



## ***Ascaris suum* Cuticle Ultrastructure Due to the In Vitro Application of Ethanol Extract to Fruits *Musa x paradisiaca* L. 'Pisang Ambon'**

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### **Abstract**

Species of *Musa* have various activities, one of which is anthelmintic activity. Part of banana plants that can be used as anthelmintic is part of the fruit, because of the phytochemical content possessed. Tannin is able to inhibit the enzyme cholinesterase, damage the membrane of the worm and can precipitate the protein. The purpose of this research is to know the content of tannin and also total tannin level on ethanol extract of *Musa x paradisiaca* L. 'Pisang Ambon' and *in vitro* effect of the extract on *Ascaris suum* cuticle. Thin Layer Chromatography (TLC) showed of ethanol extract of Ambon banana containing tannin with a total test of tannin was 54.98% w/w. In vitro at negative control (NaCl 0.9%) worm death occurred at 289 hours, positive control (pyrantel pamoate 5 mg/ml) at 1 hour, extract concentration 200 mg/ml at hour 27 and concentration 400 mg/ml at 1 hour. Histopathological examination and scanning electron microscope showed a degenerative change in ultrastructures of the worm's cuticle. From the results of this study, it can be concluded that exposure of ethanol extract to Ambon banana give a damaging effect to cuticle ultrastructure in the form of crack/break, perforation and separation of muscular from cuticle and edema on *A. suum* worm. High tannin content in the raw/green ambon banana, can be developed to eradicate ascariasis including egg stage which until now cannot be done with synthetic drugs due to the thickness and strength of layers of worm eggs consisting of layers of protein and lipids.

### **How to Cite**

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## INTRODUCTION

Worm infection is an endemic and chronic disease caused by parasitic worms with high prevalence, non-lethal, but is capable of causing a decline in nutritional and public health conditions (Iman *et al.*, 2015). *Ascaris lumbricoides* and *Ascari suum* are the most common intestinal worms found in humans and pigs. Eradication of infection of worms including *ascariasis* in Indonesia continues to be done until today. One of the methods used is by giving the anti-worm drug.

Research on the potential of plants as anthelmintic has been done. From several studies that have been done, obtained plants that have anthelmintic properties such as papaya, pare, temugiring, temu hitam, pin nut, cassava (Magdeleine *et al.*, 2010; Tiwow *et al.*, 2013). Williams *et al.*, (2014) also has research on the anthelmintic effects of cocoa beans, pecan skin, pine bark, and white clover. Based on a study conducted by Iman *et al.*, (2015), it was concluded that the concentration of Chinese leaf extract (*Cassia alata* L.) had a significant effect on mortality of adult female *A.suum*, and had 39.98% of Lethal Concentration 50 (LC<sub>50</sub>). From in vitro testing by Tiwow *et al.*, (2013), the ethanol extract of betel nut concentration of 10% able to make *A. lumbricoides* paralysis and at concentration of 20% able to make worm *Ascaridia galli* become lysis/die. Ethanol extract of betel seeds at a concentration of 30% had more effective anthelmintic power against *A. lumbricoides* worms and *A. galli* worms. Based on the results of other studies, papaya leaf extract is vermifidal to *A. suum* worms, in vitro with LC<sub>100</sub> of 13.378% and LT<sub>100</sub> of 54.706 hours. Papaya leaf extract is ovicidal to *A. suum* by direct contact in vitro. Venkatesh *et al.*, (2013) showed that banana tree extract ethanol exhibited significant anthelmintic activity against *Pheretimaposthuma* worm, at concentrations of 100, 50, 25 mg/ml indicating the time of paralysis and death respectively at 42.33±1.45 and 54.00±0.58 min, 74.33±1.20 and 101.67±1.45, 82.67±1.45 and 115.00±1.73.

Studies show that the species of *Musa* have various activities such as protecting from heartburn, immunomodulatory potential, anthelmintic activity, antidiabetic activity, antibacterial, antioxidant, anti-cancer, and anti-HIV activity (Joseph *et al.*, 2014). In a study conducted by Malini *et al.*, (2017) *Musa x paradisiaca* L also included one of the plants used by the inhabitants of Karangwangi, Cianjur, West Java in treatment. In anthelmintic activity test of the banana extract by Venkatesh *et al.*, (2013) of the phytochemical analysis showed a positive presence of tannins as

well as flavonoids, glycosides, terpenoids, and sterols. According to research conducted by Ariani and Linawati (2016) ambon banana (*Musa paradisiaca* var. *Sapientum* (L.)) was known to contain saponins, glycosides, tannins, alkaloids, and flavonoids.

Tannin content can inhibit the enzyme cholinesterase and damage the membrane of the worm. Inhibition of enzyme work can cause the process of digestion metabolism disturbed so that worms will lack nutrients, in the end, worms will die from lack of energy. The membrane of worms damaged by tannin causes paralytic worms that eventually die (Tiwow *et al.*, 2013). Traditionally, people have used *Musa x paradisiaca* L. 'Pisang Ambon' in the treatment of worms in animals. Therefore, it is critical to do the research to prove that other than to turn off the worms as anthelmintic, the ethanol extract of Ambon banana can cause damage to cuticle ultrastructure of *A. suum* worm.

Treatment by using herbs will be an alternative to prevent the occurrence of ascariasis. Banana plants are widely available in all regions in Indonesia make the treatment effort that is easy to get, lower price, and when used appropriately then the side effects caused smaller when compared with the use of synthetic drugs.

## METHOD

The research was conducted at Parasitology Laboratory of Faculty of Medicine, Gadjah Mada University (UGM) Yogyakarta, Anatomy Pathology Laboratory of Faculty of Veterinary Medicine of UGM. The examination of the secondary metabolite was done at UGM Integrated Research and Testing Laboratory, *Scanning Electron Microscope* (SEM) done at Zoology Research Center of Lembaga Ilmu Pengetahuan Indonesia (LIPI) Cibinong, from February to June 2017. The primary tools used are UV-Vis spectroscopy, microtome, floating bath, tissue processor, JEOL JSM-5310LV *Scanning Electron Microscope*.

Ethanol extract of Ambon banana which has been tested by phytochemical screening, identification of tannin with TLC and continued test of total tannin level. Adult *A. suum* worms were obtained from the slaughterhouses of Radhajaka pigs in Jagalan, Surakarta. The worms obtained were divided into five groups. The first group was used for determination, the second group as the negative control. The group when the positive control and group of 4.5 were used for the in vitro treatment of the banana extract ethanol concentration of 200 mg/ml and 400 mg/ml, were

then used in the manufacture of histopathologic pr *Scanning Electron Microscopy* for ultrastructure observation of the cuticle after treatment.

#### Identification of Tanin Compound with TLC and Tanin Level Test

The banana ethanol extract was carefully weighed, extracted with 10 ml of diethyl ether for 20 hours, dried with filter paper. The obtained residue was boiled with 5 ml of distilled water for 2 hours, then cooled and filtered. Conducted a 10 µl spot spotting on the silica gel plate and then inserted the plate into the saturated chamber of the ethyl acetate-acetic acid-formic acid (100-11-11-27) phase of motion. Brought to the limit and then dried the plate and observed under UV light then sprayed with *ferric chloride* reagent.

The tannin content of the ethanol extract of the ambon banana was determined by ultraviolet-visible spectrophotometry method using *Folin Ciocalteu* reagent based on the complex formation of *molybdenum-tungsten blue*. The hydroxyl group in the phenolic compound reacts with the Folin Ciocalteu reagent forming a *blue molybdenum-tungsten* complex and can be measured uptake at visible light areas at 760 nm wavelength with a reaction time of 30 min (Malanggi *et al.*, 2012).

#### Preparation of Histology Preparations

The worms were fixed with 10% formalin solution for 24 hours. After that worm cutting approximately one cm. Dehydration process on *tissue processor* using alcohol 80% (2 hours), 95% (2 hours), 95% (1 hour), absolute alcohol (3x1 hours). Next clearing using xylol (3x1 hours) followed by impregnation using paraffin (3x2 hours). *Embedding* is done by moving a network that is in *embedding cassette* into the *base mold* then filled with liquid paraffin, then attached to a 3x3 cm block of wood. Cutting is done using microtome with a thickness of 6 µm, taken with glass object then dried.

The stage of deparaffinization, i.e., by inserting preparations into xylol (2x5 minutes). The preparations are rehydrated by immersion into an absolute ethanol solution (2x1 min), 95% ethanol (2 x 1 min) then dyed with aquades. Stain preparations using hematoxylin and eosin. The result is observed under a light microscope (Suwiti, 2010).

#### Scanning Electron Microscopy

*Scanning electron microscopy* is performed on the worm's cuticle. Samples were fixed with absolute ethanol to be sent to the Zoology Research Center of Lembaga Ilmu Pengetahuan Indonesia

(LIPI) Cibinong. To conduct SEM, sample preparation performed there are seven stages. The first stage is the cleaning stage, the sample soaked in *cacodylate buffer* approximately 2 hours agitated in an *ultrasonic cleaner* for 5 minutes. The second stage is prefixation; the sample is put into 2.5% *glutaraldehyde* solution several hours to 2 days. The third stage is a fixation, the sample soaked in tannic acid 2% 6 hours to a few days then washed with cacodylate buffer for 15 minutes, washing repeated until 4 times followed by aquades for 15 minutes. The fourth stage is the dehydration stage, the sample is immersed in 50% alcohol for 5 minutes repeated up to 4 times, 70% alcohol for 20 minutes, 85% alcohol for 20 minutes, 95% alcohol for 20 minutes, 10 minutes absolute alcohol 2 times done at room temperature. The fifth stage is drying, the sample soaked in butanol tart for 10 minutes 2 times, then frozen in the freezer until frozen, then put into frozen drier or vacuum dryer until dry. The sixth stage is the mounting, the sample is glued to the stub specimens according to need or desire, the seventh stage of the coating with Au using the ion coater device and the scanning electron microscope JEOL JSM-5310LV (Goldstein *et al.*, 1992).

## RESULTS AND DISCUSSION

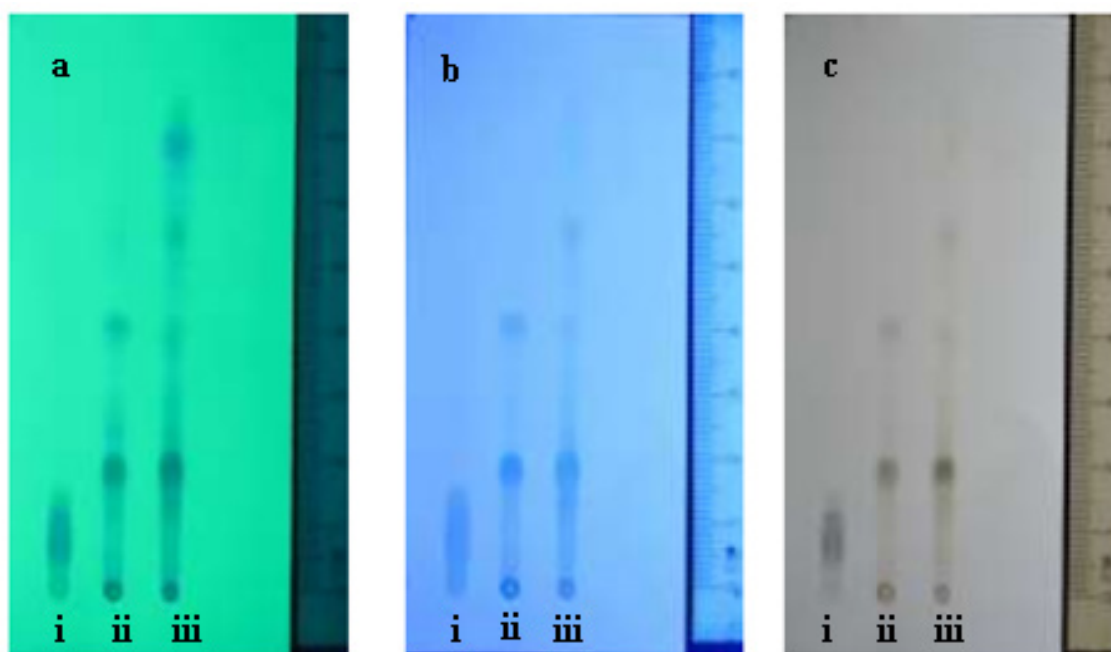
#### Identification of Tanin Compound with TLC and Tanin Level Test

The presence of tannin content shown in the TLC results seen in Figure 1, showed the same *R<sub>f</sub>* value between standard tannin and ethanol extract of *Musa x paradisiaca* L. 'Pisang Ambon'. In the total test of tannin, the ethanol extract of Ambon banana has a content of 54.98% w/w. The process of tannin extraction on bananas is based on the principle or character of the tannin itself soluble in the polar solvent. This is supported by Cannel (1998) in Askadilla *et al.*, (2015) that tannins are a mixture of polyphenols in plants that form glycosides, and tannins are polar in glycoside form, while ethanol is a polar solvent that is more effective in attracting tannins than with both semipolar and nonpolar solvents.

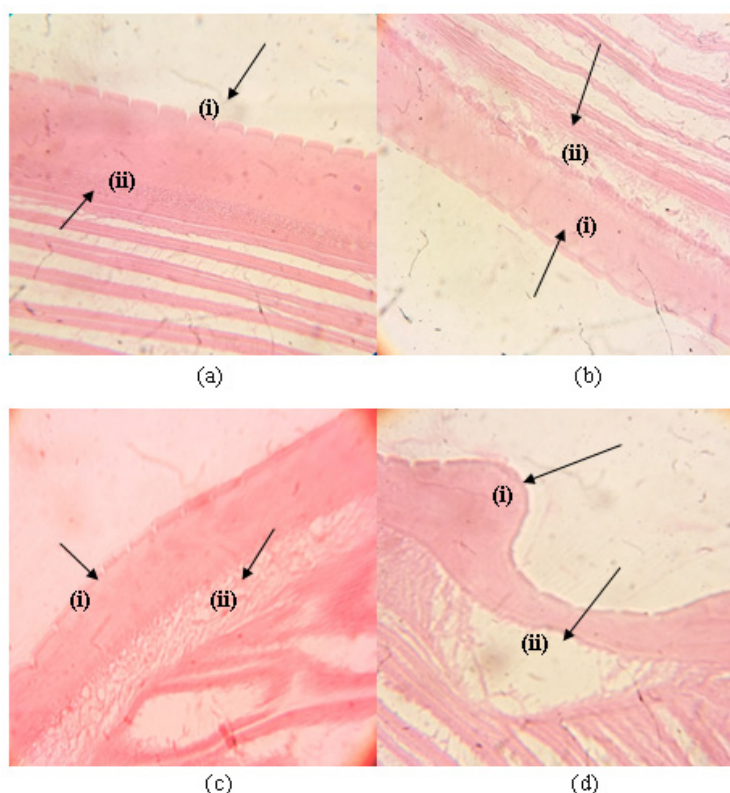
#### Histopathology Preparation

Damage of *A. suum* worm ultrastructure on negative controls that died at 289 hours, positive control at 1 hour (60 min), ethanolic extract treatment of Ambon banana concentration 200 at 27 hours and 400 mg/ml at hour to-1 (60 minutes) is shown in Figure 2.

*Ascari ssuum* exposed to ethanol extract of ambon bananas paralyzed and then died. The ef-



**Figure 1.** Tests of tannins group identification with TLC method; (a). UV readings are 254 nm, (b). Readings on UV 365 nm; (c). Visible Readings. (i): Comparator Tannic Acid; (ii): Extract (Fruit / Banana Meat); (iii): Extract (Banana Skin)



**Figure 2.** Histopathological structure of the cuticle surface of 400x magnification; (a). Negative control (NaCl 0.9%): (i) Cuticle looks flat, (ii) Cuticle seen linked with muscular; (b). Positive control (pyrantel pamoate 5 mg/ml): (i) Cuticle looks flat, (ii) Cuticle seen separately with muscular; (c). Treatment of extract 200 mg/ml: (i) Cuticle looks flat, (ii) Cuticle seen apart with muscular; (d). Treatment of the extract 400 mg/ml: (i) The cuticle looks uneven, (ii) The cuticle is seen separately with the muscular and occurs edema.

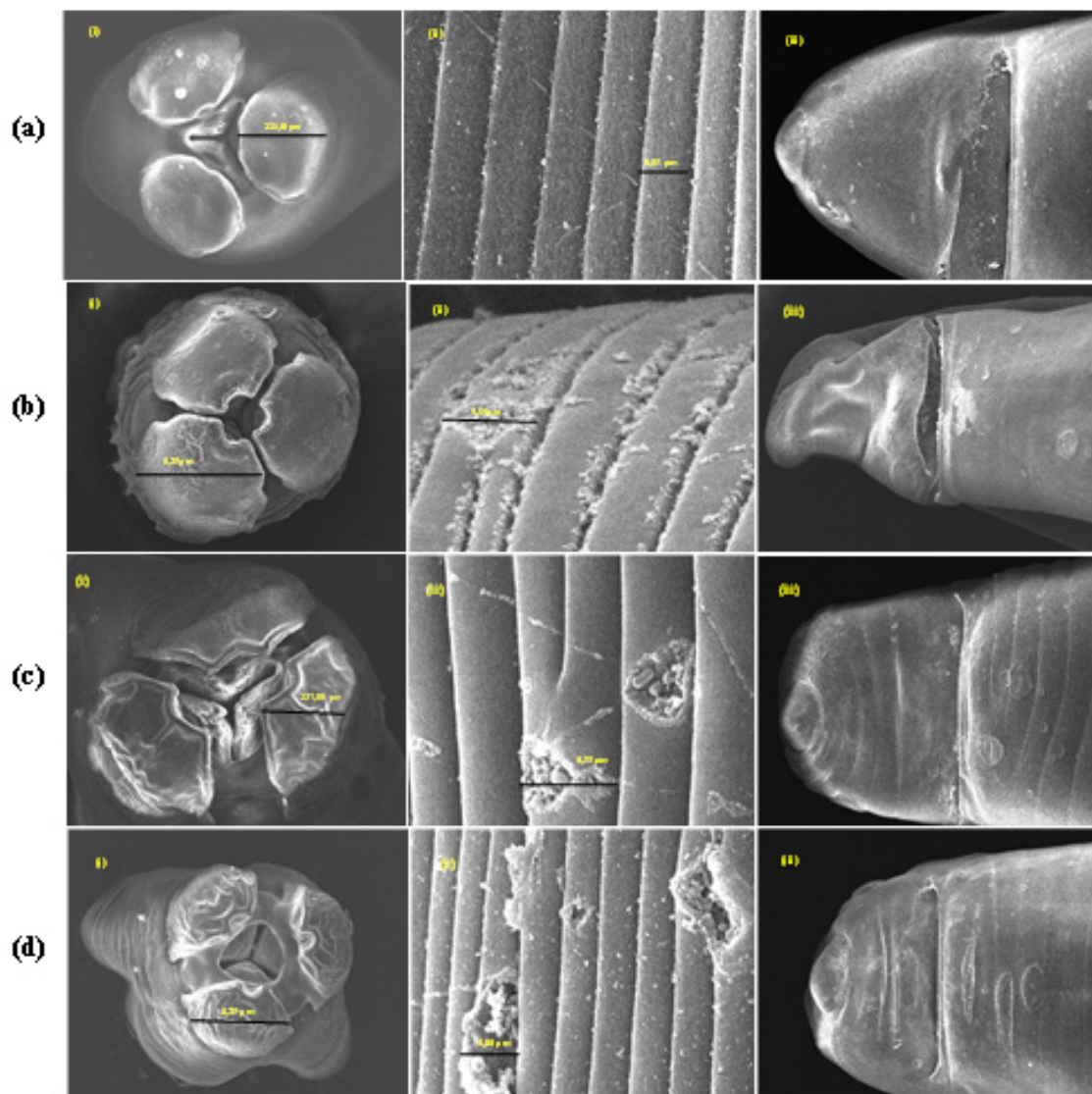
fect of exposure on the cuticle/worms of the negative control group indicates no damage, the cuticle looks smooth and flat. The positive control, cuticle looks flat but cuticle seen apart with muscular as well as treatment of extract concentration 200 mg/ml, while at level 400 mg/ml showed cuticle which is uneven, separated by muscular and also happened oedem.

The body wall of the nematode consists of the cuticle, hypodermis, and muscle wall of the body. The outermost layer is the cuticle, the polysaccharide complex structure whose function is essential for animals (Pandey *et al.*, 2013; Roberts and Janovy, 2009). Cuticle worms have an important role as a protective layer and are also used

as absorption of nutrients in the host. When the cuticle is damaged by anthelmintic treatment, it can disrupt the worm defense system.

### Scanning Electron Microscopy

*Scanning electron microscopy* of the anterior cuticle of the lips and interlabial or posterior parts of the worms seen in Figure 3. The worm cuticle on the negative controls is smooth, no damage. In contrast to worm cuticles on positive controls and worm cuticles with exposure to ethanol extract of ambon bananas, experiencing the occurrence of blisters, cracks and potholes. Cuticle damage due to exposure to extracts appears to be more widespread and more in line with the concentra-



**Figure 3.** *Scanning Electron Microscope Ascaris suum* Worm; Negative control (NaCl 0.9%): (i) Anterior lips and interlabia worms, (ii) Cuticle (iii) Posterior anal worms; Positive control (pyrantel pamoate 5 mg/ml): (i) Anterior lips and interlabia worms, (ii) Cuticle (iii) Posterior anal worms; Treatment of extract 200 mg/ml: (i) Anterior worm lips and interlabia, (ii) Cuticle (iii) Posterior anal worms section; Treatment of extract 400 mg/ml: (i) Anterior worm lips and interlabia, (ii) Cuticle (iii) Posterior anal worms section.



tion of extracts.

This study is supported by other research on the potential of various plants with its phytochemical content as anthelmintic which also causes the occurrence of surface damage of the worms/cuticle worms which are a rough extract of *Flaminga vastita* causing damage to cuticle and muscle of *Ascaris suum* body (Yadav *et al.*, 1992). The extract of the *Lasimachia ramosa* plant causes death, the visible worm body shrinks, and surface changes in *Ascaris suum* and *Fasciola buski* (Challam *et al.*, 2010) and antimmintic activity of *Trigonella Foenum-graecum* produce swelling, blisters in the *Gastrothylax crumenifer* (Swarnakar *et al.*, 2014). The efficacy of *Citrullus colocynthis* fruit extracts on *Orthocoelium scolicoelium* also show damage, blisters and cracks in the calendar, vacuolization of parenchymal cells, separation of the muscle and acetabulum (Swarnakar and Kumawat, 2014). Swargiary, 2015 also showed damage to the worm cuticle *Faciolopsis buski* possible because of bioactive content in the plant *Alpinia nigra*.

Another study by Challam *et al.*, (2012) compared with control, parasite *Raillietina echinobothrida* treated ethanol extract *Carex baccans* showed extensive distortion and topography surface topography destruction, muscle layer disorder, vacuolization of the thickened layer, and swelling. Alcohol extracts from *Acacia oxyphylla* and *Securiniga virosa* lead to morphological changes in the cestode parasite, *R. Echinobothrida* (Dasgupta *et al.*, 2013). In the same way, the surface of blisters and severe lesions were observed in *F. gigantica* when the parasite was incubated with *Siwa propolis* extract (Hegazi *et al.*, 2007). The surface change in *Fasciola hepatica* when treated with *genistein*, consists of swelling and blisters, especially in the posterior region and also the release of thorns (Toner *et al.*, 2009).

Tannin toxicity can damage the membrane, meaning tannin has the ability to cause damage when it comes to the worm's cuticle. Tanin will interfere with the permeability of the cell itself. Due to disruption of the permeability, the cell cannot perform life activities so that metabolism is inhibited. According to Pandey *et al.*, (2013) worms have no way of storing energy, so the worms will eat continuously to meet their metabolic needs. Therefore the disruption of the process of eating and also the occurrence of paralysis due to exposure to active substances that cause damage will cause the death of worms. Tannins derived from polyphenol compounds also have the ability to precipitate proteins. These findings can be developed in studies for the eradication of

ascariasis including the egg stage that until now has not been possible with synthetic drugs.

## CONCLUSION

From the results of this study, it was concluded that from exposure to ethanol extract to Ambon banana as anthelmintic gave effect to the ultrastructure damage on the cuticle of *A. suum* worm.

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