olives [10]. Moringa leaf extracts VCO and EVOO have higher total phenol levels (7.473% and 44.83%, respectively) at a 40% concentration, compared to 5.982% and 9.44%, respectively, without Moringa leaves [11]. Those scientific facts of moringa are a strong fundamental using it as the primary raw material to produce antioxidant agents.

The crucial characteristics have been carried out utilising ultrasonic-assisted extraction (UAE) to collect the phenols as the main bioactive compounds. The ultrasonic extraction method is an efficient and environmentally friendly extraction technique because it is not only able to speed up the process time and increase the concentration of secondary metabolite compounds obtained from natural materials, but is also more energy efficient, thus supporting the principle of green extraction [12]. This extraction has been completed using multiple sets of temperatures and extraction times. Following this, its chemical properties of phenols have been quantified using the UV-Visible spectrophotometry, while their functional groups of chemical structures have been confirmed using Fourier-transform infrared spectroscopy (FTIR). After all these sets of analytical study for moringa leaves formulation in edible oils to identify the pre-manufacturing formula of antioxidant products. Expectantly, it can be used to supply the local market with more sustainable, eco-friendly, and low-cost biopharmaceutical products. Additionally, the best formulation of moringa leaves in edible oils might enhance the health status of Indonesian effectively through antioxidant agents.

2. Materials and Methods

Research materials including the chemical reagents such as moringa leaves dried powder, extract virgin olive oil (EVOO) (Filippo Berio), virgin coconut oil (VCO) (Benara), aquadest, Gallic acid (Merck), ethanol PA (Smart Lab), Folin Ciocalteau reagent (Merck), Sodium Carbonate (Merck), and Potassium bromide. Next, all the instruments, such as the ultrasonic water bath, spectrophotometer UV-Visible, and Fourier Transform Infrared Spectroscopy (FTIR), were set up for experimentation in the chemical laboratory

2.1 Ultrasonic-assisted extraction (UAE)

The first step in the experiment was extraction using the sonication methodology. To begin the extraction, this study determined the best composition of the dried-powder moringa tree as many as 0, 2, 3, and 4 g in 10 mL of edible oil, either virgin coconut oil (VCO) or extract virgin olive oil (EVOO). All the samples were extracted using an ultrasonic water bath at 42 kHz for multiple sets

of times, which were 0, 10, 20, and 30 minutes, respectively. The samples were in slurry dosage form and had high viscosity, then filtrated using cheesecloth to get filtrate and stored in a dark glass bottle. After collecting the filtrate on the bottle, the proportion of the extract yields, either moringa leaves in VCO or EVOO, has been calculated using the formula Yield (%) = (weight of extract/weight of sample) x 100%.

2.2 Total phenolic contents (TPC)

The second step involved determining total phenolic content using Gallic acid as a standard. A 0.5 mg Gallic acid was dissolved in a 5 mL volumetric flask with aquadest to make a 100 ppm stock solution. From this, standard solutions of 3, 6, 12, 18, 24, and 30 ppm were prepared by diluting 0.15–1.5 mL of the stock solution to 5 mL with aquadest.

To determine the maximum wavelength, a mixture of 0.5 mL of 24 ppm Gallic acid, 2.5 mL Folin–Ciocalteau reagent, and 2 mL Na_2CO_3 was vortexed for 3 minutes and incubated for 45 minutes. UV-Vis spectrophotometry at 400–800 nm showed 759.9 nm as the optimal wavelength. For standard curve analysis, each diluted Gallic acid solution (0.5 mL) was reacted with Folin–Ciocalteau and Na_2CO_3 as above, and absorbance was measured at 759.9 nm. A linear regression ($y = ax + b$) was obtained from these values.

For sample analysis, 5 mg of moringa leaf extract in VCO or EVOO was dissolved in ethanol PA to yield a 1000 ppm solution. Then, 0.5 mL of each sample was treated with the same reagents and conditions as the standard, and absorbance was measured at 759.9 nm. Sample concentrations were calculated using the standard curve.

Separately, 40 g of turmeric powder was placed in a 100 mL flask, topped with oil to the mark, and extracted at 50–70°C for 1–6 hours. The mixture was filtered through cheesecloth and stored in a dark glass bottle.

2.3 Fourier-transform infrared spectroscopy (FTIR)

The third step involved identifying functional groups of the samples using Fourier Transform Infrared (FTIR) spectroscopy. Samples were categorized into liquid (VCO or EVOO and herbal oil) and solid (dried moringa powder) forms.

For FTIR analysis, each sample was prepared using potassium bromide (KBr) pellet technique. Liquid samples were applied to the surface of KBr pellets formed under 80 torr pressure, while solid samples were mixed with KBr and pressed into pellets. Spectra were recorded in the range of 4000–400 cm^{-1}.

2.4 Statistical analysis

The fourth step, two-way ANOVA statistical analysis, carried out the quantitative data experimental results. This analysis was conducted to identify the best formulation with the most significant chemical characteristics of total phenolic compounds.

3. Results and discussion

3.1 Phenolics extraction formulas

Extraction of dried-powder moringa leaves in edible oils, either in virgin coconut oil (VCO) or extra virgin olive oil (EVOO), was conducted in the preliminary step of this study to determine the best composition of the mixture . In addition, this step was done by using multiple sets of various sonication times (from 0, 10, 20, and 30 minutes).

Table 1. Extraction yield of dried-moringa leaves in VCO.

Weight of moringa leaves in 10 mL of oils (g)	Yield (%)			
	0 min	10 min	20 min	30 min
0	89.1776	86.1435	88.6064	87.6579
2	44.2758	42.2772	44.0001	43.2382
3	40.5640	33.0632	37.4353	35.7777
4	23.5494	21.3721	23.4876	22.1909

Table 2. Extraction yield of dried-moringa leaves in EVOO.

Weight of moringa leaves in 10 mL of oils (g)	Yield (%)			
	0 min	10 min	20 min	30 min
0	97.7478	92.5392	96.6007	96.4468
2	50.6275	47.5468	50.3748	48.2131
3	41.6732	38.8526	39.7160	39.6453
4	29.9016	23.8335	29.6653	26.5699

The extraction process combined ultrasonic-assisted extraction (UAE) with 24-hour non-thermal maceration to maximize bioactive compound recovery without damaging main components, followed by cheesecloth filtration to separate the filtrate. The currently used methodology for bioactive compound extraction is ultrasonic-assisted extraction (UAE), which involves indirect ultrasonic radiation utilized on the mixture of sample and solvent through an ultrasonic bath. In this study, the highest yield was obtained from 10 mL of EVOO without moringa leaves, as no particle interaction occurred, resulting in a significant extract yield. The yield after sonication extraction showed that the highest yield was achieved at 20 minutes, indicating that 20 minutes is the optimal time for extracting the bioactive compounds contained in moringa. However, the yield before extraction was high. This is suspected because the moringa was not fully absorbed by the oil (solvent), or because there was not yet a maximum interaction between the moringa powder and the oil, resulting in a higher yield.

3.2 Total Phenolic contents (TPC) formulas

The extraction process using sonication indicated that the formulation of moringa leaves in virgin coconut oil (VCO) or extra virgin olive oil (EVOO) has phenolic compounds as the primary secondary metabolite, which has a good impact on the health system of the human body [13].

Table 3. Total phenolic contents (TPC) of dried-moringa leaves in VCO.

Weight of moringa leaves in 10 mL of oils (g)	TPC (%) mg GAE/g			
	0 min	10 min	20 min	30 min
0	0.99 ± 0.99^{j}	1.78 ± 1.06^{hij}	3.86 ± 0.80^{ghij}	0.86 ± 1^{ij}
2	6.18 ± 0.69^{fgh}	6.64 ± 0.80^{fgh}	11.50 ± 1.06^{cde}	5.49 ± 1.84^{fghi}
3	7.10 ± 1.75^{efgh}	7.34 ± 1.06^{defg}	29.79 ± 4.17^{ab}	9.42 ± 1.75^{cdef}
4	12.43 ± 1.84^{cd}	26.09 ± 1.60^{b}	33.26 ± 1.84^{a}	13.12 ± 2.50^{c}

Information of a,b,c,d,e,f,g,h,i,j refers to the result of statistical analysis using two-way ANOVA that the samples have significantly different with $p < 0.05$

Table 4. Total phenolic contents (TPC) of dried-moringa leaves in EVOO.

Weight of moringa leaves in 10 mL of oils (g)	TPC (%) mg GAE/g			
	0 min	10 min	20 min	30 min
0	2.94 ± 0.40^{i}	3.63 ± 1.06^{hi}	7.80 ± 2.12^{fgh}	3.17 ± 1.06^{i}
2	3.17 ± 1.06^{hi}	6.64 ± 1.45^{ghi}	14.05 ± 1.45^{d}	8.26 ± 1.20^{efg}
3	6.41 ± 1.45^{ghi}	12.66 ± 2.12^{de}	21.23 ± 2.81^{b}	14.51 ± 1.20^{cd}
4	12.43 ± 2.50^{def}	23.77 ± 1.06^{b}	41.13 ± 1.06^{a}	19.14 ± 1.75^{bc}

Information of a,b,c,d,e,f,g,h,i,j refers to the result of statistical analysis using two-way ANOVA that the samples have significantly different with $p < 0.05$

Ultrasonic-assisted extraction (UAE) followed by filtration proved to be a quick, simple, and efficient method for isolating bioactive compounds. This study revealed the highest TPC at 20 minutes, with values exceeding 30 mg GAE/g in VCO and 40 mg GAE/g in EVOO, where the TPC in EVOO was about twice as high as in VCO (~7 mg GAE/g), consistent with previous findings[11] [12]. Nevertheless, at the maximum point of 20 minutes, the TPC dropped by way of the prolonged extraction time. The longer extraction time may also produce some heat, which increases the extraction temperature, causing the degradation of bioactive compounds, named thermodegradation [14]. The reduction of TPC in the current study may result from structural destruction and polyphenol decomposition. Several factors may be considered, such as exposure time to light and air, the presence of oxidative enzymes, and free radicals released during the extraction. In addition, thermodegradation might be anticipated by using a temperature of no more than 40 ^{o}C and managing the power used in sonication. Additionally, literature provides information that the power associated with the frequency values should be around 40 Watts and 20 kilo Hertz [15]. This experimental study also found that the more extraction time, the higher

the TPC values. It corresponds to the interaction time of particles inside the formula being more extensive, up to the saturation occurring and reaching the optimal extraction time.

3.3 Fourier transform infrared spectroscopy (FTIR) formulas

To confirm the chemical structure of phenolic compounds in the extract yields, the Fourier transform infrared spectroscopy (FTIR) carried out the result as illustrated in Figure 1. The phenolic compound was identified in O-H, C-H sp2, C-H (aliphatic), C=C, and C-O-C stretching. EVOO has O-H (alcoholic), C=C (aromatic), C-O (ester), C=O (aldehyde) stretching, and C-H bending. In addition, it has the spectra of C-H stretching stand for aliphatic chemical structure. While the VCO has similar spectra as EVOO but has no C=C spectra. As for the formulation of moringa leaves in edible oils, there is no significant difference, except only the formula with EVOO, which has C-H sp2 (Figure 1). Ultrasonic-assisted extraction (UAE) has successfully extracted the bioactive compound, flavonoids, which act as antioxidants, from all natural resources using the optimal composition formula of 4 g moringa leaves in 10 mL of edible oil. Regarding the spectra in Figure 1, the results of the analysis showed the presence of absorption with several functional groups typical of phenol compounds, namely O–H, stretching and bending C–H, C=O, C=C, and C–O groups.

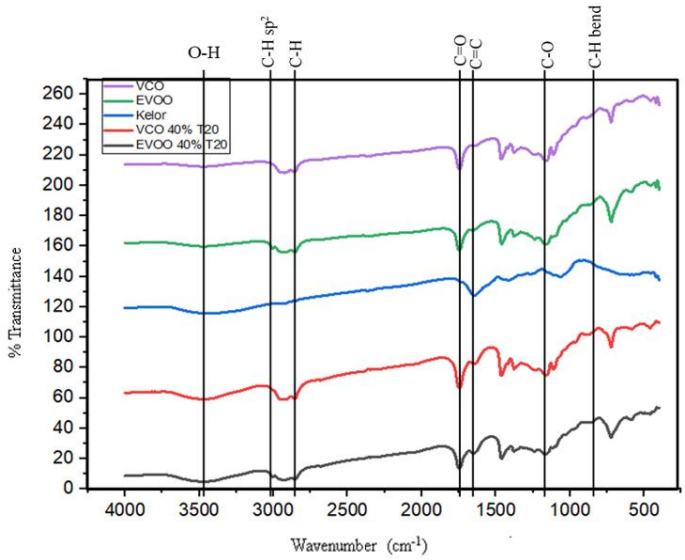

Figure 1. Spectra representation of edible oils, virgin coconut oils (VCO), extra virgin olive oils (EVOO), kelor (moringa leaves), 40% moringa leaves in VCO with 20-min extraction times, and 40%

Statistical data analysis for the concentration of total phenolic compounds using the two-way ANOVA method showed that the various TPC data are significantly different between each variable. It means that the variables used in the study, such as extraction time and composition of moringa leaves in edible oils, had a remarkable influence on the total phenolic concentration of the formulas. The ultrasonic extraction method proved more effective than the hot maceration method used in previous studies [11]. While the maximum total phenol content in hot maceration was only around 15 mg GAE/g, the ultrasonic method achieved a total phenol content of up to 41

mg GAE/g. This finding highlights that applying UAE with edible oils as green solvents provides a sustainable alternative to conventional extraction methods."

4. Conclusion

The variation in extraction time and moringa leaf powder composition significantly affected the total phenolic content of Moringa oleifera leaf extracts in extra virgin olive oil (EVOO) and virgin coconut oil (VCO). The best results were obtained with an extraction time of 20 minutes using 4 grams of moringa powder, yielding total phenolic contents of 41.13 ± 1.06 mg GAE/g in EVOO and 33.26 ± 1.84 mg GAE/g in VCO, respectively. FTIR spectra of the best-performing herbal oil samples, along with pure moringa powder, EVOO, and VCO, showed characteristic functional groups of phenolic compounds, including O–H, C–H stretching and bending, C=O, aromatic C=C, and C–O groups.

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