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Detection of Flavonoid Compounds of Daruju Root Extract (*Acanthus ilicifolius* Linn) using Thin Layer Chromatography and UV-Vis Spectrophotometry

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Abstract

This research focuses on isolation techniques and detection of flavonoid compounds in daruju root extract. The solvent used in maceration is ethanol, then concentrated with a rotary evaporator until we collected the crude extract. This crude extract was separated using a separatory funnel by adding diethyl ether and n-butanol solvents. The viscous extract was tested for phytochemical preliminaries to detect flavonoids by reacting the viscous extract with H2SO4 and Mg. The result obtained is a change in color to yellow, this proves that the extract contains positive flavonoids. After that, TLC was carried out to purify the extract and use a UV-Vis spectrophotometer to confirm the results of the purification. TLC results showed an Rf value of 0.6-0.8. Optimization results of the UV-Vis spectrophotometer with a wavelength range of 200-800 nm give maximum results at 291 nm which are thought to have detected flavonoids.

Keywords: Daruja root, Spekrofotometer UV-Vis, TLC, Flavonoid

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1 Introduction

The use of traditional medicine is a legacy from our ancestors from one generation to the next, so its existence is related to the culture of the Indonesian nation [1]. The Indonesian people have been creative and have done real work in various fields, including preparing medicinal ingredients and performing traditional treatments [2]. The development of the nation's culture goes hand in hand with the level of civilization of the nation, as well as the

development of the use of medicinal plants and traditional medicines as a health effort [3].

One of the plants that has long been used by the community as traditional medicine is Daruju (Acanthus ilicifolius Linn.), Daruju has a bitter taste and is cold [4]. The effects of this plant traditionally include anti-inflammatory, cough suppressant, and anti-neoplastic (inhibiting the growth of neoplasm or tumor cells) [5]. In addition, the roots, leaves, and seeds can be used to treat several diseases including intestinal worms, hepatitis, liver cancer and cough medicine [6].

Maceration is a simple extraction process by withdrawing the active substance from simplicia with the principle of soaking for 3x24 hours to 5x24 hours using a solvent and stirring every 1x24 hours at room temperature. The filtered fluid will penetrate the cell wall and enter the cell cavity containing the active substance that will dissolve, because of the difference in the concentration of the active substance solution inside the cell and outside the cell, the concentrated solution is pushed out. This process is repeated so that there is a balance of concentration between the solution inside and outside the cell. The solvent used can be water, ethanol, methanol, ethanol-water or other solvents. Remaceration means adding a solvent after the first filtering of the maseration and so on. The advantage of the maceration method is the method of processing and the equipment used is simple and easy to operate [7].

Chromatography techniques that are often used are paper chromatography, preparative thin layer chromatography and column chromatography, as a porous absorbent commonly used aluminum oxide, silica gel, kieselgur, cellulose and synthetic herse [8]. Paper chromatography and thin layer chromatography are generally used for identification because this method is unique and easy to do for compounds in small amounts, while column chromatography is for separating compounds in large quantities [9].

The visible Spectrophotometry Method is the absorption of visible light by a colored solution or also known as the colorimetric method. Only the solution of the compound can be determined by this method. Colorless compounds by reacting with reagents to produce colored compounds [10]

Based on the above background, the formulation of the problem that arises in this study is whether the Daruju Root (*Acanthus ilicifolius Linn*) contains flavonoids? This study aims to identify the flavonoid content of Daruju Root (*Acanthus ilicifolius Linn*) by thin layer chromatography (TLC) and UV Visible Spectrophotometry. The benefit of this research is to obtain chemical data of Daruju Root (*Acanthus ilicifolius Linn*) so that its use as a traditional medicine is not only based on experience, but has been supported by chemical data.

2 Materials and Method

This research was conducted to prove the production of Daruju root extract contains flavonoids with several methods and materials used as follows:

2.1 Sample Processing

The roots of Daruju (*Acanthus Ilicifolius Linn*) are cleaned of adhering dirt, sorted wet with running water until clean and cut into small pieces and then dried in direct sunlight.

2.2 Material Extraction

The sample of Daruju Root (*Acanthus Ilicifolius Linn*) was weighed as much as 500 g, then refluxed using methanol for 3-4 hours then filtered and treated up to 3 times until the sample was completely extracted. The filtrate was collected and then evaporated using a rotary evaporator to obtain a thick extract. 7 grams of the evaporated extract was dissolved in distilled water and methanol with a total volume of 100 ml with a ratio of 1:1 we call it methanol extract. we repeat extraction with n-Butanol (n-Butanol extract)

2.3 Flavonoid Preliminary Test

Prepare a test tube that includes each NaOH, Concentrated H2SO4, and a control tube. In each test tube, 0.1 gram methanol extract solution was added. The color of each tube is compared to the control tube, if there is a yellow color change it is positive for flavonoids.

2.4 Thin Layer Chromatography (TLC)

The n-butanol extract which had been dissolved in 95% alcohol was spotted on the lower edge of the plate. The plate is inserted into the chamber which contains the eluent, which is a homogeneous mixture of the bottom layer of the solvent between chloroform – methanol – water (13:7:2). The plate is allowed to elude until the eluent propagates up to the top margin of the plate, then it is removed and dried in the air. Observation of stains using UV lamps 254 and 366 nm. The plate was also sprayed with 10% H_2SO_4 reagent and heated at 110°C for 10 minutes to clarify the color of the stain formed. The TLC process is repeated until it gets the right results. After the results with TLC are concluded positive, then proceed with preparative TLC.

2.5 Preparative Chromatography

The conditions and methods of preparative chromatography and TLC used in this study were the same, but in preparative chromatography, the stains formed on the edge of the plate were connected by a line from one edge to the other. The inside of the line was scraped by removing the part that had been heated and dissolved with 95% alcohol as isolate and accommodated as a pure fraction.

2.6 Measurement of the sample spectrum with a UV-Visible Spectrophotometer

The isolates obtained from the preparative TLC were identified qualitatively by UV-Vis spectrophotometry. 2 ml of isolate was put into a UV-Vis spectrophotometer cuvette "Spectroquat Pharo 300" to identify the absorbance value of flavonoid compounds at the maximum wavelength. Observations were made at a wavelength of 200-800 nm.

3 Result and Discussion

From the research that has been done, the results show that in the extraction process of 1000 grams of Daruju Root samples using methanol as a solvent, 15 grams of thick methanol extract was obtained. From the extract obtained, identification was carried out using thin layer chromatography, Preparative chromatography, and UV Visible spectrophotometry and the following results were obtained.

Flavonoids contain an aromatic ring composed of 15 carbon atoms with a basic nucleus arranged in C6-C3-C6 conjugates. The presence of an aromatic ring causes the band to be strongly absorbed in the long UV-vis region. Prior to isolation and purification, chemical testing (preliminary test) was carried out using NaOH, concentrated H_2SO_4, and concentrated Mg-HCl powder to produce a yellow color and the results obtained were in accordance with the literature. Preliminary test results can be seen in Table 1

In this study, the identification of flavonoid compounds in the Daruju Root extract went through several stages, beginning with identification by thin layer chromatography (TLC) using Chloroform: Methanol: Water as eluent (13:7:2). This eluent is widely used as an eluent in the separation of flavonoids with advantages in terms of insulating ability against flavonoids and high separation speed. The results of the elution showed 3 yellow-green fluorescence stains visible in the UV at 366 nm, after spraying with 10% H_2SO_4 showed 2 green fluorescence stains visible in the UV at 366 nm. TLC test results can be seen in Tables 2 and 3 and Figure 1a

According to the literature, flavonoids appear under a UV lamp with green-yellow, pink, whitish, orange, yellow to brown fluorescence colors. In this study, yellow stains were found which appeared on thin layer chromatography and could be interpreted as flavonoid compounds. The location of the spots before spraying 10% H_2SO_4 fraction A with Rf 0.6, fraction B with Rf 0.7, and fraction C with Rf 0.8, after spraying 10% H_2SO_4 fraction A with Rf 0.6 and fraction B with Rf of 0.7 adds confidence to the content of flavonoids.

In preparative thin layer chromatography, 2 fractions were obtained, namely yellow and blue. The results obtained were interpreted as having flavonoid content, namely fraction A. Then analyzed by UV-Vis Spectrophotometer. The results of the preparative chromatography test can be seen in Table 4 and Figure 1c

From the UV-Vis spectrophotometer analysis measured at a wavelength of 200-800 nm, it gives the maximum wavelength for fraction A 291 where at that wavelength it is

suspected that there is Plavonoid in Daruju Roots. UV-Vis Spectrophotometer test results can be seen in Figure 1b.

Table 1. Preliminary test results for flavonoids

Samples	Reactor	Coloration	Interpretation
Methanol extract	NaOH+ Concentrated H_2SO_4 + Mg Powder	Yellow	+

Table 2. Identification results of Daruju Root n-Butanol extract with elution liquid Chloroform: Methanol: Water (13:7:2) before spraying 10% H_2SO_4

Spots	Retardation Factor (RF)	Rickshaw color at 366 nm UV without ammonia vapor
1.	0,6	Yellow
2.	0,7	Dark Blue
3.	0,8	Pink

Table 3. Identification results of Daruju Root n-Butanol extract with elution liquid Chloroform: Methanol: Water (13:7:2) after spraying 10% H_2SO_4

Spots	Retardation Factor (RF)	Color tricycle with ammonia vapor + UV 366 nm
1.	0,6	Green-yellow fluorescence
2.	0,7	Green-yellow fluorescence

Table 4. Results of preparative thin layer chromatography of Daruju Root n-Butanol extract

Spots	eluent composition Chloroform – methanol-water	Color stain with UV light		Interpretation
		Without H_2SO_4	additioning H_2SO_4	
1	13:7:2	Yellow	Yellow	1 Stain
2	13:7:2	Blue	Blue	1 Stain

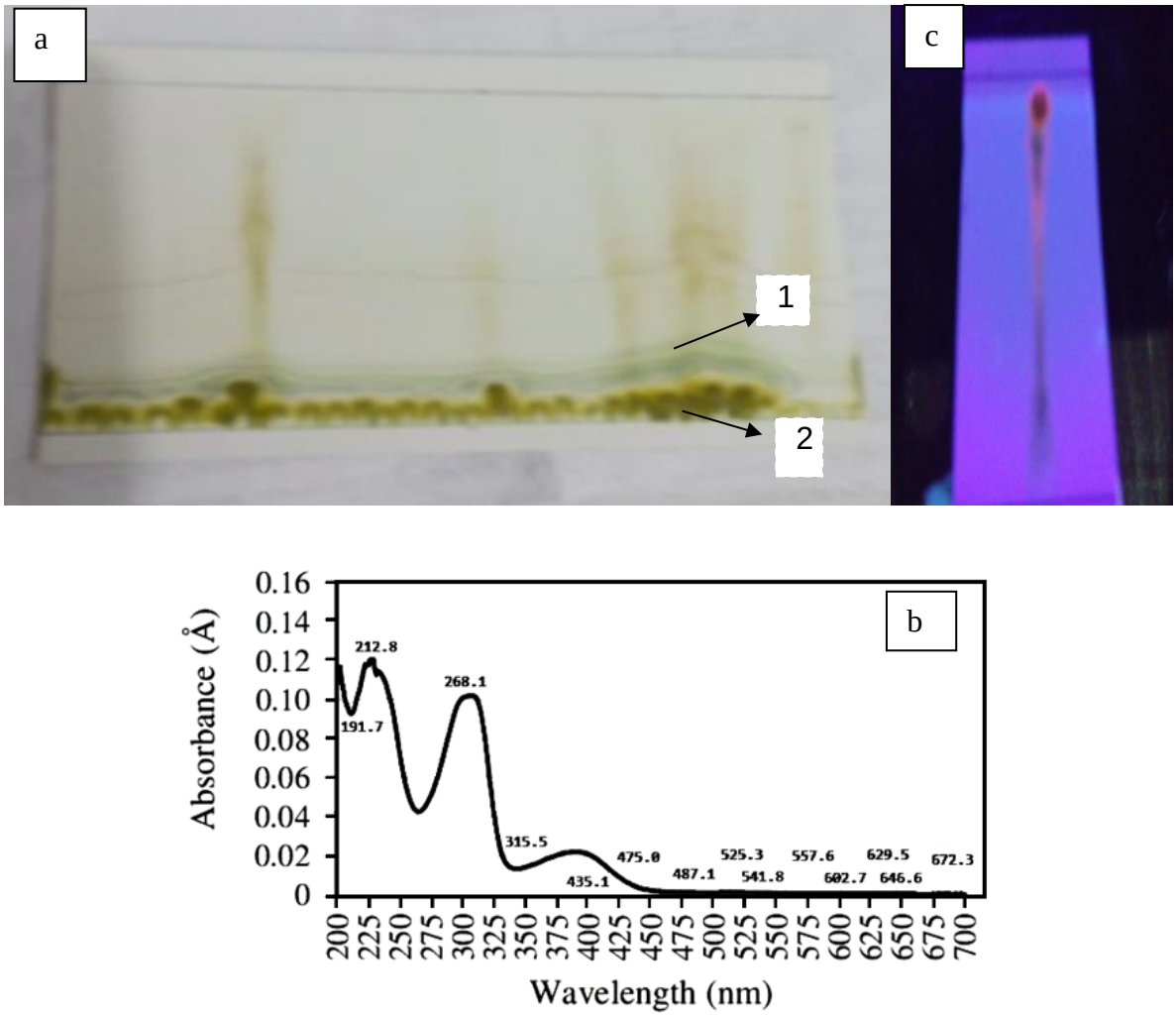

Figure 1. a) green fluorescence stains visible in the UV at 366 nm, b) UV Vis Spectrophotometer test results c) separation of TLC-P n-Butanol fraction with silica gel F254 as stationary phase, mobile phase (chloroform: Methanol: Water), developer distance 10 cm strain 1 and 2.

4 Conclusion

Based on the results of the research conducted, it can be concluded that from the preliminary test results obtained positive results are indicated by a yellow color and the results of UV-Vis spectrophotometry measurements are measured at a wavelength of 200-800 nm. It is confirmed that the roots of Daruju (*Acanthus ilicifolius Linn*) contain flavonoid compounds.

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