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Isolation and Identification of Chemical Components of Winged Bean (Psophocarpus tetragonolobus D.C) Leaf Diethyl Ether Extract

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Abstract: This study aims to identify the chemical content of winged bean leaves which were first made into extracts with variations in solvent composition and will be identified using 2 Dimensional Thin Layer Chromatography (TLC) and IR Spectrovotometry. The results process of the n butanol fraction using chloroform-methanol (8:2) developer liquid, 3 (three) spots of the compound were obtained where 3 stain bands were formed on the preparative thin layer chromatography plate. The three bands were scraped and dissolved and then purified with developer liquid on the isolates, new spots were obtained from the separation of spots of fraction C, then sprayed with 10% sulfuric acid and green-yellow-green stains were obtained with an Rf value of 0.89, while the fraction A and B obtained two stains after spraying 10% Sulfuric Acid with Rf values of 0.5 (yellow) and 0.49 (yellowish red). Band I having a maximum wavelength of 309.1 indicating the presence of a conjugated double bond at -π* and band II have a maximum wavelength of 370.2.

Keywords: TLC, IR Spectrovotometry, Winged Bean Leaves.

Introduction

Indonesia is the second richest country in the world that has biodiversity after Brazil where 30,000 species of about 40,000 plant species that exist in the world live in the Indonesian archipelago (Lukito, 2011). Among the 30,000 species that live in Indonesia, at least 9,600 plant species have medicinal properties and approximately 300 species have been used as ingredients in traditional medicine by the traditional medicine industry (Depkes RI, 2007).

One of the plants that can be used as traditional medicine to inhibit bacterial growth is the winged bean (*Psophocarpustetragonolobus D.C.*), which belongs to the Fabaceae family or the legume group, which contains chemical saponins, flavonoids and tannins. Winged bean plant parts that can be used as traditional medicine include leaves. The leaves can be used as a medicine for diseases of the eyes and ears as well as ulcer medicine (Utami, P, 2008).

Based on the description above, a research will be conducted on the determination of flavonoid chemical compounds in Winged Wings (*PsophocarpustetragonolobusD.C.*) using Infrared Spectrophotometry method. The purpose of this study was to determine the chemical content of flavonoids from winged bean leaf extract (*Psophocarpustetragonolobus D.C.*) by using the Infrared Spectrophotometry method. The benefits of the results of this study can add information about the chemical content of winged bean plants and as a reference for further research on winged bean plants. (Mursiti et al, 2017)

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Materials and Methods

The type of research conducted is experimental research which is a laboratory research using Infrared Spectrovotometry. The research design is the identification of saponin compounds from the ethanol extract of Winged Beans (Psophocarpustetragonolobus D.C) by infrared spectrophotometry. (Wibowo et al, 2017)

Tools and Materials

The tools used include stirring rods, jam bottles, chambers and cover slips, glass funnels, 500 ml separating funnels, 250 ml Erlenmeyer, 500 ml, 100 ml, 250 ml, 500 ml beakers, 25 ml measuring cup, 50 ml , 100ml, Cotton, Filter paper, Ultraviolet lamp, Glass plate, Oven, Sprayer, Electric heater, Water bath, Sprayer, Rotavavor, Maceration set, Silica gel G60F254 nm

Than The materials used include Aquadest, aluminum foil, aluminum chloride, sulfuric acid 10%, ethanol extract of winged bean (PsophocarpustetragonolobusD.C), ethanol, FeCl3, chloroform, ethanol, infrared spectrophotometry

Sample Extraction

a. Extraction with ethanol solvent.
   Winged bean leaf samples that have been prepared weighed as much as 250 g, then extracted with ethanol by maceration and left for 5 days at room temperature and protected from sunlight while stirring every day. After 5 days, it is then filtered into a container and the dregs are squeezed out after that they are separated between the dregs and the juicer. The filtrate was evaporated with a vacuum rotary evaporator to obtain a completely concentrated extract (ethanol extract).
b. Extraction with ether solvent
   The thick ethanol extract obtained was suspended with distilled water, then extracted with 50 ml of ether in a separating funnel, carried out 3 times, the ether extract was collected and evaporated. The aqueous layer was then extracted with n-butanol.
c. Extraction with n-butanol pelarut solvent
   The thick ethanol extract obtained was suspended with distilled water, then extracted with 50 ml of n-butanol in a separating funnel, carried out 3 times, the n-butanold extract was collected and evaporated. (Amir, et al, 2022)

Separation by two-dimensional thin layer chromatography

Two-dimensional TLC was carried out on a single-stain fraction with two different types of eluents with the aim of proving that the fraction was a pure compound, namely, the single fraction obtained was spotted on a 10x10 cm silica gel plate with elucycloroform liquid: methanol (8:2), to direction 1, after being eluted, it was removed from the chamber, dried and then detected with a UV light stain of 254 nm, then the plate was rotated at 900 and then eluted again with elucycloroform liquid: methanol (8:2) for direction 2, after being eluted, it was removed from the chamber and then in dried, then detected using a UV light stain viewer 254 nm. (Maulana et al, 2022)

Identification using Infra Red Spectrophotometer

The isolates obtained from the results of preparative TLC were dissolved in ethanol and centrifuged then analyzed with an IR spectrophotometer. 0.2 gram of KBr pellet was added with one drop of isolate, dried and then observed for the spectrum at a wavelength of 4000-400 cm-1 (Rizkita et al., 2021)

Results and Discussion

Based on the results of the research on the isolation and identification of the chemical components of the winged winged bean (Psophocarpus tetragonolobus D.C) extract, the extract used was maceration of 250 grams using 250 ml of ethanol solvent for 5 (five) days and 5 grams of dry ethanol extract was obtained in get the following results:

Table 1. Identification results of n butanol extract with chloroform - methanol as elution liquid (8:2).

Fraksi	Rf	**Warna bercak pada Uv254 nm tanpa H_2S0_4 10%**	**Warna bercak dengan uap H_2S0_4 10%+ Uv 254 nm**
A	0,68	Kuning	Fluoresensi kuning
B	0,5	Hijau	Fluoresensi hijau
	0,48	Merah Kekuningan	Kuning tua
C	0.89	Hijau kekuningan	Merah Kekuningan

Table 2. Identification results of n butanol extract with chloroform - methanol as elution solution (7:3) and (6:4)

No Urut Noda	Nilai Rf		Warna Pada UV	
	(7:3)	(6:4)	(7:3)	(6:4)
1	0,26	0,48	yellow	green
2	0,11	0,35	green	yellow
3	-	0,25	-	brown

Table 3. Results of two-dimensional thin layer chromatography of the n-butanol extract

Klorofor m-metanol (8:2)	Warna noda dengan sinar UV 254 nm Tanpa **H_2S0_4 10%**	**H_2S0_4 10%**	Jumlah
Band I	-	Kunin g	1 noda
Band II	-	Kunin g	1 noda

From the isolation process of the n butanol fraction using chloroform-methanol (8:2) developer liquid, 3 (three) spots of the compound were obtained where 3 stain bands were formed on the preparative thin layer chromatography plate (complete results see Figure 1). The three bands were scraped and dissolved and then purified with developer liquid on the isolates, new spots were obtained from the separation of spots of fraction C, then sprayed with 10% sulfuric acid and green-yellow-green stains were obtained with an Rf value of 0.89, while the fraction A and B obtained two stains after spraying 10% Sulfuric Acid with Rf values of 0.5 (yellow) and 0.49 (yellowish red). (Rizkita et al, 2020).

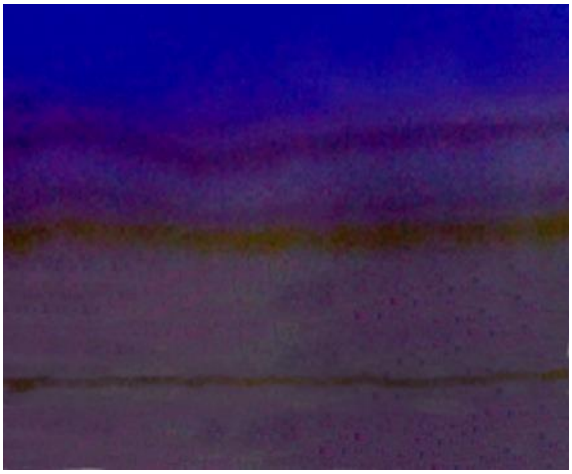

Figure 1. The results of the separation of TLC-P diethyl ether fraction with silica gel F254 as stationary phase, n-hexane mobile phase: ethyl acetate (8:2), swelling distance 20 x 20 cm

In the weight fractionation process, the diethyl ether fraction obtained was 1.11 grams from 3.38 grams of dry methanol extract. The identification results showed that the winged winged diethyl ether fraction contained chemical components after being identified by Prefabricated Thin Layer Chromatography (TLC) which was indicated by the presence of a stain (chromatogram) using Hexane - ethyl acetate as eluent (7:3) indicating that the stain separation indicated was better. (Rizkita et al, 2022)

The isolated n-butanol fraction was 1.11 grams, the eluent used was chloroform-methanol (7:3). During the isolation process, there was no change of eluent and continued to use the eluent of Chloroform-methanol (7:3). The isolated isolate was monitored for its TLC profile with Chloroform-methanol eluent (6:4). The isolation of the n-butanol fraction was then dripped with NaOH, H2SO4 and concentrated MgHCL powder to produce 3 isolates, namely isolates A, B and, C at Figure 2.

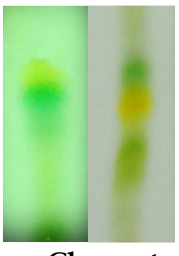

Figure 2. Thin Layer Chromatography Results of n-butanol Ekstrak Extract

From the three fractions, the purity test was continued by using two-dimensional TLC and two systems of the same solvent mixture with eluents of Chloroform-methanol (8:2) and n-hexane: ethyl acetate (8:2) which aims to extend the elution distance from the stain and provide eluents of different polarity. The three isolates gave the appearance of a stain, but only isolate C showed a single stain at 366 nm UV light, so it was assumed that the isolate (isolate C) was a pure compound at Figure 3.

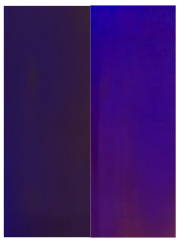

Figure 3. Two-dimensional TLC separation results of n butanol fraction with silica gel F254 as stationary phase, chloroform-methanol as mobile phase (8:2) and hexane: ethyl acetate (8:2) with developer distance 10x10 cm

The IR spectro results measured at a wavelength of 200-800 nm contained two bands, namely band I having a maximum wavelength of 309.1 indicating the presence of a conjugated double bond at -π* and band II having a maximum wavelength of 370.2 indicating a shift. double bond. so it can be presumed that the C fraction contains flavonoid compounds at Figure 4

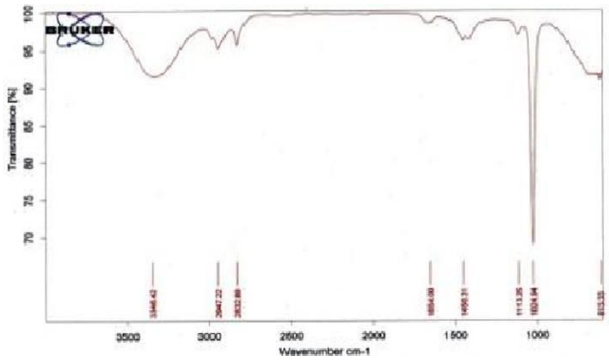

Figure 4. IR Spectrum of the n butanol fraction

Conclusions

Based on the results of the research conducted, it can be concluded that from the preliminary test results obtained positive results indicated by the presence of a yellow color and the results of Infra red (IR) spectrophotometry measurements obtained two bands, namely band I having a maximum wavelength of 309.1 indicating the presence of a conjugated double bond at -π* and band II have a maximum wavelength of 370.2, indicating that there is a shift in the double bond. So it can be assumed that fraction C contains flavonoid compounds. (Wibowo et al, 2018)

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