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Identification and analysis of chemical compounds in mulberry leaf extract (*Morus alba*) by spectrophotometer method

Dwi Kartika Wati[1], Zhahrotun Khofifah Nur Achyar[1], Nur Aziza[1], Luluk Mudzakaroh[1], Lidia Wahyu Pratiwi[1], Armanda Bella Paramitha[1], Majida Ramadhan[1*], Nafisa[1], Seftya Mahyantika[1]
[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Islam Malang
Jl. MT. Haryono No. 193, Malang, East Java, Indonesia. 65411
*Email: majida.ramadhan@unisma.ac.id

ABSTRACT. Mulberry leaves (*Morus alba* Linn.) are a plant whose potential has been widely studied because it contains phytochemical compounds such as flavonoids and phenols which have various health benefits. This plant belongs to the Moraceae family and has the characteristics of a woody trunk, branches and brown bark with a height of between 5 and 10 meters, has oval or heart-shaped leaves with tapered tips, and has oval-shaped fruit that is black when ripe. This research aims to analyze chemical compounds in mulberry leaf extract (*Morus alba* L.) using spectrophotometric methods. Phytochemical screening showed positive results for flavonoids (Shinoda and NaOH test), alkaloids (Wagner and Dragendorff test), tannins (Braymer's solution and base) test, and steroids (Salkowski test). UV-Vis spectrophotometric analysis at a wavelength of 200-800 nm shows maximum absorption at 786 nm for the 10-2 dilution sample. Analysis of total flavonoid content using standard quercetin showed concentrations of 50.06 mg/g and 52.87 mg/g for the 1000 ppm and 500 ppm samples, respectively. The extract yield was 29.086%, with a water content of 4.415% and an ash content of 0.1%. These findings indicate that mulberry leaf extract contains various bioactive compounds with potential pharmacological properties, especially flavonoids which can be useful for medicinal applications.

Keywords: ethanolic extract; flavonoid content; *Morus alba*; phytochemical screening; spectrophotometric analysis

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INTRODUCTION

Indonesia has a lot of biodiversity and potential that is widely used in the health sector. Plants in Indonesia have long been used as traditional medicines. Plants used as traditional medicines are plants that have secondary metabolite compounds (Sapitri *et al*., 2022). Secondary metabolite compounds can be flavonoids, alkaloids, saponins, polyphenols, triterpenoids and other metabolite compounds found in plants (Kopon *et al*., 2020).

One of the plants in Indonesia that has secondary metabolite compounds is mulberry leaves (*Morus alba* Linn.). Mulberry leaves (*Morus alba* Linn.) are plants whose potential has been widely studied because they contain phytochemical compounds such as flavonoids and phenols which have various benefits in the health sector (Latifah *et al*., 2022). This plant is included in the Moraceae family and has the characteristics of a woody, branched and brown bark with a height of between 5 to 10 meters, has oval or heart-shaped leaves with tapered ends, has oval-shaped fruits with black color when ripe (Badruzaman, 2021). Mulberry plants grow in tropical climates such as Indonesia. This plant can easily grow in home yards, valley areas and coastal areas. This plant can easily adapt. It can grow at an altitude of 0-3300 meters above sea level with an average annual temperature ranging from 0-40°C with an average annual rainfall of 1500-2500 mm (Purnama & Astutik, 2024). Mulberry leaves contain active compounds such as alkaloids, flavonoids, polyphenols and terpenoids. In addition, mulberry leaves have a chemical content rich in anthocyanins, phenolics and fatty acid components (Pogaga *et al*., 2020).

The content of compounds in the leaves of mulberry plants can be taken advantage of in the field of pharmacology by making extracts from the leaves of these plants (Putri, 2023). To take secondary metabolites in plants, the right solvent is needed. One solvent that can be chosen is ethanol. Ethanol

was chosen as a solvent because ethanol is a solvent that can extract secondary metabolites. attract phenolic compounds optimally in plant extracts (Kumalasari & Andiarna, 2020). One method that can be used to analyze the content of secondary metabolites in plants is the Spectrophotometer method. The spectrophotometer method has several main advantages, namely being able to measure uptake with precision, making it suitable for quantitative analysis of compounds (Lorenza *et al*., 2021). In addition, the spectrophotometer method is also a method that is classified as having a fast measurement process and allows analysis in a short time (Mutiananda, 2023). The spectrophotometric method does not require harmful reagents, making it safer to use in various applications (Putri & Asnandi, 2021).

Based on the description above, the phytochemical test of ethanol extract of mulberry leaves (*Morus alba* Linn.) using spectrophotometric method was conducted. This research was conducted to determine the content of chemical compounds and total flavonoids in mulberry leaves (*Morus alba* Linn.) that can be utilized. The findings of this study are expected to contribute to the development of natural antioxidant sources and support further exploration of mulberry leaves as potential raw materials for nutraceuticals, functional foods, or herbal formulations.

MATERIALS AND METHODS

This research is a laboratory observation research with quantitative descriptive method conducted at the Integrated Laboratory of the Islamic University of Malang in October-November 2024. The tools used in this study are test tubes, test tube racks, labels, dropper pipettes, measuring cups (Pyrx), beakers (Pyrx), hot plates, cuvettes (Eppendorf), Uv-Vis Spectrophotometer (Thermo Scientific GENESYS), and Fourier Transform Infrared (FT-IR) Spectra (Thermo Scientific). While the materials used are mulberry leaf extract, Mayer's Reagent, Wagner's Reagent, Dragendorff's Reagent, Bourchardat Reagent, 96% ethanol, concentrated HCI, Mg powder, 10% NaOH, distilled water, 10% FeCl3, 10% NH_4OH, 10% H_2SO_4, Lieberman Bourchard Reagent, 10% $AlCl_3$, Quercetin, CH_3COONa 1M (SmartLab).

Mulberry leaf sampling. Samples of mulberry leaves (*Morus alba* Linn.) were obtained from Rusunawa 2 Islamic University of Malang. The sampling method used purposive sampling technique. The leaves taken are intact leaves that are green, not brownish, and not yellowish with varying sizes (Syahara & Siregar, 2019).

Preparation of mulberry leaf simplisia. Samples were wet sorted by separating the leaves from contaminants and then washing. Mulberry leaf samples are dried by oven for 2×24 hours at 60-65°C. Then blended or ground into powder. The powder is then sieved to get a powder size of 100 mesh to expand the surface of the sample, so that the interaction with the solvent and extraction of active compounds is more optimal (Hidayatullah *et al*., 2024).

Determination of water content. Water content testing was carried out by weighing 1 gram of mulberry leaf simplisia samples and then inserted into the moisture analyzer (AnD MX-50) and waited for approximately 10 minutes until the lights went out indicating that the water content analysis process was complete. The maximum allowed moisture content limit contained in simplisia is 10% in accordance with the regulations of the Ministry and Health of the Republic of Indonesia (Ulfah *et al*., 2022).

Determination of ash content. Analysis of ash content was carried out by weighing 1 gram of careful simplisia (W1) in a silicate crew that had previously been incinerated and weighed (W0). After that, the simplisia was incinerated using a furnace slowly (with the temperature gradually increasing to 600 ± 25ºC) (Ulfah & Mulangsari, 2018). Ash content was calculated with the equation:

$$total\ ash\ content\ (\%) = \frac{W2 - W0}{W1} \times 100\%$$

Description:
W0 = Weight of silicate crucible that has been refined
W1 = Weight of initial simplisia sample

W2 = Weight of the sample after it has been quenched

Extract preparation. The extraction began by weighing 60 grams of fine simplisia and then extracted by maceration method using 96% ethanol solvent and distilled water until the simplisia was completely submerged in a ratio of 1:10 for 2x24 hours. Maceration was carried out for 24 hours in a reagent bottle while occasionally stirring in the hope that all organic compounds might be attracted to 96% ethanol solvent and distilled water. The results of maceration were filtered to obtain the filtrate and the residue which was macerated again with ethanol, this was done twice. The filtrate obtained was collected and then concentrated with a rotary evaporator until a thick extract was obtained (Dewi, 2020).

Extract characterization and yield calculation. The yield was obtained by comparing the weight of mulberry leaf extract and the weight of the simplicisa before extraction. Then the extract yield is calculated using the following formula (Ramadhan *et al*., 2019).

$$Extract\ yield = \frac{extract\ weight\ (g)}{simplified\ weight\ (g)} \times 100$$

UV-Vis spectrophotometry. UV-Visible Spectrophotometer characterization was performed to determine the absorbance peak and wavelength of the sample (Ginting & Dwandaru, 2021). Measurements were performed using a Thermo Scientific GENESYS UV-Vis spectrophotometer with a wavelength of 200-800 nm.

FT-IR characterization. FT-IR (*Fourier Transform InfraRed*) characterization was performed to identify potential biomolecules in mulberry leaf extract (Sonia, 2024). Mulberry leaf extract was characterized for its functional groups using an IR spectrophotometer. Fourier Transform Infrared (FT-IR) spectra were analyzed using a spectrometer in the range of 4000-500 cm-1. Measurements were performed using transparent KBr pellets that were pressed, then the pellets were placed on the IR and the wavelength was determined. A mixture of KBr and soursop leaf extract was also made into transparent pellets that were pressed, which were then placed in the FTIR device for measurement (Ramadhan *et al*., 2019).

Alkaloid screening. Alkaloid screening was performed using Mayer's test, Wagner's test, Dragendorff's test, and Bouchardat test. **Mayer's test:** Reaction tubes are prepared and labeled (write the sample name and test). Then, 20 drops of liquid extract from the plant are added to the reaction tube. Next, 10 drops of Mayer's reagent are added (drop by drop) to the reaction tube through the wall of the tube containing the liquid extract while gently shaking it. Observe what happens. Record the observation results. A positive alkaloid result will form a yellowish color along with a white or yellowish-brown precipitate (Tiara *et al*., 2024). **Wagner's test:** Prepare and label the test tubes (write the sample and test names). Add 20 drops of liquid extract from the plant into the test tube. Then add 10 drops of Wagner's reagent (drop by drop) into the test tube containing the liquid extract while gently shaking it. Observe what happens. Record the observation results. A positive alkaloid result will form a brownish-red precipitate (Tiara *et al*., 2024). Dragendroff's test: Prepare and label the test tubes (write the sample name and test name). Add 20 drops of liquid extract from the plant into the test tube. Then add 10 drops of Dragendorff's reagent (drop by drop) into the test tube containing the liquid extract while gently shaking it. Observe what happens. Record the observation results. A positive alkaloid result will form an orange or yellow precipitate (Tiara *et al*., 2024). **Bouchardat test:** The Bouchardat test interacts with the nitrogen atoms in the alkaloid structure. However, the reagents used and the mechanism of complex formation are slightly different. The Bouchardat reagent contains a solution of iodine in potassium iodide. When this reagent interacts with alkaloids, a blackish-brown precipitate forms. The formation of this precipitate indicates the presence of a bond between the iodine atoms in the reagent and the nitrogen atoms in the alkaloid molecule. The intensity of the color and the amount of precipitate formed can serve as qualitative indicators of the presence of alkaloids in the sample (Tiara *et al*., 2024).

Flavonoid screening. Flavonoid screening was carried out using the Shinoda test and natrium hidroksida 10% test. Shinoda test: Prepare and label the test tubes (write the sample name and 3 tests). Add 20 drops of liquid extract (isopropyl alcohol) from the plant into the test tube. Add 20 drops of absolute ethanol and then add 3 drops of concentrated hydrochloric acid. The formation of a red color indicates the presence of auron and chalcone. If no color is present, add 1 small spatula of magnesium powder to the reaction tube containing the liquid extract while gently shaking it. A positive result for flavonoids is indicated by the formation of a pinkish-red color (orange, red, or magenta indicating the presence of flavon and flavonols) (Innaya *et al*., 2024). Natrium hidroksida 10% test: Test with 10% NaOH by placing the extract in a test tube, adding 2-4 drops of 10% NaOH solution, and observing the color change until it turns yellow to brownish yellow (Innaya *et al*., 2024).

Saponin screening. Saponin screening was conducted using the foam test. The sample extract is placed in a test tube, 10 mL of hot water is added, and shaken for approximately 1 minute. Saponin is positive if stable foam forms for at least 10 minutes, reaching a height of 1 to 10 cm (Innaya *et al*., 2024). Tannin (polyphenol) screening was conducted using Braymer's test and the alkaline solution test. Braymer's test: Prepare the test tubes and label them (write the sample name and test name). Add 20 drops of liquid extract from the plant into the test tube. Then add 10 drops of 10% $FeCl_3$ into the test tube containing the liquid extract through the side of the test tube while gently shaking it. Observe what happens. Record the observation results. A positive result for tannins (polyphenols) forms a blackish-green color (Innaya *et al*., 2024). Alkaline solution test: Prepare and label the test tubes (write the sample and test names). Add 20 drops of liquid extract from the plant (both leaves and twigs) to the test tubes. Then, 20 drops of 10% ammonium hydroxide (NH_4OH) solution are added to the reaction tube containing the liquid extract through the side of the tube while gently shaking it. Observe what happens. Record the observations. A positive result for tannins (polyphenols) is indicated by the formation of a yellowish glow (Andrianto, 2017).

Steroid screening. Steroid screening was conducted using the Salkowski test. Prepare and label the test tubes (write the sample and test names). Add 20 drops of liquid extract (isopropyl alcohol) from the plant into the test tubes. Add 20 drops of chloroform to the mixture, followed by 10 drops of sulfuric acid (H_2SO_4) slowly along the wall of the test tube. Observe: if a brown ring forms in the middle of the mixture, this indicates the presence of steroids (Innaya *et al*., 2024).

Terpenoid screening. Terpenoid screening was conducted using the Liberman Bouchard test. Place 1 mg of solid ethanol sample on a drop plate, add 6 drops of anhydrous acetic acid, then stir with a spatula until dissolved. Next, one drop of concentrated H_2SO_4 is added. If a purple to orange color forms in the solution, it indicates the presence of triterpenoid compounds, while a blue or green color indicates the presence of steroid compounds (Innaya *et al*., 2024).

Total flavonoid test. The total flavonoid test was carried out through several stages, namely: (1) Finding the highest wavelength. Take 0.5 mL of quercetin solution at a concentration of 100 ppm, then add 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M CH_3COONa, and 2.5 mL of distilled water. The solution was homogenized using a vortex mixer and incubated for 40 minutes in a darkened area. The maximum wavelength was measured using a spectrophotometer with an absorbance range of 415–440 nm; (2) Preparation of quercetin standard solution. A total of 10 mg of quercetin was dissolved in 96% ethanol with a volume of 10 mL. One milliliter of the 1000 ppm stock solution was taken and then diluted with 96% ethanol to 10 mL, resulting in a test concentration of 100 ppm. Then, 2, 4, 6, 8, and 10 mL were pipetted and dissolved in 96% ethanol to a volume of 10 mL, resulting in final concentrations of 20, 40, 60, 80, and 100 ppm. At each concentration, 0.5 mL of quercetin solution was taken, then 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1M CH_3COONa, and 2.5 mL of distilled water were added. The solution was homogenized using a vortex mixer and incubated for 40 minutes in a dark place. It was then tested using a spectrophotometer at a maximum wavelength of 434 nm; and (3) Determination of total flavonoids. A total of 10 mg of test sample was dissolved in 96% ethanol with a volume of 10 mL. One milliliter of the 1000 ppm stock solution was taken and then diluted with 96% ethanol to 10 mL, resulting in a test concentration of 100 ppm. Pipette 5 mL and dissolve with

96% ethanol to a volume of 10 mL, resulting in a final concentration of 50 ppm. At each concentration, take 0.5 mL of the test sample solution, then add 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M CH_3COONa, and 2.5 mL of distilled water. The solution was homogenized using a vortex mixer and incubated for 40 minutes in a darkened area. It was then analyzed using a spectrophotometer at a maximum wavelength of 434 nm. The total flavonoid content in the extract was expressed in milligrams of quercetin equivalent (QE)/gram of fresh sample.

RESULTS AND DISCUSSION

Moisture content testing aims to prevent rapid fungal growth in the extract (Rosmayati *et al*., 2023). Based on the test results, the moisture content obtained was 4.415%. This indicates that the mulberry leaf extract produced meets the moisture content standard of below 10%, which is in accordance with the regulations set by the Indonesian National Standard (SNI) (Indonesian Ministry of Health, 2017). Meanwhile, ash content testing yielded a value of 0.1%, indicating that mulberry leaf crude drugs contain many minerals. Ash content determination was carried out to provide an overview of internal and external mineral content originating from the initial process to the formation of the extract (Mewar & As`ad, 2023).

The results of phytochemical screening and extraction yield of mulberry leaves (*Morus alba* L.) revealed several characteristics of secondary metabolites successfully identified. The extraction yield obtained was 29.086%, indicating that the extraction process using 96% ethanol solvent was sufficiently effective in extracting bioactive compounds from mulberry leaf crude material. This high yield value indicates that mulberry leaves contain compounds that are soluble in polar solvents such as ethanol (Wahyuni & Hertiani, 2016). According to Hasanah *et al*. (2017), the high yield value is also influenced by the selection of extraction methods and solvent types that are suitable for the polarity of the target compounds.

UV-Vis spectrophotometer. Analysis of bioactive compounds in plants using UV-Vis spectrophotometry is effective for quantitatively analyzing the content of metabolites in plants (Nurulhadi *et al*., 2024). The analysis was performed using wavelengths of 200–800 nm, as shown in Figure 1.

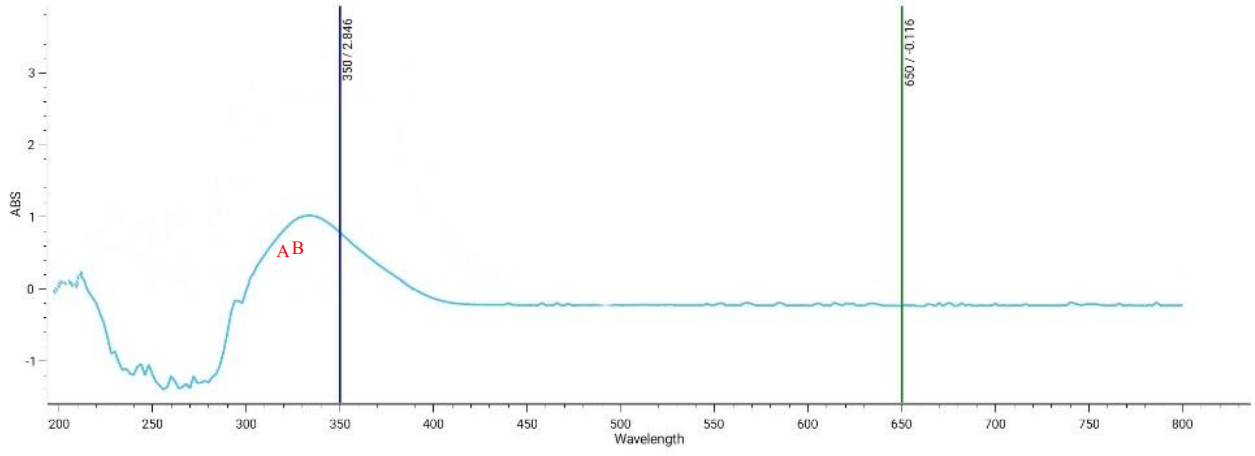

Fig. 1. Absorbance readings of mulberry leaf extract on a UV-Vis spectrophotometer with a wavelength of 200-800 nm: Sample 2 with a dilution of 10-2 (blue line)

The samples used were mulberry leaf extracts with dilutions of 10^{-1}, 10^{-2}, and 10^{-3}. The absorption results obtained from sample 2 with a dilution of 10^{-2} showed an absorption of 0.786 nm. Based on Fig. 1, the UV-Vis spectrophotometry graph of the mulberry leaf extract shows two peaks. The wavelength at peak A is 310 nm, while peak B has a wavelength of 330 nm. These results indicate

the characteristic spectrum of isoflavone flavonoids, which have two peaks in the wavelength range of 310-330 nm. (Sosang *et al*., 2016).

FT-IR test. FTIR spectroscopy is a fast, simple, and non-destructive analytical technique that allows all chemical properties in a sample to be traced and displayed in spectra. FTIR is performed in the mid-infrared wavelength range (wavelength numbers 4000–400 cm^{-1}). FTIR analysis is used to examine the absorption spectrum of each sample, where the absorption data detected at wavenumbers 4000–400 cm^{-1} is then analyzed to identify functional groups at each detected wavelength (Puspitasari *et al*., 2021). The results of the functional group identification of secondary metabolite compounds in ethanol extracts of mulberry leaves (*Morus alba* Linn.) using FTIR can be observed in Fig. 2.

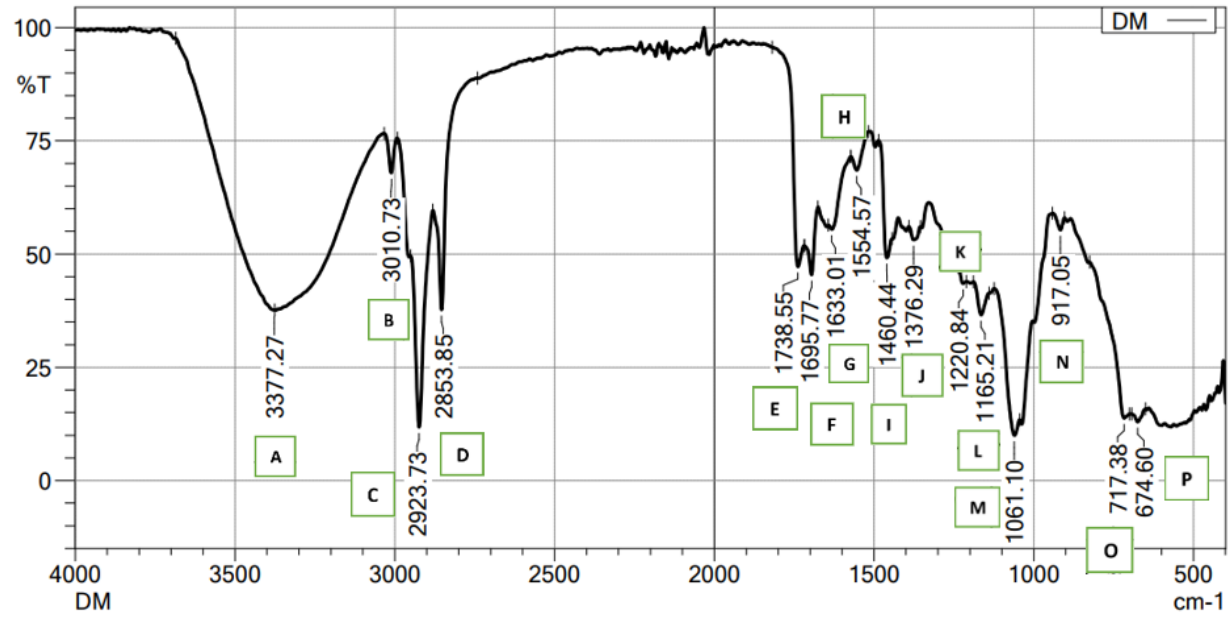

Fig. 2. IR spectrum of ethanol extract from mulberry leaves (*Morus alba* Linn.): A. 3377.27; B. 3010.73; C. 2923.73; D. 2853.85; E. 1738.55; F. 1695.77; G. 1633.01; H. 1554.57; I. 1460.44; J. 1376.29; K. 1220.84; L. 1165.21; M. 1061.10; N. 917.05; O. 717.38; P. 674.60

Based on infrared (IR) spectrum analysis of ethanol extracts from mulberry leaves (*Morus alba* Linn.), several functional groups were suspected to be present, indicating the characteristics of alkaloids, flavonoids, and saponins, as shown in Table 1.

Table 1. Interpretation of infrared (IR) data from ethanol extracts of mulberry leaves (*Morus alba* Linn.)

Code	Wave Numbers (cm^{-1})	Frequency Area (cm^{-1})	Functional Groups	Supporting Secondary Metabolites	Bonding
A	3377.27	3300 - 3500	Amina	Alkaloid	N-H
B	3010.73	3010 - 3095	Alkenes	Flavonoid	C-H
C	2923.73	2850 - 2970	Alkanes	Steroid	C-H
D	2853.85	2850 - 2970	Alkanes	Steroid	C-H
E	1738.55	1690 - 1760	Aldehydes, ketones, carboxylic acids and esters	Saponin	C=O
F	1695.77	1690 - 1760	Ketones	Saponin	C=O
G	1633.01	1610 - 1680	Alkenes	Flavonoid	C=C
H	1554.57	1500 - 1570	Nitro compounds		NO_2
I	1460.44	1340 - 1470	Alkanes	Steroid	C-H
J	1376.29	1340 - 1470	Alkanes	Steroid	C-H
K	1220.84	1180 - 1360	Amina	Alkaloid	C-N

L	1165.21	1050 - 1300	Alcohols, ethers, carboxylic acids and esters	Saponin	C=O
M	1061.10	1050 - 1300	Alcohols, ethers, carboxylic acids and esters	Saponin	C=O
N	917.05	675 - 995	Alkenes	Flavonoid	C-H
O	717.38	675 - 995	Alkenes	Flavonoid	C-H
P	674.60		Alkenes	Flavonoid	C-H

Based on Table 1, alkaloid compounds found at wavelengths of 3377.27 and 1220.84 cm^{-1}, which are classified as N-H groups of amine compounds. Flavonoid compounds are present at wavenumbers 3010.73; 1633.01; 917.05; 717.38, and 674.60 cm^{-1}, which belong to the C-H and C=C groups of alkene compounds. Steroid compounds are present at wavenumbers 2973.23; 2853.85; 1460.44, and 1376.29 cm^{-1}, corresponding to C-H groups characteristic of alkane compounds. Meanwhile, at wavenumbers 1738.55; 1695.77; 1165.21 and 1061.10 cm-^{1}, there are C=O groups of alkane compounds, indicating the presence of saponin compounds. Additionally, the FT-IR spectrum also shows the presence of double bond stretching, specifically in the C=O group of ketone compounds at wavenumbers 1738.55; 1695.77; 1165.21, and 1061. 10 cm^{-1} and aromatic C=C bonds at wavenumbers 1633.01 cm^{-1} (Puspitasari *et al*., 2021). The interpretation of the FT-IR spectrum is further supported by phytochemical screening results, where N-H or amine groups indicate the presence of alkaloid metabolites. Alkane functional groups indicate the presence of steroid metabolite compounds. Additionally, aromatic C=C groups indicate the presence of flavonoid metabolite compounds.

Phytochemical screening. To determine the compounds contained in mulberry leaves, phytochemical screening tests were conducted using flavonoids, alkaloids, steroid-terpenoids, saponins, and tannins as test parameters. The results are shown in Table 2.

Table 2. Phytochemical screening test of ethanol extract from mulberry leaves (*Morus alba* Linn.)

Phytochemical Test		Results	Description
Parameters	Test		
Flavonoid	NaOH	+	Reddish yellow color formed
	Sinoda	+	Red color formed
Alkaloid	Mayer's	+	Forms a yellow color without a white precipitate
	Wagner's	+	Formed reddish-brown color
	Dragendorff	+	Yellow precipitate formed
	Bouchardat	-	Brown color without sediment
Steroid-Terpenoids	Salkowski	+	Brown ring formed
	Lieberman Bouchard	-	Cloudy brown in color
Saponin	Foam Test	-	No foam
	Braymer's	+	Formed greenish black color
	Alkaline Solution	+	Formed fluorescent yellow color

The results of phytochemical screening tests on ethanol extracts of mulberry leaves (*Morus alba* Linn.) showed the presence of several groups of secondary metabolites, namely alkaloids, flavonoids, tannins, and steroids. In the alkaloid group, Mayer's test showed negative results, indicated by the formation of a yellow color without any white precipitate. Conversely, Wagner's and Dragendorff tests showed positive results with the formation of reddish-brown and yellow precipitates, indicating the presence of alkaloids. Meanwhile, the Bouchardat test showed negative results, as no blackish-brown precipitate formed, only a cloudy brown color. These results indicate that although there are positive indications from two tests (Wagner and Dragendorff), the Mayer and Bouchardat tests do not provide strong confirmation, suggesting that the alkaloid content in the extract is likely low or below the detection limit.

For flavonoids, the Shinoda test and the 10% NaOH test yielded positive results. The formation of a red color in the Shinoda test and a reddish-yellow color in the NaOH test indicates the presence of flavones and flavonols in the mulberry leaf extract. This is consistent with literature stating that mulberry leaves are rich in flavonoids such as quercetin and kaempferol (Latifah *et al*., 2022). These flavonoid compounds are known to have high antioxidant properties, and their presence is further supported by FT-IR results showing characteristic spectra of aromatic C=C groups and C–H from aromatic rings. Testing for saponin content using the foam test yielded negative results, as no stable foam higher than 1–10 cm was formed for more than 10 minutes. This indicates that saponin compounds were not detected or were present in very small amounts. Meanwhile, the tannin test using Braymer's yielded a blackish-green color, and the test with a basic solution produced a yellowish glow, both indicating the presence of polyphenolic compounds such as tannins. This tannin content reinforces the antioxidant profile of mulberry leaf extract, consistent with the findings of Forestryana and Arnida (2020), who also detected high polyphenol content in ethanol extracts of other tropical leaf plants**.**

The phytochemical screening of ethanol extracts from mulberry leaves (*Morus alba* Linn.) was found to indicate the presence of flavonoids, partially detected alkaloids, tannins, and steroids. These results were aligned with the findings reported by Latifah *et al*. (2022), in which flavonoids were identified as the predominant component of mulberry leaf extract, although their study exclusively focused on antibacterial activity and did not undertake a comprehensive phytochemical analysis. Furthermore, similar findings were reported by Pogaga *et al*. (2020), where ethanol extracts of mulberry leaves were demonstrated to contain flavonoids, polyphenols, and steroids. However, significant amounts of saponins and terpenoids were not detected, which corresponded with the negative results obtained in the saponin test and Lieberman-Burchard test conducted in the present study.

The positive results of the tannin test were also supported by the report presented by Forestryana and Arnida (2020), in which it was stated that complex polyphenols acting as natural antioxidants had been found in the leaves of tropical plants, including mulberry. Likewise, the positive results obtained from the steroid test using the Salkowski method were in agreement with the findings reported by Handayani *et al*. (2023), where the presence of steroid compounds had been frequently detected in ethanol extracts of aromatic and medicinal plant leaves. Consequently, the screening results were not only found to confirm previous studies but were also considered to reflect possible variations in secondary metabolite content, which might have been influenced by factors such as extraction techniques, solvent types, harvest timing, and the physiological age of the sampled leaves. A significant contribution was made by this study through the provision of more comprehensive phytochemical screening results, which were further validated by FT-IR analysis.

Total flavonoid. This test was conducted to measure the quantity of flavonoid compounds present in the extract. The total flavonoid content in plants is influenced by various factors such as sampling time, environmental temperature, light exposure, humidity, and others. According to research by Helmidanora and Sukawaty (Werdianingsih *et al*., 2022), flavonoid levels are determined using UV-Vis spectrophotometry because flavonoids exhibit absorption in the visible light spectrum due to their conjugated aromatic structure (Mahmood *et al*., 2021). Quercetin was measured at its maximum wavelength in the range of 415–440 nm. The quercetin extract prepared at a concentration of 100 ppm was tested for its absorption at the maximum wavelength of 434 nm, yielding an absorbance value of 1,200. The total flavonoid content of the extract was then calculated using the linear regression equation of the standard curve $y = ax + b$. The absorption of the quercetin standard solution yielded a linear regression equation $y = 0.0124x + 0.0339$ with a correlation coefficient $R^2 = 0.998$ (Fig. 3). These results indicate that as the concentration increases, so does the absorbance. The linear regression equation obtained was then used to calculate the total flavonoid content of mulberry leaf extract (*Morus alba*).

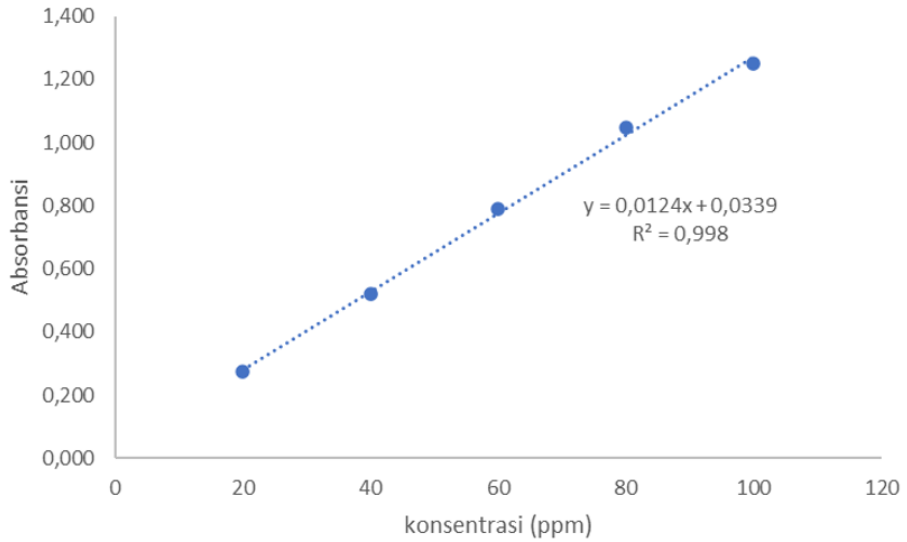

Fig. 3. Standard curve for quercetin

Each extract was prepared at concentrations of 100 ppm and 50 ppm, then analyzed for absorbance at a maximum wavelength of 434 nm, which had been previously determined using quercetin as a standard and obtained an absorption value of 1,200, then the total flavonoid content of the extract was calculated. Although the UV-Vis spectrum profile of the mulberry leaf extract (Fig. 1) showed main absorption peaks at 310 nm and 330 nm, measurements at 434 nm were maintained in accordance with the standard method for $AlCl_3$–quercetin complexation in the total flavonoid assay. This approach ensures methodological consistency and comparability with similar studies. The total flavonoid content of the samples is presented in Table 3.

Table 3. Total flavonoid test result of mulberry leaf

Sample concentration	Concentration (µg/mL)	Total Flavonoid Content (mg/g)	Mean ± SD (n = 3)
1000	50.06	50.06	0.65 ± 0.02
500	52.87	52.87	0.68 ± 0.01

The total flavonoid content in the sample is represented as QE (Quercetin Equivalent), which indicates milligrams of quercetin equivalent per gram of extract. In this study, the total flavonoid content was determined to be 50.06 mg QE per gram of extract at a sample concentration of 1000 ppm. Additionally, flavonoid content testing was also conducted at an extract concentration of 500 ppm, yielding a result of 52.87 mg QE/g of extract. Despite the lower extract concentration compared to the 1000 ppm sample, the flavonoid content was slightly higher. This is likely due to the optimal solubility of flavonoid compounds at a certain concentration or saturation in the complexation reaction with the $AlCl_3$ reagent. These findings indicate that increasing the concentration does not always result in higher flavonoid content, depending on the efficiency of the chemical reaction and the stability of the compound during testing.

CONCLUSION

Mulberry leaf extract (*Morus alba*) has characteristics that meet standards with a moisture content of 4.415% and an ash content of 0.1%. The extract yield of 29.086% indicates the effectiveness of the extraction process in extracting bioactive compounds. Phytochemical screening results indicate that mulberry leaf extract contains alkaloids (positive in Wagner's and Dragendorff's tests), flavonoids (positive in Shinoda and NaOH tests), tannins (positive in Braymer's and basic solution tests), and steroids (positive in Salkowski's test). UV-Vis spectrophotometer analysis showed two maximum absorption peaks at wavelengths of 310 nm and 330 nm, with the highest absorbance value of 0.786 at a dilution of 10^{-2}. This spectrum indicates the presence of flavonoid compounds,

particularly the isoflavone group, while testing for total flavonoids using the quercetin calibration curve method yielded total flavonoid levels of 50.06 mg/g at a concentration of 1000 ppm and 52.87 mg/g at a concentration of 500 ppm. Characterization using FT-IR revealed the presence of N-H (amine) functional groups, aromatic C=C, and aliphatic C-H, indicating the presence of secondary metabolites such as alkaloids, flavonoids, and steroids in the mulberry leaf extract. The results of this study indicate that mulberry leaves have potential as a source of bioactive compounds that can be further developed for various pharmacological applications.

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