



## The Effect of Different Solvents on Total Tannin Content of *Cengkodok* (*Melastoma malabathricum*) Leaf Extracts

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### Abstract

The use of synthetic dyes as color enhancers or generators has caused various environmental and health problems. Plant tannin biomordants offer an alternative solution to address these issues. The sample used in this study were *Cengkodok* (*M. malabathricum*) plants that grow wild in places that get enough sunlight such as shrubs. Phytochemical screening on various parts of the *Cengkodok* plant revealed that the highest tannin content is found in the leaf. However, until now there has been no scientific evidence that presents differences in tannin levels in *Cengkodok* leaf with various types of solvents. This study aims to determine the yield and total tannin content of *Cengkodok* leaf extract obtained through maceration process using methanol, ethanol, and ethyl acetate solvents. The extract yield was determined by comparing the weight of the dry extract with the powdered *Cengkodok* leaf material. The total tannin content was analyzed using UV-Vis spectroscopy at a wavelength of 755.8 nm with Folin-Ciocalteu reagent and sodium carbonate. Tannic acid was used as a reference. The research results showed that the yield values for *Cengkodok* leaf extracted with methanol, ethanol, and ethyl acetate were 5.05%, 4.72%, and 1.59%, respectively. The total tannin content of *Cengkodok* leaf extracts with methanol, ethanol, and ethyl acetate solvents was  $0.47\% \pm 0.04$ ,  $0.37\% \pm 0.01$ , and  $0.19\% \pm 0.04$ , respectively. Based on this research, it can be concluded that methanol is the most effective solvent in extraction and determination of total tannin content of *Cengkodok* leaf extracts.

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## INTRODUCTION

The textile industry is currently experiencing rapid development and has become one of the strategic sectors (Haryono et al., 2021). The growth of the textile industry in Indonesia, aside from providing positive impacts on the nation's economy, also has negative consequences in the form of environmental pollution (Chintya & Utami, 2017). During the dyeing process in the textile industry, approximately 10-15% of the dyes used are discharged as wastewater (Kant, 2012).

The utilization of natural dyes for textiles has emerged as an alternative to synthetic dyes. The use of natural dyes is believed to be safer than synthetic dyes due to their non-carcinogenic and environmentally friendly nature (Kaswinarni et al., 2019). However, natural dyes have certain drawbacks, including low intensity and stability (Riansyah et al., 2021), difficulties in sourcing raw materials, and the complexity of the manufacturing process (Munawaroh et al., 2022).

The use of natural dyes generally requires mordants, which function as color enhancers or fixatives. Mordants come in three types: metal mordants, oil mordants, and tannins. The use of heavy metals such as Sn, Cu, and Cr is prohibited due to their ecological hazards, considering the residues are directly disposed into the environment (Adu et al., 2022). Therefore, alternative materials are needed to reduce the use of alum, for example, tannin-type mordants (Ismail et al., 2014).

Biomordants are natural biological substances containing metal ions, tannins, and the like. Most biomordants are derived from plant sources and serve as mordants in the natural dyeing process. Certain parts of plants with high tannin or metal content can have mordant effects to a certain extent. Tannins play a crucial role and can act as biomordants in natural dyeing (Ismail et al., 2014). Tannin is a complex organic compound consisting of phenolic compounds that are difficult to separate, challenging to crystallize, and capable of precipitating proteins from their solutions while interacting with those proteins (Pratama et al., 2019). The use of environmentally friendly biomordants can replace metal mordants in the natural dyeing process (Rani et al., 2020). However, biomordants are less effective in providing color fastness (Periyasamy, 2022).

*Cengkodok* is one of the plants with the potential to serve as a biomordant. *Cengkodok* leaf have been utilized as a natural dye in traditional weaving in Sintang and Sambas Regencies, West Kalimantan, producing black or light brown colors (Muflihati et al., 2019). *Cengkodok* (Figure 1) is a wild plant that can be found in places with sufficient sunlight, such as mountain slopes, shrubs, moderately dry fields, or in tourist areas as ornamental plants (Noviyanty & Linda, 2020). Phytochemical screening of various parts of the *M. malabathricum* plant indicates that its leaf, fruits, flowers, and stems contain tannins, steroids, phenols, and flavonoids (Danladi et al., 2015).



Figure 1. *Cengkodok* Plant

The effectiveness of extracting a compound by a solvent largely depends on the solubility of that compound in the solvent, following the principle of like dissolves like, where a compound will dissolve in a solvent with similar properties. Some polar solvents include ethanol, methanol, acetone, and water (Verdiana et al., 2018). Ethyl acetate is a semi-polar solvent with the ability to extract both polar and nonpolar compounds (Zainudin et al., 2018). Meanwhile, ethanol has properties that allow it to penetrate cell walls, enabling cell diffusion and faster extraction of bioactive compounds (Yulianti et al., 2020). On the other hand, methanol has an advantage in dissolving polar compounds, such as phenols (Yuliarni et al., 2022).

Phytochemical screening on various parts of the *Cengkodok* plant showed that the highest tannin content was found in the leaf (Danladi et al., 2015). However, until now there has been no scientific evidence that presents differences in tannin levels in *Cengkodok* leaf with various types of solvents. As a form of exploration of biomordants from plants, a study was conducted to determine the effect of different solvents on the total tannin content of *Cengkodok* leaf extract. The aim is to determine the most effective solvent in producing the highest tannin content and to determine the potential of *Cengkodok* leaf as biomordants.

## METHOD

### Research Design

This type of research is laboratory experimental research with true experimental design using post-test only control group design which aims to determine and compare several treatments. This study used *Cengkodok* leaf samples treated with various solvents namely methanol, ethanol, and ethyl acetate. This research was conducted in the Chemistry laboratory, Faculty of Teacher Training and Education, Tanjungpura University Pontianak within  $\pm$  4 months. Experimental research is research conducted by experiments that aim to determine the effect of independent variables on dependent variables under controlled conditions that are often carried out in laboratories (Sugiyono, 2019). The method used to determine the tannin content of *Cengkodok* extract is UV-Visible spectroscopy. The instrument used in determining tannin levels is a Shimadzu 1900 brand double-beam UV-Visible spectrophotometer which has a double beam so that measurements of samples and blank solutions can be carried out simultaneously.

### Tools and Materials

The tools used in this research are glassware, analytical balance, blender, volumetric flask with various sizes (pyrex), micropipette, rotavapor, double beam UV-Vis spectrophotometer (shimadzu 1900), and personal computer. The materials used in this study are *Cengkodok* leaf samples taken in Singa Raya Village, Sambas Regency, West Kalimantan. Then the materials used were methanol, ethanol, ethyl acetate, distilled water, tannic acid, Folin Ciocalteu reagent,  $\text{Na}_2\text{CO}_3$  15%,  $\text{FeCl}_3$ , Liebermann-Buchard reagent, anhydrous acetic acid (Honeywell),  $\text{H}_2\text{SO}_4$ , and  $(\text{CH}_3\text{COO})_2\text{Pb}$ .

### Procedure

#### Collection of *Cengkodok* Leaf Samples

*Cengkodok* leaf samples were obtained from Singa Raya Village, Sambas Regency, West Kalimantan.

#### Sample Preparation and Extraction

Sample preparation and extraction refer to Fatonah et al. (2021) and Mulyani et al. (2022). *Cengkodok* leaf samples were cleaned with running water to remove dirt and dust attached to the leaf. Then dried by aerating at room temperature. The dried *Cengkodok* leaf were blended to obtain *Cengkodok* leaf powder. Powdered *Cengkodok* leaf each weighed as much as 70 grams were macerated with methanol 300 mL, ethanol 300 mL, and ethyl acetate 300 mL for 3 days. Each extract was filtered and concentrated using a rotavapor with a temperature of  $40^\circ\text{C}$  and a speed of 75 rpm to obtain a thick extract. The percentage yield of the extract was calculated using the following formula:

$$\% \text{ Yield} = \frac{\text{Weight of Extract (g)}}{\text{Weight of Initial Simplisia (g)}} \times 100 \%$$

## Phytochemical Screening and Determination of Tannin Content

### a. Phytochemical Screening

Stock solution was made with a concentration of 1000 ppm by weighing 5 mg of extract and dissolved with 5 mL of methanol. Repeat in the same way for ethanol and ethyl acetate solvents.

#### 1) Tannin Test

The sample solution was taken as much as 1 mL and then added 2 drops of 1%  $\text{FeCl}_3$  solution. The sample is positive for tannin compounds if a blackish green solution is formed (Masriani et al., 2023).

#### 2) Phenolic Test

The sample solution was taken as much as 1 mL and then added 2 drops of 5%  $\text{FeCl}_3$  solution. The sample is positive for phenol compounds if a blue-black solution is formed (Mailuhu et al., 2017).

#### 3) Flavonoid Test

The sample solution was taken as much as 1 mL and then added lead acetate ( $(\text{CH}_3\text{COO})_2\text{Pb}$ ) 10% a few drops. The sample is positive for flavonoid compounds if a yellow precipitate is formed (Yuda et al., 2017).

#### 4) Alkaloid Test

The sample solution was taken as much as 1 mL and then added Dragendorff reagent as much as 1 mL. The sample is declared positive for alkaloid compounds if a brownish red precipitate is formed (Habibi et al., 2018).

#### 5) Saponin Test

The sample extract was taken as much as 25 mg and dissolved with distilled water as much as 2 mL. The solution is then shaken quickly and vigorously. The sample is said to be positive for saponins if foam is formed which lasts for  $\pm 15$  minutes (Masriani et al., 2023).

#### 6) Triterpenoid Test

The sample solution was taken as much as 1 mL and then added with Liebermann-Bouchard reagent (anhydrous acetic acid 3 drops and concentrated sulfuric acid 1 drop). The sample is declared to contain triterpenoid compounds when a brownish red color forms on the inter-surface (Octaviani, 2022).

#### 7) Steroid Test

The sample solution was taken as much as 1 mL and then added with Liebermann-Bouchard reagent (anhydrous acetic acid 3 drops and concentrated sulfuric acid 1 drop). The sample is declared to contain triterpenoid compounds if a green-blue color is formed (Octaviani, 2022).

### b. Determination of Tannin Content

#### 1) Preparation of Parent Standard Solution

The preparation of the 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 so that a 100 ppm parent standard solution was obtained.

#### 2) Preparation of 15% $\text{Na}_2\text{CO}_3$ solution

Preparation of 15%  $\text{Na}_2\text{CO}_3$  solution refers to Hamboroputro & Yuniwati, (2017).  $\text{Na}_2\text{CO}_3$  powder was weighed as much as 15 grams and dissolved in 100 mL of distilled water.

#### 3) Determination of Maximum Wavelength ( $\lambda_{\text{max}}$ )

Determination of the maximum wavelength refers to Fatonah et al. (2021) with modifications. 2 mL of tannic acid was put into a 10 mL volumetric flask and 1 mL of Folin Ciocalteu reagent was added, then shaken and allowed to stand for 5 minutes. Into the solution was added 2 mL of 15%  $\text{Na}_2\text{CO}_3$  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  $\lambda$  400-800 nm.

#### 4) Determination of Stable 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<sub>2</sub>CO<sub>3</sub> solution, shaken and let stand again for 5 minutes. Next, distilled water was added until exactly 10 ml and shaken homogeneously. Observe the absorbance at  $\lambda_{\text{max}}$  with observation time intervals of 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 to 60 minutes.

#### 5) Preparation of Tannic Acid Standard Curve with Folin Ciocalteu Reagent

The preparation of the standard curve of tannic acid with Folin Ciocalteu reagent refers to Mulyani et al. (2022). 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, shaken and allowed to stand for 5 minutes. The solution was then added 2 ml of 15% Na<sub>2</sub>CO<sub>3</sub> solution shaken homogeneously and allowed to stand for the stable time range obtained. Observe the measured absorbance at the maximum wavelength.

#### 6) Determination of Total Tannin Content of *Cengkodok* Leaf Extract with UV-Visible Spectrophotometry

A total of 10 mg of *Cengkodok* leaf extract was dissolved with distilled water as much as 1 mL 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<sub>2</sub>CO<sub>3</sub> 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 obtained. Absorbance measurements were taken triplo using UV-Visible spectrophotometry at the maximum wavelength. The data obtained in this study are absorbance data calculated by the equation  $y = bx + a$  (Fatonah et al., 2021).

### Data Analysis

The results were expressed as the mean of three replicates  $\pm$  SD (Soenardjo & Supriyantini, 2017). The effect of treatment was analyzed using one-way ANOVA followed by the Tukey test to distinguish between treatments. P value  $<0.05$  was considered significant (Babajafari et al., 2018).

## RESULTS AND DISCUSSION

### Sample Extraction

*Cengkodok* leaf used in this study were obtained from Sambas Regency, West Kalimantan. The *Cengkodok* leaf used were wet and dry sorted. Sorting is an activity carried out to separate dirt or foreign objects from a sample. Wet sorting was carried out using running water to remove dirt and dust attached to the leaf. The leaf were then dry sorted by aerating and storing at room temperature to remove the residual moisture content of the washing. Drying is carried out at room temperature and is not carried out under direct sunlight so that the compounds contained in the sample can be maintained properly, because sunlight can cause compounds that are not able to be exposed to light to oxidize so that they can reduce their activity. After sorting, the leaf were pulverized using a blender to reduce the particle size. This is because the smaller the particle size, the more surface area so that the active substances in the leaf will be perfectly extracted. The extraction process in this study used the maceration method. This method has several advantages over other extraction methods, namely that it is the simplest, relatively cheap extraction method, and can avoid damage to thermolabile compound components (Satria et al., 2022). The solvents used are methanol, ethanol, and ethyl acetate. This method uses the

principle of like dissolve like, namely a compound will dissolve in a solvent with the same properties.

Tannins are chemical compounds that are polar and soluble in polar solvents (Nofita & Dewangga, 2021). Methanol is a solvent that is polar and able to dissolve polar compounds so it is very well used to extract secondary metabolite compounds contained in the sample (Khasanah et al., 2021). Ethanol is a polar solvent with properties that allow it to penetrate cell walls, so it is able to perform cell diffusion and attract bioactive compounds more quickly (Yulianti et al., 2020). In addition, ethyl acetate is a low-toxicity solvent that is semi-polar, has the ability to attract polar and nonpolar compounds (Zainudin et al., 2018). Each extract was filtered and concentrated using a rotavapor with a temperature of 40°C and a speed of 75 rpm to obtain a thick extract. The purpose of rotavapor is to evaporate the extraction solvent and only leave the extracted compounds (Fajriyani et al., 2022). Percent yield of *Cengkodok* leaf extract with various solvents can be seen in table 1.

Table 1. Percent Yield of *Cengkodok* Leaf with Various Solvents

| Solvent       | Weight (g) |         | Percent Yield (%) |
|---------------|------------|---------|-------------------|
|               | Dry        | Extract |                   |
| Methanol      | 70         | 3,4313  | 5,05              |
| Ethanol       | 70         | 3,3073  | 4,72              |
| Ethyl acetate | 70         | 1,1113  | 1,59              |

The highest extraction yield is obtained from the methanol solvent, while the lowest extraction yield is observed with the ethyl acetate solvent. The variation in yield among the three solvent types is due to the differing polarities of each solvent. The yield of the extract with methanol is denser compared to the extract from ethanol, which has a paste-like consistency, and the extract from ethyl acetate, which is in liquid form. This difference is attributed to the polar nature of compounds in the extract, which tends to dissolve in polar solvents. The choice of solvent type can significantly impact the resulting extract. Methanol as a solvent produces a higher yield compared to ethanol and ethyl acetate, which have lower polarities.

Based on the proportion of yields, *Cengkodok* leaf exhibit polarity that aligns closely with methanol as the solvent. The yield results in this study are in line with the research of (Verdiana et al. (2018) who calculated the yield of lemon fruit peel extract (*Citrus limon* (Linn.) Burm F.) using several types of solvents. The highest yield of lemon peel extract was obtained using 70% methanol solvent which amounted to 40.61%, while 70% ethanol extract of lemon fruit had a yield of 37.68%. Putri et al. (2015) mentioned that methanol yield was higher (17.73%) than ethyl acetate (3.51%). Likewise, research conducted by (Rafsanjani & Putri, 2015) on Bali Orange peel showed higher extracts with ethanol solvent (11.96%) than ethyl acetate solvent (9.29%).

### Phytochemical Screening

The results of phytochemical screening of methanol, ethanol, and ethyl acetate extracts of *Cengkodok* leaf show that the *Cengkodok* leaf contain tannin, phenolic, flavonoid, alkaloid, saponin, and steroid compounds which can be seen in Table 2.

Table 2. Phytochemical Screening Results of *Cengkodok* Leaf Extracts with Various Solvents

| Testing   | Solvent Variant |         |               |
|-----------|-----------------|---------|---------------|
|           | Methanol        | Ethanol | Ethyl Acetate |
| Tannin    | +               | +       | +             |
| Phenolic  | +               | +       | +             |
| Flavonoid | +               | +       | +             |
| Alkaloid  | +               | +       | +             |

|              |   |   |   |
|--------------|---|---|---|
| Saponin      | + | + | + |
| Triterpenoid | - | - | - |
| Steroid      | + | + | + |

Notes: (+) contains the tested compound

(-) does not contain the tested compound

### Tannin Test

The tannin compound test in the sample was carried out by reacting the extract with  $\text{FeCl}_3$  reagent. The test results of *Cengkodok* leaf extract with methanol, ethanol, and ethyl acetate solvents showed positive results marked by a change in color to blackish green. The phenolic group, including tannin compounds, has an aromatic ring skeleton containing hydroxyl groups (-OH).  $\text{FeCl}_3$  reagent will react with one of the hydroxyl groups on the tannin compound and cause the *Cengkodok* leaf extract to produce a blackish green color (Noviyanty & Linda, 2020). The reaction of  $\text{FeCl}_3$  reagent with tannin compounds occurs as in Figure 2.

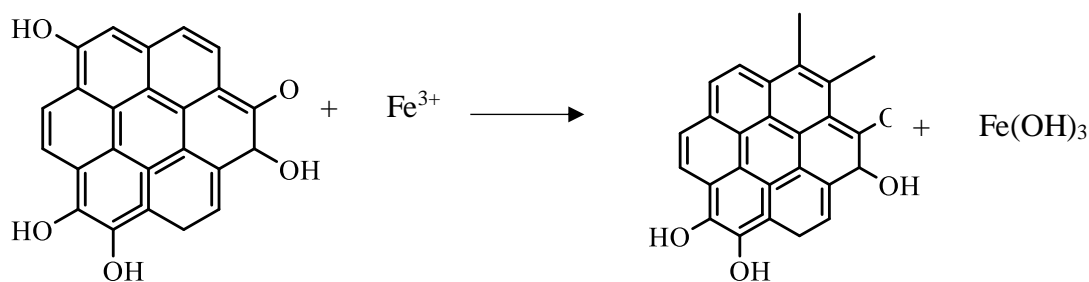


Figure 2. Reaction of Tannins with  $\text{FeCl}_3$  Reagent

### Phenolic Test

The addition of 5%  $\text{FeCl}_3$  reagent to determine the presence of phenolic content in methanol, ethanol, and ethyl acetate extracts of *Cengkodok* leaf shows a blue-black color change so it is suspected that *Cengkodok* leaf have good antioxidant activity. This is in line with the research of Mutmainnah et al. (2019) who conducted phenolic tests on cengdodok leaf that can inhibit bacterial growth on cell membranes. More specifically, the color change to blackish indicates that the sample contains catechol tannins (phenol derivative compounds) (Bayani, 2016). The reaction of  $\text{FeCl}_3$  reagent with phenolic compounds can be seen in Figure 3 (Aisyah et al., 2019).

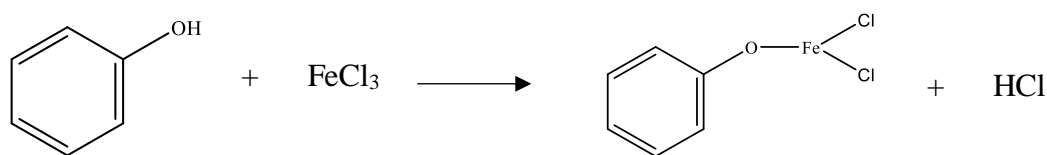


Figure 3. Reaction of Phenol with  $\text{FeCl}_3$  Reagent

### Flavonoid Test

Flavonoid compound testing on methanol, ethanol, and ethyl acetate extracts of *Cengkodok* leaf was carried out with 10% lead acetate ( $(\text{CH}_3\text{COO})_2\text{Pb}$ ) solution a few drops. Based on the test, methanol, ethanol, and ethyl acetate extracts showed the presence of flavonoids characterized by a yellow precipitate. The formation of brownish color changes and there is a precipitate on the addition of 10% Pb acetate, due to the formation of complexes between 10% Pb acetate and flavonoid compounds (Wibawa, 2021).

### Alkaloid Test

Testing for alkaloid compounds in methanol, ethanol, and ethyl acetate extracts of *Cengkodok* leaf is conducted using the Dragendorff reagent. Methanol, ethanol, and ethyl acetate extracts exhibit the presence of alkaloid content, as indicated by the formation of a reddish-brown



precipitate. Nitrogen from the alkaloid groups reacts with the Dragendorff reagent to form a coordinate covalent bond with  $K^+$ , which is a metal ion, resulting in the formation of a potassium alkaloid precipitate (Nugrahani et al., 2016). The reaction between the Dragendorff reagent and alkaloid compounds can be observed in Figure 4.

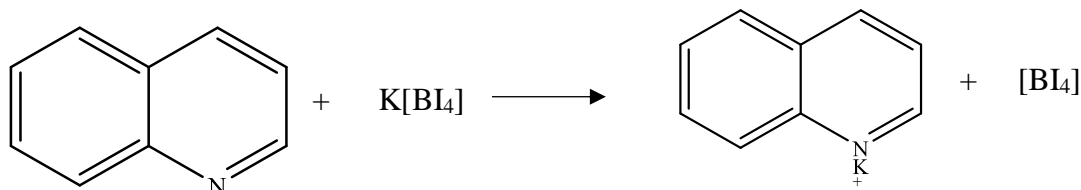


Figure 4. Reaction of Alkaloids with Dragendorff Reagent

### Saponin Test

Positive results for the saponin test are indicated by the formation of foam after shaking. Methanol, ethanol, and ethyl acetate extracts of *Cengkodok* leaf demonstrate the presence of saponin compounds, as evidenced by the formation of foam after shaking and allowing to stand for 15 minutes. The foam produced as a positive reaction in this test is due to the presence of glycosides in saponin, which undergo hydrolysis into glucose and other compounds, resulting in stable foam (Qomaliyah et al., 2023). The reaction of saponin compounds can be observed in Figure 5 (Nugrahani et al., 2016).

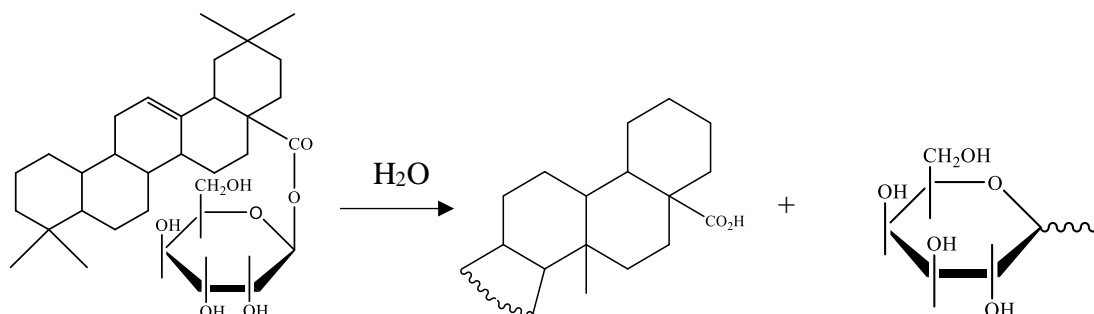


Figure 5. Reaction of Saponins with Water

### Triterpenoid Test

The triterpenoid compound test is conducted using the Liebermann-Burchard reagent. Positive results in the triterpenoid test are indicated by a color change to reddish-brown. Methanol, ethanol, and ethyl acetate extracts of *Cengkodok* leaf do not show the presence of triterpenoid compounds, as evidenced by the absence of a reddish-brown color formation (Octaviani, 2022).

### Steroid Test

The reaction of steroids with the Liebermann-Burchard reagent results in a green-blue color. In the phytochemical test using the Lieberman-Burchard reagent, a color change to bright green occurs. This is due to the oxidation reaction in the terpenoid/steroid group through the formation of conjugated double bonds (pentaenilic compounds). The results of methanol, ethanol, and ethyl acetate extracts of *Cengkodok* leaf indicate the presence of steroid compounds, as evidenced by the formation of a bright green color. The ethyl acetate extract of *Cengkodok* leaf shows a more intense green color compared to methanol and ethanol extracts. This is because steroid compounds are nonpolar and can dissolve completely in the semipolar ethyl acetate solvent (Nurjannah et al., 2022). The reaction of steroids with the Lieberman-Burchard reagent can be observed in Figure 6.



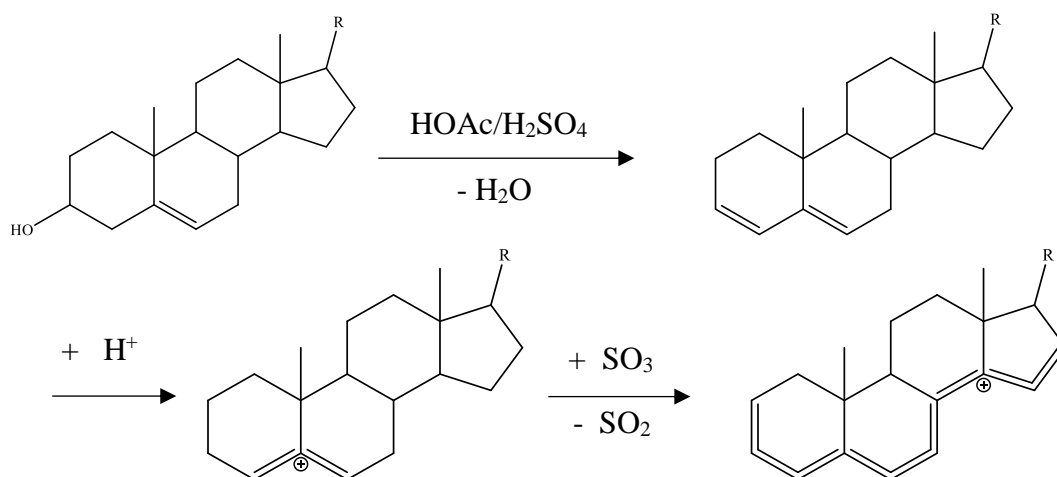


Figure 6. Reaction of Steroids with Liebermann-Burchard Reagent

### Determination of Tannin Content of *Cengkodok* Leaf Extract

Based on phytochemical screening using  $\text{FeCl}_3$  reagent, it shows that *Cengkodok* leaf extract contains tannin compounds characterized by the formation of black color. Determination of tannin content is done using UV-Vis spectroscopy method with Folin Ciocalteu reagent. The principle is the oxidation reaction of phenol compounds including tannins in an alkaline atmosphere by Folin Ciocalteu reagent produces a blue complex and gives strong absorption in UV-Visible spectrophotometry. The standard comparison solution used is tannic acid, because tannic acid is a hydrolyzed tannin group so it can be used as a comparison in measuring total tannin levels. In order for the Folin Ciocalteu reduction reaction to occur by the hydroxyl group of tannins in the sample,  $\text{Na}_2\text{CO}_3$  solution is used to create an alkaline atmosphere (Nofita & Dewangga, 2021).

The maximum wavelength ( $\lambda_{\text{max}}$ ) is the wavelength at which the absorption is maximum by reading the absorption of tannic acid standard solution. Determination of the maximum wavelength aims to determine the wavelength required for tannic acid solution to reach maximum absorption and reduce absorption reading errors that allow the influence of interference from other substances that can be dissolved (Nofita & Dewangga, 2021). In this study, the maximum wavelength of tannic acid was obtained at 755.8 nm.

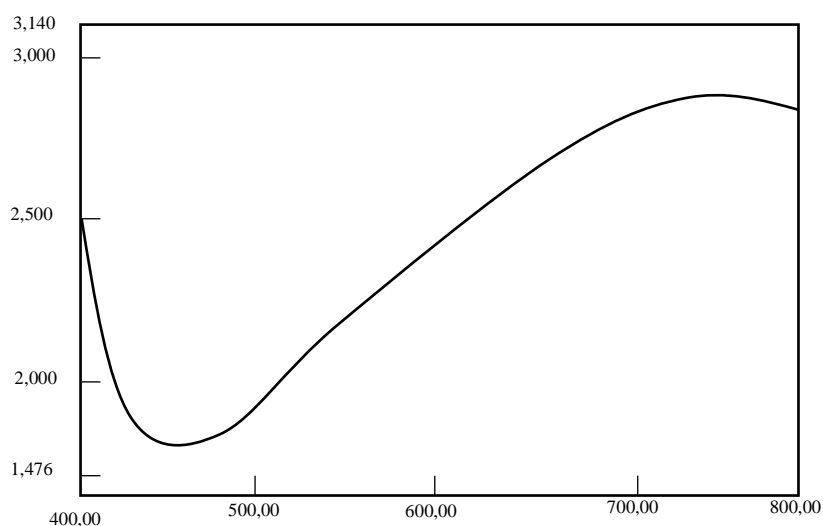


Figure 7. Maximum Wavelength of Tannic Acid

After determining the maximum wavelength, the operating time is determined. Determination of operating time is useful to know how long it takes for the analyte to react with the reagent so that it can produce maximum and stable absorbance when taking measurements (Nofita & Dewangga, 2021). The operating time obtained 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 the tannin content in *Cengkodok* leaf extract, it is necessary to measure the absorbance value of tannic acid comparison solution with varying concentrations. Based on the calibration curve (Figure 7), a linear regression equation  $y=0.0506x + 0.015$  is obtained with an average determination coefficient ( $R^2$ ) of 0.9937 and an average correlation coefficient ( $r$ ) of 99.68% of the absorbance is influenced by differences in solvents, while 0.32% is influenced by other factors such as temperature, light, and other substances. The data shows a correlation relationship between the solvent used and the absorbance. The value of  $r$  close to 1 proves that the regression equation is close to linear (Nofita & Dewangga, 2021).

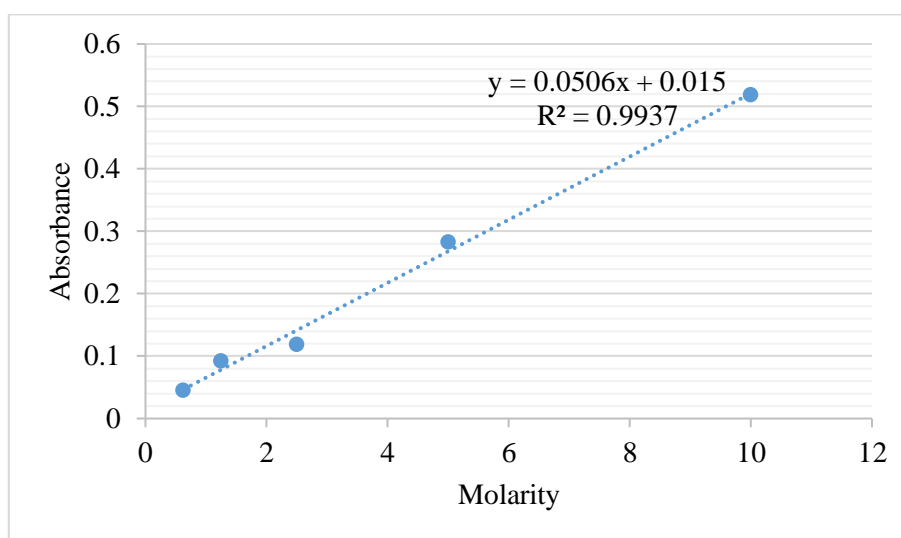


Figure 8. Calibration Curve of Tannic Acid

Testing the *Cengkodok* leaf extracts with various solvent variants reveals that the tannin content in methanol, ethanol, and ethyl acetate extracts is 0.47%, 0.38%, and 0.19%, respectively. The methanol extract of *Cengkodok* leaf shows the highest tannin content compared to ethanol and ethyl acetate extracts. This is consistent with the research conducted by Handayani, (2021), which indicates that the tannin content in methanol extracts is higher than that in ethanol extracts. A study by (Putri et al., 2015) also shows that the tannin content in methanol extracts of karamunting leaf (*Rhodamyrthus tomentosa* (Aiton) Hassk.) is higher than that in ethyl acetate extracts. Furthermore, in the research by Rafsanjani & Putri (2015), the tannin content in Bali orange peel in ethanol solvent is higher than in ethyl acetate solvent.

Based on this study, the more polar the solvent used in the extraction process, the higher the tannin content produced (Table 3). Methanol is a solvent with a higher level of polarity compared to ethanol because it has fewer C atoms, resulting in compounds bound by these solvents having different polarities. Tannin compounds are polar and tend to dissolve in polar solvents but are still soluble in semi-polar solvents. Based on the results of One-Way Anova analysis using a 95% confidence level ( $\alpha = 0.05$ ), it shows a significance value of 0.000, which is significance  $<0.05$ , so the difference in solvents has an effect on the total tannin content of *Cengkodok* leaf extract.

Table 3. Total Tannin Content of *Cengkodok* Leaf Extract

| Variant Solvent | Tannin Content   |
|-----------------|------------------|
| Methanol        | 0.47% $\pm$ 0.04 |
| Ethanol         | 0.37% $\pm$ 0.01 |
| Ethyl Acetate   | 0,19% $\pm$ 0.04 |

## CONCLUSION

This research makes a significant contribution by exploring the potential of tannins in *Cengkodok* leaf extract as natural dye dan biomordant for textile fibers and reducing dependence on synthetic dyes which have the potential to damage the environment. So, this research plays a role in supporting the development of a sustainable and environmentally friendly textile industry.

This research shows that, the highest percentage of extract using the maceration method is methanol extract with a percentage of 5.05%, *Cengkodok* leaf extract produce the most tannin in methanol solvent which shows the highest value that has been tested using standard Tannic Acid Equivalent (TAE) with a percentage of 0.47%  $\pm$  0.04 per 10 mg of extract, followed by ethanol solvent which is not much different from methanol solvent with a percentage of 0.37%  $\pm$  0.01 per 10 mg of extract. For the ethyl acetate solvent, it is not recommended to use tannin from extracts with this solvent because it shows the lowest value, namely 0.19%  $\pm$  0.04 per 10 mg, so it requires a lot of solvent and samples if you decide to use tannin with this solvent.

In conclusion, *Cengkodok* leaf extract with methanol solvent can be used as a tannin producing material with the most potential as a natural, environmentally friendly dye and biomordant for textile fibers.

## RECOMMENDATIONS

Further exploration is needed regarding the testing of biomordant tannins from *Cengkodok* leaf in the fabric dyeing process.

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