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Testing the anthelmintic activity of *Acacia mangium* pods with different levels and incubation times against the nematode worm *Haemonchus contortus* in vitro

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Abstract

This study aimed to evaluate the anthelmintic activity of *Acacia mangium* pods at varying concentrations and incubation times against the nematode worm *Haemonchus contortus* in vitro. This study used *Acacia mangium* pods and applied four treatments: P0 (100% Albendazole, positive control), P2Y (6% *Acacia mangium* pod infusion), P4Y (12% *Acacia mangium* pod infusion), and P6 (0.9% NaCl, negative control), with observations made every 60 minutes for 6 hours. The adult female nematode worm *Haemonchus contortus* test method was used to determine the anthelmintic effect of *Acacia mangium* pods in vitro. The results from in vitro the screening of adult female *Haemonchus contortus* indicate that increasing the concentration of *Acacia mangium* pod infusion significantly increases worm mortality ($P < 0.05$). *Acacia mangium* pods contain 18.98% tannin which means the anthelmintic effect can be seen from the mortality of adult worms. Based on the research findings, we conclude that *Acacia mangium* pods provide an anthelmintic effect against adult female nematode worm *Haemonchus contortus*, so it can be used in controlling nematode worm infection in the digestive tract of small ruminants.

Keywords: Acacia*,* Anthelminthic*, Haemonchus contortus*, In vitro, Tannin

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1. Introduction

Ruminants are one of the livestock species that are widely developed in livestock farming in Indonesia. However, there are still many problems faced by farmers in ruminant farming, one of which is health issues in the livestock. Animal health is a crucial aspect that needs attention because it significantly affects the success of farmers. Animal health issues are often associated with diseases that affect livestock. If not properly managed, diseases in livestock can have adverse impacts, such as a decrease in production, which can also result in losses for the farmers. Infectious diseases are one of the major health problems commonly encountered in livestock farming. One example of an infectious disease is parasitic infections. Livestock infected with parasites typically appear emaciated due to their inability to fully absorb nutrients from the feed. One parasitic disease that frequently affects small ruminants, such as sheep and goats, due to parasitic infections is haemonchosis.

Haemonchosis is a parasitic disease caused by the nematode *Haemonchus contortus*, which frequently affects ruminants, particularly small ruminants, by infecting the digestive tract. *Haemonchus contortus* is a blood-sucking nematode, with infection rates and spread reaching up to 80% (Yuswandi & Yuniar S., 2015). The disease typically manifests symptoms such as a decrease in production, anemia due to the worms sucking blood from the abomasal mucosa, and cachexia (loss of fat-free tissue mass, resulting in weight loss). In chronic infections, symptoms are more likely to include generalized edema (swelling throughout the body due to fluid accumulation). Anemia caused by *Haemonchus contortus* can range from mild to severe. Additionally, it can lead to hypoproteinemia (low protein levels in the blood), lethargy (reduced consciousness), and even death. The nematode *Haemonchus contortus* can consume approximately 0.4 ml of blood per day. Treatment for this parasitic problem is often performed by administering anthelmintic drugs (Partodihardjo et al., 2004). However, the continuous use of the same type of deworming drug can lead to resistance and reduced drug efficacy. Furthermore, the use of modern anthelmintics may result in environmental contamination and pose risks to humans consuming infected meat (Maryam et al., 2018). Therefore, there is a need for alternative deworming agents that are easily accessible and affordable for farmers. Previous studies have indicated that tannin-rich plants have the potential to control nematodes in small ruminants (Akkari et al., 2008). The use of natural deworming agents is also recommended because it helps minimize the side effects associated with chemical anthelmintics.

Tannins, which are naturally occurring polyphenolic compounds found in a wide range of plants, have been shown to possess significant anthelmintic properties, making them highly effective in controlling parasitic infections in various livestock species, including sheep and goats (Brunet & Hoste, 2006; Iqbal et al., 2007). These compounds are capable of inhibiting the development and migration of worm larvae, a key factor in reducing parasitic infestations in these animals. In particular, tannins effectively prevent the migration of worm larvae in goats, which is a crucial step in controlling the spread of parasitic diseases within herds (Alonso-Díaz et al., 2008). Furthermore, research conducted by Hadi, Handayanta, Widyawati, et al. (2021) has provided compelling evidence that shrubs with higher concentrations of tannins exhibit a much greater potential for anthelmintic activity. Their findings suggest that plants with elevated tannin levels are particularly effective in controlling the parasites that infect the digestive tracts of small ruminants, offering a natural and sustainable alternative to chemical treatments. Tannins themselves are categorized into two primary groups: hydrolyzable tannins, which can break down into simpler molecules, and condensed tannins, which tend to have a more complex structure. One example of a plant that contain these tannin compounds is the acacia plant, which has long been recognized for its tannin content and potential use in parasitic control.

According to Desta (2013), almost all types of plants contain tannin compounds, but only a select few plant species predominantly contain these compounds in significant amounts. One such species is the mangium tree. Other plant species known to contain tannins include quebracho, chestnut, mangrove, myrobalan, acacia, hemlock, and gambir. Mangium trees that are aged between 5 and 9 years have relatively high tannin content, ranging from 18% to 39%, which makes their bark a particularly valuable source of plant-based tannins. Tannins can be extracted from nearly all parts of the plant, including seeds, pods, leaves, roots, wood, and bark (Floweri, 2019). The specific acacia species that is known for its tannin content and potential applications is *Acacia mangium*. Thus, to investigate the anthelmintic effects of *Acacia mangium* pods, an anthelmintic activity test was conducted on *Haemonchus contortus*.

2. Material and Methods

2.1. Place and Time of Research

This research was conducted between April and December 2021 at two primary locations: the Jatikuwung Experimental Laboratory and the Animal Nutrition and Feed Science Laboratory. Both laboratories are part of the Animal Science Department within the Faculty of Animal Science at Sebelas Maret University, Surakarta. Additionally, the tannin content analysis was conducted at the Integrated Research and Testing Laboratory, Gadjah Mada University in 2022, further ensuring the accuracy and reliability of the data obtained for the study.

2.2. Tools and Materials of the Research

The tools used in this research are besek (woven basket), scissors, paranet (netting), label paper, stationery, scales, grinding machine, brush, tape, plastic, rubber containers for worms, tweezers, Petri dishes, timer, beaker, dropper pipette, and spectrophotometer. The materials used in this research were *Haemonchus contortus* worms, *Acacia mangium* pods, 0.9% NaCl (as the negative control), and Albendazole (as the positive control).

2.3. Design of Research

The research design in this study uses a Completely The experimental design used in this study was a Completely Randomized Design (CRD) with a factorial pattern consisting of two factors, namely incubation time (H1–H6) and treatment levels (P0, P2Y, P4Y, P6), each replicated three times. The first factor was incubation time, which consisted of six levels: H1 = 1 hour, H2 = 2 hours, H3 = 3 hours, H4 = 4 hours, H5 = 5 hours, and H6 = 6 hours. The second factor was the treatment level, which included P0 = 0.9% NaCl (negative control), P2Y = 6% *Acacia mangium* pods infusion, P4Y = 12% *Acacia mangium* pods infusion, and P6 = Albendazole (positive control). This factorial arrangement enabled the evaluation of the interaction between incubation time and treatment levels on the observed parameters.

2.4. Methods of the Research

2.4.1. Preparation of acacia pod samples infusion

The samples of Acacia plants were taken from the shrub land at the Jatikuwung Experimental Laboratory. The Acacia plant samples used included *Acacia mangium*, specifically the pods part. The *Acacia mangium* pods infusion was prepared by cutting and weighing the samples into small pieces. After the samples were cut, they were dried with the help of paranet to prevent direct sunlight exposure. Drying was performed for 10-11 days until a constant weight was achieved, followed by grinding the samples to a size of 2 mm. After grinding, proximate analysis was carried out along with the Van Soest analysis. The nutrient composition of Acacia mangium pods can be determined through proximate analysis using the methods established by the Association of Official Analytical Chemists (AOAC), which includes dry matter (DM), organic matter (OM) using the Thermogravimetric method, crude protein (CP) using the Kjeldahl method, crude fat (CF) using the Soxhlet method, and crude fiber (CF) using the Weende method (Horwitz, 2000). The content of NDF (Neutral Detergent Fiber) and ADF (Acid Detergent Fiber) can be obtained through the analysis by Van Soest et al. (1991). The Hemicellulose content was obtained from the percentage of NDF-ADF.

Afterward, the infusion was made by weighing the sample according to the concentration to be used in the testing, resulting in 6% and 12% infusions. A 6% infusion was obtained from 6 grams of Acacia pods sample dissolved in 100 ml of distilled water, while a 12% infusion was made from 12 grams of Acacia pods sample dissolved in 100 ml of distilled water. The control group used Albendazole (positive control) and 0.9% NaCl (negative control). This infusion is easy to prepare, affordable, and suitable for *Acacia mangium* pods, which are sensitive to heat.

2.4.2. Analysis of Tannin Content

The tannin content in Acacia plants is measured by adding Folin-Denis reagent to samples containing tannins. A blue-colored complex will form, and its absorbance can be measured at a wavelength of 725 nm using a spectrophotometer.

2.4.3. Preparation of the Worm *Haemonchus contortus*

Haemonchus contortus worms were obtained from the abomasum of infected sheep, sourced from the Semanggi Slaughterhouse. The abomasum was selected as the part between the rumen and duodenum. The worms were collected using tweezers and placed in a Petri dish with 0.9% NaCl to maintain their viability. Female worms were then selected, characterized by red and white thread-like coils and measuring approximately 18-30 mm in length. Female worms were chosen to help interrupt the reproductive cycle, as adult female worms can lay 5,000-10,000 eggs per day, or approximately one ovulation every 16-17 seconds.

2.4.4. Implementation Stage

The implementation stage of the research begins with placing 10 ml of 0% infusion (0.9% NaCl solution),

Table 1. Nutrient Content of *Acacia mangium* pods

Name of the sample	DM (%)	Nutrient Content (%DM)				
		CP	Cfat	CF	Ash	OM
Acacia pods (*Acacia mangium*)	26.90	8.03	6.25	29.50	5.44	94.56

Remark: Laboratory Analysis Results from the Animal Nutrition and Feed Science Department, Animal Science Study Program, Faculty of Agriculture, Universitas Sebelas Maret Surakarta 2021.

6% *Acacia mangium* pods infusion, and 12% infusion in Petri dishes. *Haemonchus contortus* worms were then immersed in the Petri dishes until submerged, with 10 worms placed in each dish. Observations of the worms mortality are made every hour for up to 6 hours. Dead worms will be motionless, showing no movement or stiffness. The worm mortality rate (%) was calculated for each treatment group using the observed dead worms. The observed variable is the percentage (%) of worms that died. The tannin content is standardized by converting the sample weight used to the tannin content in the dry matter form, allowing the tannin content at 6% and 12% doses to be determined.

2.4.5. Data analysis

The data of the tannin content were analyzed, and the death of adult female *Haemonchus contortus* worms at different doses was observed. Furthermore, the parameters of the anthelmintic test in vitro were the time of paralysis and the worm's death. Paralysis is defined as the absence of worm movement, except when stimulated with a spatula. The time of death is indicated when the worm still does not move even when immersed in the warm water of temperature 40–50°C, and the worms slowly lose their body-color (Bili Bora et al., 2014). The data obtained in this study were analyzed using analysis of variance with a Completely Randomized Design (CRD) in a factorial pattern (4x6), and were performed using R Studio version 1.0.136-©2009-2016. If the analysis of variance shows an effect of the treatment, a Duncan's Multiple Range Test (DMRT) (Gaspersz, 1991) is conducted.

3. Results and Discussion

3.1. Nutrient Content of *Acacia mangium* Pods

The results of the nutrient content analysis of *Acacia mangium* pods from proximate testing and Van Soest analysis can be seen in Table 1.

The analysis of the nutrient content of *Acacia mangium* pods shows different percentages of dry matter (DM), crude protein (CP), crude fat/lipid (CFat), crude fiber (CF), and organic matter (OM), as shown in Table 1. According to Klau et al. (2020), the nutrient content in feed affects livestock digestibility. Ongole (2008) also stated that digestibility is related to feed consumption, where digestibility is influenced by the nutrient content in the feed. Furthermore, Daryatmo and Hartadi (2010) mentioned that digestibility is closely related to the crude protein (CP) content and cell wall (NDF).

The chemical content of *Acacia mangium* pods is shown in Table 1. The crude protein (CP) content of *Acacia mangium* pods is higher than the minimum CP concentration (7%) required for microbial activity in fermentation (Crowder & Chheda, 1982). Meanwhile, the organic matter (OM) content of *Acacia mangium* pods (95.56%) is shown in Table 1., is in line with the OM concentration of *Acacia mangium* reported by Santoso and Hariadi (2007), which is 95.79%. In addition to the nutrients listed in the table above, *Acacia mangium* pods also contain other compounds, one of which is tannins, which can be used as an antiparasitic agent (Hadi, Handayanta, & Ngadyastuti, 2021). Santoso and Hariadi (2007) reported that Acacia (*Acacia mangium Willd*) contain secondary compounds such as saponins and tannins. Alonso-Díaz et al. (2008) stated that tannins have migratory inhibitory activity against worm larvae in goats. Tannins also have both in vitro and in vivo anthelmintic effects in sheep (Brunet & Hoste, 2006; Iqbal et al., 2007). The use of *Acacia mangium* pods, which contains tannins, has natural anthelmintic potential, and its availability in nature is abundant.

3.2. Fiber Fractions of *Acacia mangium* Pods

The NDF, ADF, and Hemicellulose content of *Acacia mangium* pods can be seen in Table 2. The results of the fiber fraction analysis of *Acacia mangium* pods show different percentages of NDF, ADF, and Hemicellulose, as shown in Table 2.

Table 2. NDF, ADF, and Hemicellulose Content of *Acacia mangium* pods

Name of the sample	Fiber Fraction (%DM)		
	NDF	ADF	Hemicellulose
Acacia pods (*Acacia mangium*)	54.50	29.81	24.69

Remark: Laboratory Analysis Results from the Animal Nutrition and Feed Science Department, Animal Science Study Program, Faculty of Agriculture, Sebelas Maret University Surakarta 2021.

Novika (2013) reported that NDF (Neutral Detergent Fiber) is the largest component of the plant cell wall that is insoluble in neutral detergent solutions, consisting of cellulose, hemicellulose, silica, and lignin. Meanwhile, ADF (Acid Detergent Fiber) is the substance that is insoluble in acid detergent solutions, consisting of cellulose, silica, and lignin. NDF in the rumen has a higher degradation rate compared to ADF because NDF contains hemicellulose, which is a more easily soluble fraction. Cellulose cannot be degraded by hemicellulolytic bacteria, while hemicellulose can be degraded by cellulolytic bacteria. If hemicellulose contains lignin, forming lignohemicellulose bonds, it becomes difficult to digest because microbes struggle to break it down. Therefore, the presence of ADF and NDF in feed formulation needs to be carefully considered.

The ADF content of *Acacia mangium* pods obtained (29.81%) is consistent with the ADF concentration of *Acacia mangium* reported by Hadi, Handayanta and Ngadyastuti (2021), which is 29.81%. The ADF content of *Acacia mangium* pods is also similar to the ADF concentration of Acacia angustissima reported by Hove et al. (2001), which is 29.81%. Furthermore, the NDF content of *Acacia mangium* pods obtained (54.50%) aligns

Table 3. Tannin Content of *Acacia mangium* pods

Name of sample	Tannin content	Unit	Method
Acacia pods (*Acacia mangium*)	18.98	%	UV-Vis Spectrophotometry

Remark: Integrated Research and Testing Laboratory, Gadjah Mada University 2022 with the NDF concentration of *Acacia mangium* reported by Santoso and Hariadi (2007), which is 50.77%. Meanwhile, the hemicellulose content of *Acacia mangium* pods obtained (24.69%) is consistent with the hemicellulose concentration of *Acacia mangium* reported by Syafii and Siregar (2006), which is 23.26%.

3.3. Tannin Content of *Acacia mangium* Pods

Leguminous plants have great potential as protein-rich forage for ruminant livestock in tropical regions. Several genera, such as *Leucaena, Sesbania, Gliricidia, Indigofera, Acacia*, and *Calliandra*, are well recognized as excellent protein sources for ruminants (Pazla et al., 2022). Acacia plants are among the leguminous tree species known as a good source of protein for livestock. This plant contains several methanol-extracted compounds, such as saponins, tannins, alkaloids, flavonoids, steroids, and phenolics (Sari & Sumadewi, 2019). Table 3 shows the tannin content of *Acacia mangium* pods.

The tannin content of *Acacia mangium* pods can be seen in Table 3. Tannins are one of the compounds that have anthelmintic effects both in vitro and in vivo in goats and sheep (Athanasiadou et al., 2001; Brunet & Hoste, 2006; Iqbal et al., 2007). The migration of worm larvae in goats can also be inhibited by tannins (Alonso-Díaz et al., 2008). The results of the research and testing of tannin levels in *Acacia mangium* pods showed a high content of 18.98%. This is in accordance with the statement by Malik et al. (2005), who reported that the tannin content in *Acacia mangium* ranges from 13 to 22% of DM. This result is also supported by Floweri (2019), who stated that the tannin content in *Acacia mangium* plants ranges from 18 to 39%.

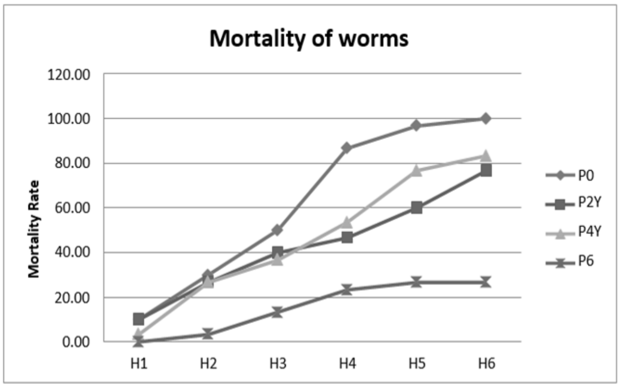
Tannins belong to the alkaloid group, which has vermifuge effects by damaging proteins in the worm's body (Maryam et al., 2018). Plants containing tannins have the potential for controlling nematodes in small ruminant livestock (Akkari et al., 2008). Condensed tannins bind to proteins in the nematode's wall, causing paralysis, making the worm inactive, and ultimately leading to its death (Min & Hart, 2003). Tannins damage the tegument, microvilli, and they exhibit ovicidial activity (Hadili, 2013; Ridwan et al., 2010; Tiwow et al., 2013). Previous studies conducted by Tiwow et al. (2013) have demonstrated that tannins exhibit ovicidial effects, inhibiting the division of egg cells. This is believed to occur as tannins bind to the outer proteinaceous layer of the worm eggs, thereby disrupting the normal cellular processes necessary for egg development and division.

3.4. Mortality of *Haemonchus contortus* Worms

The results of the observation on the mortality rate of *Haemonchus contortus* can be seen in Table 4.

The analysis in this study shows that the use of *Acacia mangium* pods infusion with different levels and incubation times significantly affected *Haemonchus contortus* ($P < 0.05$) at each level and incubation time. The mortality rate of *Haemonchus contortus* in the P2Y and P4Y treatments exhibited statistically significant differences when compared to both the negative control (0.9% NaCl) and the positive control (Albendazole), with the highest mortality observed in the P0 treatment (Albendazole). This was followed by the P4Y treatment (12% concentration), then the P2Y treatment (6% concentration), and finally, the lowest mortality was observed in the P6 treatment (0.9% NaCl). The anthelmintic efficacy of each treatment, as indicated by the respective mortality rates, is visually represented in Figure 1.

Based on the data from the use of different levels of *Acacia mangium* pods infusion, it shows that the higher the concentration or level of infusion given, the greater the effect in killing the worms, thus increasing its potential as an anthelmintic. This is in accordance with the opinion of Brunet and Hoste (2006), who stated that tannins have an in vitro and in vivo anthelmintic effect in sheep. Research by Molan et al. (2000) and Min and Hart (2003) indicated that



P0: Albendazole (positive control); P2Y: 6% level of *Acacia mangium* pods; P4Y: 12% level of *Acacia mangium* pods; P6: NaCl 0,9% (negative control)

Figure 1. Graph of the anthelmintic activity of *Acacia mangium* pods at different incubation times and levels

Table 4. Percentage of *Haemonchus contortus* Mortality by the Anthelmintic Activity of *Acacia mangium* pods at Different Incubation Times and Levels over 6 Hours

Treatments	on hour						Mean
	H1	H2	H3	H4	H5	H6	
P0 control (+)	10.00 ± 10.00	30.00 ± 10.00	50.00 ± 10.00def	86.66 ± 5.77abc	96.66 ± 5.77ab	100.00 ± 0.00a	62.22 ± 36.06a
P2Y	10.00 ± 10.00	26.66 ± 5.77	40.00 ± 0.00	46.66 ± 5.77	60.00 ± 10.00d	76.66 ± 20.81c	43.33 ± 24.00b
P4Y	3.33 ± 5.77	26.66 ± 11.54	36.66 ± 5.77	53.33 ± 5.77 de	76.66 ± 5.77c	83.33 ± 5.77bc	46.66 ± 29.30b
P6 control(-)	0.00 ± 0.00	3.33 ± 5.77	13.33 ± 15.27	23.33 ± 11.54	26.66 ± 5.77	26.66 ± 5.77	15.55 ± 13.38c
Mean (time)	5.83 ± 7.92e	21.66 ± 13.37d	35.00 ± 16.23c	52.50 ± 24.54b	65.00 ± 27.46a	71.66 ± 30.10a	

Remark: The differences in superscript on the same rows and columns indicate significant differences ($P < 0.05$)

plants containing tannin compounds can reduce the number of worm eggs, inhibit the development of nematodes, and significantly kill larvae. Furthermore, this is also in line with the findings of Hadi, Handayanta, Widyawati, et al. (2021), who studied shrubs and stated that the higher the tannin content in plants, the higher their potential as an anthelmintic. Min and Hart (2003) reported that condensed tannins bind to proteins in the nematode's wall, rendering them inactive and causing them to die.

4. Conclusion

Based on the research results and the analysis conducted, it can be concluded that *Acacia mangium* pods have an anthelmintic effect against the nematode *Haemonchus contortus* in vitro. Therefore, *Acacia mangium* pods have potential in the control of parasites in the digestive tract of small ruminant livestock. These findings suggest that *Acacia mangium* pods exhibit potential as a natural anthelmintic for controlling gastrointestinal nematodes in small ruminants. Further in vivo studies are recommended to confirm efficacy and safety.

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