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The Effect of Different Extraction Methods on Total Tannin Content of Methanol Extract of *Simpur Air* Leaves *(Dillenia suffruticosa)*

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Abstract

The use of metal mordants in fabric dyeing can harm the environment and health, so it is necessary to look for natural mordants that are more environmentally friendly. The utilization of *Simpur Air* leaves (*Dillenia suffruticosa*) as a biomordant is one of the efforts to produce a more environmentally friendly tannin mordant. *D. suffruticosa* has been reported to contain tannin compounds in all parts of the plant. However, scientific evidence of differences in the total tannin content of methanol extracts of *D. suffruticosa* leaves with various extraction methods have not been found so far. This study aims to determine the effect of different extraction methods on the yield value and total tannin content of methanol extracts of D.suffruticosa leaves using UV-Vis spectrophotometric method at a wavelength of 755.8 nm with Folin Ciocalteau reagent. *D. suffruticosa* leaves are extracted by using three methods, namely maceration, soxhletation and reflux. Tannic acid is used as a comparator. The results show that the yield value of *D. suffruticosa* leaves extracted by maceration, soxhletation and reflux methods were 8.35%; 12.26%; and 25.24%, respectively. The total tannin content of methanol extract of *D. suffruticosa* leaves with maceration method is 0.15% ± 0.007; soxhletation is 0.18% ± 0.007 and reflux is 0.21% ± 0.016. Based on the results of the data analysis, the significance value <0.05 was obtained. It can be concluded that the extraction method affects the yield value and total tannin content of methanol extract of *D. suffruticosa* leaves.

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INTRODUCTION

The impact of environmental damage and health problems arising from textile industry waste is detrimental. Synthetic dyes are one of the largest contributors to waste in the textile industry. The use of synthetic dyes can cause environmental pollution and potentially cause skin cancer and brain damage (Nuwa et al., 2018). The use of natural dyes is an alternative to synthetic dyes. Although natural dyes are believed to be safer and environmentally friendly, natural dyes also have disadvantages, namely the intensity and stability of the colors produced tend to be low and not all natural dyes can be directly used to dye fabric fibers, so they require a color reinforcing agent called a mordant (Pujilestari, 2016).

Mordants are chemicals that will form complex compounds with dyes, bind strongly to fabric fibers, and increase the affinity of natural dyes (Lestari et al., 2020). There are three types of

mordants, namely metal mordants, oil mordants, and tannin mordants. The type of mordant commonly used in the textile industry is metal mordant. According to Farida et al., (2015), alum is one type of metal mordant that is relatively environmentally friendly, but its aluminum metal content if accumulated in waste water can cause environmental pollution. Therefore, other alternative materials are needed that can be used to reduce the use of alum, one of which is tannin mordant.

Tannin mordants or commonly called "Biomordants" are natural mordants that can be obtained from plants and animals. According to Ruli et al. (2020), biomordants must contain tannins to have a good effect on fabrics. Tannins that act as mordants have a tendency to dye, so they can strengthen the overall color. Some plant parts that have high tannin compounds or metal ions can provide a mordant effect to a certain extent.

One of the plants that has potential as an alternative biomordant is *Simpur Air* leaf. *Simpur Air (Dillenia suffruticosa)* is one of the plant species included in the Dilleniaceae family of the genus Dillenia. In Indonesia, *D. suffruticosa* plants are found in Sumatra and Kalimantan (Syafriana et al., 2021). Since long time ago, the leaves and stems of *D. suffruticosa* have been used by the people of Sintang and Sambas as a natural dye for traditional weaving by producing brown color (Muflihati et al., 2019). In addition, *D. suffruticosa* is also traditionally used to treat malaria, intestinal diseases, diabetes militus, cough and constipation (Prananda et al., 2015).

The method and solvent used during the extraction process greatly affect the withdrawal of metabolite compounds from plants. The effectiveness of extraction can be assessed based on the percentage of yield produced, where the amount of yield will reflect the effectiveness of the extraction process (Febrina et al., 2015). The best extraction method is the method that can produce methanol extract of *D. suffruticosa* leaves with the highest yield and total tannin content. This study compared three extraction methods, namely maceration, soxhletation and reflux. Methanol is used as a solvent in this extraction because methanol is a polar solvent so that it can attract tannin compounds (Halimu et al., 2020).

Many studies have revealed the content of secondary metabolite compounds in the leaves of *D. suffruticosa*, including tannin compounds (Syafriana et al., 2021). *D. suffruticosa* is reported to contain tannin compounds in all parts of the plant. However, scientific evidence regarding the difference in total tannin content of methanol extracts of *D. suffruticosa* leaves with various extraction methods have not been found so far. This study aims to determine the most optimal extraction method for extracting tannins from *D. suffruticosa* leaves. The results are expected to provide valuable references for researchers and the textile industry in utilizing the potential of *D. suffruticosa* leaves as natural dyes and biomordant agents that are more environmentally friendly, which in turn can help reduce the negative impact of textile industry waste on the environment.

METHODS

Research Design

This type of research is laboratory experimental research with true experimental design using post-test only control group design. Experimental research is a research method that uses experiments with the aim of knowing the effect of independent variables on dependent variables under controlled conditions, which are generally carried out in the laboratory (Sugiyono, 2022). The research was conducted at the Chemistry Education Laboratory, Faculty of Teacher Training and Education, Tanjungpura University Pontianak. The duration of this research was carried out in a period of ± 4 months.

This study used an analytical balance, blender, glassware, micropipette, volumetric flask of various sizes (*pyrex)*, rotavapor, series of succulent tools, series of reflux tools, double-beam uv-visible spectrophotometer (*Shimadzu 1900),* personal computer and solvent methanol pa, distilled water, Folin Ciocalteu reagent, Na_2CO_3 15%, $FeCl_3$ 1% *(Merck),* $FeCl_3$ 5% *(Merck),* mayer reagent, wagner reagent, liebermann-buchard reagent, HCl *(Merck),* dragendroff reagent, chloroform *(Merck),* anhydrous acetic acid (*Honeywell),* and H_2SO_4 (Merck).

Research Procedures

Sample Collection and Preparation

Samples of *Simpur Air (Dillenia suffruticosa)* leaves were collected in August 2023 in Sintang Regency, West Kalimantan. Sample preparation and extraction refer to (Fatonah et al., 2021).

Samples of *D. suffruticosa* leaves that have been collected are then cleaned with running water to remove dirt and dust attached to the leaves. Then dried by aerating at room temperature. Dried water *D. suffruticosa* leaves are blended to obtain water *D. suffruticosa* leaf powder.

Sample Extraction

Maceration

Maceration extraction refers to (Mulyani et al., 2022) with modified. The powder of *D. suffruticosa* leaves was weighed as much as 20 grams and macerated with 150 mL methanol for 3 hours with the help of a magnetic stirrer. Then the extract was filtered and concentrated using a rotavapor at 40°C with a speed of 75 rpm to obtain a thick extract.

Soxhletation

Soxhletation extraction refers to (Puspitasari & Proyogo, 2017) with modifications. Soxletation equipment was assembled and installed, then 20 grams of *D. suffruticosa* leaf powder was wrapped in filter paper, tied with thread, and inserted into the lead. Soxhletation was carried out at 60°C with 150 ml of methanol pa solvent until the cycle droplets were clear. The liquid extract obtained was then concentrated using a rotavapor at 40°C with a speed of 75 rpm until a thick extract was obtained.

Reflux Extraction

Reflux extraction referring to (Susanty & Bachmid, 2016) with modified. The reflux equipment was assembled and installed. Then weighed 20 grams of *D. suffruticosa* leaf powder, put into a round bottom flask and added 150 ml of methanol pa solvent. Then heated at 60°C for 3 hours. The extract was then filtered using filter paper with the help of a funnel. Then the liquid extract obtained was concentrated using a rotavapor at 40°C with a speed of 75 rpm until a thick extract was obtained.

Extract yield calculation

The percentage yield of each extract of maceration, soxhlet and reflux was calculated using the following formula (Hasnaeni et al., 2019).

$$\%\ yield = \frac{weight\ of\ extract\ obtained\ (g)}{weight\ of\ initial\ simplisia\ (g)}\ x\ 100\ \%$$

Phytochemical Screening

Phytochemical screening of extracts was carried out to determine the presence of secondary metabolite compounds of alkaloids, flavonoids, tannins, triterpenoids, saponins, steroids and phenolics in the leaves of *D. suffruticosa*. Stock solution was made with a concentration of 1000 ppm by weighing 1 mg of extract and dissolved with 1 mL of methanol.

Phytochemical Screening of Alkaloid Compounds

The sample solution was taken as much as 1 mL and then added 1 mL of Dragendroff reagent. The sample is positive for alkaloid compounds if it produces a reddish-brown precipitate (Shaikh & Patil, 2020).

Phytochemical Screening of Flavonoid Compounds

The sample solution was taken as much as 1 mL and then added 3 drops of 10% lead acetate solution. The sample is positive for flavonoid compounds if it produces a yellow precipitate (Yuda et al., 2017).

Phytochemical Screening of Tannin Compounds

The sample solution was taken as much as 1 mL and then added 3 drops of 1% $FeCl_3$ solution. The sample is positive for tannin compounds if a blackish green solution is formed (Masriani et al., 2023).

Phytochemical Screening of Triterpenoid Compounds

The sample solution was taken as much as 1 mL, then added with Lieberman-burchard reagent (3 drops of anhydrous acetic acid + 1 drop of sulfuric acid). The sample is positive for triterpenoid compounds if a brownish red ring is formed (Habibi et al., 2018).

Phytochemical Screening of Saponin Compounds

The sample solution was taken as much as 1 mL, then added 1 mL of distilled water and shaken vigorously for 10 minutes until foam formed. The sample is said to be positive for saponins if foam is formed with a height of ± 1 cm (Prananda et al., 2015).

Phytochemical Screening of Steroid Compounds

The sample solution was taken as much as 1 mL, then added with Lieberman-burchard reagent (3 drops of anhydrous acetic acid + 1 drop of sulfuric acid). The sample is positive for steroid compounds if a greenish-blue ring is formed (Habibi et al., 2018).

Phytochemical Screening of Phenolic Compounds

The sample solution was taken as much as 1 mL, then added 3 drops of 5% $FeCl_3$ solution. The sample is positive for phenolic compounds if a dark green or bluish-black solution is formed (Mailuhu et al., 2017).

Determination of Tannin Content:

Preparation of Tannic Acid Parent Standard Solution

Preparation of parent standard solution refers to (Mulyani et al., 2022) which was modified. Tannic acid was weighed as much as 1 mg and then dissolved with 10 ml of distilled water to obtain a 100 ppm parent standard solution.

Preparation of Na_2CO_3 15% Solution

The preparation of Na_2CO_3 15% solution refers to the modified (Hamboroputro & Yuniwati, 2017). Na_2CO_3 powder was weighed as much as 15 grams and then dissolved with distilled water in a beaker. Then put in a 100 mL volumetric flask and added distilled water until the limit mark.

Determination of Maximum Wavelength (λmax)

Determination of the maximum wavelength refers to (Fatonah et al., 2021) with modifications. Tannic acid as much as 2 mL was put into a 10 mL volumetric flask and added 1 mL of Folin Ciocalteu reagent then shaken and allowed to stand for 5 minutes. Into the solution was added

2 mL of 15% Na_2CO_3 solution, shaken and let stand again for 5 minutes. Next, distilled water was added until exactly 10 ml and shaken homogeneously and then scanned at a wavelength of λ 400-800 nm.

Determination of Stable Time (Operating Time)

Determination of stable time refers to (Fatonah et al., 2021) with modifications. Tannic acid as much as 2 mL was put into a 10 mL volumetric flask and added 1 mL of folin ciocalteu reagent then shaken and allowed to stand for 5 minutes. Into the solution was added 2 mL of 15% Na_2CO_3 solution, shaken and let stand again for 5 minutes. Next, distilled water was added until exactly 10 ml and shaken homogeneously and then observed the absorbance at λ max with observation time intervals of 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 to 60 minutes.

Preparation of Tannic Acid Standard Curve

Preparation of tannic acid standard curve with Folin Ciocalteu reagent refers to (Mulyani et al., 2022) modified.The 100 ppm tannic acid standard solution was diluted with distilled water to obtain concentrations of 10 ppm, 5 ppm, 2.5 ppm, 1.25 ppm, and 0.625 ppm using a 10 ml volumetric flask. Into each of these measuring flasks, 1 ml of Folin Ciocalteu reagent was added and shaken and allowed to stand for 5 minutes. The solution was then added 2 ml of 15% Na_2CO_3 solution shaken homogeneously and allowed to stand for the stable time range obtained. The absorbance was measured at the maximum wavelength.

Measurement of Tannin Content of Simpur Air Leaf Extract with UV-Visible Spectrophotometer

A total of 10 mg of *D. suffruticosa* leaf extract was dissolved with 1 mL of distilled water using a microtube. The extract solution obtained was then put into a test tube and added 1 mL of Folin Ciocalteu reagent then shaken and allowed to stand for 5 minutes. Into the solution was added 2 mL of 15% Na_2CO_3 solution, shaken homogeneously and allowed to stand again for 5 minutes and added 1 mL distilled water. The sample solution was taken again as much as 1 mL and diluted with distilled water up to 10 mL and then allowed to stand in the stable time range that has been obtained. Absorbance measurements were taken in triplicate using uv-visible spectrophotometry at the maximum wavelength. The data obtained from this study is the absorbance value calculated by the equation $y = bx + a$ (Fatonah et al., 2021).

Data Analysis

The results were expressed as the mean of three replicates ± SD. The effect of treatment was analyzed using one-way ANOVA and continued with Tukey's test to distinguish between treatments. P value <0.05 was considered significant (Purnomo & Sutadji, 2022).

RESULTS AND DISCUSSION

The samples used in this study were green and healthy/fresh leaves of *D. suffruticosa*, not physically deformed caused by microorganisms and not overgrown with fungi. Samples of *D. suffruticosa* leaves were obtained in the Sintang Regency area, West Kalimantan. Samples that have been collected are then cleaned with running water to remove dirt and dust attached to the leaves. Then dried by aerating at room temperature. This drying aims to reduce the water content in the sample, so as to inhibit the enzymatic process and fungal growth and facilitate the evaporation of solvents. The dried *D. suffruticosa* leaves were then blended to obtain powder. The pulverization of the sample aims to expand the surface and help break down cell walls and membranes, making it easier for the extraction process. A 100 gram sample of *D. suffruticosa* leaves was obtained in the form of powder.

Extraction of *Simpur Air* leaf samples

Samples that have been pollinated are then extracted using three different extraction methods, namely cold method (maceration) and hot method (soxhletation and reflux). The difference in extraction methods aims to determine the effect of extraction methods on extract yields and total tannin content of the resulting extracts and determine the most effective method in attracting compounds or chemical components contained in the sample. The solvent used to extract *D. suffruticosa* leaves is methanol solvent. Tannin compounds have many OH groups which cause polar properties, so tannin compounds can dissolve in polar solvents such as methanol so that tannins can be extracted in methanol solvents (Halimu et al., 2020).

The maceration method is one of the cold extraction methods carried out by putting the sample and the appropriate solvent into a tightly closed inert container accompanied by stirring at room temperature. In this study, maceration was carried out by putting 20 grams of *D. suffruticosa* leaf samples into a glass vessel and then adding 150 ml of methanol solvent. The maceration process was carried out for 3 hours with the help of a magnetic stirrer as a stirrer. The stirring process aims to ensure that all sample surfaces can come into contact with the solvent so that the active substance can be dissolved perfectly. The results of the maceration process were filtered using filter paper and then concentrated using a rotavapor with a temperature of 40°C and a speed of 75 rpm. The maceration method has the advantage that the procedures and equipment used are simple and do not require heating, so that natural ingredients do not decompose. This cold extraction method allows many compounds to be extracted, although some compounds have limited solubility in solvents at room temperature (Puspitasari & Proyogo, 2017).

The soxhletation method is one of the hot extraction methods carried out by placing the sample powder in a cellulose sheath (filter paper can be used) into a lead that is placed above the flask and under a condenser. The appropriate solvent is then put into the flask and the temperature of the bath is set below the boiling point of the solvent (Mukhriani, 2014). In this study, socletation was carried out by putting a 20 gram sample of *D. suffruticosa* leaves into filter paper, tied with thread, and inserted into the lead. Then methanol solvent as much as 150 ml was put into a round bottom flask and heated to a temperature of 60°C. When the methanol solvent is heated, the solvent will evaporate and will form a liquid again when it hits the return cooler. Furthermore, the solvent liquid will drip on the sample, and will dissolve the active substance of the sample again. The results of the socletation process are then filtered using filter paper and concentrated with a rotavapor at 40°C and 75 rpm. This method was chosen because it has the advantages of being able to produce a larger amount of extract with the use of less solvent (material efficiency), shorter time, and more comprehensive sample extraction because it is done repeatedly (Puspitasari & Proyogo, 2017).

The reflux method is also one of the hot extraction methods carried out by putting the sample together with the solvent into a round bottom flask connected to a condenser. The solvent is then heated to the boiling point. The solvent will evaporate and condense, then return to the flask containing the sample and solvent (Mukhriani, 2014). In this study, 20 grams of *D. suffruticosa* leaf samples were put into a round bottom flask and 150 ml of methanol solvent was added. Then heated to a temperature of 60 ° C so that the solvent will evaporate towards the condenser, then turn into liquid and will drop back into the round bottom flask containing the sample, so that the sample in the round bottom flask will be submerged, so on and lasts for 3 hours. The results of the reflux process were then filtered using filter paper and concentrated using a rotavapor at 40°C and 75 rpm. This method was chosen because it is done by heating directly so that the extraction of compounds can take place efficiently and the compounds in the sample can be more effectively drawn by the solvent (Susanty & Bachmid, 2016).

The percentage yield of *D. suffruticosa* leaf extract with maceration, soxhletation and reflux methods can be seen in Table 1 as follows:

Table 1. Percentage extraction yield of water hyacinth leaves by various extraction methods

No.	Extraction Method	Weight (gram)		Percentage yield (%)
		Dry	Extract	
1.	Maceration	20	1,6710	8,35 %
2.	Soxhletation	20	2,4536	12,26 %
3.	Reflux	20	5,0491	25,24 %

Comparison of the yield results of the maceration, soxhletation and reflux methods in table 1 shows that the yield of the reflux method is greater at 25.24% compared to the yield of the soxhletation method which is 12.26% and the maceration method which is 8.35%. The yield obtained in the reflux method is greater when compared to other methods, this is because the reflux method is carried out by direct heating so that the withdrawal of compounds in the sample takes place effectively.

Phytochemical Screening

Phytochemical screening is an initial stage to identify the content of a compound contained in *D. suffruticosa* leaf extract. Chemical compounds that are the result of secondary metabolism in plants are very diverse and can be classified into several classes of natural compounds, namely saponins, steroids, tannins, terpenoids, phenolics, flavonoids and alkaloids. The results of phytochemical screening on *D. suffruticosa* leaf extract can be seen in Table 2 as follows:

Table 2. Phytochemical screening results of *Simpur Air* leaves

No.	Goals	Extraction Method		
		Maceration	Soxhletation	Reflux
1.	Alkaloid	+	+	+
2.	Flavonoid	+	+	+
3.	Tannin	+	+	+
4.	Triterpenoid	-	-	-
5.	Saponin	+	+	+
6.	Steroid	+	+	+
7.	Phenolic	+	+	+

Description:
(+) : Contains secondary metabolite compounds
(-) : Does not contain secondary metabolite compounds

Based on Table 2, it shows that *D. suffruticosa* leaf extract with maceration, soxhletation and reflux methods positively contain secondary metabolite compounds, namely alkaloids, flavonoids, tannins, phenolics, saponins and steroids. The results of phytochemical screening of *D. suffruticosa* leaf extract are in line with the results of research by Fania et al. (2023) which suggests that the extract of *D. suffruticosa* leaves positively contains alkaloid compounds, terpenoids, flavonoids, phenolics, saponins and tannins.

Testing of alkaloid compounds in *D. suffruticosa* leaf extract showed positive results, the reagent used was Dragendroff reagent. The principle of phytochemical screening of alkaloid compounds is the precipitation reaction due to the alternation of ligands. The positive result is characterized by the formation of a reddish-brown precipitate. Positive results on the Dragendroff reagent are thought to be due to the occurrence of potassium-alkaloid complexes (KI) with tetraiodobismutat ions (Prananda et al., 2015). The reaction between alkaloid compounds with Dragendroff reagent can be seen in Figure 1 as follows.

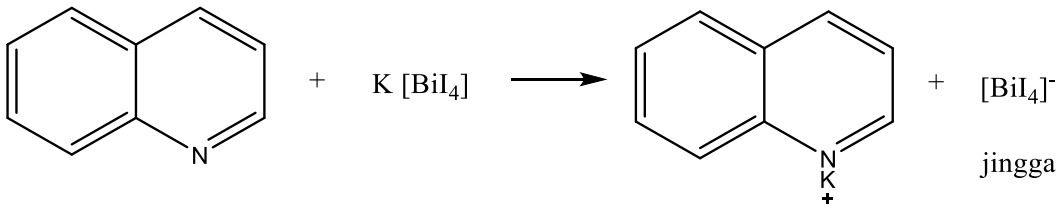


Figure 1. Reaction between alkaloid compounds with Dragendroff reagent

Flavonoid compound testing on *D. suffruticosa* leaf extract showed positive results. Flavonoid test was conducted with 10% lead acetate reagent. Positive results for the lead acetate test will produce a brownish precipitate. The formation of the color change occurs due to the complex formation between Pb acetate 10% and flavonoid compounds (Yuda et al., 2017).

Testing of tannin compounds in *D. suffruticosa* leaf extract showed positive results, the reagent used was $FeCl_3$ 1%. Positive results are characterized by the formation of a blackish green color. $FeCl_3$ reagent 1% will form a complex with one of the hydroxyl groups on tannins which causes the formation of a blue-black color on hydrolyzed tannins and a blackish green color on condensed tannins (Prananda et al., 2015). The reaction between $FeCl_3$ reagent and tannin compounds can be seen in Figure 2 as follows:

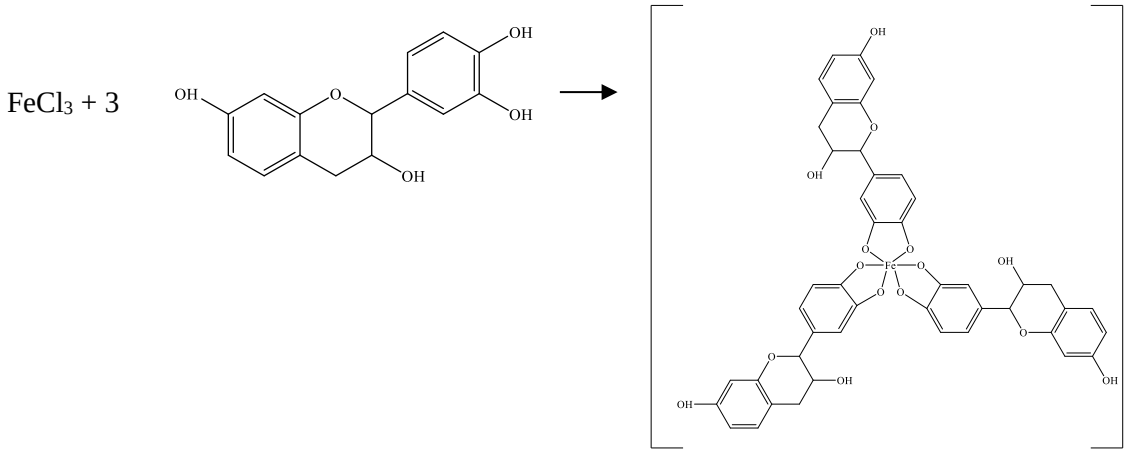
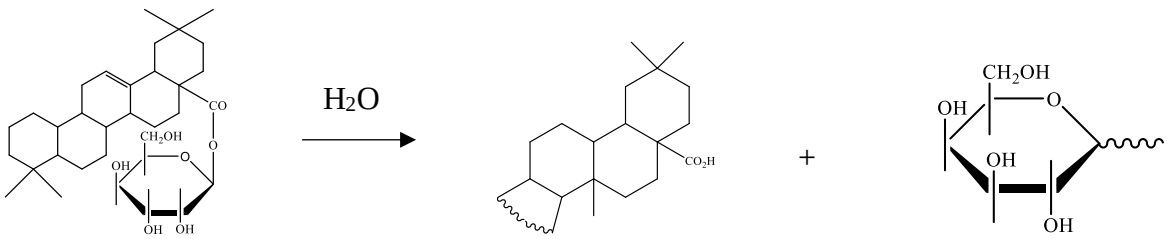


Figure 2. Reaction between FeCl3 reagent and tannin compounds

Testing for saponin compounds in the *D. suffruticosa* leaf extract showed positive results. This test was carried out by putting the *Simpur Air* leaf extract into a test tube and adding 1 ml of distilled water to it and then shaking for 30 seconds - 15 minutes. The reaction between saponins and water can be seen in Figure 3 as follows.



1-Arabinopiriosil-3β-asetil oleanolat Agligon Glukosa

Figure 3. Reaction between saponins and water

Positive results are characterized by the formation of foam as high as ± 1 - 10 cm. The foam formed is due to the presence of glycosides that have the ability to form foam in water which is hydrolyzed into glucose and other compounds (Prananda et al., 2015).

The test for steroid compounds in the *D. suffruticosa* leaf extract showed positive results. The reagent used in this test is Lieberman-Burchard reagent (glacial acetic acid (CH_3COOH) and sulfuric acid (H_2SO_4)). The principle in testing using Lieberman-Burchard reagent is based on the ability of the compound to form a color with concentrated H_2SO_4 in acetic acid solvent. Positive results in this test are characterized by the formation of a blue-green ring (Habibi et al., 2018). The reaction that occurs between steroid compounds with Lieberman-Burchard reagent can be seen in Figure 4 as follows:

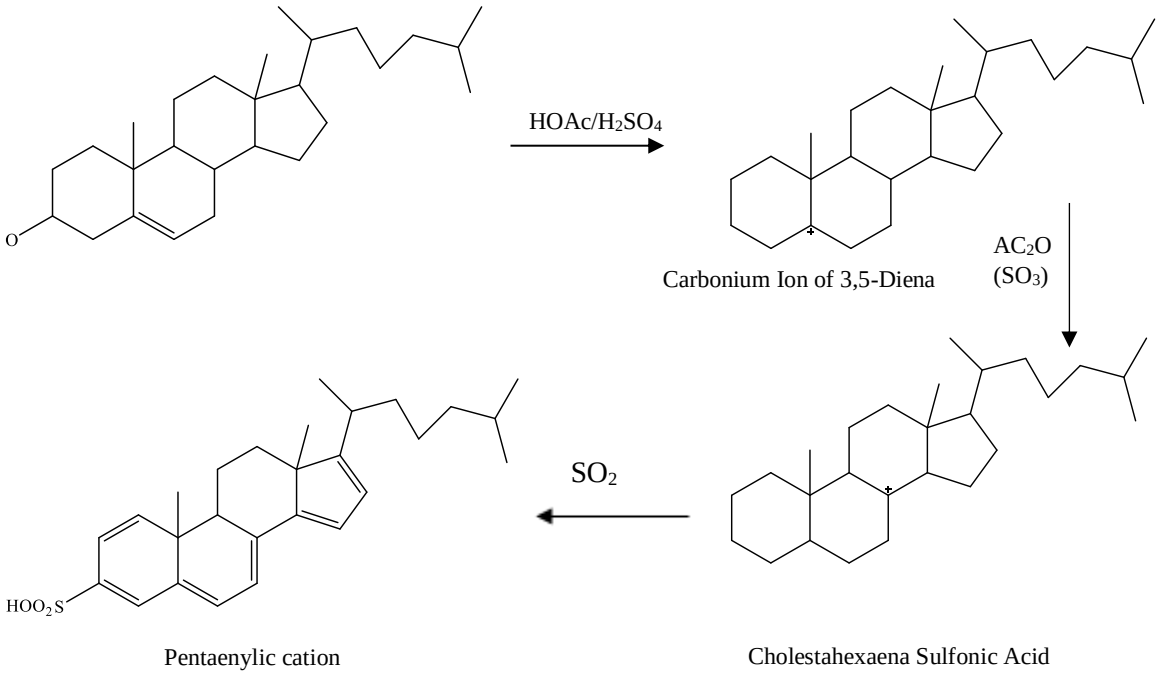


Figure 4. The reaction that occurs between steroid compounds and Lieberman-Burchard reagent

Tests for phenolic compounds in *D. suffruticosa* leaf extract showed positive results. The reagent used is $FeCl_3$ 5%. The positive result will cause the color green, red, purple, blue, or strong black (Bayani, 2016). The color formed in phenolic testing is thought to be due to the formation of the Fe^{3+} complex with phenol. Phenol will reduce Fe^{3+} to Fe^{2+}. Phenol compounds will form complexes with iron ions, causing color changes to blackish green, blackish blue or black (Prananda et al., 2015). The reaction that occurs between $FeCl_3$ reagent and phenolic compounds can be seen in Figure 5 as follows:

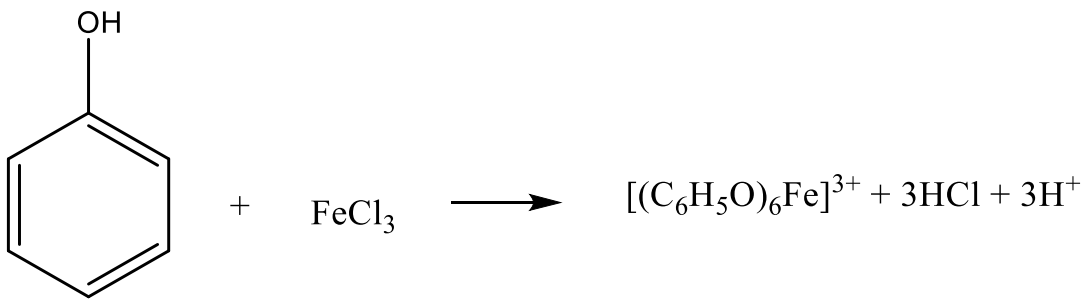


Figure 5. The reaction that occurs between $FeCl_3$ reagent and phenolic compounds

Determination of tannin content

After phytochemical screening using $FeCl_3$ reagent on *Simpur Air* leaves, it can be seen that the sample contains tannin compounds characterized by the formation of a blackish green color. Furthermore, tannin levels were determined using the uv-visible spectrophotometric method with Folin Ciocalteu as a reagent.

The formation reaction that occurs between tannin compounds and Folin Ciocalteu reagent is an oxidation-reduction reaction, where tannin acts as a reductant while Folin Ciocalteu reagent acts as an oxidizer. The result of oxidation will form a blue color that can be read at the maximum wavelength. Folin Ciocalteu is used as a reagent because tannin compounds can react with folin to form a colored solution that can be measured absorbance. The principle of the folin ciocalteu method is the formation of a blue complex compound and can be measured at the maximum wavelength with a range of λ 400-800 nm (Fatonah et al., 2021).

Tannin compounds react with Folin Ciocalteu reagent only in an alkaline atmosphere, this is done to support the dissociation of protons in phenolic compounds into phenolic ions. The way to create an alkaline atmosphere in the extract solution is by adding 15% Na_2CO_3 solution. Hydroxyl groups in tannin compounds will react with Folin Ciocalteu reagent to form a blue molybdenum-tungsten complex that can be detected by a spectrophotometer. The greater the concentration of tannin compounds, the more phenolic ions will reduce heteropoly acid (phosphomolybdate-phosphotungstate) into molybdenum-tungsten complexes so that the blue color produced is more intense. And as a comparison standard is tannic acid. Tannic acid is used as a comparison solution because tannic acid is a hydrolyzed tannin group so that it can be used as a comparison in measuring the total tannin content of *Simpur Air* leaf extract (Hartati & Shafa, 2020).

The maximum wavelength (λmax) is the wavelength at which the absorbance reaches the highest level. The maximum wavelength can be determined by measuring the absorbance of a tannic acid standard solution and varying the wavelength. Selection of an appropriate wavelength is essential to improve the quality of the analysis, provided it is not affected by interfering components or variations that may occur during the analysis process. The purpose of determining the maximum wavelength is to determine the wavelength required by the tannic acid solution in order to achieve maximum absorption. The selection of this maximum absorption wavelength is done to achieve maximum sensitivity. This maximum wavelength also minimizes absorption errors and reduces the possibility of interference from other substances dissolved in the solution (Nofita & Dewangga, 2021). The maximum wavelength curve (λmax) of tannic acid can be seen in Figure 6 as follows. It is known that the maximum wavelength of tannic acid in this study is at a wavelength of 755.80 nm with an absorbance value of 3.001.

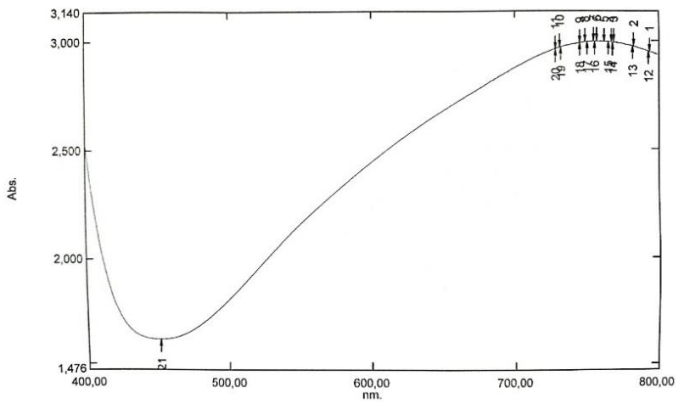


Figure 6. Maximum wavelength curve (λmax) of tannic acid

After determining the maximum wavelength, a stable time (Operating Time) is determined which aims to determine how long it takes for the analyte to react with the reagent so that it can produce a maximum absorbance and is stable when taking measurements (Nofita & Dewangga, 2022). The operating time obtained in this study is at the 47th minute. This shows that at that minute the complex compound formed is stable which is indicated by the stable absorbance value.

In determining tannin levels in *Simpur Air* leaf extract, it is necessary to measure the absorbance value of tannic acid comparison solution with varying concentrations. In this study, tannic acid solution was used with concentration variants of 10 ppm, 5 ppm, 2.5 ppm, 1.25 ppm, and 0.625 ppm. The tannic acid calibration curve can be seen in Figure 7 as follows:

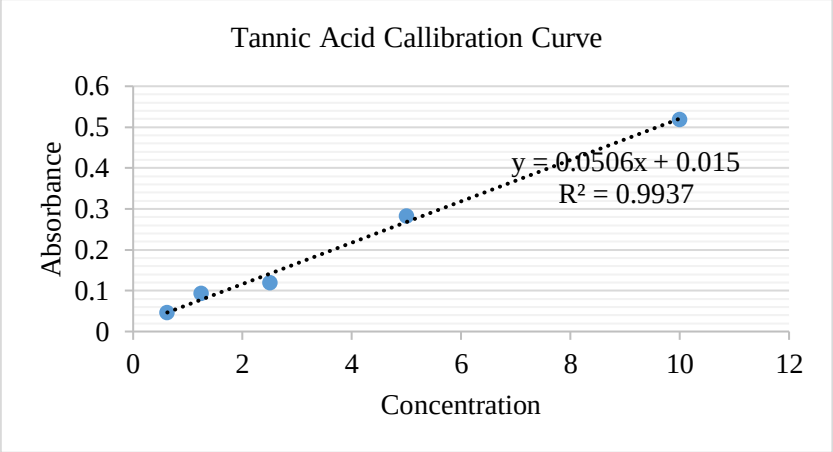


Figure 7. Linearity graph of tannic acid standard curve

Based on the calibration curve (Figure 7), a linear regression equation y = 0.0506x + 0.015 was obtained with an average coefficient of determination (R2) of 0.9937 and an average correlation coefficient (r) of 0.9968. The value of r = 0.9968 means that 99.68% of the absorbance is influenced by concentration while 0.0032% is influenced by other factors such as temperature, light, chemicals and others. The data shows a correlation relationship between concentration and absorbance, from this graph it can be directly seen that the relationship between concentration and absorbance is directly proportional, meaning that the greater the absorbance, the higher the concentration obtained (Bayani & Mujaddid, 2015). The value of r close to 1 proves that the regression equation is close to linear (Nofita & Dewangga, 2021). Data on the total tannin content of methanol extract of *Simpur Air* leaves can be seen in Table 3 as follows.

Table 3. Data on total tannin content of methanolic extract of *Simpur Air* leaves with various extraction methods

Extraction Method	Tannin Content (%w/w TAE)
Maceration	0,1512 ± 0,0073
Sokletation	0,1804 ± 0,0071
Reflux	0,2105 ± 0,0169

Comparison of the measurement results of tannin content from maceration, socletation and reflux methods in Table 3 shows that the tannin content in the reflux method is greater at 0.21% when compared to the socletation method which is 0.18% and the maceration method which is 0.15%. This shows that the reflux method provides the highest tannin content and is the most optimal extraction method for extracting tannin compounds in methanol extracts of *Simpur Air* leaves. This is also in line with several previous studies which reported that the reflux method

produces the highest yield value and compound content in the extraction process. Some previous research results can be seen in Table 4 as follows:

Table 4. Previous research on different extraction methods

Sample	Extraction Method	Yield Value	Level	Literature
Selutui puka leaf	Maceration	24,37%	-	(Apriliana et al., 2019)
	Reflux	25,76%	-	
Argania leaf	Maceration	10,64%	92,39 mg GAE/g	(Riyanti et al., 2023)
	Sokletation	24,04%	197,88 mg GAE/g	

Based on the results of data analysis using One-Way ANOVA with a confidence level of 95% ($\alpha = 0.05$), a significance value of 0.002 was obtained, which is a significance value <0.05. So it can be concluded that the difference in extraction methods can have an effect on the total tannin content of the methanol extract of *Simpur Air* leaves.

CONCLUSIONS

This research is significant in advancing the textile industry, especially in the development of natural dyes and biomordants to reduce dependence on synthetic dyes and mordants which have a negative impact on the environment. This research was carried out using three different extraction methods to determine the most efficient method for extracting tannin levels. This research can be a valuable reference for other researchers who are interested in the extraction and isolation of tannin compounds. This research used three different extraction methods to extract *D. suffruticosa* leaves with methanol solvent, this research achieved interesting results: the maceration method produced 8.35% extract, soxhletation 12.26% extract, and reflux 22.24% extract. The results of research using standard Tannic Acid Equivalent (TAE) per 10 mg sample showed that the maceration method extracted 0.1512% ± 0.0073, soxhletation 0.1804% ± 0.0071, and reflux 0.2105% ± 0.016. In conclusion, to extract more extracts *D. suffruticosa* leaves with methanol solvent, the reflux method is reliable, and in particular, the reflux method stands out as the best option for extracting more tannin content.

SUGGESTIONS

The results of this study have certain limitations, namely focusing on the study of the effect of different extraction methods on the yield and tannin content in methanol extracts from *Simpur Air* leaves. To optimize the use of *Simpur Air* leaves in the textile industry, additional research is needed that will explore the stability and fastness of tannin mordants produced from *Simpur Air* leaves. The findings from this follow-up study can provide a reference for future researchers.

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