

The Activity of Anthelmintic Areca Catechu Crude Aqueous Extract Against Haemonchus Contortus in The Goat (Capra Hircus) in Vitro

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ABSTRACT

Haemonchosis is a disease caused by the nematode Haemonchus contortus. This disease generally attacks ruminants, especially sheep and goats. The biggest economic losses due to this disease are mortality, decreased production, stunted growth, and low body weight. The existence of resistance to synthetic anthelmintics causes the development of research on alternative treatments to herbal anthelmintics to be a strategic step. This study aims to analyze the effect of Areca catechu on Haemonchus contortus in vitro so that the Lethal Concentration 50 (LC 50) can be determined. This study was divided into 9 groups. Group I was treated with 2.5% A. catechu crude aqueous extract; group II was treated with 5% of A. catechu crude aqueous extract; group III was treated with 7.5% A. catechu crude aqueous extract; group IV was treated with 10% of A. catechu crude aqueous extract; group V was treated with 12.5% A. catechu crude aqueous extract; group VI was treated with 15% A. catechu crude aqueous extract; Group VII was treated with 17.5% A. catechu crude aqueous extract, group VIII was a negative control (0.9% NaCl) and group IX was a positive control (Albendazole). The mortality of H. contortus was recorded every hour until the worm mortality was 100%. The results were then analyzed using the Reed and Muench method. Based on the in vitro test of Areca catechu crude aqueous extract against H. contortus it can cause mortality in worms with an Lethal Concentration 50 (LC 50) calculation result of 7.50%.

Keywords: anthelmintic, Areca catechu, Goat, Haemonchus contortus, LC50

INTRODUCTION

Indonesian animal breeding must have increasingly higher competitiveness in competing with similar products coming from abroad. The competitiveness might be different with breeding species such as big and small ruminants (cattle, dairy cows, water

buffalos, horses, goats, sheep, and non-ruminants such as pigs and fowls (Simatupang and Prajogo, 2004).

In addition to increasing the competitiveness the main target of goat breeders was to get a good enough profit of their animal breeding business. There were many determinant factors of the success in the goat and sheep breeding

business such as breed selection, forage, sheepfold, disease and management. The profit could result from daily body weight gain and meat quality. One of the obstacles in the animal breeding was parasitic disease. It was necessary for the animal breeding to effectively control gastrointestinal nematode in order to be successful. Disease control failure resulted in disease development, growth disruption and death. *Haemonchosis* was one of main parasitic gastrointestinal diseases affected goats in Indonesia. The biggest economic loss resulting from the disease included mortality, decrease in production, inhibited growth, and low body weight gain (Mengist *et al.*, 2014). According to Parson and Vere (1984) it was estimated that the economic loss resulting from *Haemonchosis* at goats in Indonesia was US\$4.7. The results of two studies at two provinces in Indonesia showed that the prevalence of the *Haemonchosis* on goats was up to 89.4% and it was estimated that the resulting annual loss was US\$1 million (FAO, 1991). Nematode worm infestation that often disrupted small ruminant production in tropical areas was *Haemonchus contortus*. The parasite lived in abomasum and had two living phases, including larvae phase outside hosts and parasite phase inside hosts. Adult female worms were able to lay 5000-8000 eggs per day (Troell *et al.*, 2005). Hypoproteinemia, hypocalcemia, and hypophosphatemia might take place in *Haemonchus contortus* worm-infected livestock and it caused the decrease in the carcass weight (Soulsby, 1986). Additionally, the worms could suck 0.05 ml livestock blood per livestock per day (Menzies, 2006).

Intensive use of anthelmintic has caused resistance problem. The resistance took place to all types of wide spectrum anthelmintic such as benzimidazole, imidothiazole-tetrahydropyridine and macrocyclic

lactone. The increase in the resistance and the increasing awareness of consumers of drug residuals-free products became the reason why anthelmintic study was the best approach in controlling helminthiasis.

The problem of the resistance to the anthelmintic might be solved using some species of plants containing tannin because it was reported that the plants could decrease the incidence of worm infestation. Min and Hart (2003) have proven that concentrated tannin was also able to bind protein and to inactivate nematode wall and then to kill it. It would indirectly bind plant protein in lumen to prevent microbial degradation and to increase protein intake into duodenum. The increase in the nutrient intake would decrease parasitic infection by improving host immunity. New anthelmintic development indicated that tanniferous plants could be considered as strategic and potential alternative to control nematode infestation in small ruminant livestock (Akkari *et al.*, 2008). Sandika *et al.* (2012) suggested that tannin could degrade protein so that there were only remaining collagen fibers and keratin layers that affected the change of cuticles into pale white and transparent ones, while saponin could irritate worm digestive tract mucous that nutrient absorption was disrupted and the worm became faint and dead (Dalimartha, 2009).

One of the innovations in finding new anthelmintic was the use of new biological source for the success in the effort to increase the performance of livestock production. There have been many biological sources used by breeders and researchers to improve the growth of their livestock. One of the biological sources that could be new alternative was *Areca Catechu* containing tannin, saponin and alkaloid. The study aimed at analyzing the effect of *Areca catechu* on *Haemonchus contortus in vitro* in order to find out LC50

as drug dose basis in further study *in vivo*.

MATERIALS AND METHOD

The Preparation of *Areca catechu* Crude Aqueous Extract

According to Daryatmo (2010) *Areca Catechu* simplicia was prepared by slicing *Areca Catechu* into thin slices and spreading out them in the sun to dry. The *Areca Catechu* slices were weighted for specified weight and concentration, which were 10 grams and 10%. They were poured into beaker glass. And then, 100 ml of aquades was poured into the beaker glass containing the *Areca Catechu* slices. After that, the beaker glass was put into oven at 90 °C for 15 minutes. The remaining liquid in the beaker glass was taken and filtered to obtain 10% *Areca catechu* Crude Aqueous Extract.

The Collection of *Haemonchus contortus*

Adult worms were directly collected from abomasum of naturally infected goats. The abomasum adjacent to rumen and duodenum was cut and tied using cord. Its content was carefully removed and observable parasites were collected and put into a container containing NaCl (Kuchai *et al.*, 2012).

The *In Vitro* Test of the Effect of the *Areca catechu* Crude Aqueous Extract on the *H. contortus*

The test was carried out following Peterson *et al.* (1997) cited in Alemu *et al.* (2014). There were 90 adult worms classified into 9 treatment groups with each petri dish containing 10 worms.

Each of the groups was soaked in *Areca Catechu* infuse at the following concentrations: group I at the concentration of 2.5%, group II at the concentration of 5%, group III at the concentration of 7.5%, group IV at the concentration of 12.5%, group VI at the concentration of 15%, group VII at the concentration of 17.5%, group VIII serving as negative control (NaCl 0.9%) and group IX as positive control (Albendazole). The mortality of the worms in each of the groups was observed hourly till there was a group with 100% mortality. The results were then analyzed using Reed and Muench (1938) method to obtain LC50.

Data Analysis

Data were analyzed using Reed and Muench (1938) method to obtain LC50.

RESULTS AND DISCUSSION

The mortality of the worms *H. contortus* in goats was recorded hourly till 100% mortality. The results of the study showed that the *Areca catechu* Crude Aqueous Extract at the concentration of 2.5% in 4 hours did not cause any death. The anthelmintic activity of the *Areca catechu* Crude Aqueous Extract showed that the highest mortality took place in the group with the treatment of the *Areca catechu* Crude Aqueous Extract at the concentration range of 12.5% to 17.5%. The data of the mortality of the worms resulting from the *in vitro* test were analyzed using Reed and Muench method (Table 1).

Table 1. The calculation of the mortality of the *Haemonchus contortus* in goats *in vitro* using Reed and Muench method (1938).

Concentration (%)	Dead	Live	Accumulation			Mortality ratio	Mortality percentage (%)
			Dead	Live	Total		
2,5	0	10	0	25	25	0/25	0
5	2	8	2	15	17	2/17	11.76
7,5	5	5	7	7	14	7/14	50
10	8	2	15	2	17	15/17	88.23
12,5	10	0	25	0	25	25/25	100
15	10	0	35	0	35	35/35	100
17,5	10	0	45	0	45	45/45	100

Source: Processed data of 2021

The mortality percentages above and below 50% were 11.76% and 88.23%, while the concentrations above and below 50% were 5% and 10%. The LC50 was calculated as follows:

$$\begin{aligned}
 10 - 5 &= 5 \\
 88.23 - 50 &= 38.23 \\
 88.23 - 11.76 &= 76.47 \\
 X : 5 &= 38.23 : 76.47 \\
 76.47 X &= 5 \times 38.23 \\
 X &= 2.499 \\
 \text{LC50} &= 10 - 2.499 \\
 &= 7.50 \%
 \end{aligned}$$

Areca Catechu contained tannin. The tannin could bind protein and inactivate nematode wall and kill it (Athanasiadou, 2001). The tannin contained in leguminous plants was condensed one (Min and Hart, 2003). The condensed tannin effectively controlled gastrointestinal parasites. The effect of the condensed tannin on the gastrointestinal parasites took place directly and indirectly. It took place directly through condensed tannin-nematode interaction, by affecting hatching process and infective larvae growth. It could bind protein so that the nematode wall was inactive and finally it died. It took place indirectly as the tannin bound plant protein in rumen to prevent microbial degradation and it would increase the intake of the protein into duodenum. Min and Hart (2003) have proven that the increase in the protein

nutrient would decrease parasitic infection by increasing host immunity.

The results of the study by Mubarokah *et al.* (2019) showed that the *Areca catechu* Crude Aqueous Extract concentration that gave the best result in killing the worm *A. galli* was 25% *in vitro* and the lethal concentration of the *Areca catechu* Crude Aqueous Extract was 21.18% (Mubarokah *et al.*, 2018). It was because of the tannin content of the *Areca catechu* Crude Aqueous Extract that was considered to be able to damage the protein of the worm cuticle and finally it caused the death of the worm. The results of the study of the worm protein profile showed that the worm *A. galli* treated using *Areca catechu* Crude Aqueous Extract gave less protein bands than the negative control (no treatment) (Mubarokah *et al.*, 2019).

CONCLUSION

Based on the *in vitro* test of the effect of the *Areca catechu* Crude Aqueous Extract on the *Haemonchus contortus* in sheep, the resulting LC50 was 7.50%.

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