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Effect of *Veitchia merrillii* Extract on Mortality and Tegument Structure of *Fasciola gigantica*

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ABSTRACT
This study was conducted to explore the effect of methanolic extract of *Veithcia merrillii* on mortality time and histopathological changes of *Fasciola gigantica* in vitro. This study used 25 adult flukes of *F. gigantica*, divided into five groups: C0 as negative control (0.9% PBS), C1 as positive control (0.024mg/ml albendazole), and treatment groups were P1, P2, and P3 used 10%, 25% and 50% extract, respectively. The observation was carried out every 15 minutes to monitor the motility and mortality of each treatment group. Mortality time of each group was determined by score index and analyzed descriptively. Dead flukes from each group were then prepared for histological examination. The results revealed that 50% of *V. merrillii* extract (P3) had the same mortality time as C1, where all the flukes died after 30 minutes of treatment. Group P2 had a longer mortality time at 45 minutes and P1 was at 75 minutes. Histopathological observation showed alteration on some organs for C1 and treatment groups, including separation of flukes tegument from parenchyma, vacuolization, and disintegration of testis. From the results it can be concluded that methanolic extract of *V. merrillii* at 25% and 50% concentration was effective as anthelmintic on *F. gigantica* in vitro.

Keywords: *Plant extract, Anthelmintic property, Mortality time, Histopathology examination.*

1. INTRODUCTION

Fascioliasis due to liver flukes in cattle and other livestock is primary problems in Indonesia. The infection rate is ranged between less than 10% until up to 95%, depends on area and availability of intermediate host *Lymnaea rubiginosa*. This extremely high prevalence leads *Fasciola gigantica* as one of the most important infectious agents in cattle and in human as this disease can be transmitted to human.

Fasciola gigantica is a trematode belongs to Family Fasciolidae, and resides in the bile duct and liver of ruminants, resulting in acute parenchymal hepatitis and chronic cholangitis. These flukes will interfere the metabolism of lipid, proteins, and carbohydrate which cause growth disorder, low body weight, anemia, and even death. This fluke usually infects cattle, buffalo, sheep, goat, and other ruminants [1-3]. Mehmood *et al*. [4] reported that fascioliasis caused financial losses approximately over 3.2 US$ billion/year worldwide.

The disease management for fascioliasis was originally by giving anthelmintic drugs, however repeat treatments would develop drug resistance and accumulation of drug residue in tissue organs [5-6]. Therefore a less risk treatment approach is necessary, such as using medicinal plants as anthelmintic against fascioliasis in livestock. Herbal medicine has several advantages over chemical drugs such as low cost, easy to obtain, abundant availability in nature, slight side effect, and less resistant. There are various plants which have been investigated for anthelmintic property, including *Veitchia merrillii* [7], *Curcuma aeruginosa* [8], *Cymbopogan nardus* and *Azadirachta indica* [9], *Nigella sativa* [10-11], and many more. Balqis *et al*. [7] explored the use of *V. merrillii* as anthelmintic drug on *Ascaridia galli* adult worm,

however not much information is available on the use of this plant extract on trematodes, especially on liver flukes *F. gigantica*. In this study we investigated the effect of *V. merrillii* nuts extract on motility, mortality time, and histopathological changes, with specific attention to tegument of *F. gigantica.*

2. MATERIALS AND METHODS

2.1. Plant Extraction

Nuts of *V. merrillii* were obtained from the farm in the vicinity of Universitas Syiah Kuala (USK). The nuts were peeled, washed, and dried. The dried nuts were pounded into powder and extracted with maceration using 96% methanol at room temperature. The filtrate collected was concentrated using vacuum rotary evaporator. The crude extract was diluted with 1xPBS to 10%, 25%, and 50%.

2.2. Anthelmintic Examination

Anthelmintic potency of *V. merrillii* extract was examined using five different groups, containing five flukes. Twenty five healthy, adult *F. gigantica* flukes were obtained from the liver of infected cattle in Banda Aceh abbatoir, and stored in PBS.

Flukes were placed in petri dishes and divided into P1, P2, and P3 which were given 10%, 25%, and 50% *V. merrillii* extract, respectively. The same experiment using PBS and 0.024 mg/mL albendazole were used as the negative (C0) and positive (C1) controls, respectively. The motility of flukes was recorded and scored according to Kuichi *et al.* [12] and Jiraungkoorskul *et al*. [13]: Score 3= the whole body was moving after a certain period of time, score 2= only parts of body moving, score 1= immobile but alive, and score 0= dead. Observation was conducted every 15 minutes until all flukes died. Dead flukes were further subjected to histopathological inspection.

2.3. Histopathology Observation

To understand the structure and organ changes caused by *V. merrillii* extract, histopathology examination was carried out according to Kiernan in Kmiec [14]. The flukes were fixed with 10% formalin, dehydrated in ascending concentration of ethanol. After clearing process in xylol I, II, and III, the samples were infiltrated in liquid paraffin, and embedded in paraffin block. The tissue block was cut at 5 µm thickness, stained with hematoxylin and eosin, and observed using light microscope (Olympus, Tokyo, Japan).

2.4. Data Analysis

Data were analyzed descriptively.

3. RESULTS

This experiment was done to find out the efficacy of methanolic extract of *V. merrillii* nuts on the motility, mortality time, and tegument structure of *F. gigantica in vitro*. Before treatment, the flukes were active, moving easily in PBS, and had reddish brown colour.

The methanolic extract of *V. merrillii* nuts had brownish colour and specific odour. Balqis *et al*. [7] reported greenish brown color of the extract of *V. merrillii* nuts. The slight different colour could be the results of different harvesting time.

3.1 Motility and Mortality Time of Fasciola gigantica

After 15 minutes soaking in different solution, motility of *F. gigantica* flukes was scored. In P1 group with 10% extract, the score were 2 and 1. In this group, three flukes were partly moving, and two flukes were immobilized but still alive. After 30 minutes, 4 flukes were immobilized (score 1), and after 75 minutes all the flukes were dead (score 0). For P2 with 25% extract, 1 fluke was partly moving (score 2), and 4 flukes were immobilized after 15 minutes (score 1). After 30 minutes, 1 fluke was immobilized and 4 flukes were dead. After 45 minutes all flukes were dead. In P3 with 50% extract and positive control had similar score index, where the flukes were immobilized in 15 minutes, and all were dead after 30 minutes. Motility score index of each group is presented in Table 1.

3.2 Histopathology Features of Fasciola gigantica after Treatment

The dead flukes were examined for histopathological changes of tegument and internal organs to check for abnormalities. In C0 group, the tegument was still intact and integrated with parenchyma (Figure 1.1). In treatment groups (P1, P2, P3), tegument was separated from parenchyma, the gap was farther with higher concentration (Figure 1.2-1.4). In C0 group, the sperm cells were clearly formed, while in P3, there was a decrease of sperm cell in testes (Figure 2.5-2.7), and vacuole was formed (Figure 2.8). The changes caused by *V. merrillii* extract were similar to C1 indicated by vacuoles formed within parenchyma (Figure 2.9) and by decreasing of sperm cell in testes (Figure 2.6).

Table 1 Motility score index of *Fasciola gigantica* after treatment

Treatment	Minute 15				Minute 30				Minute 45				Minute 75			
group	3	2	1	0	3	2	1	0	3	2	1	0	3	2	1	0
C0	5				5				5				5			
C1			5					5				5				5
P1		3	2			1	4				5					5
P2		1	4				1	4				5				5
P3			5					5				5				5

4. DISCUSSION

Motility and mortality time of flukes were important in determining efficacy of anthelmintic substances, as they would explain the ability of anthelmintic to deteriorate and finally kill the target flukes. From this study we assumed that the 50% *V. merrillii* extract (P3) had higher potency than other concentrations. This assumption was made based on the facts that both C1 and P3 treatments caused the flukes mortality within 30 minutes. Jeyathilakan *et al*. [15], who conducted similar research using *Allium sativum*, reported that 100 µg/ml extract of *A. sativum* was effective in inhibiting and killing *F. gigantica*. Furthermore, Vanda *et al*. [8] described that 25% and 50% *Curcuma aeruginosa* extract had the ability to kill *F. gigantica* within 48 and 60 minutes, respectively. Apparently, our results weres more comparable to the report of Vanda *et al*. [8] than to the report of Jeyathilakan *et al*. [15].

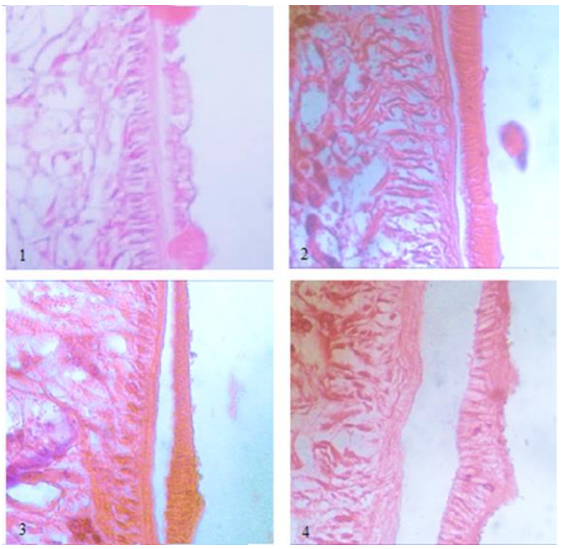

Figure 1. Tegument of *Fasciola gigantica*. 1. Negative control. 2. 10% *Veichia merrillii* extract. 3. 25% *Veitchia merrillii* extract. 4. 50% *Veitchia merrillii* extract.

Histopathological features of *F. gigantica* showed that tegument was severely affected by the extract, the spine missed and tegument disintegrated from parenchyma. Tegument plays major roles in *F. gigantica* especially for protection, respiratory regulation, nutrient absorption and secretion, and host immune system suppression [16]. Destruction of tegument will harm *F. gigantica* and lead to mortality.

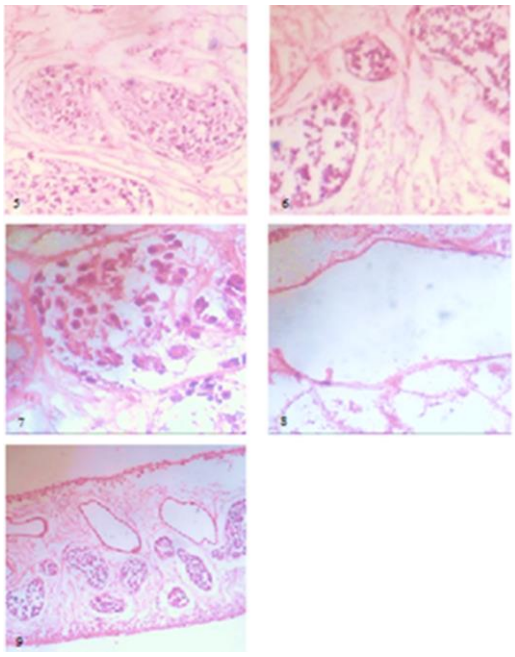

Figure 2. Testes of *Fasciola gigantica*. 5. Negative control (normal). 6. Positive control, disappearing of sperm cell. 7. 50% *V. merrillii* extract, decreasing of sperm cell. 8. 50% *V. merrillii* extract, forming of vacuole within parenchyma. 9. Positive control, forming of vacuole within parenchyma.

A study by Balqis *et al*. [7] showed that *V. merrillii* extract might cause the cuticular damage of *A. galli in vitro*. Cuticle is crucial in nematodes, functioning as protective and selective absorption. Destruction of

cuticle will then cause nematode impairment and eventually death. Cuticle in nematodes and tegument in trematodes have similar function as first line protection against host immune system.

Another organ of *F. gigantica* impacted by *V. merrillii* extract was reproductive organ. The sperm cells decreased in testes, meaning that this extract penetrated inside the flukes and damaged the internal organs as well. According to Balqis *et al.* [7], *V. merrillii* extract also had the ability to destruct female reproductive organs by dissociating and shrinkage of egg cells in the uterus of *A. galli*. Jeyathilakan *et al.* [15] reported that the use of *A. catechu* extract on *F. gigantica* caused separation of tegument from parenchyma, degeneration of epithelial cells of the intestine, and formation of vacuoles within parenchyma. They also reported that the extract of *Z. officinale* had an ability to disrupt tegument of *F. gigantica*, and the extract of *C. nardus* damaged the tegument, testes, and intestine villi.

Anthelmintic property of *V. merrillii* extract was attributed to the chemical composition which contains tannins, alkaloids, flavonoids, triterpenoids, and saponins [7]. Saponin may act as cytotoxic substance that disrupts cell membrane and interferes ionic balance which lead to cell death [17], while alkaloids may obstruct cellular membrane transport system and cause destabilization of membrane cell [18]. These two chemicals along with other components in the extract will potentiate anthelmintic activity of *V. merrillii* extract by damaging tegument and internal organs, and eventually cause death of *F. gigantica*.

5. CONCLUSION

The methanolic extract of *V. merrillii* nuts has a potential anthelmintic activity on *F. gigantica in vitro* by affecting the motility and mortality time of the fluke. The higher extract concentration given, the better anthelmintic potency. The extract also cause severe destruction on the flukes due to its ability to break tegument and harm reproductive organs. This extract is highly recommended for further study.

AUTHORS' CONTRIBUTION

HV, FA, and UB conceptualized and designed this research. The research was carried out by RAA, and MD. The manuscript was written by HV, SRA and FF.

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