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FORMULATION, EVALUATION OF PHYSICAL PROPERTIES AND ANTIOXIDANT ACTIVITY OF ETHANOL EXTRACT AND ETHYL ACETATE FRACTION GEL OF Moringa oleifera LEAVES Annisa Fatmawati1*, Rizal Fauzi1, Riza Kurniawati2, Depita Sucianingsih2, Sain Abrari2 1 Department of Pharmacy, Faculty of Health Science, Universitas Alma Ata , Yogyakarta 2 Bachelor of Pharmacy, Faculty of Health Science, Universitas Alma Ata, Yogyakarta *EmailCorresponding: annisafatma20@almaata.ac.id Submitted :..... Revised :..... Accepted:..... 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 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 tests, homogenity tests, pH measurements, spreadability tests and adhesion tests, and antioxidant activity tests using the DPPH method.

The results of the observations of physical properties on homogeneity, pH, adhesion and dispersibility met the requirements of all test groups. The test for antioxidant gel MLEE IC50: F1 MLEE 144.72 ppm; F2 MLEE 138.15 ppm; F3 MLEE 136.59 ppm when compared to extracts IC50 MLEE 23.14 ppm had a higher IC50 value, meaning that MLEE (very strong antioxidant activity). The EFML gel formulation (IC50: F1 EFML 208.81 ppm; F2 EFML 193.22 ppm; F3 EFML 182.48 ppm) gave the same results when compared to the thick fraction of Moringa leaves, which has moderate antioxidant activity. The

quercetin compound has the smallest IC50 value of 10.76 ppm with the highest antioxidant activity because this compound is a pure flavonoid compound with the maximum free radical scavenging.

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 shows 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., 2014).

Moringa leaves has antioxidant activity in ethanolic extract with IC50 22.18 ppm (Rizkayanti et al, 2017) and the IC50 value of the Moringa leaf ethyl acetat 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 test its effectiveness in regenerating skin cells topically (Almeida et al., 2015). The ethanol extract made in topical gel dosage form with HPMC base concentration has antifungal activity against M. furfur which is characterized by the formation of an inhibition zone of 24.3 mm (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 cooking 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 rutine, are antioxidants and anti-inflammatory agents and can prevent solar radiation, reduce oxidative stress, and strengthen the integrity and barrier function of human skin's mesenchymaltroma cells, and are involved in important pathways which governs cells (Almeida et al., 2015).

Antioxidants are compounds that can stop free radical propagation reactions, both those derived from metabolic by-products that occur in the body and those from the environment such as cigarette smoke, air pollution, certain drugs, ultraviolet rays, and radiation (Meigaria et al., 2016). The condition of diabetes is associated with free radicals and although free radicals are a secondary disease; in some cases, free radicals cause oxidative stress. Cellular antioxidants promote wound healing by reducing the damage caused by free radicals. Complementary antioxidants in diabetic patients are massively depleted by free radicals.

Thus, the balance between free radicals and antioxidants is very important to ensure

wound healing in diabetic patients (Gbedema et al., 2010) Studies have confirmed that medicinal plants with high antioxidant polyphenol content are effective wound healing agents in the management of diabetes, with little effect. side (Balachandar et al., 2014; Gothai et al., 2017). Based on this explanation, this study aims to formulate gel preparations from ethanol extract and ethyl acetate fraction of Moringa leaves with various concentrations of extracts and fractions.

Moringa leaf gel preparations were tested for 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 semi-solid preparation that is clear, translucent and contains active substances and is a colloidal dispersion and has strength due to the bonding of the network in the dispersed phase.

The advantages of gel preparations compared to other topical preparations are that they are easy to apply evenly to the skin without pressure, provide a cooling sensation, do not leave marks on the skin, and are easy to use. The fraction used in the gel formulation was purified extract of Moringa leaves. Purification is carried out with the aim of removing chlorophyll and other ballast substances in ethanol extracts using petroleum ether as a non-polar phase (Suryani et al., 2015). METHODOLOGY Materials Moringa leaf powder obtained from Beringharjo Market, Yogyakarta and carried out identification at the Biology Laboratory, Ahmad Dahlan University.

Ingredients for testing 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, Waterbath, Mortar and UV-Visible Thermo Scientific Evolution 201 Spectrophotometer. Plant Material, Extraction and Fractionation Moringa leaf powder from Beringharjo Market, was determined at the Biology Laboratory, Ahmad Dahlan University.

Extraction is done by weighing 2 kg of Moringa Leaf powder and then macerated with 70% ethanol (1:5), stored in a place protected from light for 3 days, macerate separated from the pulp by filtration and residual pulp was remacerated once. Filtration evaporated with a rotary evaporator then heated with water bath (Fatmawati et al., 2019). Moringa leaf ethyl acetate fraction was made by weighing 51.66 grams of EEDK, then dissolved using 500 ml of distilled water and fractionated using petroleum ether (PE) with a ratio of 1:1. PE solvent is 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 resulted 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 Methods 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, adhesion test. Antioxidant activity tests were carried out on MLEE, EFML, MLEE Gel Formula and EFML Gel Formula with quercetin compound comparison to compare efectivity of gel on regenerate skin topically. Table I.Design Formulation Gel MLEE & EFML Sample _Formula Gel MLEE _ _ _ _ _ _F1 MLEE _F2 MLEE _F3 MLEE _F1 EFML _F2 EFML _F3 EFML _Basic _ _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: _ _ _ _ _F1 MLEE _= _The formula contains MLEE with an extract weight of 1 grams _ _F2 MLEE _= _The formula contains MLEE with an extract weight of 2 gram _ _F3 MLEE _= _The formula contains MLEE with an extract weight of 3 grams _ _F1 EFML _= _The formula contains EFML with an extract weight of 0.5 grams _ _F2 EFML _= _The formula contains EFML with an extract weight of 1 gram _ _F3 EFML _= _The formula contains EFML with an extract weight of 2 grams _ _Basic _= _Basic Gel _ _ _ _ _ Organoleptic Test & Degree of Acidity Test (pH) 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 paper and the results were matched with the indicators listed on the universal pH paper packaging (Maulina and Sugihartini, 2015). Spreadability Test & Adhesion Test of Gel MLEE and EFML The spreadability test was carried out by weighing 0.5 grams of EFML Gel with various formulas on a glass-covered spherical dispersion power tester and giving a weighing load of 150 grams. After 1 minute of loading, a constant diameter measurement was carried out on the spread of the gel. The adhesion test was carried out by weighing the EFML gel sample in the amount of 0.25 grams placed between 2 objects on the adhesion power tester, then placing a load of 1 kg,

then given a load of 100 grams on the tool. Furthermore, the gel release time was recorded (Maulina and Sugihartini, 2015).

Antioxidant Activity Test of Moringa Leaf Ethanol Extract Gel (MLEE) and Moringa Leaf Ethyl Acetate Fraction (EFML) using the DPPH Method Preparation of Moringa leaf ethanol extract gel solution and Moringa leaf ethyl acetate fraction solution for antioxidant testing using the DPPH method was carried out by weighing the gel sample equivalent to 50 ppm of viscous extract and viscous fraction, each dissolved in 50.0 ml of pro-analysis ethanol in a measuring flask, was vortex with the Vortex Mixer VM-300 at medium speed and filtered with Whattman filter paper.

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 testing on each series of levels of MLEE and EFML gel solution was carried out by taking 2.0 ml of the 200, 400, 600 and 800 ppm concentration sample put into a test tube coated with aluminum foil and adding a 50 ppm concentration of DPPH solution 1.0 ml was replicated 3 times.

Furthermore, each mixture of these solutions was vortexed with a Vortex Mixer VM-300 at medium speed and incubated at 37°C for 30 minutes (Meigaria et al., 2016). 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. The analysis of antioxidant strength in the sample was carried out by finding the IC50 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).

% Inhibition = _Abs control - Abs sampel _x 100 % _ _ _Abs control _ _ _Abs : Absorbance _ _Figure 1. Inhibition Concentration Formula RESULT AND DISCUSSION Organoleptic Test 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). 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 Test Results X±SD (Second) _ _ _Color _Homogenity _ _ _ _ _F1 MLEE _Young leaf green _Homogen _5.33 ± 0.03 _5.30 ± 0.20 _4.49 ± 0.03 _ _F2 MLEE

_Dark green leaves _Homogen _5.67 ± 0.58 _5.33 ± 0.21 _5.40 ± 0.03 _ _F3 MLEE _Dark moss green _Homogen _5.00 ± 0.00 _5.40 ± 0.20 _7.48 ± 0.06 _ _F1 EFML _Dark green leaves _Homogen _6.00 ± 0.00 _5.13 ± 0.06 _57.44 ± 2.25 _ _F2 EFML _Dark moss green _Homogen _6.00 ± 0.00 _5.33 ± 0.12 _14.11 ± 0.74 _ _F3 EFML _Dark moss green _Homogen _6.00 ± 0.00 _5.17 ± 0.06 _16.71 ± 0.92 _ _Basic _Clear white bones _Homogen _6.00 ± 0.00 _5.23 ± 0.15 _12.12 ± 0.09 _ _Information: _ _ _ _ _F1 MLEE _= _The formula contains MLEE with an extract weight of 1 grams _ _F2 MLEE _= _The formula contains MLEE with an extract weight of 2 gram _ _F3 MLEE _= _The formula contains MLEE with an extract weight of 3 grams _ _F1 EFML _= _The formula contains EFML with an extract weight of 0.5

grams _ _F2 EFML _= _The formula contains EFML with an extract weight of 1 gram _ _F3 EFML _= _The formula contains EFML with an extract weight of 2 grams _ _Basic _= _Basic Gel _ _ _ _ _ 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 and Sugihartini, 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 cm2.

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 cm2 and for semifluid it is 5-7 cm2 (Garg et al, 2002). Adhesion Test Results of Gel MLEE and EFML The test results in Table II, show that F3 EFML 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). / _/ _/ _ _F1 MLEE _F2 MLEE _F3 MLEE _ _/ _/ _/ _ _F1 EFML _F2 EFML _F3 EFML _ _Figure 2. Gel of MLEE (Moringa leaf ethanolic extract) and EFML (Ethyl acetate fraction of Moringa leaves) Antioxidant Testing with the DPPH Method 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 in DPPH solution, with a maximum wavelength of 517.057 nm with an absorbance of 0.972. This shows that the DPPH compound used in this experiment has the same absorption as the theoretical maximum wavelength of DPPH (Meigaria et al., 2016). / Figure 3.

Wavelength Scan Results of DPPH Compounds on Visible Spectrophotometry
Antioxidant Activity Test of Moringa Leaf Ethanol Extract (MLEE) and Moringa Leaf Ethyl Acetate Fraction (EFML) using the DPPH Method
The results of 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 IC50 value on the MLEE gave 23.14 ppm results, while the IC50 value was 182.98 ppm for the EFML.

This shows that the IC50 value of the ethanol extract of Moringa leaves is smaller than the IC50 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., (2022), 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) _IC50 Value (X ± SD) ppm _Antioxidant Strength _ _F1 MLEE Gel _144.72 ± 3.52 _Moderate _ _F2 MLEE Gel _138.15 ± 0.93 _Moderate _ _F3 MLEE Gel _136.59 ± 1.68 _Moderate _ _F1 EFML Gel _208.81 ± 4.09 _Moderate _ _F2 EFML Gel _193.22 ± 2.53 _Moderate _ _F3 EFML Gel _182.48 ± 2.11 _Moderate _ _Moringa Extract Ethanolic _23.14 ± 2.54 _Strong _ _Ethyl Acetate Fraction Moringa _182.98 ± 2.89 _Moderate _ _Quercetin _10.76 ± 1.03 _Stong _ _ / _/ _ _Figure 4.

Antioxidant Activity Test Solution on MLEE and EFML Gel after being reacted with DPPH (2,2-difenil-1- pikrilhidrazil) reagent The results of the antioxidant activity test of Moringa leaf ethanol extract gel (MLEE) formula F1, F2 and F3 using the DPPH method are shown in Figure 4. The average IC50 value from 3 replication tests, in Table III shows that Formula 1 MLEE has an IC50 value of 144.72 ppm, Formula 2 MLEE is 138.15 ppm

and Formula 3 MLEE is 136.59 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 F3 MLEE formula followed by F2 MLEE and F1 MLEE. The smaller the IC50 value, the higher the antioxidant activity value. The results of statistical data analysis using SPSS using Duncan's test showed that there was a significant difference between F1, F2 and F3 MLEE with F1, F2 and F3 EFML gel, as well as positive control (quercetin) with sig value ($p<0.05$). Statistical analysis of the ethanol extract of Moringa leaves was also significantly different with positive (quercetin) with a sig value ($p<0.05$) even though it was included in the category of strong antioxidants.

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 IC50 value for antioxand replication testing was 3 times, in Formula F1 EFML was 208.81 ppm, F2 EFML was 193.22 ppm and F3 EFML was 182.48 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 IC50 value signifies the concentration required to scavenge 50% of the initial DPPH radicals (Gothai et al., 2017). Moringa leaf ethanol extract has antibacterial activity (Singh et al., 2014; Ajayi et al., 2015; Ehab 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%. Biological activity on diabetic human skin fibroblasts in vitro study from the Ethyl acetate Fraction is a promising complementary supplement for diabetic patients with wound healing defects. The use of Moringa leaves in the treatment of wounds is highly recommended in vivo experiments with wound models, both diabetic wounds and burns (Gothai et al., 2017). CONCLUSION The formula gel F3 MLEE has moderate antioxidant activity (IC50 136.59 ppm) is the best formulation gel based on physical properties test results. The quercetin compound has the smallest IC50 value of 10.76 ppm with the highest antioxidant activity because this compound is a pure flavonoid compound with the maximum free radical scavenging. ACKNOWLEDGEMENT The author would like to thank the Indonesian Ministry of Higher Education (RISTEK-BRIN: Hibah PDP No.B/87/E3/RA.00/2020), LL Dikti Region V, Alma Ata University , Tim Research Moringa Leaf and all participant that we can’t mention one by

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