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Determination of Total Tannin Content from Methanol Extracts of Various Species of Ferns (*Pteridophyta*)

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Abstract

The use of heavy-metal mordants in fabric dyeing is prohibited due to the resulting impacts such as environmental damage and public health, therefore environmentally friendly and non-toxic mordants such as tannin mordants (biomordants) are needed. Based on phytochemical screening on fern species (*Pteridophyta*), it is known that they contain tannins. However, scientific evidence of determining the total tannin content of various species of ferns has so far not been found. This study aims to determine the yield and total tannin content of methanol extracts of five fern species, namely *paku kawat* (*Lycopodium cernuum*), *paku resam* (*Gleichenia linearis*), *paku dayak* (*Blechnum orientale*), *paku uban* (*Nephrolepis biserrata*), *paku miding* (*Stenochlaena palustris*). Sample extraction was carried out by maceration method using methanol solvent. Extract yield was determined by weighing method. Analysis of total tannin content was carried out by UV-Vis spectrophotometric method at a wavelength of 755.8 nm using Folin-Ciocalteu reagent and sodium carbonate. The results of the research obtained the yield of methanol extracts on *paku kawat*, *paku resam*, *paku dayak*, *paku uban*, and *paku miding* are 11.271%; 8.389%; 4.810%; 3.615%; and 0.854%, respectively. Total tannin levels in methanol extracts of *paku kawat*, *paku resam*, *paku dayak*, *paku uban*, and *paku miding* were 0.024% ± 0.002; 0.256% ± 0.029; 0.272% ± 0.037; 0.143% ± 0.019; and 0.022% ± 0.012, respectively. Based on these data, it can be concluded that the methanol extract of *paku kawat* produces the highest yield while *paku dayak* produce the highest total tannin content. The results indicate that differences in fern species cause differences in tannin levels.

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INTRODUCTION

The textile industry is one of the high contributors to waste in the world. One of the wastes generated from the textile industry is liquid waste in the form of residual synthetic dyes (Hikmah & Retnasari, 2021). Synthetic waste (chemicals) from textile dyeing is one of the problems still faced by society and the fashion industry today. The increased use of synthetic dyes produces hazardous chemical waste, the waste is difficult to decompose in final disposal which has an impact on environmental damage and public health (Permatasari & Lestari, 2023). Synthetic dyes contain heavy metals, namely Cu, Ni, Cr, Hg, and Co, aromatic compounds, azo groups, chlorine, and others (Chintya & Utami, 2017). One of the efforts to reduce this impact is by replacing synthetic dyes with natural dyes (Abdurahman & Kahdar, 2021). According to Yernisa et al. (2013) natural dyes are an alternative dye that is non-toxic, renewable, easily degradable, and environmentally friendly. But on the other hand, natural dyes have unstable colors and poor color uniformity (Firyanto, 2022). In addition, the color choices are limited, and the fastness is still low (Sumarli, 2021).

Coloring the fabric is done using three main material components, namely dyestuff, water and mordant or color generating substances (Mahmudah & Achir, 2013). Natural dyes are substantive and require a mordant to adhere to the fabric, and prevent the color from fading either from exposure to light or washing. Mordant compounds function in binding natural dyes to the fabric. Mordants help the chemical reaction that occurs between the dye and the fiber, so that the dye absorbs easily (Siva, 2007). Deficiencies in color yield and low fastness encourage the search for the ideal mordant, which can increase the ability of textile fibers to absorb natural dyes (Lestari et al., 2020).

There are three types of mordants including metal mordants (metal salts), tannin mordants, and oil mordants (Siva, 2007; Saxena & Raja, 2014). Metal mordants are derivatives of metal salts such as $Al_2(SO_4)_3$ (alum), $SnCl_2$, $KcrO_4$, $CuSO_4$, and $FeSO_4$. In their use heavy metals such as Sn, Cu, and Cr are banned because they are ecologically harmful given that residues are discharged directly into the environment (Adu et al., 2022). Heavy metal compounds can cause cancer in living things and can increase COD (Chemical Oxygen Demand) and BOD (Biological Oxygen Demand) of water so that it can disrupt aquatic ecosystems (Purwati et al., 2023) (Purwati et al., 2023). According to Farida et al. (2015) alum is a relatively environmentally friendly chemical compound, but the aluminum content will accumulate in wastewater and must be treated to meet effluent water quality standards. Therefore, it is important to look for alternative materials that can reduce such use, such as tannin mordants (biomordants).

According to Kurniasari & Maharani (2015) Mordants must be selected from environmentally friendly and harmless materials so as not to cause problems to the environment. Tannin compounds have potential as mordants in natural dyeing processes. Tannins, which function as mordants, can be obtained from plant extracts. The nature of tannins that tend to give color helps strengthen the overall color intensity (Lestari et al., 2020). Tannins contain -OH groups that can form hydrogen bonds with cellulose while -COOH groups on tannins react with dyes (Chakraborty, 2010)

Ferns (*Pteridophyta*) are one of the plants that are easily found in West Kalimantan. Ferns can grow in humid habitats, on the soil surface, or on other plants (epiphytes) (Ulum & Setyati, 2015). Scientific research related to the content of chemical compounds from various species of ferns has been widely reported. Based on phytochemical tests conducted by Swandi & Salmi (2023) the methanol extract of *paku uban* leaves (*Gleichenia linearis*) contains secondary metabolites such as alkaloids, flavonoids, phenolics, tannins, saponins, triterpenoids, and steroids. The results of the phytochemical test of 96% methanol extract of *paku miding* leaves (*Stenochlaena palustris*) contain secondary metabolites including flavonoids, polyphenols, tannins, terpenoids and saponins (Sulasmi et al., 2018). Furthermore, in the research of (Shah et al. (2014) *paku uban* leaf extract (*Nephrolepis biserrata*) in methanol solvent contains anthraquinones, alkaloid flavonoids, tannins, steroids, phytosterols, saponins, and triterpenoids. In the study of Anal & Chase (2016) *paku kawat* (*Lycopodium cernuum*) contain alkaloids. Lai et al. (2010) explained that *paku dayak* leaves (*Blechnum orientale*) contain tannins, flavonoids, and terpenoids. The choice of methanol solvent is because methanol has a small molecular structure that is able to penetrate all plant tissues to draw active compounds out. Methanol can dissolve almost all organic compounds, both polar and nonpolar compounds and is also volatile so that it is easily separated from the extract (Rahayu et al., 2015). Some ferns can be used as natural dyes (Andries et al., 2022). According to Lestariningsih et al. (2021), ferns have leaf dyes that can be used as natural dyes for textile products through the ecoprint technique.

Based on the description above, it is known that some fern extracts contain tannins. However, based on the literature search, no research has been found related to the determination of total tannin content in ferns. This study will determine the total tannin content in five types of ferns, namely *paku resam* (*Gleichenia linearis*), *paku miding* (*Stenochlaena palustris*), *paku uban* (*Nephrolepis biserrata*), *paku dayak* (*Blechnum orientale*), and *paku kawat* (*Lycopodium cernuum*). This study can provide a source of information on the potential of ferns as natural dyes and biomordants that are more environmentally friendly, thus reducing the negative impact of the use of synthetic dyes in the textile industry.

METHOD

Research Desain

The design of this research is laboratory experimental research. This 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 within ± 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 independent variables in this study are *paku resam (Gleichenia linearis*), *paku miding* (*Stenochlaena palustris*), *paku uban* (*Nephrolepis biserrata*), *paku dayak* (*Blechnum orientale*), *paku kawat* (*Lycopodium cernuum*). The dependent variable in this study is the total tannin content. The method used to determine the tannin content of methanol extracts of various species of ferns (Pteridophyta) is UV-Vis spectrophotometry. The instrument used in the determination of tannin content is a double-beam UV-Vis spectrophotometer (*Shimadzu 1900*) which has a double beam so that the measurement of samples and blank solutions can be done simultaneously.

Tools and Materials

The tools used in this research are glassware, analytical balance, blender, measuring flasks of various sizes, micropipette, rotavapor, double beam UV-Vis spectrophotometer (*shimadzu 1900*) and personal computer. The materials used in this study are *paku resam* (*Gleichenia linearis*), *paku miding* (*Stenochlaena palustris*), *paku uban* (*Nephrolepis biserrata*), *paku dayak* (*Blechnum orientale*), and *paku kawat* (*Lycopodium cernuum*). Then the chemicals used are methanol, distilled water, tannic acid, Folin Ciocalteu reagent and Na_2CO_3 15%, $FeCl_3$ 1%, $FeCl_3$ 5%, Lieberman-Burchard reagent, Dragendorf reagent, $Pb(C_2H_3O_2)_2$ 10%.

Research Procedures

Samples Collection

Samples of *paku resam* (*Gleichenia linearis*), *paku miding* (*Stenochlaena palustris*), *paku uban* (*Nephrolepis biserrata*), *paku dayak* (*Blechnum orientale*), and *paku kawat* (*Lycopodium cernuum*) were obtained from Keranji Hamlet, Tanjung Mekar Village, Sambas District, Sambas Regency, West Kalimantan.

Preparation and Extraction of Samples

Sample preparation and extraction with reference to (Fatonah et al., 2021; Mulyani et al., 2022). Cleaned paku resam, paku miding, paku uban, paku dayak, and paku kawat to remove dirt and dust. Then dried by aerating at room temperature. Samples of paku resam, paku miding, paku uban, paku dayak, and paku kawat were blended to obtain powder. The powder of paku resam, paku miding, paku uban, paku dayak, and paku kawat were each weighed as much as 70 grams and macerated with 450 mL methanol solvent for 3 days. Then each extract was filtered and concentrated using a rotavapor at 40°C with a speed of 75 rpm until a thick extract was obtained. The percentage yield of the extract was calculated by the following formula:

$$\%\text{Yield}=\frac{\text{weight of the extract obtained (g)}}{\text{weight of simplisia before extraction (g)}}\times 100\%$$

(Masriani et al., 2023)

Phytochemical screening

Phytochemical screening was carried out to determine the presence of secondary metabolite compounds alkaloids, flavonoids, tannins, phenolic triterpenoids, steroids, and saponins in resam nails, miding nails, graying nails, dayak nails, and wire nails.

a. Screening of Tannin Compounds
A total of 1 mg of sample extract was dissolved with 1 mL of distilled water, then 3 drops of 1% FeCl3 solution were added. The sample is positive for tannin compounds if a green or blue-black solution is formed (Meigaria et al., 2016).
b. Screening of Phenolic Compounds
A total of 1 mg of sample extract was dissolved with 1 mL of distilled water, then 3 drops of 5% FeCl3 solution were added. The sample is positive for phenolic compounds if a strong green or blue solution is formed (Manongko et al., 2020).
c. Screening of Flavonoid Compounds
A total of 1 mg of sample extract was dissolved with 1 mL of distilled water, then a few drops of 10% $Pb(C_2H_3O_2)_2$ solution were added (Wibawa, 2021). The sample is positive for flavonoid compounds if a yellow precipitate is formed.
d. Screening of Alkaloid Compounds
A total of 1 mg of sample extract was dissolved with 1 mL of distilled water, then 1-2 mL of Dragendorf reagent was added. Positive samples contain alkaloids if a reddish-brown precipitate is formed (Shaikh & Patil, 2020).
e. Screening of Triterpenoid and Steroid Compounds
A total of 1 mg of sample extract was dissolved with 1 mL of distilled water, then added 1 mL of chloroform and then added (Lieberman-Burchard reagent) 1 mL of anhydrous acetic acid and then added 2 mL of concentrated H_2SO_4. The sample is declared to contain terpenoid compounds if a brownish or violet ring forms on the border of the two solvents, while the presence of steroids is indicated by the presence of a bluish green color (Reiza et al., 2019).
f. Screening of Saponin Compounds
A total of 25 mg of sample extract was dissolved with 2 mL of distilled water, then shaken for 1 minute. The sample is declared to contain saponin compounds if stable foam is formed (Manongko et al., 2020).

Determination of Tannin Content

a. Preparation of Parent Standard Solution
Tannic acid was weighed as much as 1 mg and then dissolved with distilled water to a volume of 10 ml so that a master solution of 100 ppm was obtained.
b. Preparation of 15% Na_2CO_3 solution
The preparation of 15% Na_2CO_3 solution refers to (Noviyanty et al., 2020). Na_2CO_3 powder was weighed as much as 15 grams and dissolved with 100 mL of distilled water.
c. 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. After 5 minutes the solution was added with 2 mL of 15% Na_2CO_3 solution then shaken and allowed to stand again for 5 minutes. Next, distilled water was added until exactly 10 mL and shaken until homogeneous then, scanning was carried out at a wavelength with a range of λ 400-800 nm.

d. 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. Next, 15% Na_2CO_3 solution was added to the solution as much as 2 mL then, shaken until homogeneous and allowed to stand for 5 minutes. Next, distilled water was added until exactly 10 mL and shaken until homogeneous. Observe the absorbance at λmax with observation time intervals of 0, 2, 4, 6, 8, 10 to 60 minutes.

e. Preparation of Tannic Acid Standard Curve with Folin Ciocalteu Reagent

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

f. Determination of Total Tannin Content of *Paku Resam*, *Paku Miding*, *Paku Uban*, *Paku Dayak*, and *Paku Kawat* Extracts by Uv-Vis Spectrophotometry.

A total of 10 mg of extracts of *paku resam*, *paku miding*, *paku uban*, *paku dayak*, and *paku kawat* were dissolved with 1 mL of distilled water using a microtube. The extract solution was then put into a test tube and added 1 mL of Folin Ciocalteu reagent then shaken and allowed to stand for 5 minutes. In the solution was added 15% Na_2CO_3 solution as much as 2 mL then, shaken homogeneously and allowed to stand for 5 minutes, then added 1 mL distilled water. The sample solution was taken back as much as 1 mL and diluted with distilled water up to 10 mL then, allowed to stand in a stable time range. Absorbance measurements were taken in triplicate using UV-Vis spectrophotometry at the maximum wavelength. The data obtained from this study is the absorbance value calculated by the equation $y = bx + a$

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

RESULTS AND DISCUSSION

Samples Preparation

The plants used as samples in this study are ferns (*Pteridophyta*) including *paku resam* (*Gleichenia linearis*), *paku miding* (*Stenochlaena palustris*), *paku uban* (*Nephrolepis biserrata*), *paku dayak* (*Blechnum orientale*), and *paku kawat* (*Lycopodium cernuum*) obtained in Keranji Hamlet, Tanjung Mekar Village, Sambas District, Sambas Regency, West Kalimantan. Samples of *paku resam* (*Gleichenia linearis*), *paku miding* (*Stenochlaena palustris*), *paku uban* (*Nephrolepis biserrata*), *paku dayak* (*Blechnum orientale*), and *paku kawat* (*Lycopodium cernuum*) were cleaned of dirt and dust and then dried by aerating at room temperature around 25-30°C. Then the sample is pulverized using a blender, this is done so that the sample size becomes smaller so as to increase the surface area of the sample and can help the process of breaking the cell membrane to remove chemical compounds in the sample (Syamsudin et al., 2022).

Samples Extraction

Samples were extracted by maceration method dissolved with methanol solvent as much as 450 mL each. The maceration method is the simplest way to perform extraction. The advantage of maceration lies in the resulting extraction, in addition to the large amount of extraction results can also prevent chemical changes in some compounds caused by heating. In addition, the extraction process using the maceration method is a commonly used method in research, because this method is easy to do and only requires simple equipment (Fatonah et al., 2021). Extraction in this maceration method is carried out to take the compound components from the sample which is carried out for 3 days. The maceration process was carried out for 3 days with the aim of optimizing the extraction of chemical compounds from leaf samples. During the soaking period, the sample is stored in a closed container and protected from direct light exposure, which aims to avoid catalysis reactions triggered by light or discoloration of the sample (Indarto et al., 2019).

The results of maceration extraction of paku resam, paku miding, paku uban, paku dayak, and paku kawat powder with 450 mL of methanol obtained dark green aqueous methanol extract. Each sample extract was filtered using filter paper, then the aqueous methanol extract sample was rotavaporized at 40°C with a speed of 75 rpm and obtained a dark green thick extract. The yield results obtained can be seen in Table 1 below.

Table 1. Yields of methanol extracts of five fern species

No	Name of Ferns	Samples Weight (g)	Weight of Extract (g)	Yield (%)
1	*Paku kawat*	70	7,890	11,271
2	*Paku resam*	70	5,872	8,389
3	*Paku dayak*	70	3,367	4,810
4	*Paku uban*	70	2,531	3,616
5	*Paku miding*	70	0,598	0,854

Based on Table 1, the yield of methanol extract of *paku kawat* obtained the highest yield value, while the yield of methanol extract of *paku miding* obtained the lowest yield value from the yield of methanol extracts of the four ferns. According to Nahor et al. (2020), the higher the yield value, indicating that the amount of extract obtained is more and more.

Phytochemical screening

Before testing tannin levels, secondary metabolite compounds were first tested. Preliminary tests or phytochemical screening on sample extracts are carried out to identify the presence of secondary metabolite compounds or strengthen the assumption that the extracts of *paku resam*, *paku miding*, *paku uban*, *paku dayak*, and *paku kawat* contain secondary metabolite compounds to be studied. The compound groups in the five fern extracts were analyzed using various types of reagents. The compound groups analyzed were tannins, phenolics, flavonoids, alkaloids, triterpenoids, saponins and steroids.

The screening results of tannin compounds show that the five species of ferns contain tannin compounds, which are indicated by the formation of a blackish green solution. Giving $FeCl_3$ 1% into the extract will result in the formation of a complex with one of the hydroxyl groups on the tannin. This causes the formation of a blue-black color on hydrolyzed tannins, and blackish green on condensed tannins (Prananda et al., 2015). The reaction that occurs between tannin and $FeCl_3$ can be seen in Figure 1 below.

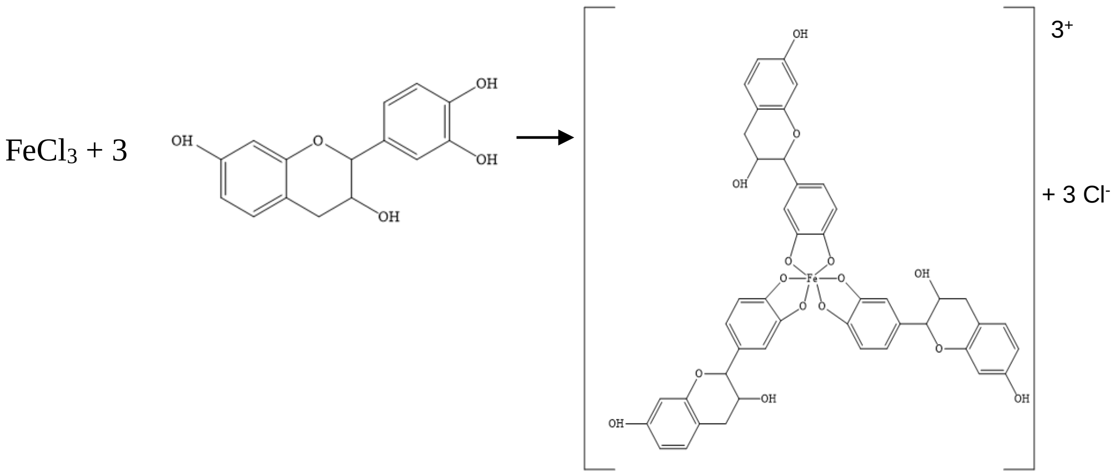

Figure 1. Reaction of $FeCl_3$ with tannin compounds

Identification of phenolic compounds is done using 5% $FeCl_3$ solution. Screening results of phenolic compounds showed that the five species of ferns contain phenolic compounds characterized by the occurrence of changes in the solution of green-black color. Phenolic reacts with $FeCl_3$ to form a concentrated red, purple, blue or black color because $FeCl_3$ reacts with aromatic -OH groups. The colored complex formed is thought to be iron (III) hexaphenolate. Fe^{3+} ions hybridize d^2sp^3 orbitals so that Fe^{3+} ions ($4s^0 3d^5$) have 6 empty orbitals filled by electron pair donors, namely oxygen atoms in phenolic compounds that have free electron pairs (Haryati et al., 2015). The phenolic reaction with $FeCl_3$ can be seen in Figure 2 below.

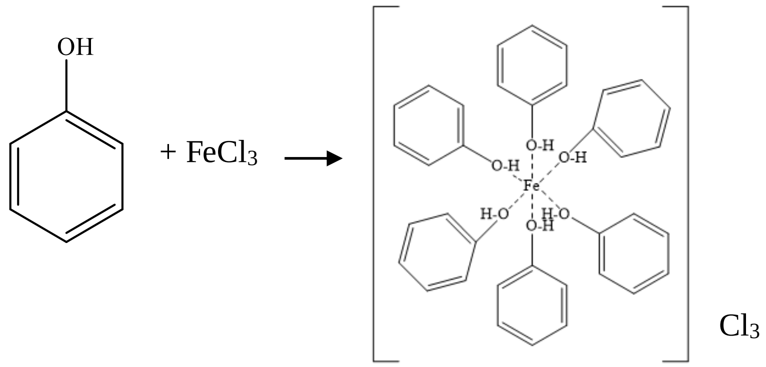

Figure 2. Reaction between phenol compounds with FeCl3

Screening of flavonoid compounds was carried out by reacting the extract with ($Pb(C_2H_3O_2)_2$) 10% solution. The screening results of flavonoid compounds show that the five species of ferns contain flavonoid compounds characterized by the presence of a yellow precipitate in the solution (Shaikh & Patil, 2020). This is due to the complex formation between ($Pb(C_2H_3O_2)_2$) 10% and flavonoid compounds (Sari et al., 2019).

Screening of alkaloid compounds was carried out using Dragendorf reagent. The screening results of alkaloid compounds showed that the five species of ferns contained alkaloid compounds characterized by brownish red color changes. Alkaloids react with Dragendorff reagent (potassium tetraiodobismutat) to produce an orange to reddish brown precipitate. In this reaction there is a ligand replacement where nitrogen which has a free electron pair on the alkaloid forms a coordinate covalent bond with K^+ ions of potassium tetraiodobismutat produces a potassium-alkaloid complex that precipitates (Haryati et al., 2015). The reaction of alkaloids with Dragendorf reagent can be seen in Figure 3 below.

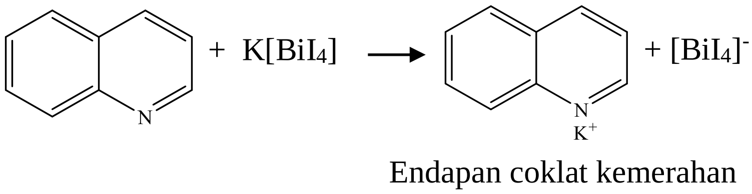

Figure 3. Reaksi identifikasi alkaloid oleh pereaksi Dragendorff

The screening results of saponin compounds showed positive results on *paku resam, paku uban*, *paku miding,* and *paku dayak* characterized by the formation of foam that lasted for 15 minutes, while on *paku kawat* showed negative results. In screening saponin compounds using the forth method, the onset of foam in the Forth test indicates the presence of glycosides that have the ability to form foam in water that is hydrolyzed into glucose and other compounds (Marliana et al., 2005). The glycoside bond component contained in saponins causes this compound to tend to be polar (Sulistyarini et al., 2020). The foam formation reaction in the saponin test is shown in Figure 4 below.

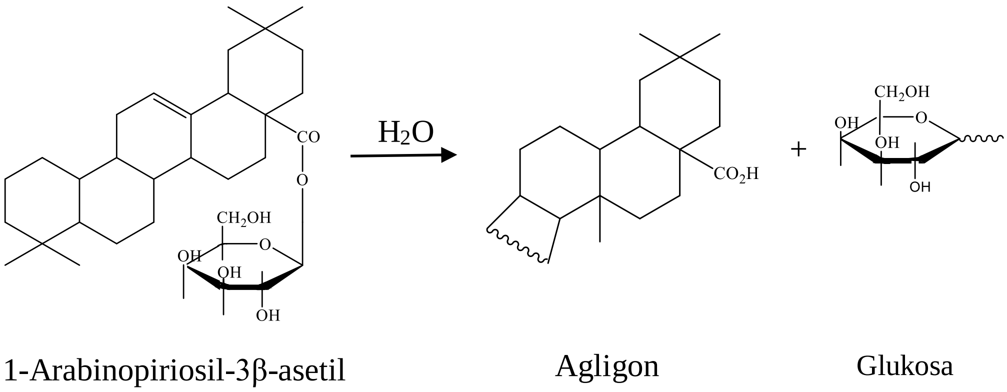

Figure 4. Hydrolysis reaction of saponins in water

In screening steroid and triterpenoid compounds using Liebermann-Bourchart reagent. Screening results showed that the five species of ferns contained steroid compounds characterized by green-blue while for triterpenoid compounds from the five species of ferns showed negative results. The extract dissolved in chloroform then added Liebermann-Bouchard reagent (anhydrous acetic acid-H_2SO_4) showed positive results with a color change to brownish red for steroids and brown-purple for triterpenoids. The reaction of triterpenoids with Liebermann reagent produces a red-purple color while steroids give a green-blue color. This is based on the ability of triterpenoid and steroid compounds to form colors by H_2SO_4 in acetic acid anhydride solvent. The difference in color produced by triterpenoids and steroids is due to differences in groups at the C-4 atom (Habibi et al., 2018). The chemical reactions that occur as in Figure 5 below.

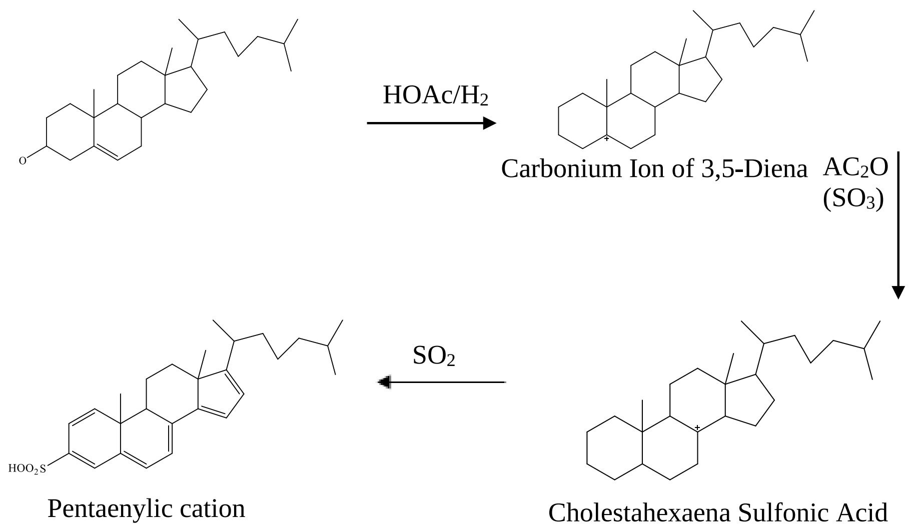

Figure 5. Steroid and terpenoid test reaction mechanism

The screening results of secondary metabolite compounds from five fern species can be seen in Table 2 below.

Table 2. Screening results of secondary metabolite compounds from five fern species

No	Name of Ferns	Screening Results						
		TAN	PHE	FLA	ALK	TRI	SAP	STE
1	*Paku kawat*	+	+	+	+	-	-	+
2	*Paku resam*	+	+	+	+	-	+	+
3	*Paku dayak*	+	+	+	+	-	+	+
4	*Paku uban*	+	+	+	+	-	+	+
5	*Paku miding*	+	+	+	+	-	+	+

Description: TAN: Tannin, Phe: Phenolic, FLA: Flavonoid, ALK: Alkaloid, TRI: Triterpenoid, SAP: Saponin, STE: Steroid, (+): Contains secondary metabolites, (-): Contains no secondary metabolites

Determination of Tannin Content

Determination of tannin content was carried out by UV-Vis spectrophotometric method using Folin Ciocalteu reagent. The formation reaction that occurs is oxidation reduction where tannin as a reductant and Folin Ciocalteu as an oxidizer. The oxidation results will form a blue color that can be read at the maximum wavelength. The principle of the Folin Ciocalteu method is the formation of a blue complex compound that can be measured at a wavelength of 755.8 nm. Folin Ciocalteu reagent oxidizes phenolics (alkali salts) or phenolic-hydroxy groups reduce heteropoly acids (Phosphomolybdate-phosphotungstate) contained in Folin Ciocalteu reagent into a Molybdenumtungsten complex (Listiana et al., 2022).

In determining tannin content, first determine the maximum wavelength. Determination of the maximum wavelength aims to determine the wavelength required for tannic acid solution to reach maximum absorption (Pratama et al., 2019). The maximum wavelength is characterized by the wavelength that can produce the highest absorption (Fatonah et al., 2021). Based on the results of the study, the maximum wavelength of tannic acid obtained with a UV-Vis spectrophotometer in the range of λ 400-800 nm is 755.8 nm. The maximum wavelength curve can be seen in Figure 6 below.

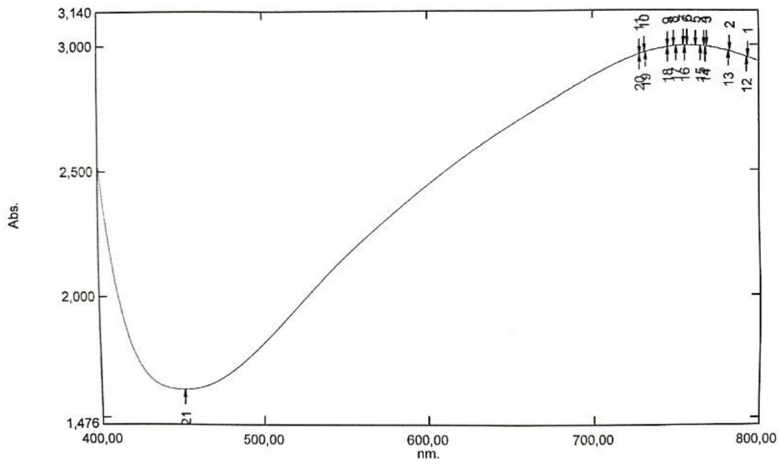

Figure 6. Tannic acid maximum wavelength curve

Determination of stable time (operating time) to determine the length of time required for tannic acid standard solution to reach constant absorption (Andriyani et al., 2010). The stable time was obtained at 47 minutes, so absorbance measurements were taken at 47 minutes.

The next test, making a curve of tannic acid standard solution with Folin Ciocalteu reagent. In determining the tannin content of some fern extracts, it is necessary to measure the absorbance value of tannic acid comparison solution with varying concentrations. Making a standard curve of tannic acid aims to determine the relationship between the concentration of tannic acid and absorbance (Andriyani et al., 2010). Standard solution was made with various concentrations, namely 10; 5; 2.5; 1.25 and 0.625 ppm. Based on the tannic acid calibration curve in Figure 7, a linear regression equation y = 0.0506x + 0.015 was obtained with an average coefficient of determination (R^2) 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 0.32% is influenced by other factors such as temperature, light, chemicals and others. From the data above, it shows a correlation relationship between concentration and absorbance. The r value is close to 1 which proves that the regression equation is close to linear. The tannic acid calibration curve can be seen in Figure 7 below.

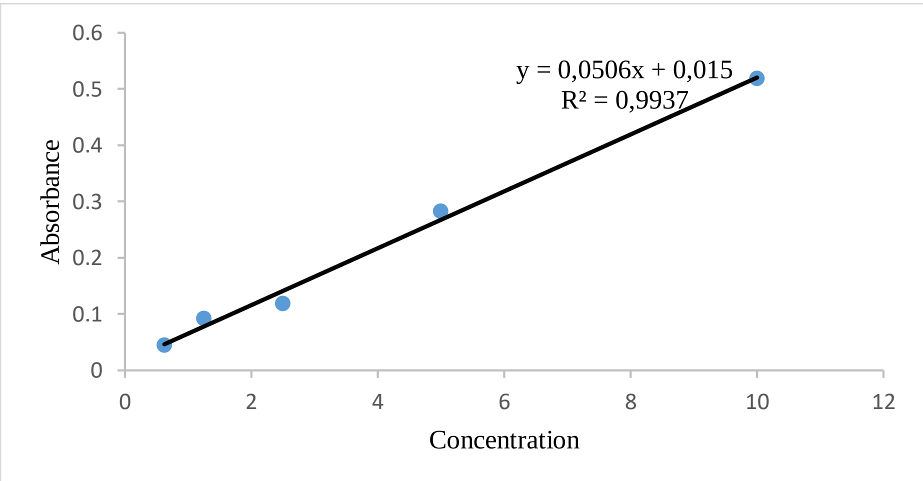

Figure 7. Tannic acid calibration curve

Determination of total tannin content of methanol extracts of *paku resam*, *paku miding*, *paku uban*, *paku dayak*, and *paku kawat* was repeated 3 times. The results of the measurement of tannin content with UV-Vis spectrophotometric method on *paku kawat*, *paku resam*, *paku dayak*, *paku uban* and *paku miding* are 0.024 ± 0.002%; 0.256% ± 0.029; 0.272 ± 0.037; 0.143% ± 0.019; and 0.022 ± 0.012, respectively. The results of total tannin content show that *paku dayak* (*Blechnum orientale*) obtained the highest tannin content while *paku kawat* (*Lycopodium cernuum*) obtained the lowest tannin content value. The table of the results of the determination of total tannin content of five species of ferns can be seen in Table 3 below.

Table. 3 Determination results of total tannin content of five fern species

No	Name of Ferns	Tannin Content (%b/b TAE)
1	*Paku kawat*	0,024%±0,002
2	*Paku resam*	0,256%±0,029
3	*Paku dayak*	0,272%±0,037
4	*Paku uban*	0,143%±0,019
5	*Paku miding*	0,022%±0,012

The results of one-way ANOVA analysis showed a significance value of 0.000, which means that the significance value <0.05, so it can be concluded that the difference in ferns species causes differences in tannin content.

CONCLUSION

This research has an important impact on environmental safety in developing fern extracts as natural dyes and biomordants to reduce dependence on synthetic dyes and chemical mordants in the textile industry. Based on the results of the research, the yield of methanol extracts on *paku kawat*, *paku resam*, *paku dayak*, *paku uban*, and *paku miding* are 11.271%; 8.389%; 4.810%; 3.615%; and 0.854%, respectively. The results of total tannin content in methanol extracts using the Tannic Acid Equivalent (TAE) standard on *paku kawat*, *paku resam*, *paku dayak*, *paku uban*, and *paku miding* per 10 mg of extract are 0.024% ± 0.002; 0.256% ± 0.029; 0.272% ± 0.037; 0.143% ± 0.019; and 0.022% ± 0.012, respectively. Based on these data, it can be concluded that the methanol extract of *paku kawat* produces the highest yield while *paku dayak* produce the highest total tannin content. The results indicate that differences in fern species cause differences in tannin levels.

RECOMMENDATIONS

This research only focuses on determining the total tannin content of various ferns species as an exploration of biomordants. To develop the potential of ferns in the textile industry, further research is needed on the range of tannin levels that can be used as biomordants in fabric dyeing.

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