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DETERMINING THE TANNIN CONTENT IN PISANG AMBON (*Musa × paradisiaca* L.) WITH THE POTENTIAL AS ANTHELMINTIC

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Abstract

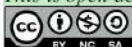
Tannins result from plants' secondary metabolism, closely associated with plant defense mechanisms against insects. Condensed tannins can disrupt the life cycle of parasitic nematodes starting from eggs, adult worms, and larvae. Currently, the antiparasitic properties of condensed tannins are being investigated as an alternative for controlling parasites. The people use the young Pisang Ambon (*Musa × paradisiaca* L.) as an anthelmintic. People generally use this part of the banana peel. Based on this, a study was conducted to determine the tannin content in the peel and fruit of Pisang Ambon (*Musa × paradisiaca* L.). The research was conducted by extracting the fruit and peel of young Pisang Ambon by maceration method using 96% ethanol as solvent. The Harborne method and Thin Layer Chromatography tested the presence of secondary metabolites. The tannin content was determined by the visible, ultraviolet spectrophotometric method. The qualitative results showed that the peel and fruit of Pisang Ambon contained condensed tannins. Meanwhile, the quantitative results showed that the tannin content of the fruit (54.98% w/w) was higher than the peel (14.32 %w/w).

Keywords: *Pisang Ambon, tannin, anthelmintic*

Introduction

Tannins result from the secondary metabolism of plants or natural materials through secondary reactions of primary organic matter carbohydrates, fats, and proteins. Tannins are also closely associated with plant defense mechanisms against insects (Schulz, 1989) and mammalian herbivores (E. & Butler, 1991). Chemically, tannins are grouped into four groups, namely hydrolyzed tannins, condensed tannins, complex tannins, and pseudo tannins. Tannins have complex biological roles ranging from protein deposition to metal chelating and functioning as biological antioxidants (Hagerman, 2002) (Trease & W.C, 1996). Condensed tannins are responsible for several adverse effects on monogastric (Vernon, 1999) and herbivorous ruminants (Aerts et al., 1999). However,

there are some advantages to the presence of condensed tannins in the diet of herbivores because they can reduce the detrimental effects of gastrointestinal parasites (Aerts et al., 1999). One is disrupting the life cycle of different parasitic nematodes, namely eggs, adult worms (Moreno-Gonzalo et al., 2013), and larvae (Niezen et al., 2002). The antiparasitic properties of condensed tannins are currently being investigated as an alternative for controlling parasites. In vitro and in vivo results show that tannins contained in plants can damage various vital biological processes of the parasitic nematode life cycle, namely (a) infecting formation of third-stage larvae (Moreno-Gonzalo et al., 2013) (Brunet et al., 2008), (b) excretion of adult worm eggs (Heckendorn et al., 2006) (Heckendorn et al., 2006) (Terril et al.,



2009) (Martínez-Ortiz-De-Montellano et al., 2010), and (c) development from nematode eggs into larvae (Niezen et al., 2002).

Adding 1 mg/mL of condensed tannins in candlenut shells in the fourth stage of *Ascaris suum* larvae will cause death and reduce motility. Observations were also made at the lowest concentration of 111 g/mL, and the results showed that condensed tannins had a potent anthelmintic effect on stage four and stage three larvae (Williams et al., 2014). The addition of 2 and 3% condensed tannins in sheep food showed a significant reduction in Faecal Egg Counts (FEC) on days 60 and 120, with the morphology of the eggs tested were *Trichostrongylus*, *H. contortus*, *Trichostrongylus* spp., and *Oesophagostomum colombianum* (Iqbal et al., 2007).

People have empirically used the natural ingredient as an anthelmintic, the young Pisang Ambon (*Musa × paradisiaca* L.). People generally use Ambon bananas to treat asthma, deworming, diabetes, hypertension, and snake bites (Hussain et al., 2010) (Imam & S. Akter, 2011) (Sable et al., 2013). Judging from the background above about the benefits of Pisang Ambon and the role of tannins as anthelmintics, this study aims to determine the tannin content of Pisang Ambon peels and fruits, which have the higher tannin content, so that they can be utilized further

Research Methods

The plant materials used were young and fresh Pisang Ambon and their peels, approximately three months old, obtained from Kecamatan Eromoko of Kabupaten Wonogiri. This plant was determined at the Biology Laboratory of the Faculty of Mathematics and Natural Sciences (FMIPA ~Ind.) of Universitas Negeri Sebelas Maret of Surakarta. Chemicals used include 96% ethanol, magnesium powder, HCl 2N, distilled water, Mayer's reagent, 10% FeCl₃, chloroform,

concentrated sulfuric acid, anhydrous acetic acid, FeCl₃, acetone, n-butane, acetic acid, distilled water, diethyl ether, ferric chloride reagent, ethyl acetate, and formic acid.

The tools used in this study included analytical balance (Ohaus), 30-mesh sieve, vacuum rotary evaporator (Buchii), kinetic maceration device, B-480 water bath (Buchi), electric water bath (Memmert), blender, UV Spectrophotometer, UV-Vis (Shimadzu), 100-1000 L and 0.5-5 mL volume micropipettes (SOCOREX), magnetic stirrer, volumetric pipette (Pyrex), Chamber, TLC plate (Thin Layer Chromatography), silica gel G 60 F254, vortex, and laboratory glasses.

Preparation of extract

Extraction was carried out by weighing the simplicia of Pisang Ambon and the banana peels as much as 500 grams each, then macerated with 5 liters of 96% ethanol at room temperature for one day, then filtered. Then, the dregs were macerated with 3.75 liters of 96% ethanol at room temperature for one day and filtered. After being filtered, the dregs were macerated again with 3.75 liters of 96% ethanol at room temperature for one day and then filtered again. The filtrate obtained was then combined and evaporated with a vacuum rotary evaporator at a temperature of 50°C, a speed of 70 rpm, and a pressure of 0.7 bars to get a thick extract of Pisang Ambon fruit and the peel.

Qualitative identification of tannin compounds

The tannins were identified using 1 mL thick extract solution of Pisang Ambon fruit and peel. The test was carried out with FeCl₃ 10% reagent. The presence of hydrolyzed and condensed tannins is indicated by the formation of blackish-blue and greenish-black deposits (Padmasari et al., 2013) (Kusumaningrum et al., 2015). Identification of tannin

Compounds by TLC was carried out at the Integrated Research and Testing Laboratory (LPPT) of Universitas Gadjah Mada. In the separation by analytical TLC, 1-gram freeze-dried Pisang Ambon peel and fruit extract were extracted with 10-mL diethyl ether for 20 hours and dried with filter paper. The residue was boiled with 5 mL distilled water for two hours, then cooled and filtered. Spotting a sample of 10 μ L on a silica gel TLC plate G 60 F254 using a microcapillary approximately 1 cm from the bottom edge of the TLC plate, then allowed drying. The TLC plate was then placed in a vessel containing the eluent. The eluent used was ethyl acetate: acetic acid: formic acid: water, with a ratio of 100: 11: 11: 27. After being eluted to the boundary line, the TLC plate was removed from the vessel and dried, the spots were observed with ultraviolet light at wavelengths of 254 nm and 366 nm, and 1% FeCl₃ spots were visible. The results were obtained using stains or marks identified as R_f (Retention factor) values from thin-layer chromatography. The distance of the mark from the spotting point was measured and recorded to obtain an R_f value for each mark.

Stage of determining tannin content using spectrophotometry

Tannin content was determined at the Integrated Research and Testing Laboratory (LPPT) of Universitas Gadjah Mada. A sample of approximately 100 mg was weighed, extracted with 10 mL diethyl ether for 20 hours, and then filtered. The remaining diethyl ether was evaporated, and aqua dest (distilled water) was added to the sample for up to 10 mL. The sample solution was taken at 0.5 mL and then added with 0.1 mL Folin Ciocalteu reagent and vortex, and waited 5 minutes. Next, added 2 mL Sodium Carbonate 20% and vortex, waited for five minutes, and added distilled water to a volume of 10 mL. The absorbance was read at λ 760 nm after being incubated for

30 min at room temperature (Chanwitheesuk et al., 2004).

Results and Discussion

Condensed tannins are polymers of flavan-3-ol units with a wide range and structural variations depending on the monomer unit and degree of polymerization. The biochemical structure of flavan-3-ol can be distinguished based on the number of phenolic groups in the B-ring, the stereochemistry of 2,3-O-methyl on the C-ring, and the presence of a galloyl group attached to the C-ring, as shown in Figure 1. Procyanidin (PC) monomers are catechins or epicatechin and their galloyl derivatives. The prodelphinidin (PD) constituents are gallocatechin epigallocatechin and their galloyl derivatives; overall, tannins are defined as polyphenols, which have the property of binding proteins or polysaccharides. Hydrolyzed tannins can bind to carbohydrates to form oxygen bridges so that tannins can be hydrolyzed by sulfuric acid or hydrochloric acid.

Differences in the biochemical structure related to the nature of the monomers were shown to modulate the biological properties of tannin condensation, particularly the condensation ability of tannins with activity in nematodes. The results of two studies also showed that prodelphinidins were more potent inhibitors of larval motility than procyanidins.

Research by Brunet and Herve (2006), which examined the interaction of extracts of four tannin-rich plants with the cuticle of L3 larvae, showed that: (i) extracts of tannin-rich plants could invade the host; (ii) the effect was not specific to the parasite species; (iii) most tannins were involved in the inhibitory process (Brunet & Hoste, 2006).

Research by Haryatmi et al. tested the effect of ethanol extract of Pisang Ambon by giving 200 mg/mL and 400 mg/mL to *Ascaris suum* worm, which caused ultra-

structural damage to the cuticle of the worm. The worm exposed to the ethanol extract of the Pisang Ambon was paralyzed and died. The histopathological structure showed a ragged cuticle separated from the muscularis, and

oedema also occurred. Damage to the cuticle was also seen to be more extensive and more numerous as the concentration of the extract increased (Haryatmi et al., 2018).

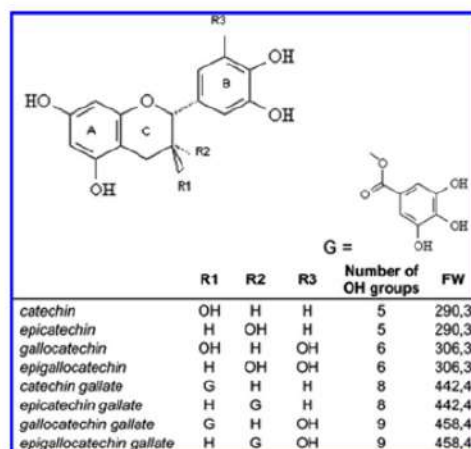


Figure 1. Chemical structure of condensed tannin monomers: flavan-3-ols and flavan-3-ol (Brunet & Hoste, 2006)

The tannin tests for the fruit and peels of Pisang Ambon were carried out with 10% FeCl_3 for three replications and were successful with several color intensities. The qualitative results of the Pisang Ambon peel and fruit test showed a positive presence of tannins. Both produced a greenish-black color which indicated the presence of condensed and hydrolyzed tannins.

Identifying the class of tannin compounds in the ethanol extract of Pisang Ambon was also carried out using

the thin layer chromatography (TLC) method. Observations of TLC results were carried out directly and under UV light with wavelengths of 254 nm and 365 nm, as shown in Figure 2. Based on the qualitative test data for samples of ethanol extract of Pisang Ambon fruit and peel using TLC, the standard color spots of tannins were obtained in the same sample as the standard which, when viewed visually, showed a blue-gray spot color with a detectable tannin R_f of 0.24.

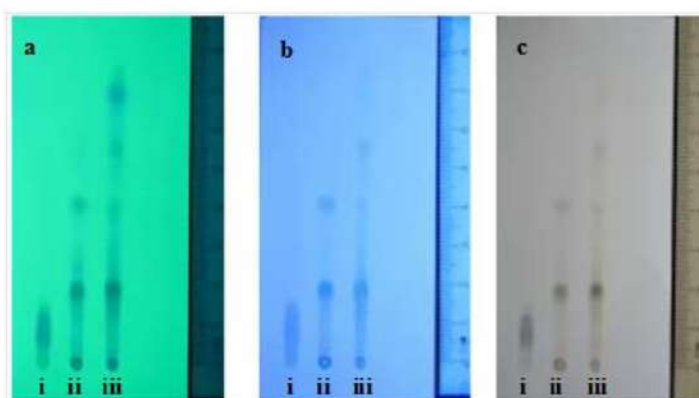


Figure 2. TLC identification of the class of tannin compounds, (a) reading at UV 254 nm; (b) reading at UV 365 nm; (c) reading at Visible; (i) Comparator Tannic Acid; (ii) Extract (Banana Fruit/Meat); (iii) Extract (Banana Peel) (Haryatmi et al., 2018).

The tannin content in Pisang Ambon fruit and peel was determined by ultraviolet-visible spectrophotometric method with Folin Ciocalteu reagent based on the formation of a blue molybdenum-tungsten complex. The hydroxyl group in phenolic compounds reacted with the Folin Ciocalteu reagent to

form a blue molybdenum-tungsten complex. Its absorption could be measured in the visible light region at a wavelength of 760 nm, with a reaction time of 30 minutes. The results of determining the tannin content in the peel and fruit of Pisang Ambon are shown in Table 1.

Table 1. The tannin content in the Pisang Ambon fruit and peel extracts

Sample	Sample Weight (g)	Result (ppm)	Average (ppm)	Result (% b/b)	Average (% b/b)
Peel extract of Pisang Ambon	0.1140	143,229.82	143,247.14	14.323	14.32
	0.1142	143,264.45		14.326	
Fruit extract of Pisang Ambon	0.1145	551,161.57	549,787.35	55.116	54.98
	0.1157	548,413.14		54.841	

The results of the determination of tannin levels using UV-Vis spectrophotometry in Table 1 show that the tannin content of Pisang Ambon is higher in the fruit (54.98%) than in the peel (14.32%). It can be because the tannins in the fruit flesh are not oxidized by direct sunlight or other oxidizing compounds such as free radicals and reactive oxygen. Based on their structure, two types of tannins are generally found in plants, condensed tannins and hydrolyzed tannins. Hydrolyzed tannins usually have lower levels in plants than condensed tannins. Ambon banana peel

contains condensed tannins, while the pulp contains hydrolyzed tannins. Condensed tannins cannot be hydrolyzed but can be condensed to produce hydrochloric acid. Condensed tannins consist of several flavonoid units (flavan-3-ol) linked by carbon bonds, as shown in Figure 1.

Cuticle damage was associated with the abundance of hydroxy groups associated with molecular weight, with the hydroxy group at R3 (Figure 1) being the hallmark of the Prodelphinidin unit. The large number of OH groups in the flavan-3-ol structure may favor binding to

larval proteins. Moreover, the presence of gallate is also involved in hydrophobic interactions. Haryatmi et al. (2018) stated that tannin toxicity could damage membranes. Tannins can cause damage when they hit the worm's cuticle. Tannins will interfere with the permeability of the cell itself. Due to the disruption of permeability, cells cannot carry out life activities, so the metabolism is inhibited. According to Pandey et al. (2013), worms have no way of storing energy, so they constantly eat to meet their metabolic needs. Therefore, the disruption of the feeding process and the occurrence of paralysis due to exposure to active substances that cause damage will cause the death of the worms (Pandey et al., 2013).

Conclusions

The results of determining tannin content using UV-Vis spectrophotometry show that the tannin content in Pisang Ambon is higher in the fruit (54.98%) than in the peel (14.32%). The peel contains tannin with a concentration of 143,247.14 $\mu\text{g/g}$, while the fruit is 549,787.35 $\mu\text{g/g}$. The tannin content in the ethanol extract of the Pisang Ambon fruit has the potential as an anthelmintic.

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