PHOTOCHEMICAL SCREENING OF GITAAN ROD ETHANOL EXTRACT (*Willughbeia firma blume*) USING THIN LAYER CHROMATOGRAPHY METHOD

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Abstract

Gitaan (Willughbeia firma Blume) is one of the forest plants in South Kalimantan, the stems of which have been trusted for generations by the people of the Halong area, Balangan district, as a medicinal plant that can treat food poisoning containing heavy metals. The benefits of the stems of the Gitaan plant are because the stems of Gitaan contain secondary metabolite compounds. No research has been found on secondary metabolites of Gitaan stem extract. To identify the content of secondary metabolites in the ethanol extract of Gitaan stems (Willughbeia firma Blume) using Thin Layer Chromatography (TLC). This study used a descriptive observational method obtained from the secondary metabolite content of the Gitaan stem ethanol extract by observing the color reaction of the spots on TLC (qualitative data) and Rf value. The results of the color test of the Gitaan stem ethanol extract were positive for secondary metabolites of alkaloids, flavonoids, terpenoids, tannins, and saponins. The best eluent for separating flavonoid compounds is ethyl acetate: chloroform (12:6), dichloromethane alkaloids: ethyl acetate (10:10), hexane terpenoid compounds: ethyl acetate (12:8), methanol tannin compounds: ethyl acetate (8 :2), and the saponin compounds are chloroform: methanol: water (15:5:1). The results of the positive spray reaction showed secondary metabolites of alkaloids, flavonoids, terpenoids, tannins and saponins. The secondary metabolite compounds in the Gitaan stem ethanol extract that have the most potential to overcome poisoning are tannins and flavonoids. The best eluent for separating flavonoid compounds is ethyl acetate: chloroform (12:6), dichloromethane alkaloids: ethyl acetate (10:10), hexane terpenoid compounds: ethyl acetate (12:8), methanol tannin compounds: ethyl acetate (8 :2), and the saponin compounds are chloroform: methanol: water (15:5:1). The results of the positive spray reaction showed secondary metabolites of alkaloids, flavonoids, terpenoids, tannins and saponins. The secondary metabolite compounds in the Gitaan stem ethanol extract that have the most potential to overcome poisoning are tannins and flavonoids. The best eluent for separating flavonoid compounds is ethyl acetate: chloroform (12:6), dichloromethane alkaloids: ethyl acetate (10:10), hexane terpenoid compounds: ethyl acetate (12:8), methanol tannin compounds: ethyl acetate (8 :2), and the saponin compounds are chloroform: methanol: water (15:5:1). The results of the

positive spray reaction showed secondary metabolites of alkaloids, flavonoids, terpenoids, tannins and saponins. The secondary metabolite compounds in the Gitaan stem ethanol extract that have the most potential to overcome poisoning are tannins and flavonoids. The results of the positive spray reaction showed secondary metabolites of alkaloids, flavonoids, terpenoids, tannins and saponins. The secondary metabolite compounds in the Gitaan stem ethanol extract that have the most potential to overcome poisoning are tannins and flavonoids. The results of the positive spray reaction showed secondary metabolites of alkaloids, flavonoids, terpenoids, tannins and saponins. The secondary metabolite compounds in the Gitaan stem ethanol extract that have the most potential to overcome poisoning are tannins and flavonoids.

Keywords: Willughbeia firma Blume, TLC, phytochemical, eluent.

Introduction

Indonesia is a country that has a large tropical forest area and a country that has many biological resources which include various types of plants. Extensive forests and a supportive tropical climate are one of the triggers for the growth of various kinds of flora in Indonesia(Noer et al., 2018). One area that has a tropical forest area is South Kalimantan. The area is often found with various plants with their respective functions that can be managed by the community, even some plants are trusted by the community as medicine(Qamariah et al., 2018).

Biodiversity in the form of plants is still a subject of research in Indonesia, because the use of plants as a treatment for a disease is only based on the experience of our ancestors who have passed down various herbal ingredients to treat diseases from generation to generation. (Rusmin, 2019). Gitaan (Willughbeia firma Blume) is a forest plant in South Kalimantan with a yellow or orange skin and a sweet taste. Gitaan stalks are trusted by local people as a medicinal plant that can overcome the problem of food poisoning. The culture of using Gitaan stems as an antidote has been carried out for generations. According to one family head who lives in the Halong area, Balangan district, about 9 out of 10 people in the area have used gitaan sticks as an antidote. How to use it is to consume boiled water from the stems of these plants. Not many people know about the efficacy of this Gitaan stem and no research has been found regarding the stem of the Gitaan plant.

Secondary metabolites are organic compounds synthesized by plants and are a source of medicinal compounds, classified into alkaloids, terpenoids, steroids, phenolics, flavonoids, and saponins (Saifudin, 2014). Maceration is one of the simplest extraction methods because it uses simple equipment and is more economical (Azwanida, 2015). Maceration was also chosen because it can extract compounds well and can prevent the decomposition of heat-labile compounds (Tursiman et al., 2012). The solvent used in this research is ethanol 96% where ethanol is used as a solvent because it is polar, universal and easy to obtain.

Thin Layer Chromatography (TLC) is a liquid chromatography method that involves two phases, namely the stationary phase and the mobile phase (eluent). The parameters on TLC used for identification are the appearance of spots when sprayed with reagents and the Rf value (retardation factor) obtained from the division of the distance traveled by the solute by the distance traveled by the solvent.(La et al., 2020).

Materials and Methods

2.1 Tools

The tools used in this study were rotary evaporator, knife, scissors, glassware, electric scale, horn spoon, stirring rod, porcelain dish, chamber, watch glass, dropper pipette, capillary tube, test tube, a set of maceration tools, a glass funnel. , water bath, filter paper, and ultraviolet lamp.

2.2 Material

The materials used in this study were Gitaan rod (Willughbeia firma Blume), TLC plate (Gel 60 F254), 96% ethanol, aquadest, 2N HCl, 5N HCl, Dragendroff's reagent, Mayer's reagent, Liebermann Buchard's reagent, ethyl acetate, chloroform, methanol, nHexane, butanol, concentrated H_2SO, magnesium powder, anhydrous acetic acid, 5% $FeCl_3$, $AlCl_3$, dichloromethane, acetic acid, acetone.

2.3 Making Simplicia

The extraction method used in this study was maceration extraction using 96% ethanol as solvent for 3 x 24 hours, and remaceration was carried out.Concentration is carried out using a rotary evaporator. Furthermore, the obtained viscous extract is calculated its yield(Usman, 2019).

$$\% \text{ Yield} = \frac{\text{Bobot ekstrak akhir (g)}}{\text{Bobot simplisia (g)}} x\ 100\%$$

2.4 Alkaloid color test

Gitaan stem extract (Willughbeia firma Blume) as much as 1 ml, then the first test tube was added 3 drops of Mayer reagent and the second test tube was added 3 drops of Dragendrof's reagent. If a white precipitate is formed after adding the Mayer reagent, it indicates the presence of alkaloid compounds (Hanani, 2014), and if the color changes to orange, approaching red after adding Dragendorff's reagent, it shows positive results for alkaloid compounds (Wardhani & Supartono, 2015).

2.5 Flavonoid color test

Gitaan stem extract (Willughbeia firma Blume) as much as 1 ml, then add 2-3 drops of ethanol, added with 0.1 grams of Mg powder and 5 drops of 5N HCl. The red to purple color that appears indicates the presence of flavonone compounds, flavonols, flavonols and dihydroflavonols (Hanani, 2014).

2.6 Terpenoid color test

Gitaan stem extract (Willughbeia firma Blume), then added 3 drops of anhydrous acetic acid and 2 drops of concentrated H_2SO. If a purple, orange, golden yellow color is formed, it indicates the presence of terpenoids (Rahmawati, 2017).

2.7 Steroid color test

Gitaan stem extract (Willughbeia firma Blume), was added with 1 ml of chloroform, 1 ml of anhydrous acetic acid and 1 ml of concentrated H_2SO slowly through the tube wall. There is a blue or green ring indicating the presence of steroids (Ulfa, 2016).

2.8 Saponin color test

Gitaan stem extract (Willughbeia firma Blume) was added with 5 ml of hot aquadest, then shaken to form a stable foam with a height of 1-3 cm for 30 seconds and after adding 2 drops of 2 N hydrochloric acid the foam did not disappear. The formation of the foam indicates a positive result of the presence of saponins (Bintoro et al, 2017).

2.9 Tannin color test

Gitaan stem extract (Willughbeia firma Blume) was added with 3 drops of 5% FeCl. Blue or greenish black color indicates the presence of tannins (Ulfa, 2016).

2.10 Thin layer chromatography (TLC)

Phytochemical tests with TLC were carried out on groups of compounds that were positive from the results of phytochemical tests with color tests. The TLC plate was previously activated in the oven at 100°C for 30 minutes. The ethanol extract of Gitaan stem (Willughbeia firma Blume) was spotted at a distance of ± 1 cm from the bottom edge of the plate with a capillary tube then dried and eluted with each mobile phase of the compound group.

1.11 alkaloid compounds

The mobile phase consists of chloroform : methanol : water (2:5:3), n-hexane : ethyl acetate : ethanol (30:2:1), ethyl acetate : methanol : water (6:4:2), ethyl acetate : chloroform (12:6), ethyl acetate : dichloromethane (8:8), chloroform: ethanol (6:4), chloroform: ethyl acetate (3:1), then put into the chamber and allowed to saturate. The TLC plate that has been smeared with extract is then put into the chamber, eluted to the mark, taken and left to dry. Then viewed under visible light, UV 254 nm and UV 366 nm. Detection was carried out using a Liebermann Burchard sprayer. The presence of alkaloids is indicated by the formation of red-brown spots after being sprayed with Dragendroff reagent (Widyaningsih et al., 2016).

1.12 flavonoid compounds

The mobile phase consists of chloroform : ethyl acetate : butanol (1:4:5), dichloromethane : ethyl acetate (2:8), butanol : acetic acid : water (6:1:3), n-hexane : dichloromethane (8:12), n -hexane: ethyl acetate (4:6), dichloromethane: ethyl acetate (10:10), after that it is put into the chamber and allowed to saturate. The TLC plate that has been smeared with extract is then put into the chamber, eluted to the mark, taken and left to dry. Then viewed under visible light, UV 254 nm and UV 366 nm. Detection was carried out using an AlCl3 spray. The presence of flavonoids is indicated by the formation of yellow spots (Sopiah et al., 2019)

1.13 terpenoid compounds

The mobile phase consisted of ethyl acetate: methanol (6:4), n-hexane: ethyl acetate (8:2), n-hexane: ethyl acetate (12:8), ethyl acetate: methanol (16:4), after it is fed into the chamber and allowed to saturate. The TLC plate that has been smeared with extract is then put into the chamber, eluted to the mark, taken and left to dry. Then viewed under visible light, UV 254 nm and UV 366 nm. Detection was carried out using a Liebermann Burchard sprayer. . After being sprayed with Liebermann Burchard reagent, then heated for 5 minutes at a temperature of 105°C. The presence of terpenoids is indicated by the formation of purplish red spots (Hanani, 2014).

1.14 tannin compounds

The mobile phase consisted of n-butanol: ethyl acetate: water (4:1:5), methanol: ethyl acetate (4:1), acetone: water (7:3), n-butanol: acetic acid: water (8 :2:10), methanol: ethyl acetate (8:2), after that it was put into the chamber and allowed to saturate. The TLC plate that has been smeared with extract is then put into the chamber, eluted to the mark, taken and left to dry. Then viewed under visible light, UV 254 nm and UV 366 nm. Detection was carried out using a 5% FeCl3 sprayer. The presence of tannins is indicated by the formation of blue-black spots(Fajriaty et al., 2018).

1.15 saponin compounds

The mobile phase consisted of chloroform: ethanol: water (10:6:1), chloroform: methanol: water (13:7:2), chloroform: methanol (3:7), ethyl acetate: ethanol: water (10:2 1:1), chloroform: methanol: water (15:5:1), after that it was put into the chamber and allowed to saturate. The TLC plate that has been smeared with extract is then put into the chamber, eluted to the mark, taken and left to dry. Then viewed under visible light, UV 254 nm and UV 366 nm. Detection was carried out using a Liebermann Burchard sprayer. The presence of saponins is indicated by the formation of brown to violet, blue or turquoise color spots (Jaya, 2010).

Results and Discussion

3.1 Yield yield of Gitaan stems (Willughbeia firma Blume)

Gitaan stem simplicia (Willughbeia firma Blume) which was used as much as 670 grams was extracted using 96% ethanol solvent to obtain a thick extract of 29.3 grams.

$$\% \text{ Yield} = \frac{\text{Bobot ekstrak akhir (g)}}{\text{Bobot simplisia (g)}} x\ 100\%$$

$$\% \text{ Yield} = \frac{29.3\ \text{g}}{670\ \text{g}} x\ 100\%$$

$$\% \text{ Yield} = 4.37\%$$

3.2 Test Results for Identification of Secondary Metabolic Compounds

Phytochemical screening aims to determine the presence of a class of secondary metabolites in the extract. The screening method is carried out by color testing using various color reagents on alkaloids, flavonoids, steroids, terpenoids, tannins, and saponins.(Simaremare, 2014).

Table 1. Phytochemical Screening Results

Compound	Reactor	Positive Results Based on Theory	Observation result	Conclusion (+/-)
Alkaloids	• Dragendor	• Orange close to red	Orange is close to red	++
	• Mayer	• Presence of white precipitate	White precipitate	++
Flavonoids	Mg + HCl 5N	Color change from red to purple	Purple	+
Steroids	Chloroform + an-hydrous acetate + concentrated H_2SO_4	There is a blue or green ring	There is no	-
Terpenoids	Anhydrous acetic acid + concentrated H_2SO_4	Formation of purple, orange, golden yellow	Deep purple	++
Tannins	FeCl 5%	Formed in blue or greenish black	Black	++
Saponins	Hot aquadest + HCl 2 N	There is a foam that lasts for 30 seconds	Foam is formed	+

Description: (+) = Contains the detected compound
(-) = Does not contain the detected compound

Based on table 3.1 of the phytochemical screening results, it can be seen that the ethanol extract of Gitaan stems (Willughbeia firma Blume) contains a class of alkaloid compounds, flavonoids, terpenoids, tannins, and saponins.

The alkaloid color test on the Gitaan stem extract was declared positive for containing alkaloid compounds as evidenced by the formation of a white precipitate after the addition of Mayer reagent (Hanani, 2014), and the orange color approached red when using Dragendorff's reagent. (Wardhani & Supartono, 2015). The chemical reaction that occurs in the alkaloid test with Mayer's reagent is thought to be because the nitrogen in the alkaloid will react with the metal ion K+ from potassium tetraiodomercurate(II) to form a precipitated potassium-alkaloid complex, and the reaction in the alkaloid test with Dragendorf's reagent, because nitrogen is used to form coordinate covalent bond with K+ which is a metal ion(Marliana & Suryanti, 2005).

The flavonoid color test on Gitaan stem extract was found to be positive for flavonoid compounds as evidenced by the formation of a red to purple color that appears (Hanani, 2014). In this study, the addition of Mg and HCl metals in the flavonoid test is to reduce the benzopyron core contained in the flavonoid structure so that red or orange flavilium salts are formed.(Ergina et al., 2014).

In the steroid color test, the extract of gitaan rods was declared negative for containing steroid compounds because there was no blue or green ring when chloroform, anhydrous acetic acid and concentrated H_2SO_4 were added (Ulfa, 2016), so that steroid research using the TLC test method on gitaan stem extract was not carried out.

Terpenoid test, gitaan stem extract was declared positive for containing terpenoid compounds as evidenced by the formation of a purple color after being dropped with anhydrous acetic acid and concentrated H_2SO. The addition of anhydrous acetic acid in this test aims to form acetyl derivatives, while the addition of concentrated H_2SO aims to hydrolyze water which reacts with acetyl derivatives to form a color solution. The color change is caused by an oxidation reaction in the terpenoid group of compounds through the formation of conjugated double bonds (Illing, et al., 2017).

The tannin color test on the gitaan stem extract was declared positive for containing tannin compounds as evidenced by the color change to black after being reacted with 5% FeCl. $FeCl_3$ can indicate the presence of a phenol group, if there is a phenolic compound, it is possible that tannins may also be present in the gitaan stem extract, because tannins are polyphenolic compounds. The black color change occurs due to the formation of complex compounds between tannins and $FeCl_3$(Ikalinus et al., 2015).
The saponin color test on gitaan stem extract was found to be positive for saponin compounds forming a stable foam for 30 seconds. The foam formed is because saponins have physical properties that are easily soluble in water and will cause foam when shaken, because saponins are surface active compounds that are easily detected through their ability to form foam.(Baud et al., 2014).

3.3 Thin Layer Chromatography Test Results

Before spraying with visible light looks faint brown. After spraying with Dragendorf's reagent, the light appears brownish red, at UV 254 dark spots are visible and at UV 366 green spots are visible. So it was concluded that the ethanol extract of Gitaan stem contained alkaloids with the formation of red-brown spots after being sprayed with Dragendroff's reagent (Widyaningsih et al., 2016).

Table 2. TLC test results for compounds Alkaloids

No	eluent	Before/after spraying	Rf value		
			visible light	UV 254	UV 366
1.	Chloroform : methanol : water (2:5:3) (Trimulyani et al., 2019)	Before	0.91	0.08 0.88	0.88
		After	0.88	0.08 0.88	-
2.	n-hexane:ethyl acetate:ethanol (30:2:1) (Murtadlo et al., 2013)	Before	-	0.06 0.08	0.08
		After	0.06	0.08	-
3.	ethyl acetate : methanol : water (6:4:2) (Abraham et al., 2014)	Before	0.91	0.75	0.75
		After	0.75	0.75	-
4.	Ethyl acetate: chloroform (12:6) (Fadhly et al., 2015)	Before	-	0.08 0.33 0.41 0.46 0.56 0.75 0.96	0.75 0.96
		After	0.16 0.33	0.08 0.33	-
5.	Ethyl acetate : Dichloromethane (8:8) (Fadhly et al., 2015)	Before	-	0.08 0.25 0.36 0.48 0.55	0.78 0.98
		After	0.25 0.1	0.08 0.25	-
6.	Chloroform : Ethanol (6:4) (Fadhly et al., 2015)	Before	0.05	0.41 0.88 0.96	0.96
		After	0.08 0.91	0.88	-

7.	Chloroform : Ethyl acetate (3:1) (Untoro et al., 2016)	Before	-	0.13 0.16 0.31 0.4 0.61	0.61 1
		After	0.01	0.16	1

Based on table 3.2 TLC test results for alkaloid compounds, the best eluents that showed the most spots in visible light were ethyl acetate: dichloromethane (8:8), chloroform: ethanol (6:4), ethyl acetate: chloroform (12:6).

Before spraying with visible light looks faint brown. After being sprayed with AlCl . reagent3, irradiated stains appear brownish yellow, the formation of color occurs due to the formation of complexes between AlCl3 with a ketone group on the C-4 atom and a hydroxy group on the neighboring C-3 and C-5 atoms of flavones and flavonols (Yulistian et al., 2015).

Table 3. TLC test results for flavonoid compounds

No	eluent	Before/after spraying	Rf value		
			visible light	UV 254	UV 366
1.	Chloroform : ethyl acetate : butanol (1:4:5) (Arel et al., 2018)	Before	0.16 0.91	0.9	0.9
		After	0.91	0.9	0.91
2.	Dichloromethane : ethyl acetate (2:8) (Haris et al., 2020)	Before	0.05	0.08 0.41 0.93	0.93
		After	-	0.08 0.41	0.93
3.	Butanol : Acetic acid : Water (6:1:3) (Iswahyudi et al., 2015)	Before	0.83	0.66 0.83	0.83
		After	0.83	0.66 0.83	0.83
4.	n-hexane : dichloro-methane (8:12) (Aisyah, Destiarti Lia, 2019)	Before	-	-	0.05 0.13
		After	-	-	0.05
5.	n-hexane : ethyl ace-tate (4:6)	Before	0.05	-	1
		After	-	-	1

	(Aisyah, Destiarti Lia, 2019)				
6.	Dichloromethane : Ethyl acetate (10:10) (Aisyah, Destiarti Lia, 2019)	Before	0.13 0.25	0.08 0.36 0.5 0.58	0.9
		After	0.13	0.08 0.36 0.5 0.58	0.25 0.9

Based on table 3.3 TLC test results for flavonoid compounds, the best eluent that showed the most spots in visible light sequentially was Dichloromethane: Ethyl acetate (10:10).

Before spraying with visible light looks faint brown. After being sprayed with Liebermann Burchard reagent, then heated for 5 minutes at a temperature of 105°C. On the stain in the light it looks purplish red(Siadi, 2012)

Table 4. TLC test results for terpenoid compounds

No	eluent	Before/after spraying	Rf value		
			visible light	UV 254	UV 366
1.	Ethyl acetate : methanol (6:4) (Putri & Raharjo, 2019)	Before	-	0.41 0.66	-
		After	-	-	-
2.	n-hexane : ethyl acetate (8:2) (Dwisari et al., 2016)	Before	-	0.33	0.33
		After	-	-	-
3.	n-hexane : ethyl acetate (12:8) (Dwisari et al., 2016)	Before	-	0.83	0.83
		After	0.08 0.83	0.08 0.58 0.83	0.83 0.91
4.	Ethyl acetate : methanol (16:4) (Dwisari et al., 2016)	Before	-	0.25 0.35	0.25
		After	0.08	0.08	0.25 1

Based on table 3.4 TLC test results for terpenoid compounds, the best eluent that showed the most spots in visible light was n-hexane: ethyl acetate (12:8).

Before spraying with visible light looks faint brown. After being sprayed with 5% FeCl3 reagent, the irradiated stain appears black, the reaction that occurs in this test is hydrolyzed tannin which reacts with FeCl3 to produce a blue-black color.(Fajriaty et al., 2018).

Table 5. TLC test results for tannin compounds

No	eluent	Before/after spraying	Rf value		
			visible light	UV 254	UV 366
1.	n-butanol : ethyl acetate : water (4:1:5) (Puspita Sari et al., 2015)	Before	-	0.75	0.75
		After	0.96	0.96	-
2.	Methanol: ethyl acetate (4:1) (Sopianti & Sary, 2018)	Before	0.25 0.75	0.75	0.75
		After	0.75	0.75	-
3.	Acetone : water (7:3) (Tresna Lestari, 2013)	Before	-	0.75	0.75
		After	0.91	0.91	-
4.	n-butanol : acetic acid : water (8:2:10) (Tresna Lestari, 2013)	Before	0.91	0.75 0.88	0.71 0.88 0.75
		After	-	0.75	-
5.	Methanol : Ethyl acetate (8:2) (Sopianti and Sari, 2018)	Before	0.13	0.13 0.71	0.71
		After	0.16 0.8	0.71	-

Based on table 3.5 TLC test results for tannin compounds, the best eluent that showed the most spots in visible light was methanol: ethyl acetate (8:2).

Before spraying with visible light looks faint brown. After being sprayed with Liebermann Burchard reagent, the stain on the light appears brown (Jaya, 2010).

Table 6. Saponin compound TLC test results

No	eluent	Before/after spraying	Rf value		
			visible light	UV 254	UV 366
1.	Chloroform: ethanol: water (10:6:1) (Firawati & Primary, 2018)	Before	0.96	0.08 0.66	0.75
		After	0.96	0.08 0.66	0.75
2.	Chloroform: methanol: water (13:7:2) (M. Agung Pratama Suharto, Hosea Jaya Edy, 2012)	Before	0.08	0.58 0.8	0.96
		After	0.08	0.58 0.8	0.96
3.	Chloroform : Methanol (3:7) (Amody & Anggreani, 2017)	Before	0.21	-	0.83
		After	0.21	0.16	0.83
4.	Ethyl acetate : Ethanol : Water (10:2:1) (Amody & Anggreani, 2017)	Before	-	-	1
		After	-	-	1
5.	Chloroform : Methanol : Water (15:5:1) (Amody & Anggreani, 2017)	Before	0.25	0.66 0.91	0.86 0.95
		After	0.25	-	0.86 0.95

Based on table 3.6 TLC test results for saponin compounds, the best eluent that showed the most spots in visible light was Chloroform : Methanol : Water (15:5:1).

From the phytochemical screening by TLC test on the ethanol extract of Gitaan stems growing in the Halong area, Balangan district, positive results were obtained containing alkaloids, flavonoids, terpenoids, tannins and saponins. The secondary metabolite compounds in the Gitaan stem ethanol extract that have the most potential to overcome poisoning are tannins and flavonoids.

Conclusion

From the phytochemical screening by color test and TLC test on the ethanol extract of Gitaan stems growing in the Halong area, Balangan district, positive results were obtained containing alkaloids, flavonoids, terpenoids, tannins and saponins alkaloids, flavonoids, terpenoids, tannins and saponins. The best eluent for separating flavonoid compounds is ethyl acetate: chloroform (12:6), ethyl acetate alkaloids: dichloromethane (8:8), chloroform: ethanol (6:4), dichloromethane: ethyl acetate (10:10), terpenoids hexane: ethyl acetate (12:8), methanol tannin compounds: ethyl acetate (8:2), and saponin compounds are chloroform: methanol: water (15:5:1).

References

Abraham, A., Fasya, A. G., Fauziyah, B., & Adi, T. K. (2014). Uji Antitoksoplasma Ekstrak Kasar Alkaloid Daun Pulai (Alstonia scholaris, (L.) R. BR) Terhadap Mencit (Mus musculus) BALB/C Yang Terinfeksi Toxoplasma Gondii STRAIN RH. *Alchemy*, *3*(1). https://doi.org/10.18860/al.v0i0.2896

Aisyah, Destiarti Lia, R. (2019). *Isolasi Dan Karakterisasi Senyawa Flavonoid Dari Fraksi Etil Asetat Batang Tumbuhan Senggani (Melastoma malabathricum L.)*. *8*(2), 61–66.

Amody, Z., & AnggreanI, K. (2017). Identifikasi Senyawa Glikosida Pada Akar Gebang (Corypha utan) Asal Desa Landayya Kabupaten Bantaeng. *Majalah Farmasi*, *14*(01), 47–52.

Arel, A., Wardi, E. S., & Oktaviani, Y. (2018). Profil Metabolit Sekunder Ekstrak Daun Berenuk (Crescentia Cujete L.)Dan Uji Sitotoksik Dengan Metode Brine Shrimp Lethality Test. *Jurnal Katalisator*, *3*(2), 82. https://doi.org/10.22216/jk.v3i2.3165

Azwanida, N. (2015) 'A Review on the Extraction Methods Use In Medical Plants Principlea, strength and Limination. Med

Baud, G. S., Sangi, M. S., & Koleangan, H. S. J. (2014). Analisis Senyawa Metabolit Sekunder Dan Uji Toksisitas Ekstrak Etanol Batang Tanaman Patah Tulang (Euphorbia Tirucalli L .) Dengan Metode Brine Shrimp Lethality Test (Bslt) Analysis Of Secondary Metabolite Compounds And Toxicity Test Of Stem Plant Etha. *Jurnal Ilmiah Sains*, *14*(2), 1–8. gracebaud1@gmail.com

Bintoro, A., Ibrahim, A. M., & Situmeang, B. (2017). Analisis dan Identifikasi Senyawa Saponin dari Daun Bidara (Zhizipus mauritania L.). *Jurnal ITEKIMA*, *2*(1), 84–94.

Dwisari, F., Harlia, & Alimuddin, A. H. (2016). Isolasi dan Karakterisasi Senyawa Terpenoid Eekstrak Metanol Akar Pohon Kayu Buta-Buta (Excoecaria agallocha L.). *Jurnal Kajian Komunikasi (JKK) UNPAD*, *5*(3), 25–30.

Ergina, Nuryanti, S., & Purtisari, I. D. (2014). Uji Kualitatif Senyawa Metabolit Sekunder Pada Daun Palado (Agave angustifolia) Yang Diekstraksi Dengan Pelarut Air Dan Etanol Qualitative Test of Secondary Metabolites Compounds in Palado Leaves (Agave. *J. Akad. Kim*, *3*(3), 165–172.

Fadhly, E., Kusrini, D., & Fachriyah, E. (2015). Isolasi, Identifikasi Senyawa Alkaloid dari Daun Rivina humilis L. serta Uji Sitotoksik Menggunakan Metode BSLT (Brine Shrimp Lethality Test). *Jurnal Kimia Sains Dan Aplikasi*, *18*(2), 67–72. https://doi.org/10.14710/jksa.18.2.67-72

Fajriaty, I., Ih, H., & Setyaningrum, R. (2018). Skrining Fitokimia Dan Analisis Kromatografi Lapis Tipis Dari Ekstrak Etanol Daub Bintangur (Calophyllum soulattri Burm. F.). *Jurnal Pendidikan Informatika Dan Sains*, *7*(1), 54–67.

Firawati, & Pratama, M. I. (2018). Isolasi dan Identifikasi Senyawa Saponin Daun Bungkus (Smilax rotundifolia) Menggunakan Metode Spektrofotometri Ultraviolet. *Jf Fik Uinam*, *6*(2), 115–121.

Hanani, E. 2014. Analisis Fitokimia. Jakarta: Penerbit Buku Kedokteran EG

Haris, M. F., Kahtan, M. I., & Widiyantoro, A. (2020). Efektivitas Ekstrak Etanol Kulit Buah Terung Ungu (Solanum melongena L.) sebagai Antimalaria terhadap Jumlah Eosinofil pada Mencit (Mus musculus) yang Diinduksi Plasmodium berghei. *Jurnal Sains Farmasi & Klinis*, *7*(2), 107. https://doi.org/10.25077/jsfk.7.2.107-114.2020

Ikalinus, R., Widyastuti, S., & Eka Setiasih, N. (2015). Skrining Fitokimia Ekstrak Etanol Kulit Batang Kelor (Moringa Oleifera). *Indonesia Medicus Veterinus*, *4*(1), 71–79.

Illing, Ilmiati, safitri, wulan, erfiana. (2017). Uji Fitokimia Ekstrak Buah Dengen. *Jurnal Dinamika*, *8*(1), 66–84.

Iswahyudi, I., Luliana, S., & Riza, H. (2015). Analisis Fitokimia Dan Profil Kromatografi Lapis Tipis Ekstrak Etanol Daun Ciplukan (Physalis angulata L.) Dengan Berbagai Metode Pengeringan Simplisia. *Jurnal Mahasiswa Farmasi Fakultas Kedokteran UNTAN*, *3*.

La, E. O. J., Sawiji, R. T., & Yuliawati, A. N. (2020). Skrining Fitokimia Dan Analisis Kromatografi Lapis Tipis Ekstrak Etanol Kulit Buah Naga Merah (Hylocereus polyrhizus). *Indonesian Journal of Pharmacy and Natural Product*, *03*(February), 45–58.

M. Agung Pratama Suharto, Hosea Jaya Edy, J. M. D. (2012). ISOLASI DAN IDENTIFIKASI SENYAWA SAPONIN DARI EKSTRAK METANOL BATANG PISANG AMBON(Musa paradisiaca var. sapientum L.). *Pharmacon*, *1*.

Marliana, S. D., & Suryanti, V. (2005). Skrining Fitokimia dan Analisis Kromatografi Lapis Tipis Komponen Kimia Buah Labu Siam (Sechium edule Jacq . Swartz .) dalam Ekstrak Etanol The phytochemical screenings and thin layer chromatography analysis of. *Biofarmasi*, *3*(1), 26–31.

Murtadlo, Y., Kusrini, D. D., Si, M., Fachriyah, D. E., Kimia, J., Sains, F., Diponegoro, U., & Soedarto, J. P. (2013). *Isolasi, Identifikasi Senyawa Alkaloid Total Daun Tempuyung (Sonchus arvensis Linn) Dan Uji Sitotoksik Dengan Metode BSLT (Brine Shrimp Lethality Test)*. *1*(1), 379–385.

Noer, S., Pratiwi, R. D., & Gresinta, E. (2018). Penetapan Kadar Senyawa Fitokimia (Tanin, Saponin dan Flavonoid) sebagai Kuersetin Pada Ekstrak Daun Inggu (Ruta angustifolia L.). *Jurnal Eksakta*, *18*(1), 19–29. https://doi.org/10.20885/eksakta.vol18.iss1.art3

Puspita Sari, P., Susanah Rita, W., & Puspawati, N. (2015). Identifikasi Dan Uji Aktivitas Senyawa Tanin Dari Ekstrak Daun Trembesi (Samanea Saman (Jacq.) Merr) Sebagai Antibakteri Escherichia Coli (E. Coli). *Jurnal Kimia*, *9*(1), 27–34.

Putri, M. A., & Raharjo, S. J. (2019). *Senyawa Metabolit Sekunder Air Perasan Daun Mimba (Azadirachta indica) Thin Layer Chromatography Profile (TLC) Secondary Metabolite Compounds Of Neem Leaves (Azadirachta indica). Muzemmila Arinda Putri , Sentot Joko Raharjo Akademi Farmasi Putra Ind*. 1–8.

Qamariah, N., Handayani, R., & Novaryatiin, S. (2018). Kajian Empiris dan Etnofarmakologi Tumbuhan Hutan Berkhasiat Obat asal Desa Tumbang Rungan Kelurahan Pahandut Kota Palangkaraya Kalimantan Tengah. *Anterior Jurnal*, *18*(1), 98–106. https://doi.org/10.33084/anterior.v18i1.424

Rahmawati.M. (2017). Isolasi Dan Identifikasi Senyawa Metabolit Sekunder Dari Ekstrak Metanol Kulit Batang Tumbuhan Mengkudu (Morinda citrifolia L.). *Journal of Chemistry*, *6*(2), 113–118.

Rusmin. (2019). Formulasi Dan Uji Mutu Fisik Supositoria Dari Ekstrak Etanol Daun Wungu (Graptophyllum pictum L). *Akademi Farmasi Yamasi Makassar*.

Siadi, K. (2012). Ekstrak Bungkil Biji Jarak Pagar (Jatropha curcas) Sebagai Biopestisida Yang Efektif Dengan Penambahan Larutan NaC. *Jurnal MIPA*.

Simaremare, E. S. (2014). Skrining Fitokimia Ekstrak Etanol Daun Gatal (Laportea decumana (Roxb.) Wedd). *Pharmacy*, *11*(01), 98–107.

Sopiah, B., Muliasari, H., & Yuanita, E. (2019). Skrining Fitokimia dan Potensi Aktivitas Antioksidan Ekstrak Etanol Daun Hijau dan Daun Merah Kastuba (Phytochemical Screening and Potential Antioxidant Activity of Ethanol Ekstract of Green Leaves and Red Leaves Kastuba). *Jurnal Balai Riset Dan Standardisasi Industri Ambon*, *17*(1), 27–33.

Sopianti, D. S., & Sary, D. W. (2018). Skrining Fitokimia Dan Profil Klt Metabolit Sekunder Dari Daun Ruku-Ruku (Ocimum tenulflorum L.) Dan Daun Kemangi (Ocimum sanctum L). *SCIENTIA Jurnal Farmasi Dan Kesehatan*, *8*(1), 44–52.

Tresna Lestari, Y. S. (2013). Isolasi Dan Identifikasi Senyawa Tanin Dari Ekstrak Air Kulit Batang Kelapa Gading (Cocos nucifera var. eburnea). *Jurnal Kesehatan Bakti Tunas Husada*, *9*.

Trimulyani, Y. W., Rokiban, A., & Sari, M. (2019). Fraksi Etanol, Kloroform, Dan N-Heksan Bunga Kamboja Putih (Plumeria acuminata L.) Sebagai Antibakteri Terhadap Escherichia coli dan Staphylococcus aureus DENGANBIOAUTOGRAFI. *JFL : Jurnal Farmasi Lampung*, *8*(2), 111–122. https://doi.org/10.37090/jfl.v8i2.147

Tursiman, Ardiningsih, P. dan N. (2012) 'Total Fenol Fraksi Etil Asetat Dari Buah Asam Kandis (*Garcinia dioica Blume*)', JKK

Ulfa. 2016. Identifikasi dan uji aktivitas antioksidan dalam bekatuk dengan menggunakan variasi pelarut. [skripsi]. Malang: Universitas Islam Negeri (UI) Maulana Malik Ibrahim.

Untoro, M., Fachriyah, E., & Kusrini, D. (2016). Isolasi dan Identifikasi Senyawa Golongan Alkaloid dari Rimpang Lengkuas Merah (Alpinia purpurata). *Jurnal Kimia Sains Dan Aplikasi*, *19*(2), 58–62. https://doi.org/10.14710/jksa.19.2.58-62

Usman, S. (2019). *Pengaruh Metode Ekstraksi Terhadap Rendemen Dan Kadar Fenolik Ekstrak Tanaman Kayu Beta-Beta (Lunasia amara Blanco) (The Effect of Extraction Method on Yield Value and Phenolic Content of Beta-Beta*. *5*(2), 175–182. https://doi.org/10.22487/j24428744.2019.v5.i2.13149

Wardhani, R. A. P., & Supartono. (2015). Uji Aktivitas Antibakteri Ekstrak Kulit Buah Rambutan (Nephelium lappaceum L.,) Pada Bakteri. *IJCS - Indonesia Journal of Chemical Science*, *4*(1), 1–6.

Widyaningsih, W., Pramono, S., & Widyarini, S. (2016). Skrining Fitokimia Ekstrak Etanol Ulva lactuca L. Dengan Metode Kromatografi Lapis Tipis. *Media Farmasi: Jurnal Ilmu Farmasi*, *13*(2), 199–211. https://doi.org/10.12928/mf.v13i2.7772

Yulistian, D. P., Edi Priyo Utomo, S. M. U., & Yusnawan, E. (2015). Studi pengaruh jenis pelarut terhadap hasil isolasi dan kadar senyawa fenolik dalam biji kacang tunggak (. *Kimia Student Journal*, *1*(1), 819–825.