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ISOLATION OF MORINGA LEAF FLAVONOIDS (*Moringa oleifera* L.) USING COLUMN CHROMATOGRAPHY

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Abstract: Preface : Moringa oleifera L. is one plant that contains secondary metabolite compounds such as alkaloids, steroids, tannins, saponins, anthraquinones, terpenoids, and flavonoids. Flavonoids donate their hydroxyl groups to inhibit free radicals. **Purpose :**This study aimed to isolate flavonoid compounds from Moringa leaf ethanol extract. **Method :** Extraction was carried out by maceration method and continued isolation using Column Chromatography method with n-butanol: acetic acid: water (4: 1: 5) eluent. Isolate results were identified by TLC, spectro, and FTIR. **Result :** FTIR analysis on fraction 5 contains flavonoid derivative compounds. **Conclusion :** It is flavonols with a purity of quercetin of 77.99%.

Keywords: *Fractionation, Total flavonoid content, FTIR, HPLC, Moringa.*

INTRODUCTION

Indonesia is a developing country rich in biodiversity. Where there are various types of plants, one of which is Moringa (Moringa oleifera L.). Moringa is a plant commonly used for spices, cosmetic oils, and various medicinal therapies (Shivani and Meenakshi, 2014). This plant is empirically used by the community to increase nutrition, treat diabetes mellitus, and as an anticancer (Krisnadi, 2015).

Almost all parts of the Moringa plant can be used as a treatment so it is known as a "miracle tree". Plants can be useful as drugs because they contain secondary metabolite compounds. One of the secondary metabolite compounds contained in Moringa leaves is flavonoids (Farooq et al., 2012). The most abundant secondary metabolites found in M. oleifera are tannins, and polyphenols that have functions in protecting plants from herbivores and regulating seed germination, as well as in plant growth. The main flavonoids in M. oleifera are biflavonyl, and delphinidine obtained through methanol extraction (Sankhalkar et al., 2016).

The content of flavonoids in plants can help the process of photosynthesis, antimicrobial, and antiviral. Antioxidation activity is also possessed by certain flavonoid compounds that can be used to inhibit bleeding and antiscoring. Flavonoids are useful for humans as antibiotic, anticancer and kidney disorders. Flavones are included in the flavonoid group that functions as a stimulant in the heart, hydroxylated works as a diuretic and as an antioxidant in fat. In addition, several types of flavonoids such as slimirin and silyburn are proven to treat liver function disorders, inhibiting prostaglandin synthesis which can work as hepatoprotectors (Ullah et al. 2020).

Test results of total flavonoid levels of ethanol extract 96% Moringa leaves more than 8 % w/b (Susanty et al., 2014). Therefore, identification research on the class of flavonoid compounds from the butanol fraction: acetic acid: water needs to be done.

METHODS

Moringa Leaf Extraction

Moringa leaf powder as much as 500 grams macerated with 96% ethanol solvent as much as 1500 mL for 3x24 hours at room temperature and occasionally stirred. Then it is filtered to obtain its filtrate. The filtrate is evaporated using a rotary evaporator at 40oC until a viscous extract is formed.

Isolation of Moringa Leaf Extract Flavonoid

The mobile phase used is n-butanol:acetic acid:water (4:1:5) and the stationary phase used is silica gel 60 GF254. The chromatographic column is filled with cotton, stationary phase that has been moistened with mobile phase. The hatching of the chromatographic column is set at a flow rate of 1 mL/min. The fractions obtained from the separation of column chromatography were then identified using Thin Lapir Chromatogration (TLC) to detect fractions containing flavonoids.

Thin Layer Chromatography (KLT) Test Flavonoids

Identification of flavonoid compounds of the mobile phase of glacial acetic acid: butanol: water (1:4:5), with the appearance of ammonia vapor stains. The positive reaction was shown to be blue after being vaporized by ammonia on observations with visible light at UV 366nm confirming the presence of flavonoid content (Putu et al., 2017).

Isolate Structure Analysis

Isolates were identified using UV-Vis, FTIR and HPLC spectrophotometers.

RESULT AND DISCUSSION

Extraction results

Moringa leaf powder extraction (Moringa oleifera L.) uses a cold extraction method, namely maceration method. The solvent used is 96% ethanol, because ethanol is polar so it is able to extract polar compounds (Hidayah et al., 2016). Phenol and flavonoid compounds are polar compounds so that they are easily extracted in polar solvents, according to the principle of like dissolve like (Kemit et al., 2010). The yield of viscous extract obtained during the extraction process was 51.75 grams with a yield yield of 10.37%. This result is in line with the research of Vongsak et al. (2012) which states that the yield of Moringa leaf extract is 9.98%-25.02%.

Isolation of Moringa Leaf Extract Flavonoid

1 g of Moringa leaf extract was dissolved with eluents, then isolated by column chromatography. The eluent used is n-butanol:acetic acid:water (4:1:5) and the stationary phase used is silica gel GF254. The resulting fraction is 12 fractions. There are 6 fractions that show spots after in KLT.

Flavonoid test results on fraction 1, fraction 2, fraction 3, fraction 4, fraction 5, and fraction 6 showed positive results for flavonoid compounds. All six fractions have a yellow-black color. The results of the flavonoid test on the results of the study are in accordance with Hanani (2017) which states that the light blue color fluoresce is suspected as a flavonoid. In addition, judging from the Rf value, the stain has an Rf value of 0.56 on figure 1, where according to research conducted by Forestryana and Arnida (2020), states that the Rf value of flavonoids is around 0.54.

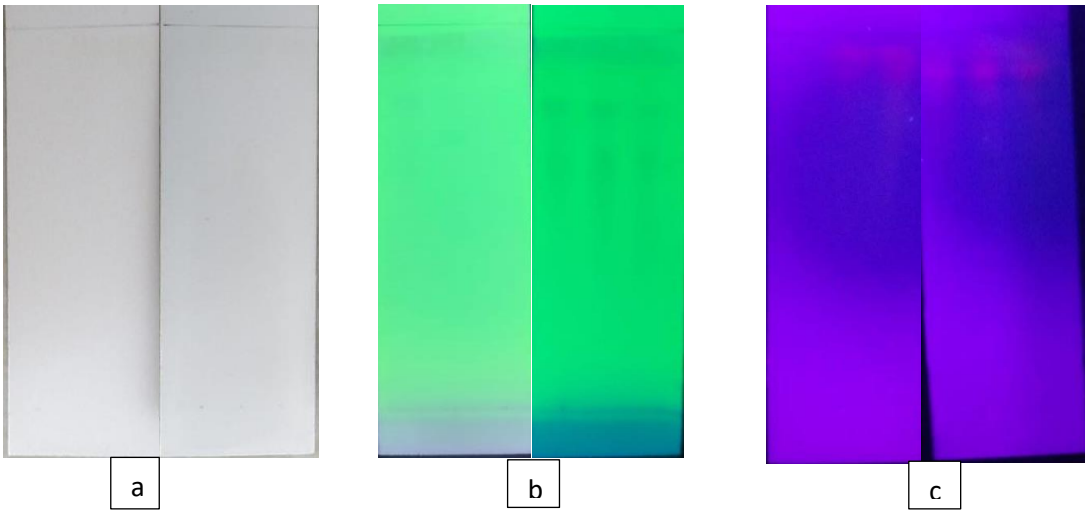

Figure 1. Fraction monitoring thin-layer chromatogram, stationary phase of silica gel GF254, n-butanol:acetic acid:water (4:1:5) mobile phase, spot appearance (a) before spraying with 10% AlCl3, (b) λ 254 nm UV light, (c) under λ 366 nm UV light after spraying with 10% AlCl3.

The analysis of the total flavonoid content was determined by the colorimetric method of aluminum chloride. The basic principle of the aluminum chloride colorimetric method is that aluminum chloride will form a stable acid complex with a keto group on the C-4 atom and also with a hydroxyl group on the neighboring C-3 or C-4 atom of flavones and flavonols (Parthasarathi and Park, 2015). In addition, aluminum chloride will form a labile acid complex with an ortho-dihydroxyl group on the A or B ring of the flavonoid structure, so that the wavelength shift towards the visible range marked by the solution results in a yellower color. The addition of CH3COOK aims to maintain the wavelength in the visible area. The complex reaction between AlCl3 and flavonoid compounds will produce a yellow color (Elfahmi et al., 2018).

The total flavonoid content carried out by Sulastri et al., (2018) and Fatriyah et al., (2020), stated that Moringa leaf extract has a total flavonoid content value of 8.90 – 10.48 mg QE/gram extract. The results of determination total flavonoid contained in fractions of Moringa leaf ethanol extract with fraction numbers 1, 2, 3, 4, 5, and 6 respectively, is 0.10; 6,35; 7,33; 3,86; 17,98; and 13.32 mg QE/gram extract. The difference in the total flavonoid content produced, is possible because the solvents used are different. This is in line with research by Yani et al. (2023) that differences in solvents affect total flavonoid levels, semipolar solvents that can attract flavonoid compounds that are polar and nonpolar. In plants, there are several free flavonoids such as flavones, flavonons, and flavonols that are easily soluble in semi-polar solvents.

The isolate chosen by the researchers was isolate 5 because it contained the highest phenolic group and the separation of a single compound. Then the isolate results were identified with UV-Vis spectrophotometers, FTIR, and HPLC.

Identification using a UV-Vis spectrophotometer is used to determine whether the isolate results obtained are flavonoid groups or not. The results of spectral analysis using a UV-Vis spectrophotometer, a solvent used methanol with a wavelength range of 200-800 nm. The results of the UV-Vis spectrophotometer obtained two wavelengths detected in isolate 5, is 274.0 nm and 653.0 nm with absorbance values of 1.669 and 0.122 respectively. Based on the measurement results, spectra 274 nm is an electron transition π→π * double bond C = C (Maharani et al., 2016). According to Markham (1988) the maximum wavelength of flavonoids in band II has a range of 230-285 nm and band I in the range of >380 nm.

Identification of compounds using FTIR aims to determine the functional group of a compound that produces the characteristic band of the compound. The molecule of a compound that intracts with energy causes a transition that causes molecular vibrations. The typical band generated from the FTIR results shows the functional group of each bond in the molecule of a compound.

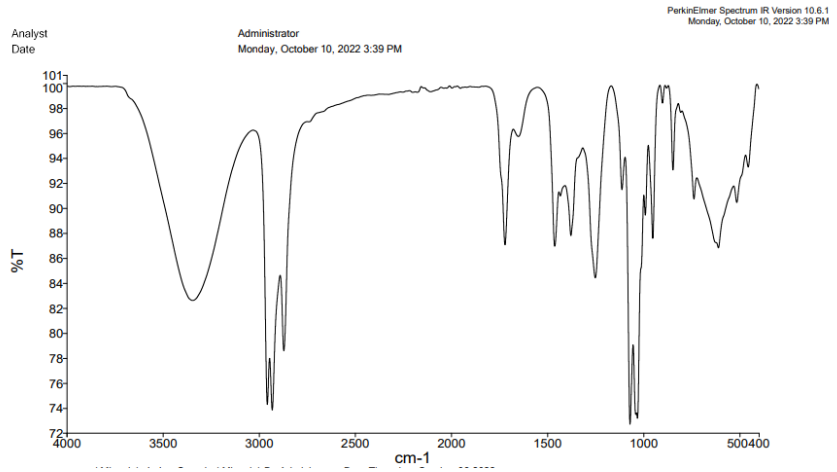

Figure 2. FTIR Isolate Spectra

Based on the interpretation in Figure 2 and Table 1. The results of the absorption band analysis of isolate 5 have the same absorption band found in O-H stretching vibrations, Csp 3 -H alkane stretching vibrations, C=C stretching vibrations, overtune absorption which shows characteristics of aromatic compounds, and C-O alcohol stretching vibrations. Indicates that the functional group identified from the FTIR wavelength result isolate 5 indicates the presence of a functional group of flavonoid compounds. Typical absorption in functional groups, namely C-H and C=C aromatics, OH, C=C, and C-O alcohols are positive results of flavonoids when identified using FTIR (Nandiyanto et al, 2019). FTIR analysis shows that Moringa leaves may contain compounds from flavonol derivatives.

Table 1. FTIR Isolate Spectra Analysis

No	Absorbance (cm^{-1})	Group	Class	Reference
1	3349.14	O-H *stretching*	Hidroksil	3550-3200
2	2873.11	C-H *stretching*	Alkana	3000-2840
3	1716,36	C=O *bending*	Karbonil	1725-1705
4	1651.89	C=C *stretching*	Aromatis	1650-1475
5	1462.29	C-H *bending*	Alkane	1500-1375
6	1251,02	C-O *stretching*	alkyl aryl ether	1300-1100
7	901,2	C=C *bending*	Alkene	980-960

Quercetin is one of the flavonol derivative compounds. Isolate is analyzed using HPLC to determine the purity of quercetin compounds obtained. Quercetin is characterized by the presence of a hydroxyl group and a carbonyl group. Research conducted by Jimenez (2017), Moringa leaves contain flavonoid compounds with quercetin type. Supported by research conducted by Lin et al, (2018), Moringa leaves contain flavonoid compounds with quercetin type.

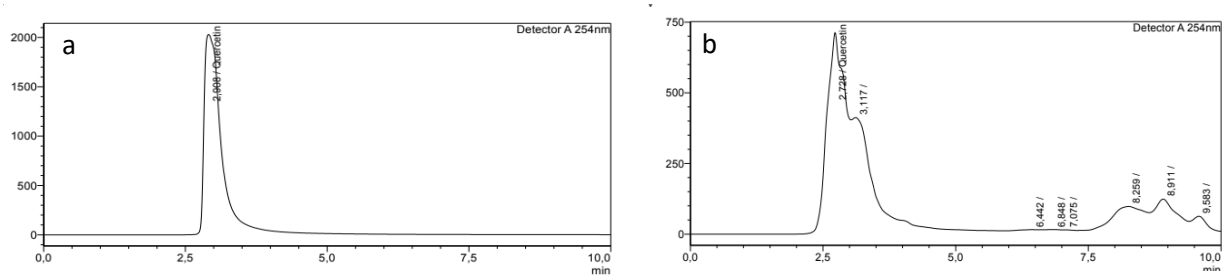

Figure 3. HPLC analysis results, (a) quercetin standard, (b) isolate 5

The chromatogram results of isolate 5 can be seen in Figure 3. which contains one acquired main peak. At the peak of this chromatogram, it also shows the retention time at the 3rd minute. Based on the results of the chromatogram standard, quercetin has an outer 44763473 area and a sample area of 17455781 with a dilution of 2 times, so that the purity of the isolate against quercetin is 77.99%. This HPLC analysis shows that Moringa leaves may contain quercetin derivatives which are characterized by the same retention time between the sample and the quercetin standard which is at the 3-minute retention time and has a peak pattern that is almost the same as the quercetin standard standard.

CONCLUSION

Isolation of flavonoids obtained there are 6 fractions. Fraction 5 was identified using a UV-Vis and FTIR spectrophotometer to obtain the result that the isolate contained flavonol-derived compounds. Compared to quercetin, the purity of isolate 5 is 77.99%.

AUTHOR CONTRIBUTION

All parties involved in the preparation of this article can be written in the author's contribution.

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