

## FORMULATION, EVALUATION OF PHYSICAL PROPERTIES AND ANTIOXIDANT ACTIVITY OF ETHANOL EXTRACT AND ETHYL ACETATE FRACTION GEL OF *Moringa oleifera* LEAVES

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### ABSTRACT

The leaves of Moringa (*Moringa oleifera* Lam) contain quercetin, a flavonoid that plays a role in the skin regeneration process with an antioxidant mechanism, can play a role in the healing process of skin wounds. The ethanol extract of Moringa leaves was fractionated using petroleum ether and ethyl acetate. The MLEE (Moringa leaf ethanolic extract) gel formula was made to vary the extract weights in the order of 2%, 4% and 6% (b/b), while EFML (Moringa leaf ethyl acetate fraction) gel preparations were made to vary the extract weights in the order of 1%, 2% and 4% (b/b). Evaluation of the physical properties of the gel preparations, including organoleptic observation, homogeneity tests, pH measurements, spreadability tests, adhesion tests, and antioxidant activity using the DPPH method. The physical properties of all preparation fulfill the requirements. The test for antioxidant gel MLEE IC<sub>50</sub>: MLEE 1 144.72 ± 3.52 ppm; MLEE 2 138.15 ± 0.93 ppm; MLEE 3 136.59 ± 1.68 ppm when compared to extracts IC<sub>50</sub> MLEE 23.14 ppm (very strong antioxidant activity). The EFML gel formulation (IC<sub>50</sub>: EFML 1 208.81 ± 4.09 ppm; EFML 2 193.22 ± 2.53 ppm; EFML 3 182.48 ± 2.11 ppm) gave the same results when compared to the thick fraction of Moringa leaves, which has moderate antioxidant activity. The formula gel MLEE 3 has moderate antioxidant activity, which is the best gel formulation based on the results of physical properties test.

**Keywords:** *Moringa oleifera* Lam, antioxidant, gel formulation, ethyl acetate fraction

### INTRODUCTION

The diverse plants in Indonesia have been studied as a traditional medicine. One of the plants that are currently popular in Indonesia, especially is moringa (Fatmawati et al., 2021). Moringa (*Moringa oleifera* Lam) is currently widely cultivated by people in Indonesia. Research showed that Moringa leaves contain quercetin, carotenoid, amino acids and alkaloids, as well as a combination of phenolic compounds (Gupta et al., 2015; Karthivashan et al., 2015). The antioxidant activity of ethanolic extract Moringa leaf is IC<sub>50</sub> 22.18 ppm Rizkayanti et al., (2017) while the ethyl acetate fraction was 18.21 ± 0.06 ppm (Gothai et al., 2017). The quercetin content of Moringa leaves has antioxidant activity, so it is necessary to study its effectiveness in regenerating skin cells topically (Almeida et al., 2015). The ethanol extract was incorporated in topical gel dosage form by using HPMC base has antifungal activity against *M. furfur* (Yusuf et al., 2017). Gel preparations with Carbopol 940 as gelling agent of Moringa Leaf Extract (*Moringa oleifera* Lamk) also have anti-inflammatory activity (Sugihartini et al., 2020).

Moringa leaves in Indonesia are used as a one of food ingredient and is a potential source of antioxidants for the human body. Natural antioxidants such as ascorbic acid or vitamin C, flavonoids, phenolic, and carotene are found in Moringa leaves. In-vitro and animal studies have shown that flavonoids, including quercetin and rutin, are antioxidants and anti-inflammatory agents that prevent sun radiation, reduce oxidative stress, strengthen the integrity

and barrier function of human skin's mesenchymal stromal cells. In addition these compounds also implicated in cells regulation ([Almeida et al., 2015](#)). Antioxidants are compounds that can inhibit free radical propagation, by transfer of a hydrogen atom, from its hydroxyl group ([Sulastrri et al., 2018](#)).

Based on this explanation, the aim of this study is to formulate gel preparations containing various concentration of ethanol extract and ethyl acetate fraction of Moringa leaves. Moringa leaf gel preparations were evaluated physical properties, tested for antioxidant activity of the extract and the fraction of Moringa leaf ethyl acetate. Topical cosmetics and traditional medicine can be formulated in dosage forms such as creams, ointments or gels. Gel is a transparent semi-solid preparation and contains active substances as colloidal dispersion and entrapped in the gel matrix. The advantages of gel preparations compared to other topical preparations are easy to apply into the skin without pressure, provide a cooling sensation, and do not leave marks on the skin. The fraction used in the gel formulation was purified extract of Moringa leaves. Purification was done to remove chlorophyll and other substances in ethanol extracts using petroleum ether as a non-polar phase ([Suryani et al., 2015](#)).

## MATERIALS AND METHODS

### Materials

Moringa leaf powder obtained from Beringharjo Market, Yogyakarta and carried out identification at the Biology Laboratory, Ahmad Dahlan University. The materials used in this study are Alcohol 70%, Alcohol 96% Pro Analysis, Petroleum Ethers, Ethyl Acetate, Aquadest, HPMC, Propylene glycol (Brataco), DPPH (Sigma Aldrich) and Quercetin (Sigma Aldrich). The equipment used is a glass tool (Pyrex), Rotary Evaporator (IKA RV 10), Waterbath (Memmert), Mortar and UV-Visible Thermo Scientific Evolution 201 Spectrophotometer.

### Preparation of Moringa Leaves Ethanol Extract (MLEE) and Ethyl Acetate Fraction Moringa Leaves (EFML)

Moringa leaves powder from Beringharjo Market, was determined at the Biology Laboratory, Ahmad Dahlan University. Extraction was done by weighing 2 kg of Moringa leaves powder and then macerated with 70% ethanol (1:5), protected from light for 3 days. The macerate separated from the pulp by filtration and the residue was remacerated again. The filtrate was evaporated by using rotary evaporator at 60°C for 8 hours ([Fatmawati et al., 2019](#)). MFLE was made by weighing 51.66 grams of MLEE, then soaked in 500 mL of distilled water and fractionated using petroleum ether (PE), with a ratio of 1 distilled water : 1 PE solvent. This solvent was used to control the ingestion or extraction of compounds with non-polar properties using a separating funnel. Fractionation with ethyl acetate solvent was carried out until the addition of ethyl acetate in a separating funnel resulting in a clear color or no dark green color in the ethyl acetate phase. The ethyl acetate phase was evaporated using a rotary evaporator at a temperature of 60°C at a speed of 60 rpm. The semi-viscous extract was put into a porcelain cup and evaporated over a water bath ([Sulistyawati et al., 2017](#)).

### Formulation of Moringa Leaves Ethanol Extract (MLEE) and Ethyl Acetate Fraction Moringa Leaves (EFML) Gel

The MLEE and EFML formulas were prepared based on the research of [Yusuf et al., \(2017\)](#) with modification of extract weight and with the addition of 0.1% methylparaben ([Ardana et al., 2015](#)). The design of the MLEE gel formulation and EFML gel formulation in this study is shown in [Table I](#). Evaluation of the physical of MLEE and EFML gel preparations includes, organoleptic observation, homogeneity test, pH measurement, spreadability test, and adhesion test. Antioxidant activity tests were carried out on MLEE, EFML, MLEE Gel Formula and EFML Gel Formula with quercetin compound comparison to compare effectivity of gel on regenerate skin topically.

**Table I. Design Formulation Gel MLEE & EFML**

Sample	Formula Gel MLEE						Gel Base
	MLEE 1	MLEE 2	MLEE 3	EFML 1	EFML 2	EFML 3	
MLEE (g)	1	2	3	-	-	-	-
EFML (g)	-	-	-	0,5	1	2	-
HPMC (%)	2	2	2	2	2	2	2
Propilen Glikol (g)	6	6	6	6	6	6	6
Methyl paraben (%)	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Aquadest ad (g)	50	50	50	50	50	50	50

Information:

- MLEE 1 = The formula contains MLEE with an extract weight of 1 gram  
MLEE 2 = The formula contains MLEE with an extract weight of 2 grams  
MLEE 3 = The formula contains MLEE with an extract weight of 3 grams  
EFML 1 = The formula contains EFML with an extract weight of 0.5 grams  
EFML 2 = The formula contains EFML with an extract weight of 1 gram  
EFML 3 = The formula contains EFML with an extract weight of 2 grams  
Gel Base = Gel Base Gel

### Physical Evaluation

The organoleptic test results of the MLEE gel obtained with object and cover glass, 0.5 mg sample put on the glass and observed with a microscope. The test for the degree of acidity of the MLEE gel was carried out by taking the MLEE gel sample and dissolving it with 1.0 mL of distilled water in a porcelain dish. Degree of acidity measurements were carried out using universal pH and the results were matched with the indicators listed on the universal pH packaging (Maulina, L., & Sugihartini, N., 2015).

### Spreadability Test & Adhesion Time

The spreadability test was carried out by weighing 0.5 grams of EFML Gel -, placed on a glass-object in spherical form, covered it by the other glass-object and put a load 150 grams. After 1 minute, the diameter of gel was measured. The adhesion time was evaluated by weighing the EFML gel sample about 0.25 grams and placed between 2 objects on the adhesion tester, then put 1 kg of load, then given a load of 100 grams. Furthermore, the gel release time was recorded (Maulina, L., & Sugihartini, N., 2015).

### Antioxidant Activity Test of Moringa Leaf Ethanol Extract Gel (MLEE) and Moringa Leaf Ethyl Acetate Fraction (EFML) using the DPPH Method

Antioxidant activity of Moringa leaf ethanol extract gel solution and Moringa leaf ethyl acetate fraction solution were carried out by using the DPPH method. Each sample was weighed and diluted in ethanol to yield an equivalent concentration 50 ppm. The solution was vortex by using Vortex Mixer VM-300 at medium speed and filtered. The concentrations of MLEE and EFML main gel solutions were obtained 1000 ppm, then a series of solution levels were made, each with a concentration of 200, 400, 600, and 800 ppm with pro-analysis ethanol in a 10 mL measuring flask (Meigaria, et al., 2016).

Antioxidant activity of each sample MLEE and EFML gel was carried out by mixing 2.0 mL of each 200, 400, 600, and 800 ppm sample with 1.0 mL of 50 ppm f DPPH. Each procedures was replicated 3 times. Furthermore, each mixture of these solutions was vortexed with a Vortex Mixer VM-300 at medium speed, covered with aluminium foil and incubated at 37°C for 30 minutes (Meigaria, et al., 2016). The analysis of antioxidant strength in the sample was carried out by finding the IC<sub>50</sub> based on the percent free radical scavenging by the sample and by the quercetin standard calculated using the equation in

Figure 1 (Meigaria, et al., 2016). The percentage decrease in DPPH from Moringa Leaves Gel was calculated by the % inhibition equation, then followed by linear regression. The IC<sub>50</sub> value is calculated by using a linear regression equation ( $Y = bx + a$ ) and entering the number 50 in the letter Y of the equation (Fatmawati et al., 2022).

$$\% \text{ Inhibition} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sampel}}}{\text{Abs}_{\text{control}}} \times 100 \%$$

Abs : Absorbance

**Figure 1. Inhibition concentration formula**

### Data analysis

The data obtained in this study were divided into three things, namely, the antioxidant power of the MLEE and EFML gel compared to the antioxidant power of the extract, the ethyl acetate fraction of Moringa leaves and the quercetin standard. IC<sub>50</sub> data were analyzed by Duncan's SPSS test.

## RESULT AND DISCUSSION

### Physical Properties

Organoleptic test results for MLEE Gel are shown in Table II. The observation results of the MLEE gel homogeneity test in Formula 1, 2 and 3 obtained a homogeneous formula as shown in Table II. The gel base formula with HPMC 2% and propylene glycol is able to form a homogeneous gel formula based on the research of Yusuf et al., (2017).

Figure 2 shows that MLEE F1 (Young leaf green), F2 (Dark green leaves), and F3 (Dark moss green) gel preparations with gel from fraction, EFML F1 (Dark green leaves), F2 (Dark moss green), and F3 (Dark green leaves).

**Table II. Physical Properties Results for Gel Formulation MLEE and EFML**

Formula Gel (n=3)	Organoleptic Test		pH (X±SD)	Spreadability X ±SD (cm)	Adhesion Time X±SD (Second)
	Color	Homogeneity			
MLEE 1	Young leaf green	Homogen	5.33 ± 0.03	5.30 ± 0.20	4.49 ± 0.03
MLEE 2	Dark green leaves	Homogen	5.67 ± 0.58	5.33 ± 0.21	5.40 ± 0.03
MLEE 3	Dark moss green	Homogen	5.00 ± 0.00	5.40 ± 0.20	7.48 ± 0.06
EFML 1	Dark green leaves	Homogen	6.00 ± 0.00	5.13 ± 0.06	57.44 ± 2.25
EFML 2	Dark moss green	Homogen	6.00 ± 0.00	5.33 ± 0.12	14.11 ± 0.74
EFML 3	Dark moss green	Homogen	6.00 ± 0.00	5.17 ± 0.06	16.71 ± 0.92
Gel Base	Clear white bones	Homogen	6.00 ± 0.00	5.23 ± 0.15	12.12 ± 0.09

Information:

MLEE 1	=	The formula contains MLEE with an extract weight of 1 grams
MLEE 2	=	The formula contains MLEE with an extract weight of 2 gram
MLEE 3	=	The formula contains MLEE with an extract weight of 3 grams
EFML 1	=	The formula contains EFML with an extract weight of 0.5 grams
EFML 2	=	The formula contains EFML with an extract weight of 1 gram
EFML 3	=	The formula contains EFML with an extract weight of 2 grams
Gel Base	=	Gel Base

### Degree of Acidity Test (pH)

Replication of pH measurements was carried out three times for each formula with the results shown in Table II. The results of the pH test on the MLEE & EFML gel (

Figure 2) preparations showed that the F1, F2, F3 formulas and the negative control gel base met the pH requirements of a good topical preparation, namely a pH range of 4.5 – 6.5 (Maulina, L., & Sugihartini, N., 2015).

### Spreadability Test Results

Table II results of the spreadability test shows F1, F2, F3 and Negative Control (gel base) can fall into the range of 5-7 cm<sup>2</sup>. The spreadability test is a requirement to enter into the important requirements of the gel preparation. If a preparation has a high spreadability, it means that the area of distribution is greater so that the active substances contained will be distributed evenly and are more effective in producing a therapeutic effect (Ulfa et al., 2016). The spreadability of semisolid is divided into 2, namely semistiff and semifluid. Semistiff is a semisolid preparation that has a high viscosity while semifluid is a semisolid preparation with a low viscosity. In semistiff, the dispersion power requirement is 3-5 cm<sup>2</sup> and for semifluid it is 5-7 cm<sup>2</sup> (Garg et al., 2002).

### Adhesion Test Results of Gel MLEE and EFML

The test results in Table II show that EFML 3 has good adhesion compared to F1, F2 and gel base. The EFML F1 gel formula has the longest adhesion time of 57.44 seconds compared to F2 (14.11 seconds) and F3 (16.71 seconds).



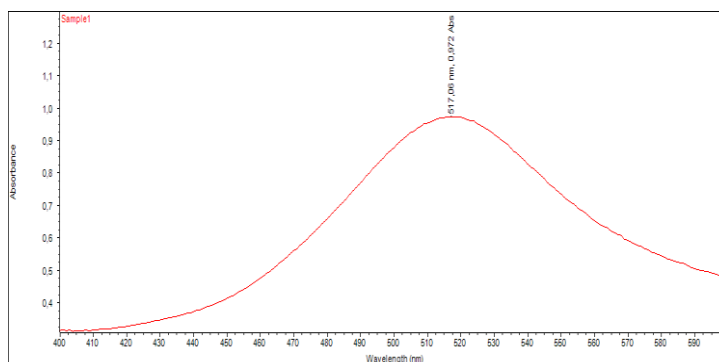
**Figure 2.** Gel of MLEE (Moringa leaf ethanolic extract) and EFML (Ethyl acetate fraction of Moringa leaves)

### Antioxidant Activity

#### Determination of the Maximum Wavelength of DPPH Solution

Antioxidant testing of extracts, fractions, MLEE, and EFML gels was carried out using the DPPH method which is a 1,1-diphenyl-1-picrihydrazyl absorption method. This method is used for antioxidant testing because it is simple, fast, easy and uses a small amount of sample in a relatively short time, is accurate and practical. DPPH compounds in visible spectrophotometric methods show strong absorption at a maximum wavelength of 517 nm (Meigaria et al., 2016). Figure 3 is a scan of wavelengths between 400-600 nm of DPPH solution, with a maximum absorption (0.972) at wavelength of 517.057 nm. The results showed that the DPPH compound used in this experiment has the same absorption with the theoretical maximum wavelength of DPPH (Meigaria et al., 2016).





**Figure 3. Maximum wavelength of DPPH**

The antioxidant activity test for the ethanol extract of Moringa leaves (MLEE) and the ethyl acetate fraction of Moringa leaves (EFML) using the DPPH method are shown in Table III. The average IC<sub>50</sub> value on the MLEE gave 23.14 ppm results, while the IC<sub>50</sub> value was 182.98 ppm for the EFML. This shows that the IC<sub>50</sub> value of the ethanol extract of Moringa leaves is smaller than the IC<sub>50</sub> of the ethyl acetate fraction of Moringa leaves, meaning that the ethanol extract of Moringa leaves has a higher antioxidant activity when compared to the ethyl acetate fraction of Moringa leaves (Table III). MLEE antioxidant activity is included in very strong antioxidant intensity (<50 ppm), while EFML has moderate antioxidant intensity (100-250 ppm) with an indicator of the level of antioxidant strength. This study is also in line with the research of Hasanah et al., (2017), with the results that the antioxidant activity of Moringa leaf gel is included in the category of moderate antioxidant strength.

**Table III. Antioxidant Activity of Formula Gel MLEE, EFML Gel, Moringa Leaves Extract, Ethyl Acetat Fraction Moringa Leaves and Quercetin**

Sample (n=3)	IC <sub>50</sub> Value (X ± SD) ppm	Antioxidant Strength
MLEE 1 Gel	144.72 ± 3.52 <sup>*a</sup>	Moderate
MLEE 2 Gel	138.15 ± 0.93 <sup>*b</sup>	Moderate
MLEE 3 Gel	136.59 ± 1.68 <sup>*</sup>	Moderate
EFML 1 Gel	208.81 ± 4.09 <sup>*a</sup>	Moderate
EFML 2 Gel	193.22 ± 2.53 <sup>*a</sup>	Moderate
EFML 3 Gel	182.48 ± 2.11 <sup>*a</sup>	Moderate
Moringa Extract Ethanolic	23.14 ± 2.54 <sup>*a</sup>	Strong
Ethyl Acetate Fraction Moringa	182.98 ± 2.89 <sup>*a</sup>	Moderate
Quercetin	10.76 ± 1.03 <sup>a</sup>	Stong

Duncan Test: <sup>\*</sup>IC<sub>50</sub> value is significantly different from quercetin positive control; <sup>a</sup>the IC<sub>50</sub> value was significantly different from the MLEE 3 gel preparation (p<0.05); <sup>b</sup>the IC<sub>50</sub> value was not significantly different from the MLEE 3 gel preparation (p>0.05)

The average IC<sub>50</sub> value from 3 replication tests, in Table III shows that Formula 1 MLEE has an IC<sub>50</sub> value of 144.72±3.52 ppm, Formula 2 MLEE is 138.15±0.93 ppm and Formula 3 MLEE is 136.59±1.68 ppm. The antioxidant activity of MLEE gel is included in moderate antioxidant intensity (100-250 ppm) with the indicator of the level of antioxidant strength shown in Table III. The comparison of the antioxidant activity of the MLEE gel which has the highest antioxidant activity is the MLEE 3 formula followed by MLEE 2 and MLEE 1. The smaller the IC<sub>50</sub> value, the higher the antioxidant activity value. The results of SPSS analysis of IC<sub>50</sub> Duncan's test showed that there were significant differences between extracts, fractions, MLEE F1, F2 and F3 gels with EFML gels F1, F2 and F3, as well as positive control (quercetin) with sig values (p<0.05 ). Table III also shows that the IC<sub>50</sub> value of the MLEE 3

gel preparation is not significantly different from that of the MLEE 2 gel, meaning that based on the IC<sub>50</sub> value, the MLEE 3 gel has the same antioxidant activity as the MLEE 2 gel.

The results of the antioxidant activity test for the ethyl acetate fraction of Moringa leaves formula F1, F2, F3 with the DPPH method are shown in Table III. The average IC<sub>50</sub> value for antioxidant replication testing was 3 times, in Formula EFML 1 was 208.81±4.09 ppm, EFML 2 was 193.22±2.53 ppm and EFML 3 was 182.48±2.11 ppm. This shows that the antioxidant activity of EFML gel has a moderate antioxidant intensity (100-250 ppm) with an indicator of the level of antioxidant strength. The scavenging activity increased in a concentration-dependent manner due to the scavenging capacity of the fraction and was comparable to quercetin. The IC<sub>50</sub> value signifies the concentration required to scavenge 50% of the initial DPPH radicals (Gothai et al., 2017). The quercetin compound has the smallest IC<sub>50</sub> value of 10.76 ppm with the highest antioxidant activity because this compound is a pure flavonoid compound with the maximum free radical scavenging.

Moringa leaf ethanol extract has antibacterial activity (Singh & Tafida, 2014; Ajayi & Fadeyi, 2015; Fouad et al., 2019), water and ethanol extracts Moringa have antioxidant activity (Rizkayanti et al., 2017). Based on the research results of Ulfa et al., (2016), it is known that Moringa leaf extract gel (*Moringa oleifera* Lam) can reduce edema up to 47.07%. The content of flavonoid active compounds in Moringa leaves functions as antioxidants that can help neutralize and stabilize free radicals so that they can maintain healthy cells and tissues (Susanty et al., 2019). The bad effects of free radicals on the skin can be reduced in the presence of antioxidant compounds (Hasanah et al., 2017).

## CONCLUSION

The antioxidant activity of the ethanol extract of Moringa leaves was 23.14 ± 2.54 ppm has the strong and the ethyl acetate fraction of Moringa leaves was 182.98 ± 2.89 ppm. The formula gel MLEE 3 (6% MLEE) has moderate antioxidant activity (IC<sub>50</sub> 136.59 ± 1.68 ppm) which is the best gel formulation based on physical properties test. Based on the results of this study, it was concluded that the ethanol extract and the ethyl acetate fraction of Moringa leaves could be formulated in an antioxidant gel preparation.

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