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ORIGINAL ARTICLE

IDENTIFICATION AND DETERMINATION OF SAPONIN CONTENT FROM EXTRACT OF *Embelia borneensis* BARK

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Abstract *Embelia borneensis* known sekilang used by the Dayak Kenyah tribe of Long Temuyat Village, Malinau, North Kalimantan as a fish poison and leech repellent. Sekilang bark extract contains alkaloids, tannins, saponins and flavonoids compounds. The purpose of this study was to determine the type and content of saponins in the methanol extract based on different maceration, reflux, and Soxhlet extraction methods. Saponin content was determined by the gravimetric method. The results showed that the type of saponin contained in sekilang bark extract was triterpenoid saponin. The average saponin content with maceration method was 24.87%, reflux method was 28.97 and soxhlation method was 29.99%. The results of data analysis with one-way anova statistical test showed that there was a significant difference between extraction methods on saponin content of sekilang stem bark with sig value of 0.009 with a significant level of 0.05 (5%).

Keywords: *Embelia borneensis* Schiff · saponins · maceration · reflux · sokhlet

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Introduction

Sekilang (*Embelia borneensis* Scheff.) is a plant that grows in the Ampan Iban forest, Malinau, North Kalimantan. Empirically, the Dayak Kenyah in Long Temuyat Village use it as a poison when catching fish and can also be used as a leech repellent (Liman, 2019). The bark of sekilang creates foam when shaken into river water and it makes fish easy to catch. The formation of foam can be attributed to the presence of saponin compounds, which are secondary metabolite compounds that when reacted with water and shaken can cause foam.

Research by Supriningrum et al. (2021) concluded that simplisia and ethanol extracts of Sekilang bark contain alkaloid, tannin, saponin and flavonoid chemical compounds. Research by Saptowo et al. (2021) reported that sekilang bark extract gave positive results for saponins with the formation of stable foam as high as 8 cm.

Saponins can reduce the surface tension of water and cause the formation of foam on the surface of the water after shaking (Nurzaman et al., 2018). Saponins are toxic to cold-blooded animals, such as fish, because they can damage the gills and liver of fish (Fisesa, 2017). Saponin is a glycoside consisting of a glycone in the form of sugar that binds to an aglycone in the form of sapogenin. Based on the aglycone structure, saponins can be divided into two types, namely steroidal and triterpenoidal types. Steroidal saponins are used for the treatment of syphilis, rheumatism, psoriasis, eczema, diabetes, gastritis, and impotence. Triterpenoid saponins are used as emulsifying compounds, expectorant stimulants in antifungal, antibacterial and anti-inflammatory (Evans, 2002).

Secondary metabolites in plants can be obtained by extraction. There are two types of extraction methods, namely cold and hot methods (Kiswandono, 2017; Pardede et al., 2020). One of the cold extraction methods is maceration and hot methods include reflux and sokhlet. The principle of maceration is to soak the simplisia in the liquid with the help of stirring. Reflux is a continuous extraction method. The liquid will continuously distill the active substances in the simplicia with the presence of back cooling. While sokhletasi is done under continuous heat conditions continuously, the sample is placed in the extraction chamber (timble).

Analysis of saponin content in plants can be done by gravimetric method (Minarno, 2016; Mien et al., 2015). The gravimetric method is based on weighing the constant weight of the compound after dissolving the sample, adding reagents, filtering, washing, drying, and weighing the precipitate until it is constant. Determination of saponin content in this study using gravimetric method to extract the results of maceration, reflux and sokhletasi with methanol solvent. The choice of solvent is based on the solubility of saponin, which is easily soluble in methanol compared to other solvents (Harborne, 1987). The purpose of conducting variations in extraction methods for determining saponin content is to determine the extraction method that can produce optimal saponin levels. The extraction method can affect the amount of compound content in the extract.

Materials and Methods

Sekilang bark, methanol, distilled water, chloride acid, chloroform, Lieberman Bouchard reagent, n-hexane, ethyl acetate, 1-butanol, ether, Meyer reagent, Dragendorf reagent, Bouchardat reagent, $FeCl_3$, amyl alcohol, sulfuric acid, Mg powder.

Simplicia Preparation and Extraction Process

The bark was washed, cut into pieces, and dried by drying under the sun until dry. Simplicia was pulverized and then extracted by three methods.

Maceration Method

Weighed 50 g of simplisia then macerated with 500 ml of methanol solvent, stirring continuously for ± 6 hours using a macerator and allowed to stand for ± 18 hours. Then filtering was carried out, the obtained macerate was evaporated until a thick extract was obtained. This method was replicated 3 times.

Reflux Method

Weighed 50 g of sekilang bark simplisia powder, put into a round bottom flask, added 500 ml of methanol solvent then heated at 60oC for 3 hours, then filtered using a buchner funnel. The liquid extract obtained is evaporated until a thick extract is obtained. Replication was carried out 3 times (Wijaya, et al., 2018).

Sokhlet Method

Weighed 50 grams of simplisia powder, wrapped in whatman paper, tied both ends with thread, then put into a soxhlet tube (thimble), added 150 mL of methanol solvent. The round bottom flask was filled with 350 mL of methanol. The extraction process is carried out at 40 ° C until the solvent droplets are faded (± 6 cycles).

Identification of Secondary Metabolite Compound

Extract of sekilang bark as much as 0.5 g, dissolved in a mixture of methanol: water (1: 1), the solution was then used for the following tests (Harborne, 1987).

Alkaloid (Mayer Reagent)

A total of 5 drops of solution added 2-3 drops of HCl 2N and 2 drops of reagent mayer. When formed white or yellow precipitate indicates the presence of alkaloid compounds.

Alkaloid (Bouchardat reagent)

A total of 5 drops of solution added 2-3 drops of HCl 2N and 2 drops of Bouchardat reagent. When formed brown to black precipitate indicates the presence of alkaloid compounds.

Alkaloid (Dragendroff reagent)

A total of 5 drops of solution, added 2-3 drops of HCl 2N and 2 drops of Dragendroff reagent. When formed orange to red brown precipitate indicates the presence of alkaloid compounds.

Flavonoid Compound Test

A total of 5 ml of solution, added a little Mg powder and 1 ml concentrated hydrochloric acid, 2 ml amyl alcohol, shaken and allowed to

separate. Flavonoids are positive if the formation of red, yellow, or orange color in the amyl alcohol layer (Harborne, 1987).

Tannin Compound Test

A total of 10 drops of solution, added to distilled water until the color fades, added 1-2 drops of iron (III) chloride reagent, when formed blue-black or green-black color indicates the presence of tannin compounds in the sample (Atmoko and Ma'ruf, 2009).

Saponin Compound Test

A total of 10 drops of solution, added 5 drops of hot water, then cooled, shaken vigorously for 10 seconds. If a lot of foam is formed for 10 minutes as high as 1 cm to 10 cm and does not disappear with the addition of hydrochloric acid 2N 1 drop, it indicates the presence of saponin content (Ministry of Health RI, 1989; Pardede et al., 2013).

Identification of Saponin

A total of 0.5 grams of extract was put into a test tube containing 10 ml chloroform, heated for 5 minutes on a water bath while shaking. Then added a few drops of Lieberman bouchard reagent. If a brown or violet ring is formed, it indicates the presence of triterpene saponins, while green or blue color indicates the presence of steroidal saponins (Suharto et al., 2012).

Gravimetric Analysis of Saponin Content

A total of 0.25 g of extract was dissolved into 10 ml of n-hexane and heated using a hotplate at 60ºC-80ºC for 30 minutes while stirring using a magnetic stirrer at 400 rpm until it evaporated. After cooling, the residue left behind was dissolved in 10 ml of ethyl acetate. The ethyl acetate solution was then poured and the residue left behind was dissolved with 10 ml of 1-butanol. The 1-butanol solution was poured again and the residue left behind was dissolved with 5 ml of methanol then this solution was dripped with 10 ml of ether while stirring. The precipitate formed in the mixture was poured on filter paper of known weight. The precipitate on the filter paper was dried for ± 2 hours at room temperature and then weighed until the weight remained. The experiment was repeated 3 times.

Results and Discussion

Simplisia and Extract Preparation

The bark was washed and cut into pieces with the aim of increasing the surface area, so that the drying process is faster. The steam bark is dried in the sun. It aims to reduce the water. During the drying process, water evaporation occurs, which causes damage to cells, as a result of water release and makes it easier for metabolite compounds to be extracted. Simplisia is mashed into shavings with the aim of increasing the surface area, so that the extraction process is more optimal (Supomo et al., 2019).

Simplisia powder is extracted using three extraction methods, namely maceration, reflux, and sokhlet. The liquid extract obtained was evaporated until a thick extract was obtained, and the yield was calculated. The extract yield is calculated based on the ratio of the weight of the extract produced to the weight of the simplicia used multiplied by 100%. The yield value is also related to the amount of bioactive content contained in a plant/animal (Dewatisari, et al., 2018). The solvent used to extract sekilang bark samples is methanol solvent. Methanol solvent is used because this solvent is polar and volatile so that the evaporation process can help accelerate the drying of the extract. According to Suharto, (2012) more saponins are produced if extracted using methanol solvents because saponins are polar so they dissolve easily with methanol compared to other solvents.

Table 1. Yield of Methanol Extract of Sekilang Stem Bark

Extraction methode	Extract weight (g)	Yield (%)	Average (%)
M1	10,77	21,54	
M 2	6,81	13,62	16,18
M3	6,69	13,38	
R1	6,04	12,08	
R2	5,75	11,44	13,88
R3	9,06	18,12	
S1	3,79	7,58	
S2	3,75	7,5	7,80
S3	4,16	8,32	

M1, M2, M3 (maceration I, 2, 3), R1, R2, R3 (refluks 1, 2, 3), S1, S2, S3 (soxhlet 1, 2, 3)

Table 1 shows that the maceration extraction method produces greater yields than the reflux and sokhlet extraction methods. This may be due to the absence of a heating process on the extract, so that compounds that are both resistant to heating and not resistant to heating can be extracted. It is also suspected that the effect of the length of extraction time carried out, namely 1x24 hours, which allows the process of withdrawing compounds to be maximized compared to reflux and sokhlet methods. According to Andika (2018) the effectiveness of the extraction process is influenced by the amount of solvent, type of solvent used as a distiller, particle size of simplisia, temperature, method, and duration of extraction. The yield of a sample is very necessary because it is to know the amount of extract obtained during the extraction process (Wisdawati et al., 2019).

Table 2. Secondary Metabolite Identification Results of Extract of Sekilang Bark

Extraction methode	Phytochemical screening			
	Alkaloid	Flavonoid	Tanin	Saponin
M1	+	+	+	+
M2	+	+	+	+
M3	+	+	+	+
R1	+	+	+	+
R2	+	+	+	+
R3	+	+	+	+
S1	+	+	+	+
S2	+	+	+	+
S3	+	+	+	+

(+) Positive for secondary metabolite compounds

Alkaloid test is carried out with the addition of 2N HCl aims to withdraw alkaloid compounds from the extract. Alkaloids are alkaline, so that the addition of HCl will form salt. Then the precipitation reaction is carried out using three reagents. Meyer reagent obtained positive results characterized by the presence of a white precipitate. Bouchardat reagent obtained positive results on all samples characterized by the presence of brown precipitate. Dragendorff reagent obtained positive results on all samples characterized by the presence of orange precipitate (Muthmainnah, 2017).

The Flavonoid test was carried out by adding a little Mg powder, concentrated hydrochloric acid, and amyl alcohol to the sample to form a red color in the amyl alcohol layer. This indicates that all samples of the methanol extract of the bark of sekilang are positive for flavonoid secondary metabolites. The purpose of adding Mg powder and concentrated HCl is to reduce the glycoside bond. For flavonoids to be identified, the glycoside bond in flavonoids must be broken

by reducing the bond. This group of compounds is easily extracted in polar solvents such as ethanol, which has polar properties due the presence of hydroxyl groups, so that hydrogen bonds can be formed. The main benefits of flavonoids in the human body are that they are antioxidant, antibacterial, and anti-inflammatory. Flavonoids can also be used as antibacterial, antiallergic, cytotoxic, and antihypertensive agents (Pardede et al., 2017).

Tannin test was carried out with the addition of 1% $FeCl_3$ reagent, and a blackishgreen color was formed. This indicates that all positive samples contain tannin secondary metabolite compounds. The color change to blackish green occurs due to the reaction between tannins and FeCl3 to form complex compounds, and the tannins formed are condensed tannins. Tannins have properties as astringents, antidiarrhea, antibacterial, and antioxidants (Supriningrum, 2019).

The Saponin test was carried out with the addition of distilled water, which had previously been heated, the sample was then shaken strongly for ± 10 seconds, foam was formed, and added HCl 2N foam remained stable. The height of the saponin foam formed can be seen in Table 3.

Table 3. The Height of The Saponin Foam Formed

Extraction methode	Foam height (cm)	Average (cm)
M1	6	
M2	4	6
M3	8	
R1	1	
R2	6	5
R3	8	
S1	4	
S2	6	5,7
S3	7	

M1, M2, M3 (maceration I, 2, 3), R1, R2, R3 (refluks 1, 2, 3), S1, S2, S3 (soxhlet 1, 2, 3)

Based on the table 3, there is one sample, R1, which is only 1 cm high foam. This is thought to be because when shaking the sample is less than optimal so that it produces little foam, the shaking process may be maximized if done using a special tool that can guarantee the uniformity of shaking in the identification of saponin foam height. Saponins are natural surfactants and will form a stable foam when shaken vigorously. Research conducted by Marpaung and Romelan (2019), states that foam is formed because saponins are compounds that have hydrophile and hydrophobic groups. When cornered, the hydrophile group will bind to water while the hydrophobe binds to air to form foam. The addition of 2N HCl will increase the polarity, so that the hydrophile group will bind. more stable and the foam formed becomes stable.

In this study, all samples showed that the methanol extract of sekilang bark from the three extraction methods positively contained saponin compounds. According to Supriningrum et al. (2021), positive samples contain saponin compounds, characterized by the formation of stable foam with a height of 1-10 cm. The foam that arises in the sample indicates the presence of glycosides that could form foam in water, which is hydrolyzed into glucose and aglycone compounds. The formation of foam shows that saponins are macromolecular compounds that have the property of reducing the surface tension of water.

Identification of Saponin

The type of saponin contained in the methanol extract of Sekilang stem bark is saponin-triterpenoid (table 4), which is characterized by a brown ring in the sample.

Table 4. Results Identification of Saponin

No.	Extraction method	Saponin type	
		Steroid	Triterpenoid
1	M1	-	+
2	M2	-	+
3	M3	-	+
4	R1	-	+
5	R2	-	+
6	R3	-	+
7	S1	-	+
8	S2	-	+
9	S3	-	+

(+) Positively contains secondary metabolite compounds, (-) Negative contains secondary metabolite compounds

Determination of the type of saponin in methanol extract of sekilang stem bark using LB (Lieberman-Bouchard) reagent. LB reagent is a mixture of acetic acid andhydride with concentrated sulfuric acid used for color reactions in identifying the type of saponin in an extract. Triterpenoid saponins are compounds that are acidic, crystalline, colorless, high melting point, contain oxygen, have 1 to 6 monosaccharide molecules, and contain several aliphatic acid compounds in the form of esters. The acidic nature of saponins is characterized by the presence of one or two carbonyl groups in aglycone or sugar molecules. The presence of oxygen content in saponins may contain hydroxyl, aldehyde group. This type of saponin has hemolytic activity with different strengths depending on the type or type of substitution. Triterpenoid class compounds show significant pharmacological activities, such as antiviral, antibacterial, antiinflammatory, and as anticancer (Nola et al., 2021).

Determination of Saponin Content of Sekilang Stem Bark Extract

Quantitative analysis of saponin content determination of methanol extract of bark was carried out by gravimetric method. One of the advantages of the gravimetric method is that it does not require a comparator substance (standard saponin) and is the simplest way of analysis compared to other methods of analyzing levels. The amount of substance resulting from the analysis of the gravimetric method is determined by directly weighing the mass of the substance separated from other substances through the separation process in a mixture (Adawiyah, 2017). The process that occurs in the gravimetric determination of saponin content of methanol extract of sekilang bark is the precipitation process (Darma and Marpaung, 2020). The analysis is carried out by separating saponin compounds from several other secondary metabolite compounds that are not needed. The goal is to purify the analyzed compounds, so that the fixed weight can be known (Marpaung and Romelan, 2019).

The methanol extract of sekilang stem bark was first dissolved with n-hexan solvent with the aim of attracting nonpolar compounds contained in the extract. Furthermore, the residue left behind was redissolved with ethyl acetate which has a higher polarity level than n-hexane to attract semi-polar compounds. The residue left behind is then added to n-butanol solvent whose polarity level is higher than ethyl acetate with the aim of attracting polar compounds and then poured. The last step is to dissolve the remaining solution from the previous solvent with methanol (the level of polarity is higher than n-butanol) and the addition of ether which functions as a precipitating agent for saponin compounds. Saponins are insoluble in ether, so ether can precipitate saponins. The precipitate formed in the mixture is poured on filter paper to separate the saponin precipitate with other impurities (compounds other than saponin), the filter paper used must be known by weight so that the amount of saponin content can be calculated. Filter paper is allowed to stand at room temperature for ± 1-2 hours and then weighing is carried out until a fixed weight is obtained, the difference in the weight of filter paper before and after filtering is determined as the weight of saponin. Furthermore, the percent of saponin content is calculated based on the data that has been obtained (Adawiyah, 2017; Etika and Giyatmi, 2022). In determining the saponin content of sekilang bark extract, each

extraction method was replicated 3 times. So that in each method there are 9 measurements of saponin content.

Table 5. Results of Saponin Level Calculation

No.	Extraction Method	Saponin content (%)	Average (%)
1	M Ia	22,91	
2	M Ib	20,26	
3	M Ic	24,22	
4	M IIa	26,68	24,87
5	M IIb	22,80	
6	M IIc	25,40	
7	M IIIa	28,89	
8	M IIIb	24,75	
9	M IIIc	27,94	
10	R Ia	33,13	
11	R Ib	35,76	
12	R Ic	33,06	
13	R IIa	27,29	
14	R IIb	23,38	28,97
15	R IIc	25,41	
16	R IIIa	29,54	
17	R IIIb	27,36	
18	R IIIc	25,83	
19	S Ia	33,28	
20	S Ib	34,95	
21	S Ic	30,35	
22	S IIa	29,40	
23	S IIb	25,13	29,99
24	S IIc	30,15	
25	S IIIa	28,33	
26	S IIIb	31,53	
27	S IIIc	26,81	

Based on the table above, the saponin content of the sokhlet extraction method is greater than the maceration and reflux extraction methods. The possibility of saponin compounds is maximally extracted by the sokhlet method with the addition of 40^{o}C temperature, compared to the maceration method which is only at room temperature and to the reflux method with a temperature of 60^{o}C there is a decrease in levels. Chairunnisa et al. (2019) stated that increasing temperature greatly affects saponin levels, the higher the temperature increase, the greater the saponin content obtained. However, excessive temperature increases tend to decrease saponin levels which indicates the solute is saturated. This is also supported in the research of Vongsangnak et al. (2004) that the heating process with a temperature of 50^{o}C quantitatively produces higher saponin levels of 125 mg/g compared to without the heating process which is as much as 71 mg/g. However, at an extraction

temperature of 80ºC, the saponin content tends to decrease, which is as much as 86 mg/g.

The results of the ANOVA statistical test of saponin levels based on differences in extraction methods show a significance value (0.009) < 0.05, so the conclusion is that H0 is rejected which proves that there is a significant difference between the three extraction methods. The test was continued with the Tukey HSD mean difference test to determine which extraction method was significantly different (Purnomo and Syamsul, 2017), the results of the three extraction methods that were significantly different, were the maceration method rather than the reflux and sokhlet methods. This can be attributed to the effect of high heating temperatures in reflux and sokhlet methods compared to maceration, resulting in the compound withdrawal process or extraction process being more optimal than the maceration method which does not experience heating (Prasetyo and Vifta, 2022).

Conclusion

The methanol extract of sekilang bark identified as triterpenoid type saponin. There is a significant difference between extraction methods on the saponin content of sekilang bark with a sig value 0.009 with a significant level of 0.05 (5%).

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

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