THIN LAYER CHROMATOGRAPHY PROFILE OF SECONDARY METABOLITES IN TAMPALA TRUNK EXTRACT (*Spatholobus littoralis hassk*)

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Abstract

Bajakah Tampala Trunk *(Spatholobus littoralis hassk)* is a plant that is used as a traditional medicine that is useful for the treatment of rheumatic pain, dysentery, and wound medicine in the community empirically or for generations in Central Kalimantan. The purpose of this study was to determine the Thin Layer Chromatography Profile, the mobile phase or the best eluent in the separation of secondary metabolites in the Bajakah Tampala stem extract *(Spatholobus littoralis hassk)*. The research method on making extract of Bajakah Tampala trunk *(Spatholobus littoralis hassk)* by maceration method, concentrated by rotary evaporator to obtain thick extract, identification by color reaction. Separation of secondary metabolites by TLC method from various ratios of eluent or mobile phase. The result of Bajakah Tampala trunk (*Spatholobus littoralis hassk*) extract was Positive secondary metabolites with color reactions are alkaloids, flavonoids, saponins, tannins, and terpenoids. The TLC profile showed the presence of mobile phase or the best eluent in the separation of secondary metabolites, namely alkaloids with a ratio of acetate: dichloromethane (8:8). Flavonoids with a ratio of n-hexane: ethyl acetate (8:12). Saponins with a ratio of Chloroform: Methanol: Water (15:5:1). Tannins with a ratio of butanol: acetic acid (8:2). Terpenoids with a ratio of n-hexane: ethyl acetate (12:8). In Conclusion, The best eluent or mobile phase was found in the separation of metabolite compounds from the extract of Bajakah tampala trunk (*Spatholobus littoralis hassk*) with TLC method.

Keywords: Thin Layer Chromatography, eluent, mobile phase,, Tampala Bajakah trunk (*Spatholobus littoralis hassk)*.

Introduction

Indonesia is a tropical country so it has many natural biological resources in the form of abundant medicinal plants. Indonesia has the second largest potential for medicinal plants in the world. A total of about 40,000 types of medicinal plants that have been known in the world, 30,000 of which are allegedly located in Indonesia, of which 940 species have been declared medicinal, of which about 78% are still obtained through direct extraction from the forest (Jamun et al, 2020).

The island of Kalimantan, which is located in Indonesia, has a diversity of medicinal plants, in that area there is a Bajakah Tampala plant (*Spatholobus littoralis hassk*) as a traditional medicine. Bajakah Tampala (*Spatholobus littoralis hassk.)* is located in the village of kampuri, Mihing Raya sub-district,

Gunung Mas district, Central Kalimantan province. People in Kampuri village use the Tampala Bajakah trunk (*Spatholobus littoralis hassk*) as a traditional medicine that is useful as a treatment for aches and pains, dysentery and wound medicine for generations. In other areas, precisely in the East Nusa Tenggara area, research has been carried out on the usefulness of the Bajakah Tampala plant *(Spatholobus littoralis hassk)* as a treatment for diarrhea (Kusumanegara A et al, 2020). The Bajakah Tampala plant (*Spatholobus littoralis hassk*) contains secondary metabolites that have medicinal effects including alkaloid compounds, polyphenols including flavonoids, and terpenoids (Julianto, 2019).

Identification of secondary metabolites using the Thin Layer Chromatography (TLC) method, namely by separating chemical compounds based on the distribution of two phases, namely the stationary phase and the mobile phase (Aladin, 2017).

The method of separating compounds using Thin Layer Chromatography (TLC) with various variations of eluent or mobile phase. This aims to be able to determine the eluent or mobile phase that is good in separating a compound that is determined with a good Rf value and sprayed with specific reagents, this is aimed at determining the class of secondary metabolite compounds (Wulandari, 2011).

In the Bajakah Tampala (*Spatholobus littoralis hassk*) plant, thin layer chromatography profile variations have not been carried out on secondary metabolites, so it is necessary to identify secondary metabolites using the Thin Layer Chromatography method. This is to be able to determine the mobile phase or a good eluent with a variety of solvents or eluents based on the level of polarity so as to find a Thin Layer Chromarography profile with good separation. Based on this description, researchers need to conduct research on the identification of secondary metabolites in the trunk extract of Bajakah Tampala (*Spatholobus littoralis hassk*) with a thin layer chromatography profile solvent variation method.

Material and Methods

The materials used in this study were Bajakah Tampala trunk simplicia *(Spatholobus littoralis hassk)*, 96% ethanol, $FeCl_3$, 1% $FeCl_3$, 5% $FeCl_3$, 10% gelatin, acetic acid, chloroform, dichloromethane, 2N HCl, concentrated HCl, N- butanol, methanol, n-hexane, ethanol, water, acetone, ethyl acetate, benzene, Mayer's reagent, Dragendorff's reagent, Lieberman burchard's reagent, Mayer's reagent, ammonia, H_2SO_4 10%, concentrated H_2SO_4, glacial acetic acid, Mg powder.

The tools used in this study were a jar, measuring cup, Rotary evaporator, Chamber, funnel, stirring rod, beaker, erlenmayer, filter paper, dropper, scissors, tube rack, test tube, horn spoon, TLC plate silica gel GF. 254, spray bottle and UV lamp.

Method

The method used in this research is descriptive observation which is carried out to determine secondary metabolites and good solvent or eluent variations using the Thin Layer Chromatography (TLC) method.

Sample Preparation

Sample preparation was carried out from the manufacture of simplicia, then to maceration with 96% ethanol solvent and concentrated with a rotary evaporator.

Identification of Secondary Metabolites With Chemical Reagents.

Alkaloid Test

The trunk extract of Bajakah Tampala *(Spothalobus littoraslis hassk)* as much as 1-2 mL was dissolved with 5 mL of 2N HCl. The solution obtained was divided into 2 test tubes. Bajakah Tampala (Spothalobus littoralis hassk) trunk extract was added with 3 drops of Mayer and Dragendroff reagents. Positive results for the presence of alkaloids are formed when a white precipitate is formed with Mayer's reagent and Orange with Dragendorff's reagent (Fadhly, et al., 2015).

Flavonoid Test

Bajakah Tampala trunk extract (*Spothalobus littoralis hassk*) as much as 0.5 grams was put into a test tube, added with one gram of Mg powder and concentrated HCL solution. A change in the color of the solution to pink/orange indicates the presence of flavonoid compounds (Noviyanti and Linda, 2020).

Saponin Test

Bajakah Tampala *(Spothalobus littoralis hassk*) trunk extract as much as 1 gram was put into a test tube, added 10 mL of hot water, cooled then shaken vigorously for 10 seconds, was positive for saponins if 1-10 cm high foam was formed, not less than 10 minutes and on addition 1 drop of 2 N HCl, the foam does not disappear (Muthmainnah, 2017).

Tannin Test

The tannin/polyphenol test was carried out by adding a 31% FeCl solution into 1 mL of Bajakah Tampala trunk extract *(Spothalobus littoralis hassk)*. A positive result of tannins/polyphenols is the formation of a dark blue or greenish black color in the test sample (Purwati et al., 2017).

Terpenoid and Steroid Test

The thick extract of Bajakah Tampala trunk *(Spatholobus littoralis hassk)* was first dissolved with n-hexane. Then, put a little into a test tube which is then added 1 mL of glacial acetic acid and 1 mL of concentrated HS_2O_4 solution.

If there is a reddish brown ring formed on the border of the two solvents, it indicates the presence of terpenoids, whereas if a blue or green ring is formed, it indicates the presence of a group of steroid compounds (Fajriaty, 2018).

Identification of Secondary Metabolites by Variation of Eluent Method

Silent Phase Selection

Selection of stationary phase for identification or separation of compounds using silica gel TLC plate GF 254 with a length of 8 cm and a width of 2 cm, then activated in the oven for 10 minutes at a temperature of 100.

Mobile Phase Selection

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/eluents will give good identification or separation of substances. Selection of mobile phase/eluent for identification or separation of a secondary metabolite compound to be studied, namely:

Table 1. Variation of Eluent/Mobile Phase

Secondary metabolite compounds	**Eluent/mobile phase**	**Rasio**	**References**
Alkaloids	Ethyl acetate : Dichloromethane	(8:8)	Fadhly, dkk., 2015
Flavonoids	*n*-hexane:ethyl acetate	(8:12)	Aisyah, dkk., 2019
Saponins	Chloroform : Methanol : Water	(15: 5:1)	Amody dan Anggreani, 2017
Tanin	*n*-butanol : acetic acid	(8:2)	Sopianti dan Sari, 2018
Terpenoid	*n*-hexane : ethyl acetate	(12:8)	Dwisari, dkk., 2016

Result and Disscusion

Making simplicia is done to facilitate the extraction process by maceration method using 96% ethanol as solvent. The maceration results were filtered and the filtrate was taken and then concentrated with a rotary evaporator with a temperature of 65 ℃ to produce a concentrated extract of Bajakah Tampala trunk (*Spatholobus littioralis hassk*)

Phytochemical identification is a qualitative test that is useful for identifying the content of active compounds contained in Bajakah Tampala *(Spatholobus littoralis hassk)* trunk using compound reagents. Then, continued with the Thin Layer Chromatography (TLC) method to emphasize the secondary metabolite compounds contained in plants and determine the best mobile phase in the separation of compounds which are then visualized using three kinds of light, namely visible light with a wavelength of 400-700 nm, 254 nm UV light which is classified as UV C light, 366 nm UV light including UV A (BMKG, 2021). The results of identification of secondary metabolites with color reagents and identification of compound separation by TLC method can be seen in the following table..

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
Saponins	10 mL of water and 1 drop of 2 N . HCl	Positif
Steroids	1 mL glacial acetic acid + 1 mL H_2SO_4 solution	Negatif
Terpenoids	1 mL glacial acetic acid + 1 mL H_2SO_4 solution	Positif
Tannins	$FeCl_3$ 1%	Positif

Table 3. Separation of Compounds by TLC Method

Compound	Eluent and Reagent	Number of Stains	Visible Light (Rf)
Alkaloids	Ethyl acetate : Dichloromethane (8:8) (Fadhly, dkk., 2015) (Reagen Dragendorff)	4	0,05
			0,25
			0,40
			0,55
Flavonoids	n-Hexane : Ethyl acetate(8:12) (Aisyah, dkk., 2019) (Reagen Amonia)	8	0,03
			0,20
			0,26
			0,46
			0,71
			0,83

			0,93
			1
Saponins	Chloroform : Methanol : Water (15:5:1) (Amody dan Anggraeni., 2017) (Reagen H2SO4)	5	0,03
			0,06
			0,13
			0,88
			0,96
Terpenoids	n-Hexane : Ethyl acetate (12:8) (Dwisari, dkk., 2016) (Reagen Lieberman Burcard)	4	0,13
			0,40
			0,50
			0,90
Tannins	Butanol : Acetic acid (8:2) (Sopianti dan Sari, 2018) (Reagen FeCl3 5%)	5	0,05
			0,35
			0,46
			0,63
			0,83

Alkaloids

Identification of alkaloids in the sample of Bajakah Tampala trunk extract *(Spatholobus littoralis hassk)* added with 2N HCl. The function of adding 2N HCl (normality) is that the alkaloids are alkaline so they are usually extracted with acid-containing solvents (Harborne, 1987). Then, each extract was tested by adding a specific reagent for alkaloids, namely Mayer's reagent giving a positive

result in the presence of a white precipitate and Dragendroff's reagent giving a positive result with an orange/brick red precipitate.

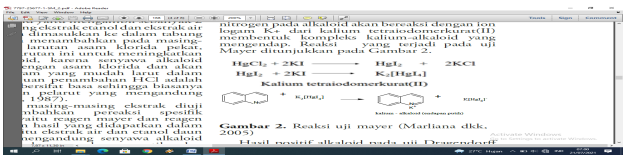
The principle of this method is the precipitation reaction caused by the change of the ligand. The nitrogen atom which has a lone pair of electrons in the alkaloids can replace ions in the reagents that occur in Mayer's reagent and Dragendorff's reagent. The nitrogen atom which has a lone pair of electrons in the alkaloids can replace ions in the reagents that occur in Mayer's reagent and Dragendorff's reagent. Alkaloids contain nitrogen atoms that have lone pairs of electrons so that they can be used to form coordinate covalent bonds with metal ions.

Alkaloids have a nitrogen atom that has a lone pair of electrons so that it can be used to form coordinate covalent bonds with metal ions. In the alkaloid test with Mayer's reagent, it is estimated that nitrogen in the alkaloids will react with K^+ ions from potassium tetraiodomercurate(II) to form a precipitated potassium-alkaloid complex (Ergina, 2014) which can be seen in Figure 4.1.

$$HgCl_2 + 2KI \longrightarrow HgI_2 + 2KCl$$

$$HgI_2 + 2KI \longrightarrow K_2[HgI_4]$$

Kalium tetraiodomerkurat(II)



Source: (Ergina, 2014)

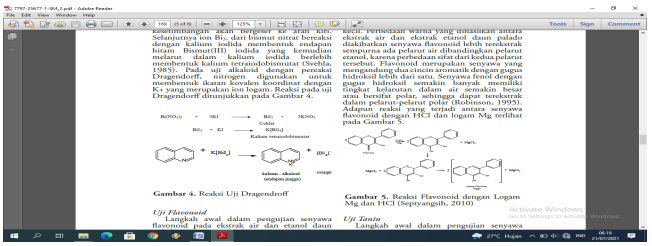
Figure 1. Reaction Structure of Alkaloids with Mayer's Reagent

The identification of alkaloids by Dragendroff's test also indicated a light brown-orange/brick red precipitate. The precipitate is a potassium alkaloid. In the identification of alkaloids with the Dragendorff reagent test, nitrogen is used to form a coordinate covalent bond with K+ which is a metal ion (Ergina, 2014) which can be seen in Figure 4.2.

$$Bi(NO_3)_2 + 3K \quad BiI_3 + 3KNO_3$$

Brown

$$BiI_3 + KI \quad K[BiI_4]$$



Source: (Ergina, 2014)

Figure 2. Reaction Structure of Alkaloids with Dragendorff's Reagent

The next test was to separate the compounds using the TLC method and the best eluent or mobile phase was ethyl acetate: dichloromethane (8:8). There were four stains indicating the alkaloid group in visible light, marked with an orange stain on a yellow background on the TLC plate after being sprayed with Dragendorff's reagent (Harborne, 1996). Separation occurs marked by the formation of spots that have an Rf value due to the eluent or mobile phase used 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 and chloroform (Julianto, 2019). The color change to orange with Dragendorff's reagent is because most alkaloids have a tertiary amine group RN. This group of compounds can react similarly to ammonia (NH^3) and act as a base reacting with acids to form ammonia salts.

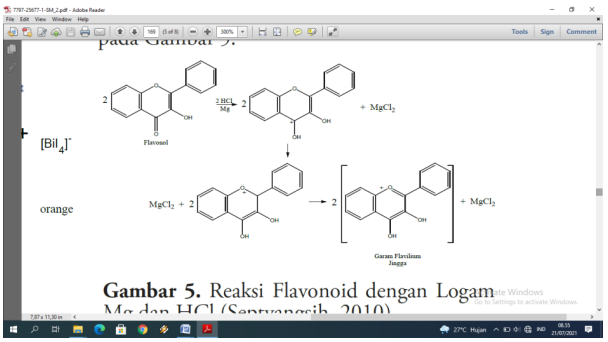
R3N + HX [R3NH]+ + X^-

Description: (X = acid anion = Cl^-, NO^{3-}, HsO^{4-}, CH_3COO^-,...)

This happens depending on the nature of the alkaloid (tertiary amine), this ion pair has a yellow to red orange to brown color. Secondary amines will produce less intense colors. (Baerheim-Svendsen and Verpoote 1983; Popl et al. 1990; Pedersen 2006 in Raal., et al, 2020).

Flavonoids

Identification of flavonoids in Bajakah Tampala *(Spatholobus littoralis hassk)* trunk extract was carried out by adding Mg powder and concentrated HCl solution to the sample extract, the color changed to orange which indicated that the sample extract was positive for flavonoids. 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) which can be seen in Figure 4.3.

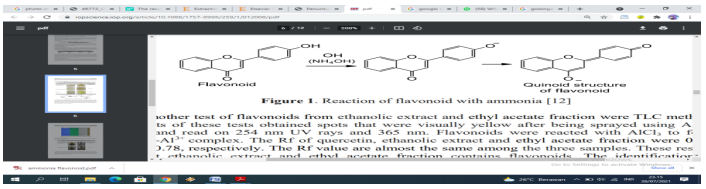


Source: (Ergina, 2014)

Figure 3. Reaction Structure of Flavonoids with Mg and HCl

The next test was carried out by separating the compounds using the TLC method, the best eluent or mobile phase was n-hexane : ethyl acetate (8:12) there were eight stains indicating the flavonoid group in visible light with a yellow-brown stain marked in visible light and two blue stains on UV light at 366 nm after being evaporated with ammonia (Harborne, 1996).

The flavonoid compounds in the Bajakah Tampala trunk *(Spatholobus littoraalis hassk* have the most spots on the eluent or mobile phase which are semi-polar groups where the flavonoid compound in the Bajakah Tampala trunk *(Spatholobus littoraalis hassk)* has a flavonoid structure that can dissolve in semi-polar solvents. Then the formation of a thick yellow stain after being sprayed with ammonia means that it contains flavonoid compounds due to the formation of quinoids in the -ring which contains a longer conjugated bond (Warsi and Sholichah, 2017) which can be seen in Figure 4.4.



Source: (Warsi and Sholichah, 2017).

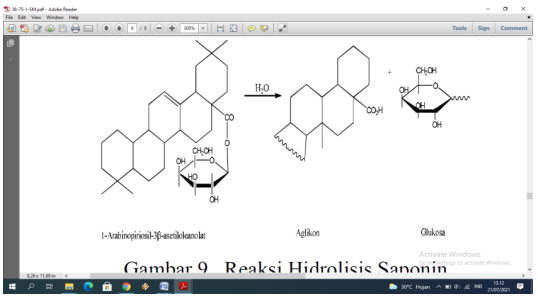
Figure 4. Reaction Structure of Flavonoids with Ammonia

Furthermore, in UV light at 366 nm after evaporation of ammonia, there was a color change or no color change or light blue fluorescence on the two stains, the type of flavonoid that may be related is isoflavones that do not contain free 5-OH (Yuda, et al., 2017).

Saponins

Identification of positive saponin compounds containing saponins because foam is formed that does not disappear indicates the presence of glycosides that have the ability to form foam in water which is

hydrolyzed into glucose and other compounds (Muthmainnah 2017; Nugrahani, et al., 2016). The reaction that occurs can be seen in Figure 4.4



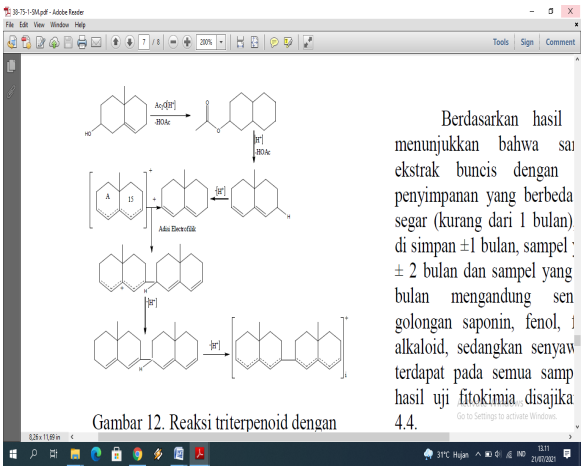
Source: (Nugrahani, et al., 2016)

Figure 5. Reaction Structure of Saponins with H_2O

The next test was carried out by separating the compounds using the TLC method. The best eluent or mobile phase was chloroform: methanol: water (15:5:1). There were five stains indicating the saponin group in visible light, marked with purple stains after being sprayed with 10% H_2SO_4. (Fajriaty, 2017). Good separation of compounds in the separate extract in the ratio of eluent or mobile phase which is more polar due to the presence of water. Saponins are compounds that are soluble in polar solvents and saponins are also nonpolar due to the presence of a hydrophobic group, namely an aglycone (sapogenin) (Agustina, et al., 2017). Then the formation of a purple stain due to the acidic nature of H_2SO_4 can reveal an invisible stain. H_2SO_4 has oxidizing properties so that if the stain that is not visible in the UV lamp will appear on spraying H_2SO_4, this happens because the structure of the chemical components is broken down so that the bond changes (Wahyuni, et al., 2018).

Steroids/Terpenoids

The results obtained were positive containing the presence of triterpenoids with the formation of a reddish brown ring at the border of the two solvents and negative for steroids because no blue or green rings were formed (Fajriaty, 2018). The principle of the reaction in the reaction mechanism of the triterpenoid test is the condensation or release of H2O and incorporation with carbocations. This reaction begins with the acetylation of the hydroxyl group using glacial acetic acid. Then there is the release of the hydrogen group along with the electrons, so that the double bond moves. The compound undergoes resonance acting as an electrophile or carbacation. The attack of the carbacation causes electrophilic addition, followed by the release of hydrogen. Furthermore, the hydrogen group along with its electrons is removed, then the compound undergoes conjugation extension which shows the appearance of a brownish ring (Nugrahani, et al., 2016) which can be seen in Figure 4.6.

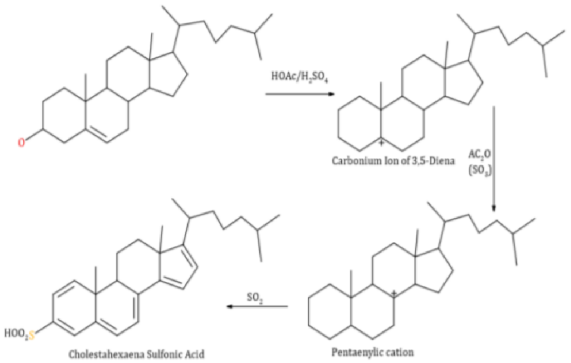


Source: (Nugrhani, et al., 2016)

Figure 6. Reaction Structure of Triterpenoids with Liebermann-Burchard Reagent

The next test was carried out by separating the compounds using the TLC method on triterpenoid compounds, the best eluent or mobile phase was n-hexane: ethyl acetate (12:8). Lieberman-burchard reagent (Fajriaty, 2017).

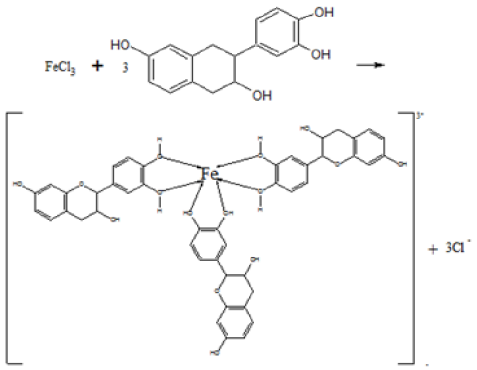
The best separation of triterpenoids is the eluent or mobile phase which is nonpolar. This is in accordance with the general nature of terpenoids which are soluble in organic solvents and usually insoluble in water (Julianto, 2019). Then the stain changes to purple due to the reaction of triterpenoids with the Liebermann-Burchard reagent to produce a red-purple color. This happens because of the ability of triterpenoid compounds to form color by H2SO4 in acetic anhydride solvent. The difference in color produced by triterpenoids is due to differences in groups on the C-4 atom (Habibi, et al., 2018 in Iskandar, 2020), as shown in Figure 4.7.



Source: (Iskandar, 2020)

Figure 7. Triterpenoid Test Reaction Structure with Liebermann-Burchard Reagent
Tannins

The tannin compound test used 1% $FeCl_3$ which showed positive results due to a blackish green color change. The tannin identification test using $FeCl_3$ was used to determine whether the sample contained phenol groups. The presence of a phenol group is indicated by a blackish green or dark blue color after being added with $FeCl_3$ giving positive results, it is possible that in the sample there are polyphenol compounds. This is confirmed by (Harbone, 1987) the classic way to detect simple phenolic compounds is to add the extract with 1% $FeCl_3$ solution in water, which gives a strong green, red, purple, blue or black color. Formation of blackish green or dark blue color in the extract after being added with $FeCl_3$ because tannins will form complex compounds with Fe^{3+} ions (Ergina, et al., 2014) as can be seen in Figure 4.8



Source: (Ergina, et al., 2014).

Figure 8. Reaction Structure of Tannins with $FeCl_3$

Furthermore, the identification method of TLC identification was carried out with the same specific reagents or reagents, resulting in a good separation of compounds in the ratio of mobile phase or butanol eluent: acetic acid (8:2). visible after being sprayed with 5% $FeCl_3$ (Yuda, 2017). The separation occurs because the mobile phase used is more polar, so the identified tannins are hydrolyzed tannins which have an OH group and when sprayed with $FeCl_3$ will be black and the reaction principle is the same as the color test (Julianto, 2019).

Conclusion

The best eluent or mobile phase was found in the separation of metabolite compounds from the extract of Bajakah Tampala trunk (Spatholobus littoralis hassk) with TLC method.

Acknowlegements

Thank you to Sari Mulia University for allowing me to conduct research Thin Layer Chromatography Profile Of Secondary Metabolites In Tampala Trunk Extract (*Spatholobus Littoralis Hassk*) and also

thanks to Kunti Nastiti as supervisor 1 and Ikna Urwatul Wusko as supervisor 2 who have always provided input and guidance in conducing this research.

Declaration of Interest Statement

The authors declare that they have no conflict of interest

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