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Determination of Tannin Content in Matoa Plants (*Pometia pinnata*) Based on Variations in Leaf Age

Uci Ulandari, Masriani*, Risya Sasri, Ersando
Department of Chemistry, Faculty of Mathematics and Natural Science, Tanjungpura University Pontianak, Indonesia
* Corresponding Author e-mail: masriani@fkip.untan.ac.id

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Abstract
The growth of the textile industry contributes significantly to environmental pollution from dye waste. The use of natural dyes from plants could be a solution to this problem, although they have weaknesses such as easy fading and less stable colors. Therefore, to improve color stability, a mordant is needed as a color fixative. Biomordants are mordants derived from plants that can be used as substitutes for metal mordants to bind dye to fabric fibers. Matoa leaves are one of the plants with the potential to be used as a biomordant because they contain tannin compounds. This study aims to determine the tannin content of methanol extracts of matoa leaf with various leaf age variations that can be used as a biomordant. The old and young matoa leaves were macerated with methanol for 3 days. The tannin content was determined using the Folin Ciocalteu method using a spectrophotometer Ultra Violet (UV) dual beam with positive control of tannic acid at a wavelength of 755.8 nm. The yield of methanol extract from old matoa leaves was 5.42% and from young matoa leaves was 8.58%. Phytochemical screening of methanol extract of old and young matoa leaves revealed the presence of secondary metabolite content of tannins, phenolics, flavonoids, saponins, triterpenoids, and alkaloids. The total tannin content of methanol extract from old matoa leaves was 0.37% ± 0.03, lower than that of young matoa leaves, which was 0.44% ± 0.06. Based on this research, it can be concluded that matoa leaves contain tannins and have the potential to be used as a biomordant.

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INTRODUCTION

Based on the data from the Central Statistics Agency (BPS) in the first quarter of 2019, the growth of the textile and clothing industry reached 18.98%. Textile significant contribution to water pollution (Guntama *et al*., 2023). Disposal of textile industrial wastewater into the environment results in water pollution. Textile waste contains colorants and hazardous heavy metals (Alfian Maulana *et al*., 2017). The characteristics of textile waste include strong coloration even though the low concentration can inhibit light from entering thewater, is toxic and carcinogenic (Yanto *et al*., 2023).

One way to reduce textile waste is by using natural dyes from plants (Enrico, 2019). These natural dyes have the advantage of being non-toxic, renewable, easily degradable, and environmentally friendly (Permatasari & Lestari, 2023). However, the weakness of natural dyes is their tendency to fade easily, less color intensity, and instability to heat, sunlight, and pH (Pujilestari, 2015). To overcome the weaknesses in the process of dyeing fabric using natural dyes, mordants are used. Mordant is a binding agent used to perfectly bind the color of the fiber to the cloth to prevent it from fading easily. Mordant functions as a binder between natural dye and the fabric fiber, thus increasing the color fastness and producing better colors (Munawaroh *et al*., 2022). Generally, there are three types of mordants: oil mordants, metal salt mordants, and tannin mordants. Tannin mordants, also known as biomordants, are obtained through the extraction process of plant parts. Biomordants are solutions used as color binders from more natural and safe materials (Gumulya, 2021). Biomordants are produced through plants containing tannin, which affects dyeing (Aji Priambudi *et al*., 2020). Matoa (*Pometia pinnata*) is one of the plants with potential as a biomordant. The secondary metabolite content in matoa leaf extract contains flavonoids, phenolics, saponins, tannins, and steroids (Hajar *et al*., 2021). Tanin is a very complex organic compound consisting of phenolic compounds found in various plants such as the bark, stems, fruits, and leaves. Matoa leaves are one part of the plant that has bioactivity. Matoa leaves consist of two parts, namely old and young. The old matoa leaves are dark green in color, with a size of 20-30 cm located in the fifth row from the stem, while the young matoa leaves are colored from red to light green, with a size of 8-15 cm located at the top of the first row downwards (Tehuayo & Ulfa, 2023).


Figure 1. Matoa leaf (*Pometia pinnata*)
Source : Personal Documentation

Based on the phytochemical screening on the ethanol extract of matoa leaves, it indicates the presence of tannins, alkaloids, coumarins, and flavonoids (Kuspradini *et al*., 2016). According to the research by Suparyanto and Rosad (2021) on the phytochemical analysis of ethanol extract with various concentrations of 50% ethanol, 70% ethanol, and 96% ethanol, matoa leaves contain tannins. Based on the study by Kharismawati *et al*. (2009) regarding the tannin content of other plants, it is found that the older the leaves, the higher the tannin content. However, up to this point, there is no literature regarding determining the total tannin content in matoa leaves of different ages. This serves as an exploration of the biomordant form of the plant. The purpose of this research is to determine the effect of age difference in Matoa leaves the total tannin content and as an alternative to the use of metal mordants which cause environmental pollution.

METHOD

Research Design

This study is an experimental laboratory with post-test only control group design conducted with the aim of comparing the age difference of matoa leaves. This study was carried out at the Chemistry Laboratory 2 of the Faculty of Teacher Training and Education, Tanjungpura

University, Pontianak for 4 months.

Tools and Materials

The equipment used in this study includes an analytical balance, blender, volumetric flask (*Pyrex*) 10 mL, micropipette, rotary evaporator, glassware, spectrophotometer Ultra Violet (UV) dual beam, and a computer. The materials used in this study include mature matoa leaves and young matoa leaves, methanol, distilled water, 15% Na_2CO_3, tannic acid, Folin-Ciocalteu reagent, $FeCl_3$, Liebermann-Burchard reagent, HCl, NaOH, Dragendroff reagent, and chloroform, H_2SO_4.

Procedure

Collection and Extraction of Matoa Leaf Samples (*Pometia pinnata*)

Samples of old and young Matoa (*Pometia pinnata*) leaves were obtained from Dusun Sidomulyo, Limbung Village, Sungai Raya Subdistrict, Kubu Raya Regency, West Kalimantan. A total of 50 grams of dried powder of old and young Matoa leaves was macerated with 250 mL of distilled methanol for 72 hours. Subsequently, each extract was filtered and concentrated using a rotavapor at a temperature of 40°C with a speed of 75 rpm until the final concentrated extract was obtained. The percentage yield of the obtained extract was calculated using the formula.

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

Phytochemical Screening of Methanol Extracts

Phytochemical analyses were conducted to each extract using specific reagents according to (Masriani et al., 2023)

Tannin Screening

Matoa leaf extract is dissolved in aquadest then heated and then added FeCl3 1% solution. The sample tested positive for tannin compounds if a brownish-green or bluish-green color was formed **Saponins Screening**

Matoa leaf extract is added in a test tube added 1 mL of aquadest and shaken vigorously for 10 minutes until foam forms. Matoa leaves are said to contain positive saponins formed if foam with a height of 1-10 cm that lasts for 15 minutes

Phenolic Screening

A total of 1mL of ethanol extract of matoa leaves was reacted with $FeCl_3$ 1%. Samples containing phenol are characterized by the formation of blackish-green to solid black.

Flavonoid Screening

Each 10 mg of condensed ethanol extract of matoa leaves was dissolved with 1 mL of 96% ethanol. The solution is then added magnesium powder and two drops of HCl 2 N. Matoa leaves are declared to contain flavonoids when an orange solution is formed.

Alkaloid Screening

The alkaloid test was carried out by means of a thick extract of matoa leaves dissolved with 1 mL of 96% ethanol then added HCl 2N. The solution is divided into two, tested with Mayer's reagent and Wagner's reagent, respectively. Positive samples contain alkaloids if Mayer's reagent shows a yellow color change accompanied by a white precipitate and with Wagner's reagent to an orange- red color accompanied by the formation of an orange to brownish-yellow precipitate.

Triterpenoid Screening

A total of 1 mL of matoa leaf ethanol extract was added with 2 mL of chloroform and three drops of H_2SO_4 concentrated. Matoa leaves are stated to contain triterpenoid compounds when a brownish- red color is formed between surfaces

Steroid Screening

The sample solution was taken as much as 1 mL and then added Lieberman-Burchard reagent as much as 3 drops. The sample is positive if a green-blue color is formed.

Determination of Tannin Content
Preparation of Tannic Acid Standard Solution

mg tannic acid was dissolved into 10 mL aquadest to give concentration of 100 μg/mL then stored in room temperature.

Determination of Maximum Wavelength (λ_{max})

The standard solution of tannic acid was taken as much as 2 mL and put into a 10 mL volumetric flask.10 mL and then added 1 mL of folin ciocalteu reagent. Then shaken and allowed to stand again for 5 minutes. Furthermore, 2 mL of 15% Na_2CO_3 solution was added to the solution, shaken and allowed to stand again for 5 minutes. Next, distilled water was added until the limit mark and shaken until the solution was homogeneous. Then scanning was carried out at a wavelength of 400-800 nm.

Determination of Operating Time

The standard solution of tannic acid was taken as much as 2 mL and put into a 10 mL volumetric flask and then added 1 mL of folin ciocalteu reagent then shaken and allowed to stand for 5 minutes. Next, 2 mL of 15% Na_2CO_3 solution was added to the solution, shaken and allowed to stand again for 5 minutes. Next, distilled water was added until the limit mark and shaken until the solution was homogeneous. Then the absorbance was observed at (λ_{max}) with observation time intervals of 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 to 100minutes.

Preparation of Tannic Acid Calibration Curve with Folin Ciocalteu Reagent

100 m tannic acid standard solution was taken as much as 2 mL and put into a 10 mL volumetric flask to get several concentrations of 10 ppm, 5 ppm, 2.5 ppm, 1.25 ppm and 0.625 ppm. Then, each into a measuring flask added 1 mL of reagent folin ciocalteu then shaken and allowed to stand for 5 minutes. Next, 2 mL of 15% Na_2CO_3 solution was added to the solution, shaken and allowed to stand in the stable time range obtained. Observe the measured absorbance value.

Determination of Total Tannin Content of Old and Young Matoa (Pometia pinnata) Leaf Extracts with Uv-Vis Spectrophotometer

A total of 10 mg of old and young matoa 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 1 mL of folin ciocalteu reagent was added. Then, shaken and allowed to stand for 5 minutes. In the solution was added 15% Na_2CO_3 as much as 2 mL then shaken and allowed to stand for 5 minutes, then added 1 mL distilled water. The sample solution was taken again as much as 1 mL and diluted with distilled water to 10 mL and then allowed to stand in a stable time range. Absorbance measurements were carried out in triplicate using UV-Vis spectrophotometry at the maximum wavelength. The data obtained from this research is the absorbance value calculated by equation

$$y = bx + a.$$

Data Analysis

Data are presented as mean ± SD from three replication . The analysis began with a normality test if the data was normally distributed (*p> 0.05*) then the data continued with the Independent T- test. The difference in tannin levels in old and young matoa leaves is declared significant if (*sig. 2-tailed > 0.05*) then there is no significant difference between old and young leaves.

RESULTS AND DISCUSSION

Sample Extraction

The dried of old and young matoa leaves (50 grams) was extracted for 72 h using methanol and followed by evaporating using rotary vacuum evaporator to methanol extracts of old matoa leaf (2,71g) and young matoa leaf (4,29g).These results showed that the highest content of

secondary metabolites was in methanol extract of young matoa leaf (Table 1). In this study, methanol was chosen as solvent to extraction of matoa leaves because it is universal solvent which can dissolve all secondary metabolits found in matoa leaves, both polar and non-polar. Masriani et al (2023) have tested the effect of solvents on the tannin content of methanol, ethanol and ethyl acetate extracts of *cengkodok* leaves and found that the methanol extract had the highest tannin content. There is a dependence of the tannin content in cengkodok leaves on the polarity of the solvent. The more polar the solvent used, the higher the tannin content in cengkodok leaves. Tannin is a polar compound so it is more soluble in polar solvents. The maceration process lasts for 72 hours to ensure that the bioactive compounds in the leaves can be dissolved optimally in methanol solvent.

Extraction by maceration was chosen in this study because maceration extraction is very favorable for isolating active compounds from natural materials because besides being cheap, the process is also simple. This maceration extraction does not require heating, so the risk of damage to secondary metabolites in plants is minimal (Susanty & Bachmid, 2016). The principle of maceration is to soak the simplisia powder in a solvent at room temperature and protected from exposure to sunlight (Tantrayana & Zubaidah, 2015).

After the maceration process is complete, the solution containing matoa leaf extract is filtered to separate the filtrate and residue. The filtrate was then further processed using a rotavapor at 40°C with a speed of 75 rpm to evaporate the methanol solvent, so that a concentrated extract was obtained. The use of rotavapor in this condition aims to maintain the stability of active compounds that are susceptible to high temperatures.

Table 1. Rendement results methanol extracts of matoa (*Pometia pinnata*) leaves

Methanol extracts	**Weight**	**Rendement (%)**
Old matoa leaf	2.71	5.42
Young matoa leaf	4.29	8.58

Initial weight of matoa (*Pometia pinnata*) leaves = 50 gram

Phytochemical screening towards each extract were carried out using specific reagents. Based on phytochemical analysis, methanol extracts of old and young matoa leaves showed the presence of tannins, phenolics, flavonoids, saponins, triterpenoids, and alkaloids. Previously, the ethanol extract of matoa leaf indicated presence of flavonoid dan tannin (Martiningsih et al., 2016).

Table 2. Phytochemical screening result for methanol extracts of old and young matoa leaves using specific reagents

secondary metabolites	old matoa leaf	young matoa leaf
Tannin	+	+
Saponin	+	+
Phenolic	+	+
Flavonoid	+	+
Triterpenoid	+	+
Alkaloid	+	+
Steroid	-	-

Tannins are natural compounds that have phenol hydroxy groups that form stable bonds. Tannins are known to function as natural antioxidants, metal binders and free radical catchers in the body (Noer *et al*., 2020). However, tannins are also commonly found in plant parts such as leaves, fruit, skin and stems which act as biomordants (Listiana *et al*., 2022). The results of phytochemical screening of methanol extracts of old and young matoa leaves showed positive results marked by blue-black discoloration. The reaction of tannin compounds with $FeCl_3$ occurs as shown in Figure 2.

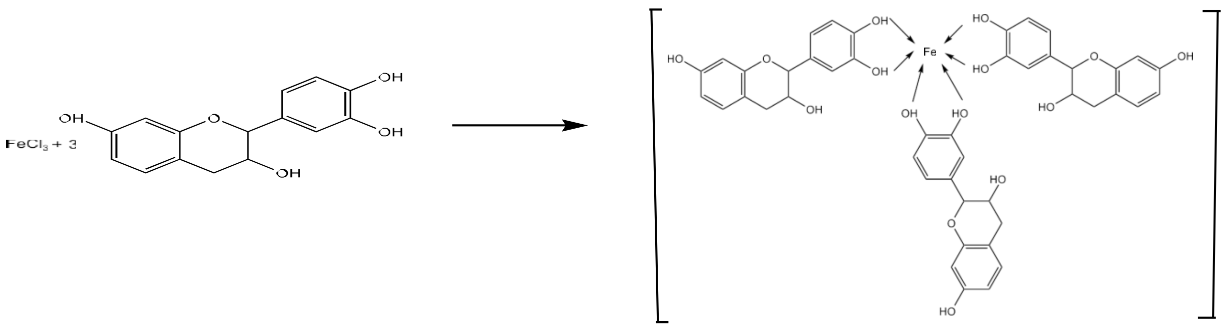

Figure. 2. Reaction of tannin compounds with $FeCl_3$ (Rossalinda *et al*., 2021)

Saponins are glycoside compounds that have soap-like properties when dissolved in water. Saponins are complex glycoside compounds with high molecular weight produced mainly by low-level marine animal plants and some bacteria. The results of phytochemical screening of methanol extracts of old and young matoa leaves showed positive results with the formation of foam when after the mixture was diluted with water.the formation of foam at the time after the mixture is shaken. The reaction between saponins and water presented in Figure 3.

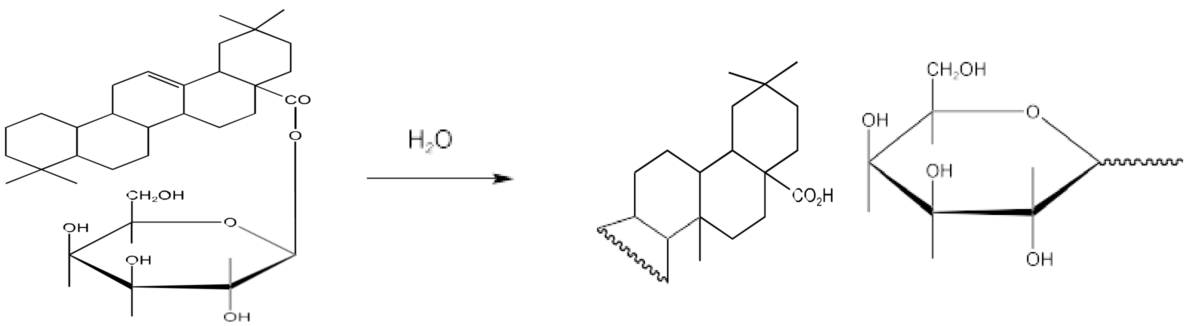

Figure 3. Reaction of Saponins with water (Setiabudi & Tukiran, 2017)

Phenolic compounds are a large group of organic compounds known for their antioxidant activity. Phenolic compounds are aromatic compounds and the presence of one or more hydroxyl groups (OH), OH groups in the structure of phenolic compounds donate H atoms as free radical donors, making phenolic compounds have various benefits such as antidiabetic, anti- inflammatory, anticancer, antimicrobial and immunoregulation (Mahardani & Yuanita, 2021). The screening results of methanol extracts of old and young matoa leaves showed positive results for phenolics with a color change to blackish green in the sample. The reaction between phenolic with $FeCl_3$ can be seen in Figure 4.

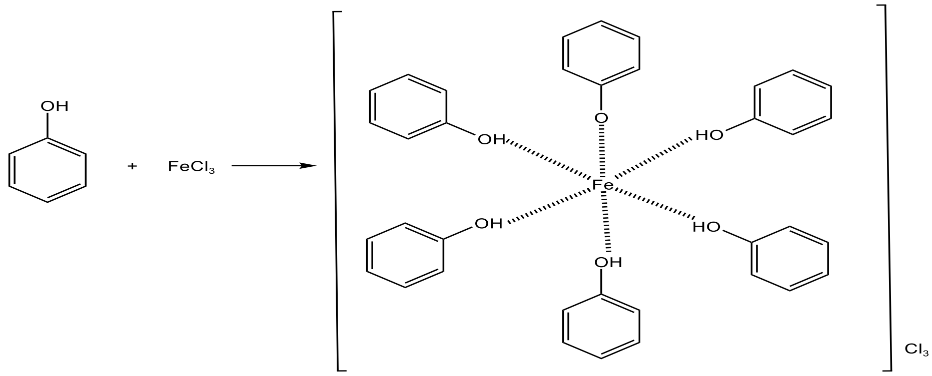

Figure 4. Reaction Phenol with $FeCl_3$ (Ramayani *et al*., 2021)

Flavonoids are secondary metabolite compounds included in the group of phenol compounds whose benzene structure is substituted with OH groups (Susila Ningsih *et al*., 2023). Organic compounds contained in plants function to capture free radicals. Flavonoids have a basic carbon skeleton consisting of 15 carbon atoms in which two benzene rings (C6) are bound by a propane chain (C3) (Noer *et al*., 2020). Flavonoids are known to affect antioxidants in the human body and have several biological activities such as antibacterial, antiviral, anti- inflammatory, antiallergic and anticancer (Ahriani, 2021). The results of phytochemical screening of methanol extracts of old and young matoa leaves showed positive flavonoids indicated by a color change to orange-reddish in the sample. The reaction flavonoids and NaOH reagent presented in Figure 5.

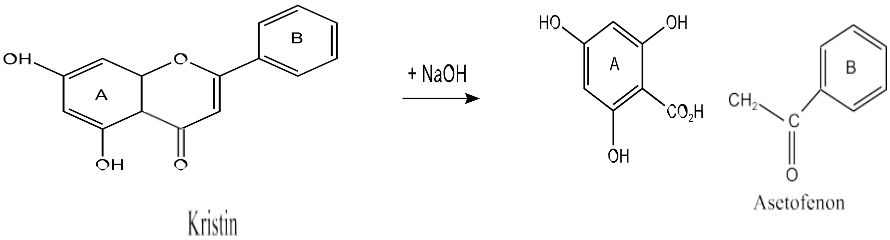

Figure 5. Flavonoid reaction with NaOH reagent (Suharyanto & Prima, 2020)

Alkaloids are known to have various biological activities such as antimicrobials (Kuspradini *et al*., 2016). The results of phytochemical screening of methanol extracts of old and young matoa leaves with Dragendroff reagent showed the presence of alkaloid content in matoa leaves which was characterized by the formation of an orange precipitate. The reaction between alkaloids and Dragendroff reagent can be seen in Figure 6

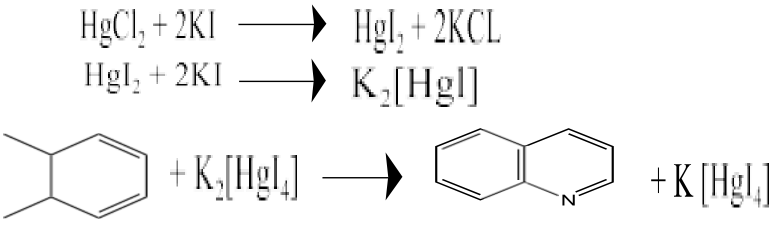

Figure 6. Alkaloid reaction using Dragendroff (Dewi, 2020)

Triterpenoids are terpenoid-derived secondary metabolite compounds whose carbon skeleton is derived from six isoprene units (2-methylbuta-1,3-diene), namely the carbon skeleton built by six C5 units and derived from acyclic C30 hydrocarbons, namely skualena (Balafif *et al*., 2013). Based on research from Nassar *et al*., (2010) stated that triterpenoids have biological compound activities such as antiviral, antibacterial, anti-inflammatory as inhibition of cholesterol synthesis and as anticancer. Test triterpenoid compounds using Liebermann- Burchard reagent. Positive results in this test are characterized by a change in color to reddish brown. The results of phytochemical screening on methanol extracts of old and young matoa leaves showed positive results can be seen in Figure 7.

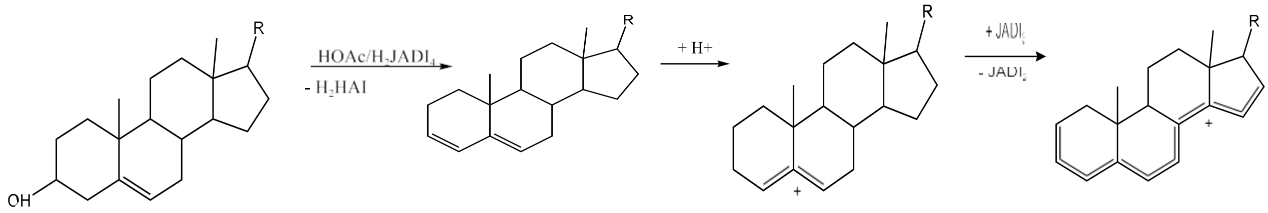

Figure 7. Triterpenoid reaction using Liebermann-Burchard (Zaini & Shofia,2020)

Steroids are terpenoid lipids known for their four-ring carbon skeleton, the structure of the compounds is quite diverse. The difference is due to the presence of oxidized functional groups bound to the ring and the oxidation of the carbon ring. Steroids are known to play an important role for the body in maintaining salt balance, controlling metabolism and improving organ function and other differences in biological functions (Mustofa, 2017). The reaction of steroids with Liebermann- Burchard reagent produces a green-blue color. In the phytochemical test using Liebermann-Burchard reagent, the color change to bright green occurs due to oxidation reactions in terpenoid/steroid groups that form conjugated double bonds (pentaenilic compounds). The results of phytochemical screening on methanol extracts of old and young matoa leaves showed negative steroids. Because there is no change in the sample.

Determination of Tannin Content in Old and Young Matoa (*Pometia pinnata*) Leaves

Based on phytochemical screening, it was found that methanol extracts of old and young matoa leaves contained tannin compounds characterized by the formation of a blue-black color when reacted with $FeCl_3$ reagent. Determination of tannin content was carried out using a Uv-Vis spectrophotometer with folin cioclalteu reagent and Na_2CO_3 as a base atmosphere giver. The positive control used in the determination of tannin levels is tannic acid because tannic acid is a hydrolyzed tannin so it can be used as a positive control when measuring tannin levels.

The maximum wavelength and operating time used in this study refer to the research of Masriani *et al.* (2023) with the maximum wavelength of tannic acid at 755.8 nm and the operating time for measuring tannic acid is at the 47th minute. The maximum wavelength is the wavelength at which the maximum absorption reads the absorption of tannic acid standard solution. The purpose of knowing the maximum wavelength is to know the amount of wavelength needed to achieve maximum absorption (Nofita & Dewangga, 2022). The maximum wavelength of tannic acid at 755.8 nm shows the optimal absorbance peak for this compound.

Based on the calibration curve (Fig. 8), 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 99.68%. The value of r which is close to 1 proves that the regression equation (Nofita & Dewangga, 2022). In determining the tannin content of the methanol extract of matoa leaves, it is necessary to measure the absorbance value of the tannic acid comparison solution with varying concentrations. The goal is to know the relationship between tannic acid concentration and absorbance (Andriyani *et al*., 2010).

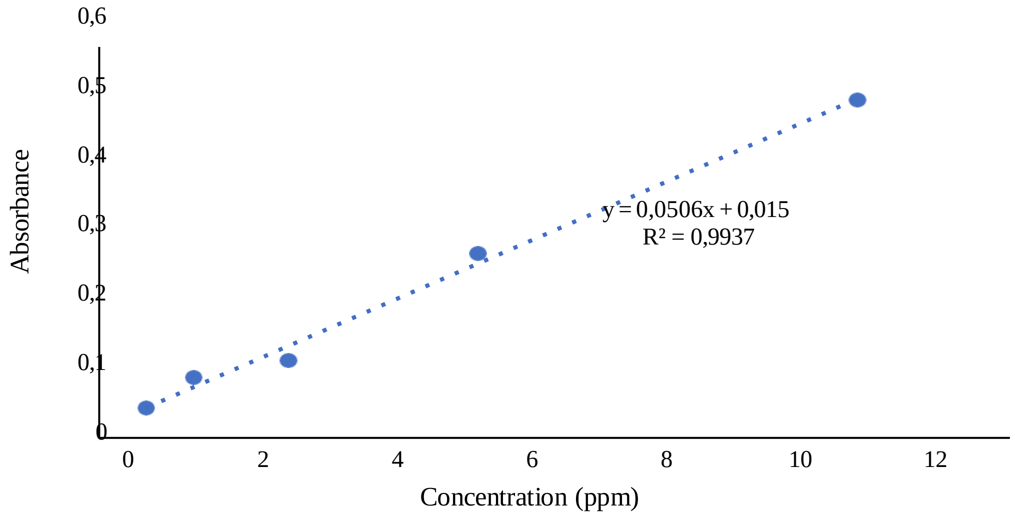

Figure 8. Tannic Acid Calibration Curve

The results of determining the tannin content of methanol extracts of old and young matoa leaves found that the tannin content of young matoa leaves was higher at 0.44% ± 0.06 compared to old matoa leaves 0.37% ± 0.03. This difference is caused by variations in compound content influenced by growth factors and leaf maturity where leaf age affects the antioxidant properties and types of polyphenols in plants (Fatimah, 2022). The results of research from Amanto *et al*., (2020) also stated that tannin levels in tin leaf herbal tea derived from young leaves were higher than tannin levels in tin leaf herbal tea derived from old leaves. In addition, research by Izzreen & Fadzelly, (2013) stated that polyphenol levels in young tea leaves are higher than levels in old tea leaves. According to (Procházková *et al*., 2009) old leaves, the content of secondary metabolites will decrease due to the degradation of metabolite content as a result of the leaf aging process. Therefore, in Fatimah's research, (2022) showed that the content of secondary metabolites in young leaves is more than old leaves where as many as 23 compounds are found in young leaves in keji plants while in old leaves there are only 18 compounds.

Research conducted by Hasan *et al*., (2017) states that luteolin levels in old leaves (lower leaves) the longer harvested luteolin levels in the leaves decreases this occurs because the content of secondary metabolites in the leaves is influenced by differences in age and plant parts. This is in accordance with the research of Mahmoudi *et al*., (2016) which states that the older the age of a leaf, the tannins contained in the leaves will decrease. However, based on the research of Rumsarwir *et al*., (2020) stated that differences in the levels of secondary metabolites in a plant are also influenced by temperature, intensity, light nutrients absorbed by soil and microorganisms.Based on the results of this study, it shows that the total tannin content in the methanol extract of young matoa leaves is higher than the tannin content of the methanol extract of old matoa leaves, which has the potential to become a biomordant in the fabric dyeing process. This is supported by Hidayati, Wahyuningsih, (2021) which states that biomordants are mordants derived from natural materials containing tannins. Because tannins can increase the absorption and binding power of dyes into cotton fabrics.

Based on research from Adu *et al*., (2022) stated that woven fabrics using biomordant tannins show better fastness values compared to woven fabrics using arbor and alum so that tannins are very effective in binding dyes and cellulose fibers from woven fabrics. In addition, the results of research from Lestari *et al*., (2020) found that cotton fabrics given tannin mordant produced the best color aging compared to alum. Because, the higher the tannin content in a plant, the color aging increases. This is due to the large number of tannins that enter the fabric fibers and are bound to the fabric fibers more and more. So from some literature that discusses tannin calm as a biomordant, therefore methanol extracts of old and young matoa leaves have the potential to become biomordants because they contain tannin compounds that can be used as biomordants as dye binders.

Analysis of data results of methanol extracts of old and young leaves begins with a normality test where the value ($p>0.05$) is declared normally distributed data. After that, it was continued with the Independent T-test which showed the value (*sig. 2-tailed> 0.05*) that between the two tannin levels of old and young matoa leaf extracts there was no significant difference.Thus, although there is a numerical difference in tannin levels, statistically this difference is not significant. These results indicate that both old and young matoa leaves have potential as a source of tannins for biomordant application in the fabric dyeing process matoa There is no significant difference between young and old.

CONCLUSION

The leaves of matoa (*Pometia pinnata*) contained steroid, triterpenoid, flavonoid and polyphenolic. Young matoa leaves contain the highest levels of tannin. Based on the results, the leaves of matoa is potential as a source of biomordant. With this research, alternatives to the use of metal mordant, which is a factor in environmental pollution, can be replaced with environmentally friendly biomordant.

RECOMMENDATIONS

Further exploration is needed regarding the testing of biomordant tannins from old matoa leaves and young matoa leaves in the fabric dyeing process.

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