

IDENTIFICATION OF SAPONINS IN BAY LEAF (*Syzygium polyanthum*) EXTRACT USING THIN LAYER CHROMATOGRAPHY METHOD

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A B S T R A C T

Background: Plants are generally able to produce two types of compounds, namely primary metabolites and secondary metabolites. Primary metabolites play an important role in the growth and development of plants, whereas secondary metabolites function more as protective compounds, attractants, or energy storage. Bay leaves are not only widely known as a food seasoning but are also believed to contain various active compounds that offer benefits, one of which is saponin. **Objective:** This study was conducted to identify the presence of saponin compounds in bay leaf extracts based on several eluents using the Thin Layer Chromatography (TLC) method. **Method:** This experimental research aims to identify saponin compounds in salam leaf (*Syzygium polyanthum*) extract using thin-layer chromatography. Extraction was carried out by the maceration method using methanol and chloroform solvents to separate its components. The extraction process was performed using the maceration method with methanol and chloroform solvents. **Results:** phytochemical tests showed that the methanol extract of salam leaves tested positive for saponins. The Liebermann-Burchard test also produced a brown color or brown ring, indicating the presence of triterpenoid compounds. Furthermore, TLC analysis using methanol:chloroform eluents in ratios of 1:1 and 2:1 showed R_f values corresponding to saponin standards. However, the use of eluents with a 1:2 ratio did not yield proper spot separation, thus not supporting optimal saponin identification.

Background: Plants in general are capable of producing two types of compounds: primary metabolites and secondary metabolites. Primary metabolites play a crucial role in the growth and development of plants, while secondary metabolites mainly function as protective compounds, attractants, or energy reserves. Bay leaves are not only widely known as a food flavoring, but they are also believed to contain various active compounds with beneficial properties, one of which is saponin. **Objective:** This study was conducted to identify the presence of saponin compounds in bay leaf extract using several eluents through Thin Layer Chromatography (TLC). **Method:** This experimental study aimed to identify saponin compounds in bay leaf extract (*Syzygium polyanthum*) using Thin Layer Chromatography (TLC). Extraction was carried out using the maceration method with methanol and chloroform solvents to separate the components. The extraction process was conducted using the maceration method with methanol and chloroform solvents. **Results:** The phytochemical test showed that the methanol extract of bay leaves positively contained saponins. The Liebermann-Burchard test also resulted in a brown color or brown ring, indicating the presence of triterpenoid compounds. Furthermore, TLC analysis using methanol:chloroform eluents (1:1 and 2:1) showed R_f values consistent with the saponin standard. However, the use of a 1:2 ratio eluent did not produce the appropriate separation of spots, thus not supporting optimal saponin identification..

INTRODUCTION

The development of technology in Indonesia today actually affects people's lifestyles. Some people choose to return to a natural way of life by using traditional ingredients to maintain health and treat various illnesses. (Sidoretno & Oktaviani, 2018). Traditional medicine is a preparation used based on empirical experience and originates from various sources, such as plants, animals, minerals, or galenic preparations, and even mixtures of these materials. These medicines can be processed into various forms of preparations (Dewi et al., 2019). Bay leaves (*Eugenia polyantha*), also known as

Syzygium polyanthum, are a traditional medicinal plant popular in Indonesia. Besides being used as a cooking ingredient, bay leaves contain secondary metabolite compounds with pharmacological potential, making them useful in traditional medicine (Harismah & Chusniatun, 2016). In addition, bay leaves are known to contain flavonoids, alkaloids, phenolics, steroids, terpenoids, and saponins. In lowland areas with sunlight and high temperatures, the vitamin C in bay leaves is more easily oxidized into dehydroascorbic acid, resulting in lower levels (Sarni et al., 2020). The compounds contained in the leaves are likely also present in the bark cortex of salam (*Syzygium polyanthum*) (Habibi et al., 2018).

Plants generally produce two types of compounds, namely primary metabolites and secondary metabolites. The chemical compounds in plants are products of their secondary metabolism, which exhibit a very wide range in terms of both quantity and type. Secondary metabolites, based on their chemical structure, can be divided into several groups, one of which is saponins. (Anggraeni Putri et al., 2023). Nuraeni & Darwiati, (2021) Saponin is an active surface compound that is easily detectable due to its ability to form foam. The polar property of saponin is caused by the presence of glycosidic bonds in its structure. The presence of saponin can be observed through the formation of foam 1-10 cm high within 10 minutes in a test sample in a test tube. Meanwhile, according to Bintoro et al., (2017), stated that in the color test, the sample mixed with chloroform and heated for 5 minutes will undergo a color change after being treated with the Lieberman-Burchard (LB) reagent.

Determination of the chemical content in bay leaves is carried out through qualitative phytochemical analysis, which is the first step in identifying the chemical compounds contained in the sample. Foam tests and color tests are used as part of phytochemical screening, which is an initial stage to identify the presence of certain compounds in the tested herbal material or plant. (Teheni et al., 2023). Saponin is a type of complex glycoside commonly found in plants and is characterized by its ability to produce foam. Therefore, when dissolved in water and shaken, saponin will form foam. (Gunawan, 2018).

Based on research by Mustiqawati & Yolandari, (2022), , that bay leaves contain saponins, tannins, steroids, and flavonoids, with higher saponin levels in young plants. These saponin compounds have great potential in the pharmaceutical field, including their use as antibiotics, antifungals, and antitumors, offering various benefits for human health (Hanindita & Yety, 2023).

Based on the above explanation, the author wants to identify saponin compounds in bay leaves (*Syzygium polyanthum*) using several solvents through thin-layer chromatography methods.

METHOD

Research Design

This laboratory experimental research aims to identify saponin compounds present in the extract of salam leaves (*Syzygium polyanthum*). Thin layer chromatography techniques were applied in this research process

Time and Place of Research

This research was conducted in May-June 2025 at the Mandala Waluya Laboratory.

Tools and Materials

The chemicals used in this study included 2 N hydrochloric acid, chloroform, methanol, Lieberman-Burchard (LB) reagent, and bay leaf samples (*Syzygium polyanthum*). The equipment used included aluminum foil, stirring rods, porcelain dishes, glass funnels, chambers, Erlenmeyer flasks, scissors, measuring glasses, beakers, filter paper, dropper pipettes, pencils, water baths, spatulas, test tube racks, test tubes, analytical balances, glass jars, and rotary evaporators.

Work Procedure

Preparation of concentrated bay leaf extract

Bay leaves are first cleaned of dirt, then dried for three days by airing them. This drying process aims to reduce the water content in the bay leaf samples, making the extraction process easier. Once dry, the bay leaves are ground using a blender into powder (bay leaf simplicia) so that the extraction process yields a higher result. The finer the sample size, the easier it is for the solvent to penetrate the cells to extract active compounds. Extraction is carried out using the maceration method with methanol as the solvent. Methanol is chosen because of its polar properties, which correspond to the characteristics of saponins, which are also polar compounds. (Syafriah, 2021).

The bay leaf simplicia sample was placed in an Erlenmeyer flask and soaked with 600 milliliters of methanol solvent. For three days, the Erlenmeyer was covered with aluminum foil. After

this process, the soaking result was filtered to produce filtrate I. Next, the extraction was continued with 400 milliliters of methanol solvent and left for two days, with occasional stirring using a stirring rod.

Saponin Identification

Foam test

Saponins were identified by weighing 0.5 g of methanol extract of bay leaves, which was then dissolved in 10 mL of hot water and cooled. After that, the solution was shaken vigorously and one drop of 2 N HCl was added. If a stable foam forms that does not disappear within a few seconds, the sample is considered to contain saponins. (Oktapiya et al., 2022).

Color test

As much as 0.5 grams of dried sample powder was placed into a test tube, then 5 mL of chloroform was added. The mixture was shaken over a water bath and cooled. Next, a few drops of Lieberman-Burchard reagent were added to the mixture. A positive result for saponins is indicated by a color change starting from red, pink, purple, or blue, which then slowly changes to green. (Kartikasari D., Rahman R, I., 2022). According to Ngginak et al., (2021), Qualitative testing of saponins with the Lieberman-Burchard reagent produces a color change to brown, green, or blue, indicating the presence of triterpenoid and steroid saponins.

Identification of saponins using TLC

In the TLC test, it will be made into three fractions, with eluents made from two solvents, namely polar and non-polar. The polar solvent consists of methanol, and the non-polar solvent consists of chloroform. The first fraction consists of a mixture of methanol and chloroform in a ratio of 1:2, the second fraction consists of methanol and chloroform in a ratio of 2:1, and the third fraction consists of methanol and chloroform in a ratio of 1:1.

After the fraction is prepared, the spotting process begins. Three Merck GF254 silica plates, each measuring 7 cm in length and 3 cm in width, are prepared. Using a capillary tube, the previously obtained concentrated extract is spotted at designated points at the bottom edge of the TLC plates. The extract is then allowed to dry in the open for a while.

The eluents used consisted of a mixture of methanol and chloroform in a ratio of 1:2 for the first fraction, 2:1 for the second fraction, and 1:1 for the third fraction. Each plate was then placed in a chamber containing the labeled eluent and allowed to elute until the specified distance was reached. After that, the plates were removed and air-dried. The height of the spots or the migration distance was determined using a UV lamp at wavelengths of 254 nm and 366 nm.

Data analysis

The retardation factor (RF) is a parameter used to describe the movement of compounds in Thin Layer Chromatography (TLC). The RF value represents the position of the spot on the stationary phase after the separation process (Wulandari, 2011). Fath (2016) stated that the RF value for saponin standards ranges from 0.57 to 0.92. The RF value can be calculated using the following formula:

$$R_f = \frac{\text{(distance traveled by the compound)}}{\text{(distance traveled by the solvent)}}$$

RESULTS AND DISCUSSION

Extract yield is an important indicator to assess the efficiency of an extraction process. In this study, extraction was carried out using the maceration method with methanol as the solvent. The yield calculation was done by comparing the weight of the obtained thick extract with the initial weight of the dry simplicia used in the extraction process. The extract yield values of bay leaves can be seen in Table 4.1.

Yield presentation can be calculated using the formula below:

$$\frac{\text{weight of extract}}{\text{weight of sample}} \times 100\%$$

Table 1. Presentation of the extract yield of bay leaves (*Syzygium polyanthum*)

Sample weight (g)	Bay leaf extract weight (g)	Yield %
3895 g	19,8 g	0,5%

Source: Primary data, 2025

The maceration results obtained were then filtered to obtain filtrate I, while the residue was further macerated to obtain filtrate II. The results of filtrate I and filtrate II were combined to obtain a thick extract using a rotary evaporator. The thick extract obtained, amounting to 19.8 grams, will then be used for phytochemical tests and thin-layer chromatography tests.

Phytochemical Identification of Saponin Compounds

Phytochemical testing on bay leaf samples was conducted using foam test and color test parameters.

The changes that occurred in the foam and color can be seen in Figure 1.

Table 2. Phytochemical test results of bay leaves

Sample	Reagent	Observation Result Parameters	Description
Bay leaf	HCl Foam Test	Stable foam is formed	+
	Color test LB	Shaped like a brown ring	+

Source: Primary data, 2025

Note: (+) : contains saponins



Figure 1. Results of phytochemical tests A. foam test (HCl) B. color test (Liebermann-Burchard).

Phytochemical tests were conducted using the foam test and the color test. For the foam test, ten milliliters of hot water were added to the sample. After cooling, the sample was vigorously shaken for about ten seconds until foam formed. Stable foam in the sample could be obtained by adding 1 drop of 2 N HCl to the test tube. The presence of saponins could be indicated by foam reaching a height of approximately 4 cm that persisted for more than 10 minutes, as shown in Figure 1A. For the color test, the sample was extracted using 10 mL of chloroform and heated in a water bath for about 5 minutes. After cooling, a few drops of Liebermann-Burchard reagent were added, producing a brown ring, as shown in Figure 1B. Kartikasari et al. (2022) It is also stated that the identification of saponins can be done by dissolving 0.5 grams of sample in 2 mL of distilled water, then shaking it for 10 seconds. A positive result is indicated by the formation of stable foam that lasts at least 10 minutes. Saponins are triterpenoid glycosides that are polar due to the glycosidic bonds in their structure.

The results of the methanol extract of bay leaves can be seen in Figures 2, 3, and 4, as follows:

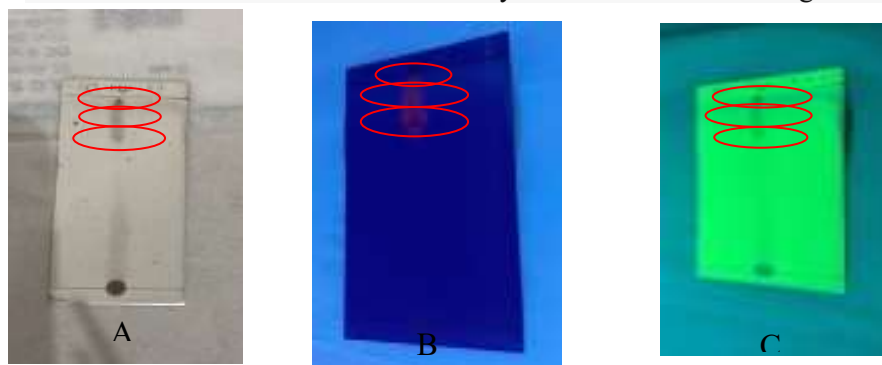


Figure 2. Saponin test using TLC A before UV irradiation B and C after UV irradiation with wavelengths of 366 nm and 254 nm in methanol-chloroform 1:1 solvent.

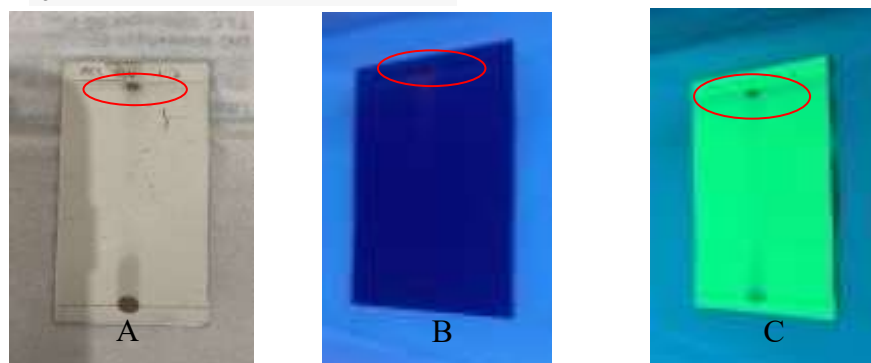


Figure 3. Saponin test using TLC A before UV irradiation, B and C after UV irradiation with wavelengths of 366 nm and 254 nm in methanol-chloroform 1:2 solvent

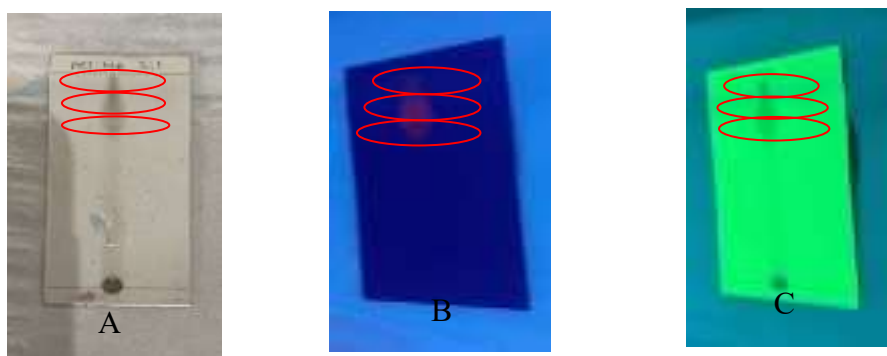


Figure 4. Saponin test using TLC A before UV irradiation, B and C after UV irradiation with wavelengths of 366 nm and 254 nm in methanol-chloroform 2:1 solvent

The testing that has been carried out using the thin-layer chromatography method will subsequently determine the Rf value by calculating the distance of the spot using the formula below:

$$R_f = \frac{\text{distance traveled by the compound}}{\text{distance traveled by the solvent}}$$

Table 3. Results of TLC identification of saponin compounds from bay leaf extract using methanol:chloroform eluent.

Comparison of energies Methanol:chloroform	Travel distance (cm)		Spot results	Rf
	Compound	Solvent		
1:1	Stain 1 (5,8)	6	Red	0,96
	Stain 2 (5,4)	6	Blue	0,9
	Stain 3 (4,8)	6	Blue	0,8
1:2	5,8	6	Red	0,96
2:1	Stain 1 (5,7)	6	Red	0,95
	Stain 2 (5,1)	6	Blue	0,85
	Stain 3 (4,5)	6	Blue	0,75

Source: Primary data, 2025

The results of the study showed that the TLC test of saponin compounds from bay leaves using a methanol:chloroform eluent produced several spots, as shown in Table 2, with a 1:1 ratio. The first spot, with an Rf value of 0.96, turned red when sprayed with Liebermann-Burchard reagent; according to color theory, the color indicating a positive presence of saponins is blue, so this separated spot is not a saponin compound or is another compound. Meanwhile, the second and third spots had Rf values of 0.9 and 0.8, respectively, both showing a blue color when sprayed with Liebermann-Burchard reagent, indicating that both are positive for containing saponins. These two spots are saponin compounds separated from other compounds in bay leaves, in accordance with the standard Rf value range for saponins, which is 0.57–0.92 (Fath, 2016). In the second eluent, with a ratio of 1:2, as shown in Figure 3, a single red spot formed after being treated with Liebermann-Burchard reagent and had an Rf value of 0.96. According to theory, the blue color indicates the presence of saponins, so this red spot does not contain saponins. Therefore, the eluent with a 1:2 ratio is not suitable for saponin identification. However, in the third eluent with a 2:1 ratio, three spots with different colors appeared. After adding the Liebermann-Burchard reagent, the first spot with an Rf value of 0.95 produced a red color. According to theory, the red color indicates that the spot does not contain saponins, while the blue color indicates the presence of saponins. The second and third spots, with Rf values of 0.85 and 0.75, respectively, each showed a blue color after being treated with the Liebermann-Burchard reagent, which according to theory indicates that these Rf values are positive for the presence of saponin compounds. The third can be seen in Figure 4.

CLOSING

Phytochemical test results showed that the methanol extract of bay leaves contains saponin compounds that have positive effects. In addition, the Liebermann-Burchard test produced a brown color or brown ring, indicating the presence of triterpenoid compounds. Analysis using Thin Layer Chromatography (TLC) with methanol:chloroform eluent variations (1:1 and 2:1) showed Rf values corresponding to the standard Rf values for saponins. Meanwhile, the eluent with a 1:2 ratio did not provide suitable results for saponin identification.

This research is limited by the availability of materials and equipment to be used, which impacts the delay in completing the research and affects the smoothness of data collection, result analysis, and the optimal achievement of research objectives.

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REVERENCES

- Anggraeni Putri, P., Chatri, M., & Advinda, L. (2023). Karakteristik Saponin Senyawa Metabolit Sekunder Pada Tumbuhan. *Jurnal Serambi Biologi*, 8(2), 251–258.
- 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.
- Dewi, R. S., Illahi, S. F. N., Fina Aryani, E. P., & Agustini, T. T. (2019). Persepsi Masyarakat Mengenai Obat Tradisional Di Kelurahan Simpang Baru Kecamatan Tampan Kota Pekanbaru. *Jurnal Penelitian Farmasi Indonesia*, 8(2), 75–79. <https://doi.org/10.51887/Jpfi.V8i2.782>
- Gunawan, D. H. (2018). Penurunan Senyawa Saponin Pada Gel Lidah Buaya Dengan Perebusan Dan Pengukusan Decreasing Saponin Compounds On Aloe Vera Gelwith Boiling And Steaming. *Jurnal Teknologi Pangan*, 9(1), 2597–436.
- Habibi, A. I., Firmansyah, R. A., & Setyawati, S. M. (2018). Skrining Fitokimia Ekstrak N-Heksan Korteks Batang Salam (*Syzygium Polyanthum*). *Indonesian Journal Of Chemical Science*, 7(1), 1–4.
- Hanindita, N., & Yety, N. (2023). Penetapan Kadar Saponin Dalam Gambas (*Luffa Acutangula L.*) Hasil Pengeringan Matahari Dan Pengeringan Oven Menggunakan Metode Gravimetri Determination Of Saponin Content In Gambas (*Luffa Acutangula L.*) Resulting From Sun Drying And Oven Drying Using T. 10(1), 57–64. <https://doi.org/10.33508/Jfst.V10i1.4648>
- Harismah, K., & Chusniatun, D. (2016). Pemanfaatan Daun Salam (*Eugenia Polyantha*) Sebagai Obat Herbal Dan Rempah Penyedap Makanan. *Rozhledy V Chirurgii*, 19(2), 110–118.
- Kartikasari D., Rahman R, I., R. A. (2022). Uji Fitokimia Pada Daun Kesum (*Polygonum Minus Huds.*) Dari Kalimantan Barat. *Jurnal Insan Farmasi Indonesia*, 5(1), 35–42. <https://doi.org/10.36387/Jifi.V5i1.912>
- Mustiqawati, E., & Yolandari, S. (2022). Identifikasi Senyawa Saponin Ekstrak Daun Jeruk Nipis (*Citrus Aurantifolia S.*) Dengan Kromatografi Lapis Tipis. *Jurnal Promotif Prefentif*, 5(2), 66–73.
- Ngginak, J., Apu, M. T., & Sampe, R. (2021). Analisis Kandungan Saponin Pada Ekstrak Seratmatang Buah Lontar (*Borassus Flabellifer Linn*). *Bioedukasi (Jurnal Pendidikan Biologi)*, 12(2), 221. <https://doi.org/10.24127/Bioedukasi.V12i2.4451>
- Nuraeni, Y., & Darwiati, W. (2021). Pemanfaatan Metabolit Sekunder Tumbuhan Sebagai Pestisida Nabati Pada Hama Tanaman Hutan. *Jurnal Galam*, 2(1), 1–15. <https://doi.org/10.20886/Glm.2021.2.1.1-15>

- Oktapiya, T. R., Pratama, N. P., & Purnamaningsih, N. (2022). Analisis Fitokimia Dan Kromatografi Lapis Tipis Ekstrak Etanol Daun Rosella (*Hibiscus Sabdariffa* L.). *Sasambo Journal Of Pharmacy*, 3(2), 105–110. <https://doi.org/10.29303/Sjp.V3i2.181>
- Sarni, S., Hamzah, H., Malik, A., A. I. I., & Khadijah, K. (2020). Analisis Kandungan Vitamin C Daun Kelor (*Moringa Oleifera* Lam) Pada Ketinggian Berbeda Di Kota Baubau. *Techno: Jurnal Penelitian*, 9(1), 337. <https://doi.org/10.33387/Tjp.V9i1.1719>
- Sidoretno, W. M., & Oktaviani, I. (2018). Edukasi Bahaya Bahan Kimia Obat Yang Terdapat Didalam Obat Tradisional. *Jurnal Pengabdian Masyarakat Multidisiplin*, 1(2), 117–123. <https://doi.org/10.36341/Jpm.V1i2.453>
- Syafriah, W. O. (2021). Identifikasi Saponin Pada Ekstrak Metanol Daun Pepaya (*Carica Papaya* Linn) Dengan Metode Kromatografi Lapis Tipis. *Journal Of Health Quality Development*, 1(2), 103–108. <https://doi.org/10.51577/Jhqd.V1i2.361>
- Teheni, M. T., Nurwanti, R., & Syafriah, W. O. (2023). Penetapan Kadar Saponin Ekstrak Daun Pepaya (*Carica Papaya* Linn) Menggunakan Metode Gravimetri. *Jurnal Ners*, 7(1), 738–743. <https://doi.org/10.31004/Jn.V7i1.14209>