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Analysis of Total Phenolic Levels in Moringa Leaf Extract (*Moringa Oliefera* Lamk.) in Vegetable Oil

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Abstract: *Moringa leaf (Moringa Oliefera Lamk.) vegetable oil extract is an herbal oil that is potential as a medicine. The study aimed to determine the content of secondary metabolites through photochemical tests and the total phenolic content of Moringa leaf extract in extra virgin olive oil and virgin coconut oil. The extraction method applied is hot maceration with various doses of Moringa leaves. The best amount is used by extraction with variations in temperature and heating time. The functional groups of herbal oils were identified using an FTIR spectrophotometer. Phytochemical test results reveal that the vegetable oil extract of Moringa leaf contains secondary metabolites in the form of flavonoids, phenolics, terpenoids, alkaloids, steroids, and tannins. The highest total phenolic content in Moringa leaf extract in virgin olive oil at a concentration of 40%, extraction temperature of 50°C with a heating time of 2 hours with a total phenol content value of 15.78% GAE (Gallic Acid Equivalent). The results of the FTIR interpretation reveals that herbal oils have O-H, C-H, C=O, C=C aromatic, C-O, and aliphatic C-H functional groups.*

Keywords*: herbal oil, moringa leaf, extra virgin olive oil, extra coconut oil, hot maceration*

INTRODUCTION

The use of herbs from many natural ingredients as drugs has proven efficacious and has a lower risk of irritation and allergies (Aburjai & Natsheh, 2003; Almeida et al., 2008). Moringa leaves are part of the moringa plant which is often used for traditional medicine (Farooq, 2012; Taher, 2017). Flavonoids, phenolic compounds, carotenoids, alkaloids, steroids, tannins, saponins, and terpenoids are the metabolite compounds in moringa leaves that cause moringa leaves to have potential as antioxidants, anti-inflammatories, and antibacterials (Tekle et al, 2015; Moyo et.al, 2013; Nweze, et al., 2014; Manguro & Lemmen, 2011). The sources of polyphenols in moringa leaf are flavonoids including myricetin, quercetin, kaempferol, isorhamnetin, or rutin, as well as phenolic acids which have been shown to have a good effect on human skin and have succeeded in replacing synthetic materials to protect the skin from free radicals (Moyo, 2012; Singh et al., 2017). Creams containing moringa oleifera leaf extract show efficacy in preventing UV radiation and increasing skin moisture (Ali, 2013). Moringa leaf extract in water provides a significant protective value, with an SPF of 2, and shows no potential for irritation (Baldisserotto, 2018). Phytochemical compounds in moringa leaf extract can increase the number of macrophages which can speed up the wound healing process due

to increased Epidermal Growth Factor (EGF) and Transforming Growth Factor β (TGF-β) (Miftah, 2020). Rubbing crushed moringa leaves on the temples can relieve headaches, stop bleeding from shallow wounds, has an antibacterial and anti-inflammatory effect when applied to wounds or insect bites, and can cure skin diseases caused by fungi and bacteria (Sandeep, 2019).

Olive oil (*olea europaea*) is rich in antioxidants, contains mono unsaturated fatty acid, and contains 30 phenolic compounds (Muzzalupo et al., 2012). These phenolic compounds are useful for dealing with dry skin and treating skin diseases because they show antimicrobial activity against viruses, bacteria, yeast, and fungi (Dağdelen, 2016). The oleic acid in olive oil plays a role in moisturizing the skin and reducing scars (Badiu & Rajendram, 2021; Danby et al., 2013). The content of phenolic compounds in virgin coconut oil is 59.88 µg/mL (Pranata et al., 2021) where 90% are saturated fatty acids, namely lauric acid (53%) and caprylic acid (7%), both of which are medium chains fatty acid which easily enters the skin layer to maintain skin elasticity. High lauric acid in virgin coconut oil can act as an antioxidant, antiviral, antibacterial, and antiprotozoal (Dc & Sio, 2021) and reduces oxidative stress due to UV exposure (Aulia et al., 2014).

Herbal oil is called herbal oil extract with a combination of antioxidants and nutrients, which is obtained from plants and is a good base for ointments or creams (Mikaili et al., 2012). Herbs infused with vegetable oils are a great way to harness the oils and active compounds from the plants themselves. The use of vegetable oil solvents is included in the class of bio-based solvents (renewable resources), which have a positive impact on health and the environment (non-toxic, biodegradable, and no emission of volatile organic compounds) (Li et al., 2017). The advantages of extraction using oil solvents include not being volatile at high temperatures, safe, economical, and environmentally friendly (Yara-Varón et al., 2017). The antioxidant activity of thyme-enriched corn oil was higher than without due to the presence of phenolic compounds such as thymol and hydrocarbons such as terpinene and p-cymene (Karoui et al., 2016). Moringa contains nonpolar active compounds so it will dissolve in vegetable oils with the same polarity. The type of extraction used is the hot maceration method, namely immersing Moringa powder in vegetable oil as a solvent using the principle of osmosis. The advantage of the maceration method is that it requires equipment that is simple, cheap, and easy to do (Istiqomah, 2013). Vegetable oils can become more competitive in terms of economics, food safety, and environmental friendliness and theoretically and experimentally have proven their potential as alternative solvents (Li et al., 2013).

RESEARCH METHODS

Materials and tools

Moringa leaf powder obtained from CV. Berkah Banyuwangi Moringa, Borges extra virgin olive oil, and Benara brand virgin coconut oil, concentrated ammonia (Merck), sulfuric acid (Merck), concentrated HCl (Merck), acetic anhydrous acid, $FeCl_3$, Folin-Ciocalteu reagent, Mg metal, Mayer's and Dragendorf's reagents, gallic acid, distilled water.

A measuring cup, test tube rack, magnetic stirrer, test tube, Erlenmeyer flask, stir bar/spatula, suction ball, a hot plate measuring flask oven bottle, dark glass bottle, *cheesecloth*, analytical balance (Ohaus), thermometer, stopwatch, bath, hot plate, UV Vis spectrophotometer (Perkin Elmer Lambda 25), Fourier Transform Infra-Red (Shimadzu IR Prestige21).

Procedures

Extraction of Moringa in Vegetable Oil with Variation of Doses

Moringa leaf powder was added to vegetable oil (virgin olive oil; virgin coconut oil) with varying doses of moringa, namely 0;10;20;30;40% (gr/mL), and then heated at 50°C for 2 hours. The extract obtained was then filtered. Then the filtrate was put into a dark glass bottle and stored in a dark room.

Extraction of Moringa in Vegetable Oil with Variation of Temperature and Heating Time

30 grams of Moringa leaf powder were mixed into 100 cc of vegetable oil (virgin olive oil; virgin coconut oil) and heated for 1;2;4;6 hours at a temperature of 50;60;70°C. The filtrate was then stored in a dark glass bottle after the extract solution was filtered.

Qualitative Phytochemical Screening

Identification of Alkaloid content

1 mL of herbal oil in a test tube and 5 drops of concentrated ammonia, filtered, added 2 mL of 2 N sulfuric acids, and shaken until two layers are formed and separated. The first tube added 1 drop of Dragendorf's reagent and the second tube added 1 drop of Mayer's reagent.

Identification of Phenolic content

3 drops of herbal oil were dropped on the porcelain pellet and added to methanol, then stirred until homogeneous, and added a few drops of $FeCl_3$.

Identification of Terpenoids and Steroids

1 mL of herbal oil plus 0.5 mL of anhydrous acetic acid and 1-2 mL of concentrated sulfuric acid.

Identification of Saponin content

As much as 1 mL of herbal oil in a test tube is added to 2 mL of distilled water, then shaken until homogeneous and heated for 2-3 minutes then cooled and shaken vigorously.

Identification of Flavonoid content

Herbal oil as much as 1 mL in a test tube added 5 drops of ethanol and shake until homogeneous. Then added Mg metal and 5 drops of concentrated hydrogen chloride.

Identification of Tannin content

Put 1 mL of herbal oil into a test tube and add 2-3 drops of 1% $FeCl_3$ solution.

Determination of Total Phenol Levels

Herbal oil into a 25 mL volumetric flask and then add methanol solvent up to the mark. As much as 0.5 mL of the extract and the standard solution were added to 5 mL of Folin-Ciocalteu reagent and allowed to stand for 3 minutes. After that, 4 mL of 10% Na_2CO_3 was added and incubated for a while in the dark and at room temperature. The concentration of the oil solution was entered into the regression equation of the standard gallic acid solution and the total phenol content was obtained as expressed in milligrams of gallic acid equivalent per gram of oil (mg GAE/g oil). Calculations using the formula

$$y = ax + b. \qquad \text{(Eq 1)}$$

Identification of Functional Groups in Samples Using Fourier Transform Infra-Red (FTIR)

Herbal oil sample was dripped a little on one part of the sodium chloride plate, then the sodium chloride plate was added so that the liquid was evenly distributed on the surface and pressed for 10 minutes with a pressure of 80 torrs to produce pellet plates. The pellets were identified at wave numbers 400-4000 cm^{-1} using FTIR.

RESULTS AND DISCUSSION

The results of the extraction of moringa leaf samples in vegetable oil are dark green in color because of the diffusion process of moringa leaves with oil solvents so that the cell membrane is split and the secondary metabolites in the cytoplasm are pressured to come out and dissolve in the oil solvent. The results of extracting moringa leaves in coconut oil and olive oil can be seen in Figure 1.

Figure 1 Extraction results of Moringa leaf powder in pure coconut oil (left) and virgin olive oil (right) with various concentrations

Moringa leaves extracts of virgin coconut oil (VCO) and extra virgin olive oil (EVOO) obtained were subjected to qualitative analysis to determine the content of secondary metabolites/active compounds contained in the herbal oil.

Table 1 Phytochemical Test Results of Moringa Leaf Extract in Vegetable Oil

	The Concentration of Moringa Leaves									
	Virgin Coconut Oil					***Extra Virgin Olive Oil***				
Phytochemical test	**0%**	**10%**	**20%**	**30%**	**40%**	**0%**	**10%**	**20%**	**30%**	**40%**
Phenolic	+	+	+	+	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+	+	+	+	+
Steroids	+	+	+	+	+	+	+	+	+	+
Alkaloids (Mayer)	+	+	+	+	+	+	+	+	+	+
Alkaloids (Dragendoff)	-	+	+	+	+	-	+	+	+	+
Saponins	-	-	-	-	-	-	-	-	-	-
Tannins	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	+

Note: (-) : no color or foam is formed
(+) : light color or slightly foamy

Based on the results of Table 1, shows that Moringa leaf extract with virgin coconut oil and extra virgin olive oil is positive for phenolic compounds, alkaloids, tannins, flavonoids, terpenoids, and steroids, and negative for saponins. Based on the concept like dissolves like, non-polar compounds dissolve in non-polar solvents (Yara-Varón et al., 2017). Alkaloids are also called secondary metabolites which are semi-polar in nature so that samples are easily extracted by polar and non-polar solvents. Identification of alkaloids was negative with dragendroff reagent in extra virgin olive oil and virgin coconut oil but positive for all doses of moringa leaf oil extract. Steroids have a basic structure of four carbon rings called the steroid core. Meanwhile, triterpenoids have a cyclic structure, such as aldehydes, alcohols, or carboxylic acids which are non-polar, so they are easily extracted in non-polar solvents. Several flavonoid compounds tend to dissolve easily in non-polar compounds such as flavones, isoflavones, and flavanones. Negative results for the saponin test because saponin compounds tend to have polar properties such as sapogenins and glycosides so they are easily extracted in polar solvents as well.

Total Phenol Content of Moringa Leaf Extract in Olive Oil (EVOO) and Coconut Oil (VCO)

Moringa leaf extract in virgin coconut oil and extra virgin olive oil produced higher levels of total phenols than vegetable oil. The higher the dose of moringa in the sample from a dose of 20% -40%, the higher the total phenol level. This shows that the total phenol content in Moringa leaves is extracted in vegetable oil. The total phenol content in extra virgin olive oil was higher than virgin coconut oil which indicated that extra virgin olive oil had a better ability to extract phenolic compounds than virgin

coconut oil. Extra virgin olive oil contains phenols namely oleuropein glycoside, ligstroside and ortho-diphenol. The ester group of elenolic acid with 3,4' - dihydroxyphenylethanol (hydroxytyrosol) is oleuropein while the ester group of elenolic acid with 4-hydroxyphenylethanol (tyrosol) is ligstroside. Orthodiphenol is a group of phenols that have two adjoining hydroxyl groups that form a ring-like structure of hydroxytyrosol and oleuropein. The presence of phenol provides stability from oxidation in vegetable oils (Vissers et al., 2004). The best total phenol content results in the concentration variation treatment, namely 40%.

Table 2 Total Phenol Content of Moringa Leaf Extract in Concentration Variations of Virgin Coconut Oil and extra virgin olive oil

The Concentration of Moringa Leaf	Total Phenol Content (GAE)	
	VCO	EVOO
0%	5,982	9,443
10%	6,345	10,551
20%	6,902	12,919
30%	7,241	13,386
40%	7,473	15,777

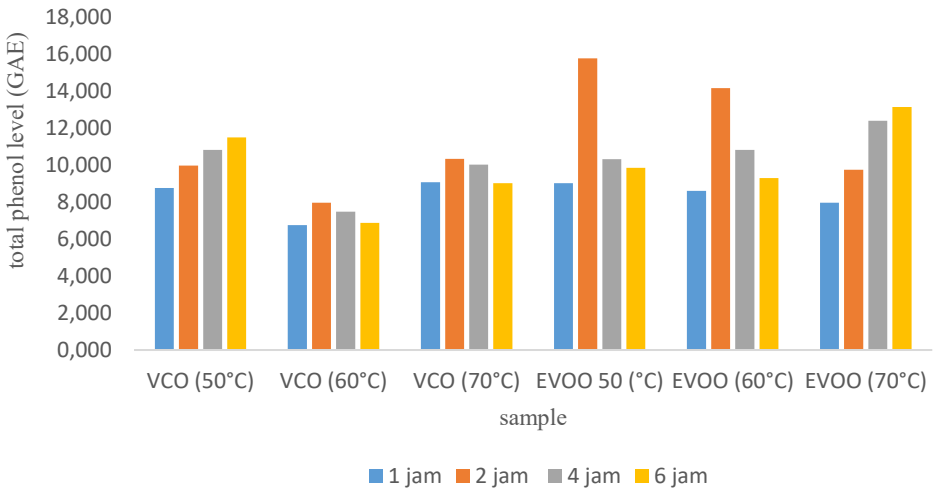

Figure 2 Total Phenol Levels in Moringa Leaf Extract in Pure Coconut Oil and Pure Olive Oil with Variation of Extraction Time and Temperature

The presence of total phenol levels in VCO and EVOO is influenced by temperature and heating time. Based on the results of Figure 2, it shows that the longer the extraction time of Moringa leaves in oil and the higher the temperature in the

extraction process, the total phenol content will increase and the heating time and certain temperature will decrease. In VCO with increasing heating time at 50 °C, the total phenol value increased while at 60 °C and 70 °C heating it increased for 2 hours heating time and then decreased until 6 hours heating time. In EVOO, with increasing heating time at 70 °C, the total phenolic value increased, while at 50 °C and 60 °C, it increased for 2 hours of heating, then decreased until 6 hours of heating. Based on research by Juliantari et al., (2018) the higher the temperature used to extract, the higher the phenol content obtained, at high temperatures, it will be easier the release phenol compounds that are in the sample cell walls but high temperatures can cause damage to polyphenol compounds on the herbs to be extracted. The degradation of phenolic compounds was caused by the extraction temperature which was too high causing a decrease in phenol content. In the VCO moringa extract, the best phenol content was obtained at an extraction temperature of 50 °C and a heating time of 6 hours, namely 11.51 GAE. Whereas in the EVOO moringa extract, the best phenol content was obtained at an extraction temperature of 50 °C and a heating time of 2 hours, namely 15.78 GAE. Statistical analysis showed that there was an effect of total phenol levels of moringa leaf extract in virgin coconut oil and extra virgin olive oil with variations in extraction time and temperature

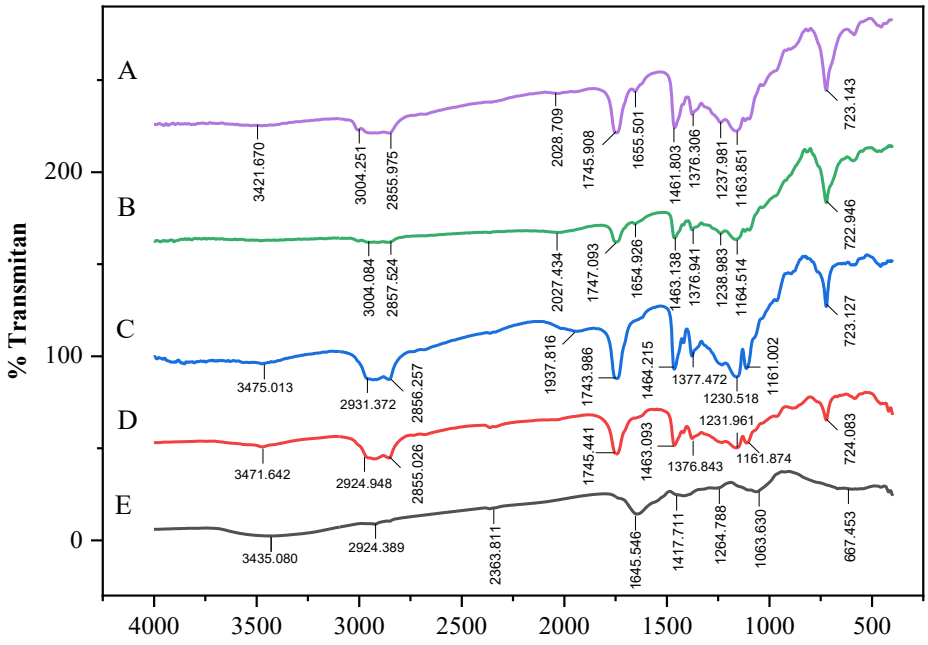

Figure 3. FTIR spectra (A) Moringa leaf extract EVOO 50ºC, 2 hours; (B) EVOO; (C) Moringa leaf extract VCO 50ºC, 6 hours; (D) VCO; (E) Moringa leaf powder

Table 3 Interpretation of the functional groups of the FTIR spectrum of Moringa Vegetable Oil Extract

Range (cm^{-1}) (Socrates, 2001)	EVO 50°C 2 hours (cm^{-1})	EVOO (cm^{-1})	VCO 50°C 6 hours (cm^{-1})	VCO (cm^{-1})	Moringa Powder (cm^{-1})	Functional groups
4000-3200	3421	-	3475	3471	3435	O-H *stretching*
3000-2800	2855	2857	2931	2924	2924	C-H *stretching*
1870-1550	1745	1747	1743	1745	1645	C=O *stretching*
1600-1450	1461	1463	146	1463	-	C=C aromatic *bending*
1460-1150	1376	1376	1377	1376	1417	CH_2 *bending*
1310-1020	1237	1238	1230	1231	1264	C-O-C *stretching*
995-670	723	722	723	723	667	C-H deformation

Based on Table 3, all samples showed that there was an absorption pattern of the stretching vibration of the OH group at wave numbers 3420-3471 cm^{-1}. There is an asymmetric/symmetric stretching vibration of the CH_2- (methylene) group at wave numbers 2924-2940 cm^{-1}. At absorption wave numbers 1645-1747 $cm^{-1,}$ there is an absorption pattern of C=O stretching of the carbonyl ester functional group from triglycerides. In wave numbers 1417-1461 cm^{-1} it shows a stretching vibration C=C while in wave numbers 1376-1417 cm^{-1} the absorption pattern is caused by a CH_2 bending vibration. In the wavenumber region 1230-1264 cm^{-1} there is a stretching C-O vibration. There is an absorption pattern at wave number 3004 cm^{-1} which is a stretching vibration of the cis olefinic (C=CH) double bond in the extra virgin olive oil spectra. The typical spectrum of flavonoids is shown by the presence of functional groups C=C aromatic, C=O carbonyl, O-H, bonded CH aromatic, aliphatic C-H, and C-O alcohol (MP, 2018).

CONCLUSION

Moringa leaf (*moringa oleifera* Lamk.) extract of extra virgin olive oil and virgin coconut oil have the potential as raw materials for cosmetics and medicine because through phytochemical tests they contain compounds that are antioxidants. The use of hot maceration extraction with an extraction temperature of 50°C with a heating time of 2 hours obtained the highest total phenol content. The use of vegetable oil as a solvent in herbal extraction is not limited to the use of extra virgin olive oil and virgin coconut oil, but other vegetable oils such as sunflower seed oil, avocado oil, etc.

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