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Isolation and Identification of Flavonoid Compounds from Saga (*Abrus Precatorius*) Leaf Extract

Azkia Nurul Fajriah[1]*, Tesalonica Maria Supit[1], Nayla Intan Pasha[1], Hamka George Marly Hairil Opur[1], Fajar Maulana[1]

[1]Faculty of Pharmacy, Universitas 17 Agustus 1945 Jakarta, North Jakarta, Indonesia, 14350
*Corresponding author: tesalonica.ms@gmail.com

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Abstract
Saga leaves (*Abrus precatorius* L.) are traditional medicinal plants known to contain various secondary metabolites, including flavonoids that exhibit bioactive properties such as anti-inflammatory and antioxidant activities. However, information regarding the isolation and characterization of flavonoid compounds from saga leaves remains limited. This study aimed to isolate and identify flavonoid compounds present in saga leaves as a basis for the development of natural materials with pharmaceutical potential. The methods employed included extraction using Ultrasonic-Assisted Extraction (UAE) with a methanol:water (9:1) solvent system, followed by phytochemical screening, liquid–liquid fractionation, column chromatography, and preparative thin-layer chromatography (PTLC). Compound identification was carried out using UV-Vis spectrophotometry, along with melting point determination to evaluate the physical characteristics of the isolate. The results showed that UAE extraction produced a yield of 4.68%, and phytochemical screening confirmed the presence of flavonoid compounds. The ethyl acetate fraction was obtained as the target fraction with a yield of 15.4%. Purification using column chromatography and PTLC yielded a flavonoid isolate that exhibited a maximum absorption at a wavelength of 282 nm and a melting point of approximately 127°C. These results indicate that the isolated compound possesses flavonoid characteristics with a moderate level of purity. This study concludes that saga leaves have potential as a natural source of flavonoid compounds that can be further developed in the pharmaceutical and health fields.

Keywords: Saga leaves, Flavonoid, Ultrasonic-Assisted Extraction

Introduction

Vine saga is a type of plant that belongs to the *leguminosae* family, with the genus *Abrus* and the species *Abrus precatorius* (1). This plant grows by climbing and is traditionally known to be effective in treating diseases, such as mouth ulcers and toothaches. In traditional medicine, the most widely used part is the leaves, either consumed directly or brewed to treat mouth ulcers and toothaches, vine saga leaves are also known to be effective in treating inflammatory diseases, rheumatism, headaches, and stomach aches (2).

The content of saga leaves includes alkaloids, flavonoids, and saponins which have antibacterial functions (3). However, in this research the focus of the study is only on flavonoid compounds, considering that flavonoid compounds are secondary metabolites of polyphenols, found widely in plants and foods and have various bioactive effects including antiviral, anti-inflammatory (4), cardioprotective, antidiabetic, anticancer (5), antiaging, antioxidant (6).

Flavonoid compounds are polyphenol compounds that have 15 carbon atoms arranged in a C6-C3-C6 configuration, meaning that the carbon skeleton consists of two C6 groups (substituted benzene rings) connected by a three-carbon aliphatic chain (7). The flavonoid content in medicinal plants can also be used as an alternative treatment to prevent and reduce inflammation. Studies show that flavonoids, especially compounds from the flavone group, can express anti-inflammatory activity through modulating the expression of pro-inflammatory genes such as cyclooxygenase-2, inducible nitric oxide synthase, and several cytokines. This study aims to isolate and identify flavonoid compounds from saga leaves (*Abrus precatorius L.*) as a basis for developing the potential of saga leaves as a source of natural bioactive compounds.

In this study, flavonoid metabolite compounds contained in saga leaves will be isolated and identified using the UAE extraction method. UAE is a method that uses ultrasonic waves and is faster in the extraction process. This is because the ultrasonic-assisted extraction process can increase cell wall permeability, causing spontaneous bubbles (cavitation) in the liquid phase below its boiling point and increasing cell damage (8).

Materials and Method

Materials

Saga leaves (*Abrus precatorius*), methanol (Lab Grade), aquadest, concentrated HCl, Mg rod, ethyl acetate, chloroform, silica gel, silica plate.

Methods

Extraction of Saga Leaves Using the UAE Method

The dried plant material was ground using a blender until a fine dry powder was obtained and then mixed with 2.5 L of methanol:water solvent (9:1). The mixture was extracted using an ultrasonicator at a frequency of 20 kHz for 30 minutes and subsequently filtered through a funnel lined with filter paper. The resulting liquid extract was evaporated using a rotary evaporator to obtain a thick saga leaf extract, and the extraction yield was calculated.

Screening of Flavonoid Compounds in Saga Leaves

A total of 1 mL of thick saga leaf extract was placed into a test tube, then 2 drops of concentrated HCl were added and shaken until dissolved. Small pieces of magnesium ribbon were then added to the mixture. The appearance of yellow, blue, orange, or red color indicated a positive result (9).

Liquid-Liquid Fractionation
The thick extract was dissolved in water and then successively partitioned with n-hexane and ethyl acetate in a 1:1 ratio until clear fractions were obtained. Each fraction was separated and concentrated using a rotary evaporator to remove the solvent. The yields of the concentrated fractions were then calculated.

Column Chromatography
Mobile phase optimization was carried out using TLC on the ethyl extract of saga leaves, employing silica gel plates as the stationary phase and chloroform:methanol as the mobile phase in ratios of 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9 until a chromatogram with a single spot was obtained. One gram of the thick extract was then mixed with silica gel and loaded into the column as the stationary phase. The optimized mobile phase was prepared and used to elute the column to achieve separation.

The column chromatography eluates were collected in vials for each fraction. TLC analysis was then performed for each fraction by spotting with a capillary tube onto a 7 × 1 cm silica plate and eluting with chloroform:methanol (7:3). The TLC plates were observed under UV light at 254 nm and 366 nm, and the Rf values were calculated.

Preparative Thin-Layer Chromatography (PTLC)
The chromatogram showing the best band separation was selected for further isolation using PTLC. A 20 × 20 cm silica gel plate was prepared as the stationary phase with chloroform:methanol (7:3) as the mobile phase, and the ethyl acetate fraction extract was applied along the plate and eluted to the solvent front limit. The developed plate was observed under UV light at 254 nm and 366 nm, after which the separated band was scraped off and dissolved in methanol.

Identification of Maximum Absorption Wavelength Using a UV-Vis Spectrophotometer
The band obtained from PTLC was scraped off and dissolved in methanol. The resulting solution was transferred into a cuvette for analysis. The sample was then measured using a UV-Vis spectrophotometer at a wavelength range of 200–400 nm.

Crystal Characterization Test
The band obtained from PTLC was scraped off and dissolved in methanol before being transferred into a vial. The solution was then filtered to obtain a clear filtrate. The filtrate was evaporated until crystals formed, and the percentage yield was calculated.

Melting Point Test
The melting point test was performed using a Digital Melting Point Apparatus by placing the sample into a capillary tube and inserting it into the instrument slot. The sample was heated gradually, initially at a faster rate and then more slowly as it approached the expected melting point. The temperatures at which the first droplet appeared and when the entire sample completely melted were observed through the lens and recorded.

Results and Discussion

In this study, the sample used was saga leaves (*Abrus precatorius L.)*. The extraction process was carried out using the UAE method with a methanol:water (9:1) solvent system for 30 minutes at an ultrasonic frequency of 20 kHz. The UAE method was selected because of its ability to accelerate the extraction process through ultrasonic waves that generate cavitation, leading to disruption of plant cell walls and facilitating the release of secondary metabolites.

Table I. Result of extraction, evaporation, and % yield

Results	
Initial Weight	412 g
Volume of Solvents	2.5 L
Weight of Extract	19.3 g
% Yield	4.68 %

In Table 1, based on the data obtained, the extraction of 412 grams of powdered material using methanol:water (9:1) solvent with a volume of 2.5 liters produced a total of 19.3 grams of thick extract after the evaporation process. The percentage yield showed a value of 4.68% of the initial powder weight, indicating the amount of compounds successfully extracted and dissolved in the solvent used.

Phytochemical screening is a qualitative analysis conducted to determine the presence of secondary metabolites in a sample. The results obtained from the screening of saga leaves (*Abrus precatorius L*.) indicate the presence of secondary metabolites belonging to the flavonoids. A positive result for flavonoid test was indicated by the formation of a yellow solution after the addition of HCl and Mg.

Heating the sample in flavonoid testing helps dissolve the flavonoid compounds, as they are soluble in hot water. The addition of Mg and HCl reduces the benzopyrone nucleus in the flavonoid structure. This reduction results in a change in the conjugation system, allowing the flavonoid compounds to absorb light in the visible region (10).

The liquid–liquid fractionation step was carried out to separate compounds based on their polarity using n-hexane and ethyl acetate as solvents (11). A total of 3 grams of the ethyl acetate fraction was obtained and selected as the target fraction because flavonoids are generally semipolar, allowing them to be more readily distributed into ethyl acetate than into nonpolar solvents. The percentage yield of 15.4% indicates that the fractionation process successfully enriched the flavonoid content in the ethyl acetate fraction.

Purification of flavonoid compounds was continued using column chromatography with silica gel as the stationary phase and chloroform:methanol (7:3) as the mobile phase. This eluent system was selected based on TLC optimization results that showed good spot separation (12). The fractions obtained from column chromatography were further

analyzed by TLC under UV light at 254 nm and 366 nm to identify the fractions containing the target compound.

Figure 1. Results of column chromatography

In PTLC, silica gel was used as the stationary phase and a chloroform:methanol (7:3) mixture as the mobile phase. Prior to elution, the chromatography chamber was saturated with the mobile phase to ensure uniform solvent distribution on the stationary phase and achieve efficient compound separation. The isolate obtained from column chromatography was spotted onto the PTLC plate, placed into the saturated chamber, and the separated spots were observed as an intense green spot under UV light at 254 nm and a reddish-purple spot at 366 nm.

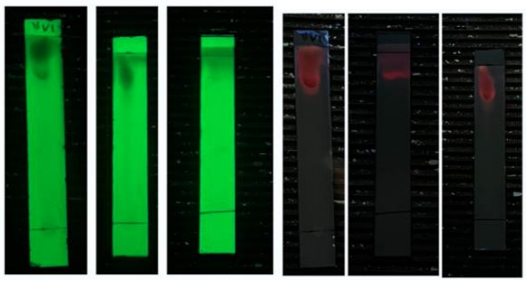

Figure 2. TLC test results of column chromatography under the UV light at 254 nm (left) and 366 nm (right)

The fraction exhibiting a Rf value parallel to that of the reference standard was scraped off from the plate for further analysis. The isolated compound was subsequently subjected to maximum absorbance measurement. This step was performed to further characterize the purified flavonoid compound.

Figure 3. UV-Vis Spectrum Results

Flavonoid compound identification was carried out using UV-Vis spectrophotometry in methanol as the solvent. The spectrum showed a maximum absorption peak at 282 nm, which is characteristic of flavonoid absorption bands. The maximum wavelength of flavonoids is around 282 nm due to the electronic transition $\pi \rightarrow \pi^*$ in the aromatic ring system (benzoyl system/Band II) of ring A which produces strong absorption in the UV region 270–290 nm (13).

The isolate obtained from saga leaves (*Abrus precatorius L.*) through PTLC was dissolved in methanol and then evaporated until crystals were formed. The percentage yield of the crystals obtained was 0.002%. This indicates that the target compound is contained at very low levels or that it is lost during the extraction and purification process so that the amount of pure compound obtained is relatively small compared to the starting material.

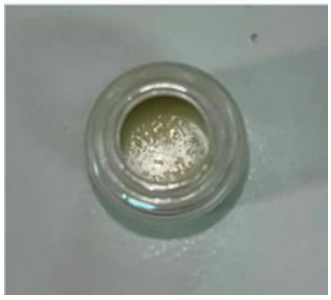

Figure 4. Saga Leaves Crystals

The melting point test results showed a temperature range of approximately 127°C, which falls within the typical melting point range of flavonoid compounds around 120–180°C (14). The relatively broad melting range and the presence of slight decomposition indicate that the isolated compound has moderate purity. This statement is supported by the figure shown below.

Figure 5. Crystal melting point test results

Conclusion

Based on the results of the study, it can be concluded that saga leaves (*Abrus precatorius L.)* contain flavonoid compounds. The isolation and purification process using the UAE method followed by liquid–liquid fractionation, column chromatography, and PTLC successfully produced flavonoid compounds with good purity. Identification using UV-Vis spectrophotometry revealed characteristic absorption typical of flavonoids, and these findings support the potential of saga leaves as a source of natural bioactive compounds for further development in the pharmaceutical and health fields.

Conflict of Interest Statement

The authors declare no conflict of interest related to this work.

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