

Cek Plagiasi jurnal tambahan Haris 3

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Submission date: 26-Sep-2025 09:50AM (UTC+0700)

Submission ID: 2762328698

File name: 3._Jurnal_Haris_Tambahan.pdf (1.2M)

Word count: 4560

Character count: 24054

Identification of Phytochemical Compounds and Determination of Flavonoid and Tannin Levels in Wedelia (*Sphagneticola trilobata* (L.) Pruski)

Andika Gundawa Hidayat¹, Diah Kartika Wardani², Ulinuha Farah Makhabati², Yumna Nabillah Iffatuzzahra², Haris Setiawan^{2*}

¹Chemical Engineering Study Program, Faculty of Industrial Technology, Universitas Ahmad Dahlan, Yogyakarta, Indonesia

²Department of Biology, Faculty of Applied Science, Faculty of Technology, Universitas Ahmad Dahlan, Yogyakarta, Indonesia

¹2200020045@webmail.uad.ac.id; ²diah2100017050@webmail.uad.ac.id; ²2238017030@webmail.uad.ac.id; ²yumna2100017034@webmail.uad.ac.id;

²haris.setiawan@bio.uad.ac.id*

*corresponding author

ARTICLE INFO

Article history

Received: January 9th 2025

Revised: February 3rd 2025

Accepted: March 20th 2025

Keywords

Ethanol Extract

Flavonoids

Screening Phytochemical

Wedelia Leaf

ABSTRACT

Wedelia plant, which has the scientific name *Sphagneticola trilobata* (L.) Pruski, is one of the plants classified as weeds and is found in various agricultural areas. The largest phytochemical compound content in wedelia leaves consists of flavonoids, tannins and alkaloids. The purpose of this study was to identify phytochemical compounds contained in wedelia leaves and to determine the levels of existing compounds. This research method consists of wet sorting, making extracts by maceration method using 96% ethanol. The ethanol extract was then analyzed using Fourier Transform InfraRed (FT-IR), High Performance Liquid Chromatography (HPLC), and UV-Vis Spectrophotometer. Data analysis to determine the line equation and correlation value using statistical analysis application SPSS Version 30.0 and analysis of phytochemical compound components using descriptive analysis. Based on the results of the analysis of phytochemical compounds using the FTIR method, several functional groups were obtained that were very identical to antioxidant compounds in the form of flavonoids, tannins, and alkaloids. After that, further tests on flavonoid levels using the HPLC method obtained levels of 215.435 mg/g (%b/b), and determination of tannin levels using a UV-Vis Spectrophotometer obtained levels of 128.35 mg/g (%b/b).

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How to Cite: Hidayat, A.G., Wardani, D.K., Makhabati, U.F., Iffatuzzahra, Y.N., & Setiawan, H. (2025). Identification of Phytochemical Compounds and Determination of Flavonoid and Tannin Levels in Wedelia (*Sphagneticola trilobata* (L.) Pruski). *Journal of Biotechnology and Natural Science*, 5(1):01-10.

1. Introduction

Indonesia is one of several countries included in the tropics. Various uniqueness and biodiversity that have high potential benefits can be managed to trigger various benefits obtained from the plants in it (Dewi et al. 2021). Biodiversity in Indonesia is supported by high rainfall, where there are around 74 types of typical natural ecosystems, 31.750 newly discovered plant species, 15.000 medicinal plants, and 7.000 species used as medicinal plants (Setiawan, 2022). Indonesian people since decades ago, have many beliefs about plants that are believed to be a source of traditional medicine that can provide healing from various diseases (Kurniawati 2017). One of the medicinal plants used in traditional medicine is Bandotan (*Ageratum conyzoides* L.) to treat paraquat exposure (Listina et al., 2024) and Songgolangit (*Tridax procumbens* L.) which is used to treat respiratory tract damage due to exposure to cigarette smoke (Nurrohiim et al., 2023).

Sphagneticola trilobata (L.) Pruski is one of the asteraceae family which is a family of sunflowers or daisies. The plant has a creeping nature on the ground and is spread in tropical and subtropical forest ecosystems (Maimunah et al., 2020). Many of these species come from Mexico, Central America and one of them is Indonesia. These plant species are usually introduced as ornamental plants, or arrow cover plants in the yard and develop vegetatively. The plant very quickly forms a dense ground cover that prevents other plant species from regenerating (Sushama 2019). The plant grows flower seeds and is classified as a herbaceous plant, Autotrophic, similar to a shrub. Wedelia plants can reach 70 cm in height, have shiny green leaves, pale green undersides, and serrated edges, while the stems are round, rooted, and stolons can reach a length of 2m or more (Pham et al., 2023). Wedelia (*S. trilobata* L. Pruski) plants in Indonesia are rarely used for herbal medicine because the content of phytochemical compounds from these plants is unknown. Some research in testing phytochemical compounds in these plants is still limited to testing using tube method by looking at color changes due to the addition of reagents. Therefore, further testing is needed to determine the levels of phytochemical compounds contained in the plant extract including flavonoids and tannins as antioxidant compounds that have many health benefits, so that the levels of phytochemical compounds contained can be a reference reference in several studies that will be conducted in the future. Several studies in Indonesia have utilized these plants in traditional medicine including as antibacterial against *S. typhi* and *E. coli* (Mardina et al., 2020) and as anti-infertility in cases of obesity (Iffatuzzahra et al., 2024). However, these studies have not analyzed the levels of phytochemical content in these plant.

Some previous research that has been done states that the wedelia plant has phytochemical compounds contained flavonoids, alkaloids, terpenoids, saponins and also tannins (Firmansyah & Pusparani, 2019). The role of phytochemical compounds in the health sector includes flavonoids sourced from Songgolangit plants as in Nurrohm et al., (2023) acts as an anti-oxidant in reducing Reactive oxygen species (ROS) due to exposure to cigarette smoke, tannin compounds as anti-diarrhea, anti-oxidant, antibacterial, and astringent (Sunani & Hendriani, 2023). Alkaloid compounds as anti-amyloid, anti-inflammatory, anti-oxidant, and anti-depressant (Hussain et al., 2018). As well as Saponin and Terpenoid compounds as antioxidants because the compounds are able to reduce superoxide through the formation of superoxide intermediates so as to prevent biomolecular damage by free radicals (Putri et al., 2023). The purpose of this research is to determine the content and levels of phytochemical compounds contained in these plants so that they have potential in further development as herbal medicines. Various treatments using medical drugs cause many side effects that can worsen health problems. So the potential of some phytochemical compounds from plant extracts can be used as herbal remedies. compounds found in plants can be used in the development of new drugs (Yuan et al., 2016). It is hoped that the results of this study will be able to provide a scientific basis regarding phytochemical compounds and the total levels contained in wedelia plants which are very rarely utilized, besides that it is hoped that further research can be carried out on the potential of phytochemical compounds contained in wedelia plants for the development of the world of health.

2. Methods

2.1 Wet Drying and Sorting Process

Wedelia leaves were prepared as much as 15 kg which will be used as samples, taken from the back garden of campus 4 of Ahmad Dahlan University, Yogyakarta. The treatment consists of 1). Wet sorting is carried out by separating impurities in Wedelia plants by taking only the leaves and those discarded include damaged leaves, stems, roots, and flowers then followed by washing using running water until clean, 2). Dried using a cabinet dryer at 50°C for 2-3 days using a container that has been coated with aluminum.



Figure 1. Morphology of Wedelia (*Sphagneticola trilobata* (L.) Pruski) (Description: a. leaves and stems, b. leaves and c. ethanol extract paste of wedelia leaves)

2.2 Extraction Method

Prepared as much as 1.5 kg of crushed wedelia leaf symphilia, put into each glass jar weighing 500 grams, then extracted by maceration method using 96% ethanol solvent for 3x24 hours with a solvent:sample ratio of 3:1. After the maceration process is complete, followed by filtering using filter paper and concentrating using a rotary evaporator at a temperature of 50°C and a pressure of 175 bar. The thick extract that has been obtained is then weighed and stored in a closed tube so as not to be contaminated.

2.3 Analysis of phytochemical compounds using the FTIR (Fourier Transform Infra Red) method

Wedelia viscous extract paste samples were taken as much as 1 gram, then placed on a pellet in a horizontal position by making the detector in direct contact with the sample, then the irradiation area occupied a diameter of about 0.2 mm. Then the sample is read using an FTIR tool with a wave range of 4000-500 cm⁻¹ at a resolution of 2/cm, then the resulting chromatogram is compared with the IR table as a database to determine the functional groups in the sample (Kresnamurti et al., 2022).

2.4 Preparation of Quercetin Standard Standard Solution

Weighed as much as 10 mg of Quercetin standard with Sigma-Aldrich brand produced by PT. New Praktika Alkesindo, diluted using 96% methanol in a 10 mL volumetric flask to obtain a concentration of 100 ppm, then Quercetin standard solution was taken as much as 0.6; 0.8; 1.0; and 1.2 mL and diluted in a 10 mL flask for each concentration; 8; 10; and 12 ppm. Then 10 mg of wedelia leaf extract was taken and diluted using 96% methanol in a 10 mL volumetric flask. Then each solution was filtered using a 0.45 µm filter membrane and put into a 10 mL vial (Sukmawati et al., 2019).

2.5 Quercetin content determination of Wedelia leaf ethanol extract using HPLC (High Performance Liquid Chromatography)

Determination of the maximum wavelength of Quercetin was carried out using a concentration of 100 ppm using a UV-Vis Spectrophotometer with the Thermo Scientific GENESYS™ 140/150 Vis/UV-Vis Spectrophotometers model made by PT. Mora Anugerah Berkas at a wavelength of 300-500 nm. so that the maximum wavelength was 371 nm (Mizzi et al., 2020). The solution was inserted into the HPLC device as much as 20 µL with the mobile phase in the form of methanol: distilled water (60:40), flow rate of 1 mL/minute. Then each chromatogram was recorded and the measurement results in the form of the area obtained were recorded, the calculation of levels was carried out using the linear regression equation $y = ax + b$ (Husnia & Budiarti, 2021). In HPLC analysis, a Shimadzu LC 2030 branded device equipped with a UV detector made by PT Karunia Duta Medika was used with a C18 column that has specifications of 4.6 mm inner diameter, 250 mm length, 5 µm particle size, 100 Å pore size, 0.624 cm³/gr pore volume, made of stainless steel.

2.6 Determination of tannin content using UV Vis Spectrophotometer

In the analysis using the UV-Vis spectrophotometer using a device with the specifications of the Thermo Scientific GENESYS™ 140/150 Vis/UV-Vis Spectrophotometer Model made by PT. Mora Anugerah Berkas, each concentration used was made into 3 repetitions with the results of the average abortion ± standard deviation. A total of 1 mg of tannic acid was dissolved using a glass beaker and included in a 10 mL volumetric flask and sufficient volume, used as a 100 ppm parent standard solution. Preparation of standard series with concentrations of 4; 6; 8; 10; and 12 ppm, by pipetting 0.4; 0.6; 0.8; 1.0; and 1.2 mL of raw tannate is inserted into a 10 mL volumetric flask, plus 50% concentration of folin denis reagent as much as 2 mL and 15% Na₂CO₃ solution as much as 2 mL, then the volume is

sufficient to the limit mark, and incubated at room temperature and in the dark for 15 minutes, and measuring the maximum wavelength using the 6 ppm series in the range of 690-820 nm. The sample solution was dissolved as much as 10 mg made 3 replications using a 10 mL volumetric flask of distilled water and added 2 mL of 50% folin denis reagent and 2 mL of 15% Na₂CO₃ solution, then the volume was sufficient to the limit line (Arifah et al., 2023).

2.7 Research Data Analysis

The research data obtained is then analyzed, where in FTIR data, comparisons are made from several libraries, such as comparing the wavelength obtained from the sample against a reference wavelength that has the same spectral band as in the chromatogram results. Furthermore, HPLC data including quercetin standard at 32 extracts obtained were plotted against the area value in the SPSS Version 30.0 application to obtain a linear regression line and the resulting equation to calculate the flavonoid content in the sample, besides the Tannic Acid standard absorbance data and samples from several replicates were calculated standard deviation using the SPSS Version 30.0 application then continued to plot the standard absorbance so as to obtain a linear regression line and equation to calculate the tannin content contained in the sample.

3. Results and Discussion

3.1 Results of FTIR analysis

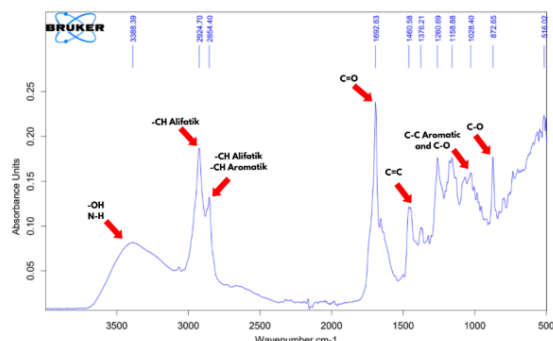


Figure 2. Test Results of Wedelia Leaf Extract using FTIR (Caption: cluster name (wave number/cm); -OH and N-H (3388.39/cm); -CH Alifatik and -CH Aromatic (2924.70 and 2854.40/cm); C=O (1692.63/cm); C=C (1450.58 and 1376.21/cm); C-C Aromatic and C-O (1028.40 and 872.65/cm).

Based on the results of the extract spectrum test, it shows the presence of (-OH) and (N-H) functional groups at wave numbers 3388.39 cm⁻¹ derived from alcohol and water where these functional groups are components of flavonoids and tannins, besides that in the extract there are also functional groups (-CH Aliphatic) with wave numbers 2924.70 cm⁻¹, (-CH Aliphatic and Aromatic) with wave numbers 2854.40 cm⁻¹ which are nitrile compounds (Go 35), (C=O) group at wavelength 1692.63 cm⁻¹, (C=C) group was also detected at wavelength 1460.58 cm⁻¹ and 1376.21 cm⁻¹, and (C-O and C-C Aromatic) group at wavelength 1028.40 cm⁻¹ and 872.65 cm⁻¹. The results of the functional groups detected showed similarities to bioactive compounds, namely flavonoids including (-OH), (-CH Aliphatic and Aromatic), C=O and C=C (Feliara et al., 2018). Functional groups identical to tannins include (-OH), (CH Aliphatic), C=O, C-C, and C-O (Hayati & Fasyah, 2010) Functional groups identical to alkaloid compounds are (C-H), (C=O), (C-C), (N-H), and (-CH Aliphatic) (Gamah et al., 2023). The detected functional groups are strongly suspected to be flavonoids and tannins where the presence of the detected functional groups with several band characteristics indicates that the wedelia extract is positive for flavonoids and tannins, in this case the compounds present have biochemistry mostly as antioxidant

compounds that inhibit the formation of free radicals and reduce tissue damage due to inflammation. The structure of bioactive compounds in the form of flavonoids and tannins is shown in Figure 3.

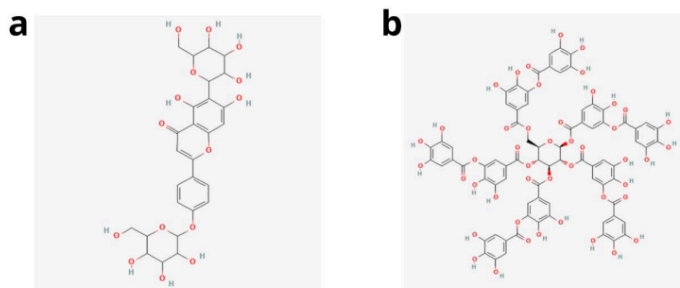
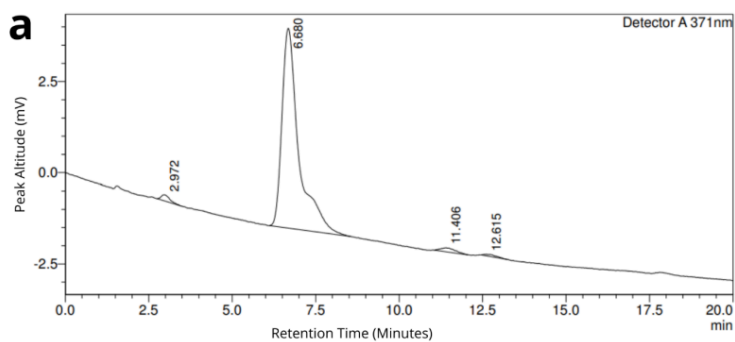
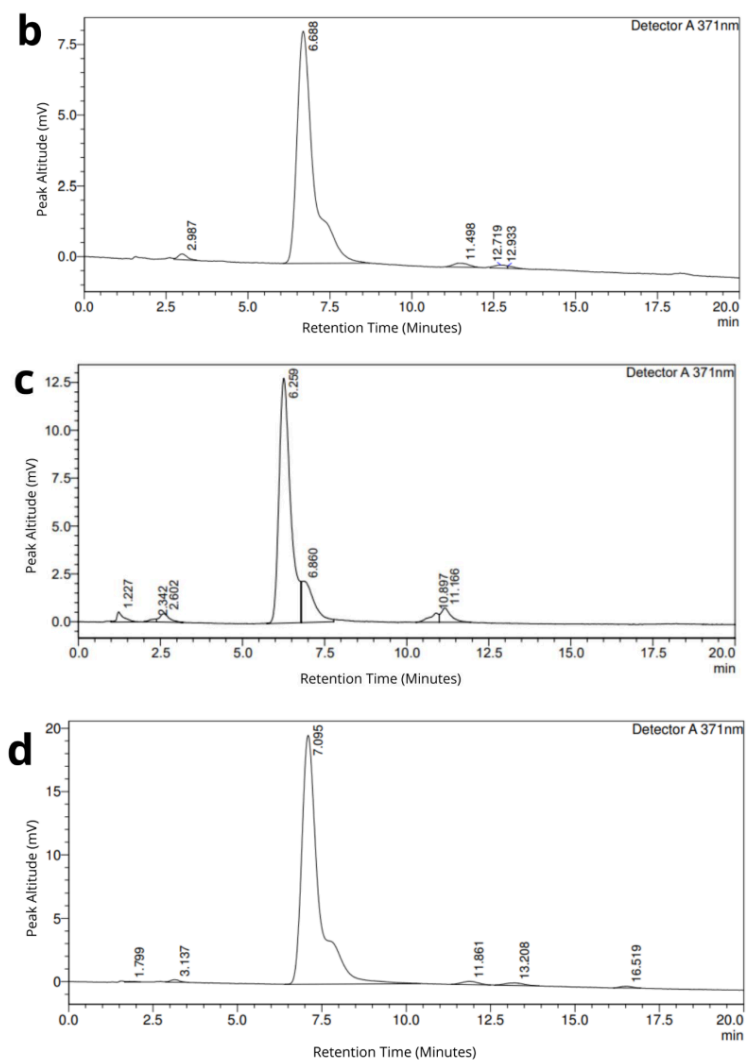


Figure 3. Structure of bioactive compounds (Description: a.Flavonoids; b.Tannins)

Based on the picture of the bioactive compound structure in Figure 3 above, it shows that each bond in each bioactive compound has the same functional group characteristics as the results detected in the FTIR tool, where the FTIR tool reads the wavelength of each functional group contained therein.

3.2 Results of HPLC analysis





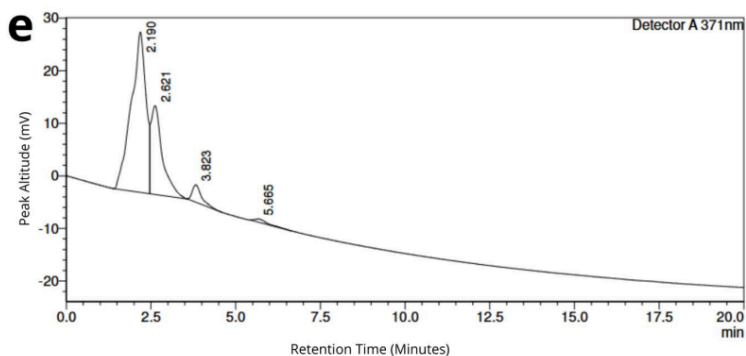


Figure 4. Spectrum of HPLC analysis results (Description; a. 6 ppm; b. 8 ppm; c. 10 ppm; d. 12 ppm; and e. Wedelia leaf extract).

Based on the results of the pictogram data from the testing of standard solutions and Quercetin standard solutions using HPLC instruments, area data is obtained as in table 1.

Table 1. HPLC Results of Quercetin Raw Area

Concentration (ppm)	Area
6	191211
8	290234
10	398952
12	693303

In determining the area under the standard peak, integration is performed, where peak integration is a mathematical operation performed by HPLC chromatography software to measure the area under the peak. The area measurement is based on integration which hypothetically divides the area under the peak into several rectangles which are summed to give the total area under the peak, so that the area number on the HPLC device can appear from the HPLC software.

The standard curve is a curve obtained from various series solutions that are still in linearity so that a linear regression can be made. The purpose of the standard curve is to provide information on the concentration of the sample solution (Fahira et al., 2021). The standard curve shows how the concentration of the solution (x-axis) is related to (y-axis). The regression equation of this curve is $y=a+bx$, where y is the dependent variable, x is the independent variable, a is the intercept, and b is the regression coefficient or slope (Santoso et al., 2021). Based on the Quercetin standard curve, the line equation $y=80750x-333322$ was obtained with a correlation value (R^2)=0.9217 with an area (y) in the wedelia leaf sample (*Sphagneticola trilobata* (L.) Pruski) of 14503, the obtained Quercetin content was 215.435 mg/g (%b/b). The results of flavonoids in the form of quercetin in wedelia plants have very high levels when compared to plants from the same family (asteraceae), namely purslane (*Portulaca oleracea*) which only has levels of 3.27 mg/g (Budiawan et al., 2021).

3.3 Results of Uv-Vis Spectrophotometer Analysis

Measurement of the absorbance value of the solution with UV-Vis Spectrophotometer was carried out at the maximum wavelength of 754 nm, after which the absorbance measurement of the tannic acid equivalent standard solution was carried out so that the data presented in table 2.

Table 2. Tannic Acid Absorbance Value Measurement Results

Concentration (ppm)	Absorbance
6	0.240±0.001
8	0.306±0.000
10	0.239±0.001
12	0.720±0.003

The results of the determination of the standard curve of tannic acid obtained the equation $y=0.0776x-0.2704$ with a correlation value (R^2)=0.9053, where the correlation coefficient value is close to one, proving that the relationship between tannic acid concentration and absorbance value is linear. Determination tannin content in ethanol extract of Wedelia leaves was carried out 3 times replication and measured at a wavelength of 754 nm and obtained an absorbance value of 0.726. The results of the calculation and determination of tannin content obtained the average content in the ethanol extract of Wedelia leaves is 128.35 mg/g. With the high tannin content contained in Wedelia extract has high potential bioactivity for health, including accelerating blood clotting, reducing blood pressure, lowering serum lipid levels, and maintaining the firmness of mucous membranes (Hoque et al., 2024).

4. Conclusion

Based on the results of screening phytochemical compounds using the FTIR method, it was found that the functional groups were very identical to bioactive anti-oxidant compounds in the form of flavonoids and tannins, besides further testing of the bioactive content including flavonoid and tannin compound levels using the HPLC method and UV-Vis Spectrophotometer obtained flavonoid levels of 215.435 mg/g (%b/b) and tannin levels of 128.35 mg/g (%b/b). Based on the test results of bioactive levels, it shows that the ethanol extract of wedelia leaves contains high antioxidant compounds. With the presence of these high antioxidant compounds, ethanol extract of wedelia leaves has great potential in the development of traditional medicine in the future, especially as a therapeutic agent in counteracting free radicals and preventing degenerative diseases. Broader applications of these findings include opportunities to develop more effective herbal medicine formulations for several diseases, further research on the mechanism of action of its bioactive compounds, as well as exploration of the potential for commercialization in the pharmaceutical and cosmetic industries. Further studies are also needed to test the bioavailability, toxicity, and effectiveness of these compounds in in vivo models to ensure their safety before widespread application.

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