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66 **Microscopic Identification and Determination of Total Flavonoid Content of Moringa Leaves Extract and Ethyl Acetate Fraction (Moringa oleifera L.) Annisa Fatmawati***, Depita Sucianingsih, Riza Kurniawati, Muhammad Abdurrahman **Department of Pharmacy, Faculty of Health Science, Universitas Alma Ata**, Yogyakarta, Indonesia Submitted 02 November 2021; Revised 14 December 2021; Accepted 14 December 2021; Published 30 December 2021 *Corresponding author: annisafatma20@almaata.ac.id **Abstract This research was conducted to identify simplicia microscopically, phytochemical screening and determination** total content extract ethyl fraction Moringa Moringa oleifera L.)

using Spectrophotometry The design in study to microscopic of leaf simplicia, 96% 70% **ethanol extract and ethyl acetate fraction of** Moringa leaves from 70% ethanol extract, then carry out screening determination total content quercetin Phytochemical screening on **the ethyl acetate fraction of** Moringa leaves included tests for the content of saponins, and The of identification Moringa leaf showed presence calcium crystals the of mesophyll and The of of flavonoid in ethanol was \pm (w/w), ethanol was \pm (w/w), leaf acetate $14 \pm$ (w/w). highest flavonoid was in 96% extract Moringa in with Indonesian Pharmacopoeia that thick of Moringa leaves containing no less than 6.30% (w/w) total flavonoids was calculated as quercetin.

Keywords: quercetin, Moringa oleifera , microscopic, flavonoid Identifikasi Mikroskopis dan Penentuan Kandungan Flavonoid Total Ekstrak Daun Kelor **dan Fraksi Etil Asetat** (Moringa oleifera L.) Abstrak Penelitian dilakukan mengidentifikasi secara skrining dan penetapan flavonoid ekstrak fraksi asetat daun (Moringa oleifera L.) dengan metode Spektrofotometri UV-Vis. **Rancangan percobaan yang digunakan dalam penelitian ini** dengan melakukan identifikasi mikroskopis pada simplisia serbuk daun kelor, membuat

ekstrak etanol 96% 70% fraksi asetat kelor ekstrak 70% dilakukan fitokimia dan penetapan kadar flavonoid total dengan standar kuersetin.

Skrining fitokimia pada fraksi etil daun meliputi kandungan saponin, dan Hasil mikroskopis simplisia daun kelor menunjukkan adanya kristal kalsium oksalat berbentuk roset, mesofil dan Hasil kadar total ekstrak 96% 16,69 0,74 (b/b), etanol sebesar \pm % fraksi asetat kelor \pm % (b/b). flavonoid paling terdapat ekstrak 96% kelor dengan Farmakope Herbal Indonesia 2017, bahwa ekstrak kental daun kelor mengandung flavonoid total tidak kurang dari 6,30 % (b/b) dihitung sebagai kuersetin. Kata Kunci: kuersetin, Moringa oleifera , mikroskopis, flavonoid 67 1.

Introduction Moringa (Moringa oleifera) is a species of the genus Moringa belonging to the family which easy grow in areas as Moringa can grow in tropical and subtropical areas on all types of soil and is resistant to dry seasons within months. leaves widely used by the community as traditional medicine, vegetables, beauty and fatty foods. One the of leaves beauty as antiaging aging), moisturizing and overcoming dry skin. 1 Moringa extract has activity², tyrosinase inhibitor ³, antibacterial ⁴, antioxidant, and arthritis.⁵

Moringa contain and are rich in pro vitamins A, C, E, especially carotene which will be converted into vitamin in body significantly affect The of glucocyanate and isothiocyanate compounds in plants known the as hypotensive, anti-cancer, inhibiting bacterial and fungal activity. ⁶ Determination of levels and studies on the content profile of secondary metabolites the group be used as the basis for determining the isolation of potential compounds as antidiabetic, antioxidant, or compounds that can become active raw materials for drugs.⁷ Based previous Moringa leaves contain secondary metabolites including flavonoids, and saponins. ⁹ Flavonoids are one of the largest natural antioxidant and are found in all plants, so it can be ascertained that are in plants.

Phytochemical compounds in Moringa leaves that as antioxidant are so the flavonoid content Moringa can determined using a UV-Visible spectrophotometer. ¹⁰ Determination the flavonoid of extract natural can quercetin a Natural are reacted AlCl₃ and complex whose can determined by visible spectrophotometry. ^{10,11} The reaction standard quercetin and AlCl₃ is shown in Figure 1. This study to the flavonoid content the and ethyl fraction of Moringa leaves. ⁸ **2. Materials and methods** 2.1.

Materials **The tools used in the research were microscope, maceration vessels, separating funnels, electric stoves, laboratory glassware (pyrex), scales, rods, ml measuring pipettes, test tubes, aluminium foil, rotary evaporator, water baths, porcelain crucibles, filter oven, containers, universal ruler, adhesive dispersible equipment and**

their weights, cuvette and UV-Vis spectrophotometer Scientific Evolution 201). The materials used in this study Moringa (Moringa oleifera from Market Yogyakarta, ethanol, ethanol, acetate, ether, (Sigma Aldrich), 10% AlCl₃ and 5% acetic acid. 2.2.

Research sites **This research was conducted** at the Integrated Chemistry Laboratory and Phytochemical Laboratory, Bachelor of Pharmacy Study Program, Alma Ata University Yogyakarta in May 2021. Figure 1 . Complex Reaction Between Quercetin and AlCl₃ 8 68 2.3. Microscopic of leaf simplicia Moringa leaf powder is placed on a glass object, given a solution of chloral hydrate and covered with a cover glass. Then heated on a bunsen with clamps kept boiling, and heated to dry. The preparations were then placed on a microscope and observed with a magnification of 10 times. 12,13 2.4.

Moringa leaf extract Moringa powder weighed much 2000 for ethanol and grams 96% were into different glass jars then added ethanol solvent according to concentration variations **as much as 10** L for 70% ethanol solvent and 2.5 L for 96% to submerged stored for 3x24 hours at room temperature and stirred occasionally to speed up the process of dissolving compounds. extract obtained was then filtered and separated from the residue, then the residue or dregs obtained were for hours the same and (1:5). filtrate obtained was **then concentrated using a rotary evaporator** a of until thick extract obtained.

thick extract weighed obtain extract yield.2 2.5. Fractionation of 70% ethanolic **Moringa leaf extract Moringa** ethyl fraction made weighing grams EEDK, then dissolved using 500 ml of distilled water and using ether and ethyl acetate in a ratio of 1:1. The ethyl acetate phase containing the moringa leaf extract fraction was evaporated **using a rotary evaporator** at a temperature of 60°C at a speed of 60 rpm. Next, the semi-viscous extract was put into a porcelain cup and evaporated over a water bath. The formula for calculating the yield the acetate of leaves = of ethyl fraction/Weight thick of leaves) x100%). 11 2.6.

Moringa ethyl fraction phytochemical screening Phytochemical **tests were carried out** to determine the class of compounds contained the extract Moringa leaves and ethyl fraction (FEDK). examination carried namely examination alkaloid polyphenols flavonoids, saponins. examination carried by one ml EEDK with FeCl₃ The reaction is positive if a blackish or dark blue is For examination flavonoids by taking 1.0 ml of EEDK, adding a drops 10% the will show a positive flavonoid if there is a specific color And examination carried by 1.0 of with hot water and shaking. Positive reaction of saponins when a long-lasting foam is formed.11,12 2.7.

Determination **of total flavonoid content of Moringa leaf extract and ethyl acetate**

fraction 2.7.1. Determination of maximum wavelength 1.0 of solution taken as as mL with mL 10% AlCl₃ 8 of acetic then UV-Vis spectrophotometer readings with a wavelength of 400-500 nm were taken.10 2.7.2 Determination of operating time Determination of operating time is done by a ppm solution as as mL with mL 10% AlCl₃ and 8 ml of 5% acetic acid, the solution obtained is measured by the absorbance of the obtained intervals 0-60 minutes until the absorbance is obtained stable.10 2.7.3

Determination of the standard curve of quercetin The ppm solution made in series with concentrations of **20, 40, 60, 80 and 100** ppm, 1 mL of each concentration was taken into a volumetric flask, added 1 mL of AlCl₃ 8 of acetic allowed to stand for the optimum time, then 69 Absorbance readings were carried out using a spectrophotometer the wavelength.10 2.7.4. Determination of total flavonoids The extract ethanol and ethyl fraction Moringa leaves were **weighed as much as** 100 mg and added 100 ml of each solvent concentration of ethanol (1000 ppm concentration), then taken 1,0 and 1,0 of AlCl₃ acid 8 mL of 5% acetate, vortexed and allowed to stand for the optimum time and read the absorbance the wavelength repeated 3 times.10 2.8.

Research data analysis The content calculated by 70% extract, ethanol and **the ethyl acetate fraction of** Moringa (Moringa oleifera L.) and then calculated using **the linear regression equation** = + 10 The statistical test using LSD Significance test used test average between the 3 treatment groups using SPSS 16th software, this was to determine the significant difference in total flavonoid between of ethanol extract, 96% ethanol **and ethyl acetate fraction of** Moringa leaf. 10 3. Result 3.1. Moringa simplicia identification Microscopic of Moringa powder to the of identification fragments using a microscope.

The chloral hydrate solution aims to remove cell content such as protein and starch, so that the cell identification on leaves be clearly observed with a microscope. 12 Table 1 the results Moringa leaf simplicia, showing that the presence of calcium oxalate crystals in the form of rosettes, mesophyll and stomata is in accordance with previous research and **the Indonesian Herbal Pharmacopoeia Edition II** (2017). 13 3.2. Extract yield **and ethyl acetate fraction** The of leaf thick extract (EEDK) and Moringa leaf ethyl acetate fraction is in 2.

on **the Indonesian Herbal Pharmacopoeia Edition** the of leaf Microscopic Parameter Observation Indonesian Herbal Pharmacopoeia Reference 13 Rosette-shaped calcium oxalate crystals13 Mesofil 13 Stomata13 Table 1. Moringa Leaf Simplicia Powder Fragment (Moringa oleifera L) 70 extract **is not less than** 9.2% so that all extracts obtained meet the requirements. 13 3.3. Phytochemical screening moringa leaf **extract and ethyl acetate fraction** After the phytochemical screening was carried out to

determine the class of secondary metabolite compounds the This is qualitative of chemical compounds in Moringa plant.

The screening tests carried out were flavonoids, saponins tanins because this test already represented several groups of compounds contained in the Moringa plant. results that the extract Moringa was positive flavonoids, saponins and tanins as shown in Table 3. 9 3.4. **Determination of total flavonoid content of Moringa leaf extract and ethyl acetate fraction** Analysis the flavonoid in this study was carried out using **a standard solution of quercetin** in Table 4. The readings were carried out at a wavelength of 413.15 nm with an operating time of 30 minutes according to previous studies. 10 The determination of quercetin wavelength in Figure 2.

Determination of operating time in this study aims to determine the reaction time flavonoid with can react perfectly so that it is optimum Method Thick Extract Rendemen Maceration Moringa Leaf Ethanol Extract 70% (MLEE 70%) 16.09 % Maceration Moringa Leaf Ethanol Extract 96% (MLEE 96%) 23.80 % Fractionation Moringa Leaf Ethyl Acetate Fraction (EFML) 17.89 % Table 2 . Calculation **of Moringa Leaf Extract** and Fraction Yield Screening MLEE 70 % MLEE 96 % EFML Treatment Results Flavonoid + + + Ammonia Vapor Intensive Ye I I ow Saponin + + + Shaking Stable Foam 30 second Tanin + + + FeCl₃ 1% Formed blackish green color Alkaloid + + + Mayer Sediment Table 3 . Phytochemical Screening Moringa Leaf **Extract and Ethyl Acetate Fraction** Figure 2 . Determination of Quercetin Wavelength in **Determination of Total Flavonoid Levels** 71 when the absorption is read with Visible Spectrophotometry.10 Figure 3 showed the standard curved of quercetin. The of total content shown in Table 5 resulted in the highest content in ethanol of leaves to ethanol and fraction of leaves.

flavonoid obtained the extract Moringa leaves 70%, extract 96% the fraction Moringa is 10.84%; and This that flavonoid contained MLEE 70%, MLEE 96% and **the ethyl acetate fraction of the** 70% **ethanol extract of Moringa leaves** can said be high and have the potential to be developed and explored related biological especially as an antioxidant. 6 Moringa plants the characteristics of an oval shape with **all parts of the** same width, the upper part of the leaf is light green, the edges are split, the underside of the leaves is rounded, the leaf edges are flat, the leaf surface is rough and the leaf spines are pinnate. The cross-sectional sections of the leaves consisted of the lower epidermis, bundle vessels, collenchyma, spongy tissue, upper epidermis and palisade.

13 is extraction that carried by immersing simplicia powder using a solvent that is suitable for the immersion process, stirring extract certain The solvents used in this study were 70% and 96% ethanol. Ethanol is a solvent that is polar, non-toxic and has the advantage of being able to more compounds methanol and water. 14 The choice of

solvent used is based on the nature of the compound to be taken dissolves meaning that if the compound to be taken is polar, the solvent used must be polar so that the active substance will be completely dissolved into the solvent.¹⁵ According to research by Siluh et al.,¹⁶ previously that compounds, flavonoids, be completely in solvent.

The stirring process in the maceration method aims to take a certain substance by breaking the walls and cell membranes due to the difference pressure and the cell.¹⁷ use solvents different Quercetin standard (ppm) Absorbance 20 0.173 40 0.281 60 0.401 80 0.534 100 0.655 Table 4 . Quercetin Standard Curve for Determination of Total Flavonoid Level Figure . of Standard Regression on of Flavonoid Level 72 concentrations is carried out to determine differences the of metabolite in extract the higher the concentration of a solution, the more substances be so that the percent yield value of the extract will be greater.¹⁸ Concentration of the filtrate using a vacuum rotary evaporator for 1 hour at a temperature 60°C a of rpm produces thick which be further.

Calculation of the yield of a sample is related to the active compounds contained in the so the the value, the more active compounds are taken.¹⁹ Figure 3, the curve shows that the higher the concentration of the solution, the higher the absorbance value obtained, with the linear regression $y = 0.0061x +$ and the value of $R = 0.9999$. The calculated R value $> R$ table = 0.8783 (degrees of freedom 3; $<$ so linear equation can used calculate total content in this study.²⁰ The limit of detection (LOD) is ppm the of quantification (LOQ) value is 1.396 ppm.

The limit detection states smallest amount of analyte in the sample that can be detected still a response compared the While limit quantization is smallest of analyte in the sample that still meets the criteria carefully and thoroughly and can be quantified with good accuracy and precision.²¹ Determination total levels with compounds quercetin added $AlCl_3$, in complex compounds shown in Figure 1. This test was carried out using UV-Vis spectrophotometry with principle complex formation reactions, in which there was a shift in the reading of visible light waves marked by the color of the solution.

yellowish and the addition of acetic acid to maintain the wavelength in the visible light region.¹⁰ The hydroxy contained flavonoid will with to complex 8 hydroxyl group in in almost always located on ring B at the 3' and positions, also with $AlCl_3$ form complexes. research can be an informative basis that the flavonoid group in Moringa leaves plays a role in producing medicinal raw materials derived from natural ingredients with standardized levels of identity compounds.^{7 4.}

Conclusion The total content was in 96% extract Moringa (16.69 0.74% in accordance with the 2017 Indonesian Herbal Pharmacopoeia, the extract Moringa contained less

6.30% (w/w) total flavonoids calculated as quercetin. 5. Acknowledgement The author would like to thank the APTFI Perguruan Farmasi Indonesia), Ministry Higher Education DIKTI), Dikti Region V, Alma Ata University, Tim Research Table 5 . Results of **Determination of Total Flavonoid Levels of** MLEE and EFML Sample Replication
Flavonoid Total (% b/b) X ± SD (% b/b) MLEE 70% 1 10.60 10.84 ± 0.49 2 10.84 3 11.09
MLEE 96% 1 16.66 16.69 ± 0.74 2 16.34 3 17.07 EFML 1 13.63 14.15 ± 0.90 2 14.45 3
14.37 73 Moringa Leaf and all participant that we can't mention one by one.

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