PHYTOCHEMICAL SCREENING ON LEAF ETHANOL EXTRACT Ba'balik Angin (*Alphitonia Excelsa*) WITH THIN-LAYER CHROMATOGRAPHY METHOD USING SOLVENT VARIATIONS

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Abstract

The leaves of Ba'balik Angin (*Alphitonia Excelsa*) are one of the plants used by everyday people to bathe because it can produce foam to remove dirt and dust and has benefits as an antiseptic. The Purpose This study to know the metabolite compounds used to be contained in the leaves of Ba'balik Angin (*Alphitonia Excelsa*) and know the phase of motion or eluen the best compounds. Method making Ba'balik Angin leaf extract (*Alphitonia Excelsa*) using the maceration method and using Rotary Evaporator until obtaining a viscous extract is then done identification of secondary metabolites to obtain color reactions and thin layer chromatography. The Results based on the results of research on the identification of secondary metabolite compounds Ba'balik angin leaf extract (*Alphitonia Excelsa*) obtained alkaloid compounds, flavonoids, tannins, terpenoids and saponins. It can be concluded that the alkaloids that have the best separation are Ethyl Acetate : Chlorofrom (12:6), Flavonoids that have the best separation is n-Heksan: dichloromethane (8:12), Tannins that have good separation are Methanol : Ethyl Acetate (8:2), Terpenoids that have the best separation and many stains are n-Hexane: Ethyl Acetate (12:8), Saponins that have the best separation and many stains are Ethyl Acetate :Ethanol:Water (10:2:1). In Conclusion From this study has been found the best phase of motion and eluent in separating the metabolite compounds of Ba'balik Angin leaves.

Keywords: Thin Layer Chromatography, Eluent Variation, Secondary Metabolites, Leaf Ba'balik Angin (Alphitonia Excelsa)

Introduction

In developing countries, one of them is Indonesia which has the largest tropical forest in the world which has potential as a medicinal plant. Of the approximately 40,000 thousand types of medicinal plants that have been known in the world, there are 30,000 of them allegedly located in Indonesia. However, there are only 1,200 types of plants that have been used as raw materials for herbal medicines (Nugroho and Ningsih., 2017).

Islands in Indonesia that have abundant natural resources, one of which is the island of Kalimantan, precisely in the province of South Kalimantan, in the Kotabaru area, Cantung village, downstream left, Kelumpung Hulu, which has Ba'balik wind plants that can grow in forest areas, shrubs, and steep slopes., et al., 2016). This plant is usually used by people for bathing because it can produce foam to remove dirt and dust. This Ba'balik leaf has a distinctive mint smell. How to use the leaves of Ba'balik Angin, namely by washing the leaves and then placing them in a container that already contains water and then rubbing it until it produces foam. It turns out that from Australian research according to (Tarannum Naz, 2013) there are efficacy of the Ba'balik Angin plant (Alphitonia Excelsa) as an antiseptic on the skin and contains secondary metabolites in the plant. The secondary metabolites of Ba'balik Angin leaf include flavonoids, saponins, and tannins where these compounds have medicinal effects. The part in the treatment of strong positive young leaves contains saponins because the characteristic of saponins (sapo) is to produce foam.

Identification of secondary metabolites can be done by using chemical reagents to see secondary metabolites from various reagents and using the Thin Layer Chromatography method.

Thin Layer Chromatography (TLC) is the separation of chemical compounds between two phases, namely the mobile phase and the stationary phase. The mobile phase is a single solvent or a mixture in which the extract undergoes separation while the stationary phase is a TLC plate coating material from selika gel powder (Lade et al, 2014). Thin Layer Chromatography is a qualitative analysis of a sample that can be detected to separate sample components based on differences in polarity levels, while the working principle is to separate samples based on differences in polarity between the sample and the solvent used. This technique usually also uses a stationary phase from the form of a silica gel plate and a mobile phase that has been adjusted to the type of sample to be separated. The solution or mixture of solutions to be used is called the eluent. The closer the polarity between the sample used and the eluent, the more the sample will be carried away by the mobile phase. Based on the description above, the reason for this research is to detect chemical compounds using the Thin Layer Chromatography method using a variety of solvents on the leaves of Ba'balik Angin (Alphitonia Excelsa).

Material and Methods

The material used this study in Leaf Ba'balik 96% ethanol, aquadest, FeCl3, Mayer and Dragendorff reagents, Concentrated HCL, Methanol, N-Butanol, Chloroform, N-Hexane, Acetic Acid, Benzene, Ethyl acetate, Dichloromethane.

The tools used in this scissors, basin, jar, stirring rod, chamber, beaker, pipette, ultraviolet lamp, test tube, filter paper, cloth, test tube rack, TLC plate silica gel GF 254.

The method used in this research is descriptive qualitative research to see the description or chromatographic profile of secondary metabolites with the best eluent variation.

Sample Preparation

The sample used is the Ba'balik Angin Leaf (Alphitonia Excelsa) obtained in the city area Kotabaru Cantung kiri hilir,kelumpung hulu and conducted research at the Pharmaceutical Technology Laboratory, Department of Pharmacy, University of Sari Mulia Banjarmasin.

Identification of Secondary Metabolites With Chemical Reagent.

Flavonoid Test

Flavonoid testing will be carried out by taking 1-2 ml of extract and adding one gram of Mg powder and concentrated HCL solution. If it produces a red-orange color it contains positive flavonoids (Noviyanti and Linda, 2020).

Alkaloid Test.

The alkaloid test will be carried out by taking 1-2 ml of extract which has been dissolved with 5 ml of HCI2N. The solution obtained was divided into 2 test tubes. Extraction of the leaves of Ba'balik Angin was added with 3 drops of Mayer and Dragendrof reagents. A positive result is the presence of alkaloids when a white precipitate is formed with Mayer's reagent and Orange with Dragendrof's reagent (Fadhly, ddk, 2015).

Terpenoid Test

Terpenoid testing will be carried out by taking 1-2 ml of leaf extract which has been dissolved with n-hexane. Then, put a little into a test tube which then add 1 ml of glacial acetic acid, 1 ml of concentrated H2SO4. If there is a reddish brown ring formed at the boundary of the two solvents, it indicates the presence of terpenoids (Fajriaty, 2018).

Tannin Test

Tannin testing will be carried out by taking 1-2 ml samples with a few drops of 1% FeCl3 added. If the solution has a greenish color or a blue-black color is formed, it is positive that it contains tannins (Purwati et al., 2017).

Saponin Test

A total of 1 gram of leaf extract was put into a test tube, added 10 mL of hot water, then shaken vigorously for 10 seconds containing saponins to form a foam as high as 1-10 cm for not less than

10 minutes and with the addition of 1 drop of 2 N HCL, the foam did not disappear. (Muthmainnah, 2017).

Identification of Secondary Metabolites by Variation of Eluent Method

Silent phase

In selecting the stationary phase for the identification or separation of compounds using a TLC gel plate GF 254 for all compounds and activated in an oven at 105 for 30 minutes.

Mobile phase

The selection of the mobile phase/eluent is based on the chemical nature of a secondary metabolite compound to be studied, namely the level of polarity of the substance. The combination of various mobile phases or eluents will result in good identification or separation of substances, while the mobile phase or eluent is used for the identification and separation of a secondary metabolite compound to be studied.

Table 1. Varition of Eluent/Mobile Phase

Secondary metabolite compounds	Eluen/ mobile phase	References
Flavonoids	n-hexane : dichloromethane (8:12)	Aisyah, dkk., 2019
Alkaloids	Ethyl acetate : Chloroform (12 :6)	Fadhly, dkk., 2015
Terpenoids	n- hexane : Ethyl acetate (12:8)	Dwisari, dkk., 2016
Saponins	Ethyl acetate : ethanol :water (10 : 2 : 1)	Amody and Anggreani., 2017
Tanin	Methanol: ethyl acetate (8:2)	Sopianti and Sari., 2018

Result and Discusion

This research was conducted to determine the best eluent variation from Ba'balik Angin Leaf Extract (Alphitonia Excelsa). The simplest was made to facilitate extraction by maceration method with 96% ethanol solvent, the choice of 96% ethanol solvent because it has a broad absorption capacity so that all compounds can be pulled out of the simplicia at a temperature of 65°C in a rotary evaporator. Thin Layer Chromatography is a method used to separate a mixture of chemical compounds based on their distribution between two phases, namely the stationary

phase and the mobile phase. Before spotting the sample, the stationary phase to be used is activated in the oven for 30 minutes at a temperature of 105°C. This aims to remove the water content on the plate so that the absorption capacity of the plate is maximized. After that, make the mobile phase solvent according to the ratio that has been set, before inserting the TLC plate into the chamber, saturate it first with the aim that the illusion on the eluent is not bent and so that the illusion is stable and rises up well. Then smear the sample using a capillary tube on the TLC plate, place the TLC plate that has been smeared with the sample into the saturated chamber, let stand and wait for the election to rise to the upper limit. Phytochemical identification of secondary metabolites tested by the test tube method included alkaloids, flavonoids, saponins, terpenoids and tannins and the separation of compounds by thin layer chromatography method.

Table 2. Identification of Secondary Metabolites by Color Reaction

Compound	Reagent	Result
Alkaloids	Mayer's reagent	Positif
	Dragendorft's reagent	Positif
Flavonoids	Concentrated Mg and HCl powder	Positif
Tannins	$FeCl_3$ 1%	Positif
Terpenoids	1 mL glacial acetic acid + 1 mL H_2SO_4 solution	Positif
Steroids	1 mL glacial acetic acid + 1 mL H2SO4 solution	Negatif
Saponins	10 mL of water and 1 drop of 2 N . HCl	Positif

Table 3. Separation of Compounds by TLC Method

Compound	Eluent and Reagent	Number of Stains	Visible Light (Rf)
Alkaloids	Ethyl acetate : Chloroform (12 :6) (Fadhly, dkk., 2015) (Reagen Dragendorff)	2	0,06
			0,2
Flavonoids	N-hexane : dichloromethane (8:12) (Aisyah, dkk., 2019) (Reagen Amonia)	4	0,01
			0,05
			0,08
			0,83
Tannins	Methanol: ethyl acetate (8:2) (Amody dan Anggraeni., 2017) (Sopianti dan Sari, 2018) (Reagen FeCl3 5%)	6	0,03
			0,13
			0,46
			0,66
			0,73
			0,9
Terpenoids	n-Hexane : Ethyl acetate (12:8) (Dwisari, dkk., 2016) (Reagen Lieberman Burcard)	4	0,03
			0,08
Saponins	Ethyl acetate : ethanol :water (10 : 2 : 1)	6	0,05
			0,33

(Sopianti dan Sari, 2018)	0,45
(Reagen FeCl3 5%)	0,51
	0,66
	0,76

Alkaloid

Testing for alkaloids using Mayer's reagent showed a white precipitate if the positive result contained alkaloids, a white precipitate would form with Mayer's reagent (Fadhly et al., 2015). For the formation of a white precipitate in Mayer's reagent, it is estimated that nitrogen in the alkaloids will react with metal ions K+ from potassium tetraiodomercurate(II) to form a precipitated potassium-alkaloid complex (Ergina, 2014).

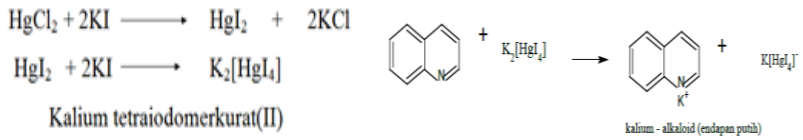

Figure 1. Structure of Mayer and Dragendroff (Ergina, 2014)

While Dragendroff got an orange color, if the positive result contains alkaloids, an orange precipitate will be formed with Dragendroff's reagent (Fadhly, et al, 2015). Dragendorff's reagent contains bismuth nitrate and potassium iodide in a solution of glacial acetic acid (potassium tetraiodobismuthate (III), an orange precipitate is formed, the precipitate is potassium alkaloid. In the alkaloid test with Dragendorff's reagent, nitrogen is used to form a coordinate covalent bond with K+ which is a metal ion (Ergina, 2014).

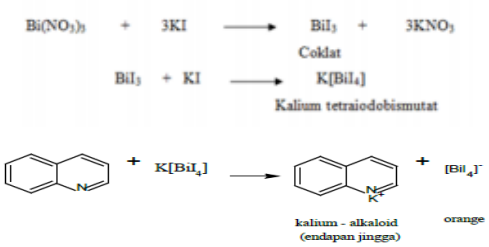

Figure 2. Structure of Alkaloids with Dragendroff (Ergina, 2014)

Then the TLC test was carried out on the separation of the best mobile phase alkaloid compounds in this compound, namely Ethyl acetate: Chloroform (12:6), producing two stains in visible light after being sprayed with Dragendroff's reagent producing an orange color. Separation occurs by marked the presence of spots that can be formed that have an Rf value because the eluent and mobile phase to be used are in accordance with the general physical

properties of alkaloids which are slightly soluble in water and soluble in non-polar organic solvents such as ethyl acetate: chloroform (Julianto, 2008). 2019). The color changes to orange because the alkaloids have a tertiary amine group RN, this group can react similarly to ammonia (NH3) and act as a base that can react with acids to form ammonia salts. R3N + HX → [R3NH] = + X-

Description (X = acid anion = CI-, NO3-, HsO4-, CH3COO) An ion exchange reaction occurs between ammonia and potassium tetraiodobismuth which leads to the formation of an insoluble complex salt. [R3NH+X- + K[BiI4] → [R3NH]+[BiI4]- + KX. This can occur depending on the nature of the tertiary amine alkaloids will produce intensive colors that have a yellow to orange (orange) to red to brown color after being sprayed by Dragendroff (Baerheim-Svendsen and Verpoote 1983; Popl et al. 1990; Pedersen 2006 in Raal. a 2020).

Flavonoid

Flavonoid testing is used Mg and HCL powder until a red-orange color is obtained. If it produces a red-orange color it contains positive flavonoids (Noviyanti and Linda, 2020). The purpose of adding Mg and HCl is to reduce the benzopyron core contained in the flavonoid structure so that red or orange flavilium salts are formed (Ergina, 2014).

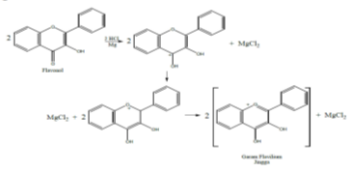

Figure 3. Basic structure of Mg and HCI (Ergina, 2014)

Then the TLC test was carried out on the separation of flavonoid compounds, the best mobile phase of this compound was hexane: dichloromethane (8:12), producing four spots in visible light after evaporation, UV 366 light contained one spot after evaporation. The flavonoid compounds in the leaves of Ba'balik Angin have the most stains in non-polar solvents. Furthermore, a dark yellow stain is formed after being evaporated with ammonia, which means that flavonoids compounds are formed. This happens because flavonoids are reacted with ammonia to form quinoids in this test. The yellow color occurs due to the formation of quinoids in the ring (Warsi, A R Sholichah, 2017).

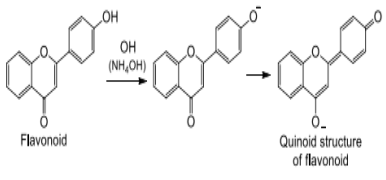

Figure 4. Structure of flavonoids and ammonia (Warsi, A R Sholichah, 2017).

UV 366 light appears light blue fluorescence and after being evaporated with ammonia a yellow green or blue green fluorescence appears. The types of flavonoids are flavones and flavonones that do not have free 5-OH or flavonols that do not have free 5-OH, but have substitutions on 3-OH (Hanani, 2014).

Tannin

Tannin testing is used FeCl3 5% until a black color is obtained, if the solution a greenish color is formed or formed blue-black color means that it is positive for tannins (Purwati, et al., 2017). The presence of a phenol group is indicated by a blackish green or dark blue color after the addition of FeCl3, if the secondary metabolite test is positive, it is possible that the sample contains phenolic compounds and it is possible that one of them is tannins because tannins are polyphenolic compounds. The classic way to detect simple phenolic compounds is to add the extract with 5% FeCl3 solution water which causes green, purple, red, blue colors. The formation of blackish green/blue ink in the extract after being added with FeCl3 will form a complex compound with Fe3+ ions (Ergina, 2014). Then the TLC test when sprayed with FeCl3 will produce a black color where the tannins are hydrolyzed.

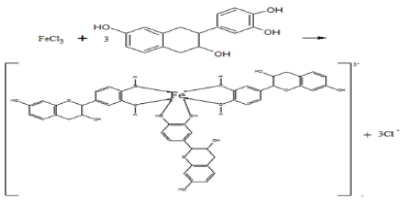

Figure 5. Basic structure of Tannins and FeCl3 (Ergina, 2014)

Then the TLC test was carried out on the separation of tannin compounds using a FeCl3 spray to produce a black color, the best mobile phase was Methanol:Ethyl acetate (8:2), producing six stains after spraying. Ba'balik Angin leaves have the most stains on the mobile phase which is non-polar because it is in accordance with the nature of hydrolyzed tannins which have OH groups.

Terpenoid

Terpenoid testing is using glacial acetic acid and concentrated H2SO4 solution, the results obtained are a reddish brown ring is formed on the border of the two solvents. The terpenoid test reaction is the condensation or release of H2O and incorporation with carbocations. This reaction begins with the acetylation of the hydroxyl group using acetic anhydride. The acetyl group which is a good leaving group will be separated, thus forming a double bond. Subsequently, the transfer of the double bond group occurs. These compounds undergo resonance acting as electrophiles followed by elongation releases as electrophiles or

carbocations. The carbocation causes electrophilic addition, followed by the release of conjugate extensions that appear brownish rings (Nugrahani, 2016).

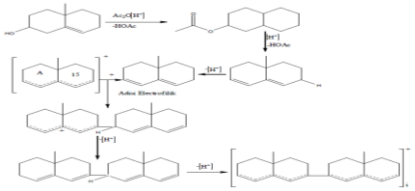

Figure 6. The basic structure of the reaction of triterpenoids with Liembermann-Burchard reagent (Nugrahani, 2016)

Then the TLC test on the separation of terpenoid compounds using a Lieberman Burchad sprayer produced the best mobile phase purple color for this compound, namely n-Hexane: Ethyl acetate (12:8), producing two stains in visible light after spraying, indicating there was a triterpenoid group marked by color purple (Fajriaty, 2017). Most stains in the mobile phase are non-polar because they are in accordance with the general nature of terpenoids which are soluble in organic solvents and usually insoluble in water (Julianto, 2019). The purple color change is due to the ability of terpenoid compounds to form color by H2SO4 in anhydride solvents.

Saponins

In the saponin test, which is using hot water and cooled then shaken vigorously for 10 seconds to form a foam as high as 1-10 cm. , the foam does not disappear (Muthmainnah, 2017). The appearance of foam indicates the presence of glycosides which have the ability to form foam in water which is hydrolyzed to glucose (Nugrahani, 2016).

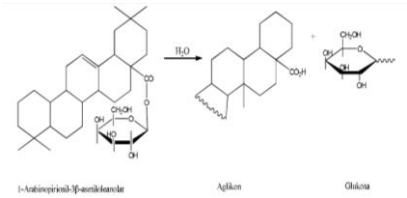

Figure .7 The basic structure of the hydrolysis reaction and water (Nugrahani, 2016)

Then the TLC test was carried out on the separation of saponin compounds using a sprayer with H2SO4 H2SO4 reagent to produce a yellow color, the best mobile phase for this compound was Ethyl acetate: Ethanol: Water (10:2:1), producing six stains after being sprayed in the light, it was marked with visible stains. purple on the TLC plate (Fajriaty, 2017). The most stains in the mobile phase are polar groups because they have a hydrophobic group, namely an aglycone (sapogenin) (Agustina, et al, 2017). There is a change in the color of the stain due to the nature of H2SO4 or sulfuric acid has oxidizing properties so that if the stain is not visible in UV light it will be visible after spraying H2SO4 because the structure of the chemical components is broken down so that the bond changes (Wahyuni, et al, 2018).

Conclusion

The selection of the best eluent variation was found in the separation of secondary metabolites from the extract of Ba'balik Angin Leaf (Alphitonia Excelsa) using the Thin Layer Chromatography method.

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Declaration of Interest Statement

The authors declare that they have no conflict of interest

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