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Anthelmintic Activity Test of Young Areca Nut (*Arecha catechu L.*) Extract Against *Fasciola sp.* Worm Eggs in Goat Feces In Vivo

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ABSTRACT

Worm infections in livestock can have a huge risk impact such as decrease in growth, body weight, meat quality and death. This study aims to determine the anthelmintic activity of young areca nut extract (*Areca catechu L.*) against Fasciola sp. worm eggs in goat feces in vivo and to determine at what concentration the young areca nut extract (*Areca catechu L.*) is effective in providing anthelmintic effects against worm eggs. The study used an experimental method with a paired t-test of 5 treatments and 4 replications. The treatments given were P0 (negative control), P1 (10% areca nut extract), P2 (20% areca nut extract), P3 (30% areca nut extract) and P4 (positive control using kalbazen worm medicine). Parameters were reduction in the number of dead worm eggs, lethal concentration, effectiveness of areca nut extract against worm eggs. The results of the study showed that there were *Fasciola sp*. worm eggs in all tested goats. Treatment with young areca nut extract (*Arecha catechu L.*) can significantly reduce the number of *Fasciola sp*. eggs ($P < 0.05$), with an LC50 result of 26.75%. The best Fecal Egg Count Reduction Test (FECRT) result in the administration of areca nut extract with a concentration of 30% (P3) of 56.75% was the same as that given treatment using kalbazen worm medicine and the lowest FECRT result in the administration of areca nut extract with a concentration of 20% (P2) of 29%.

Keyword: Anthelmintic, Areca Nut Extract, *Fasciola sp.*, Goat, Worm Egg

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1. Introduction

Animal husbandry is one of the sectors that plays an important role in the lives of Indonesian people. Ruminant livestock such as goats have an important role for human food needs. Goats also have a high economic value in life, and have many benefits, namely being able to meet the needs of animal protein, milk producers, and their feces can be used as fertilizer. In addition, animal husbandry also has an important role in national economic development, this is due to the increasing demand for animal protein along with the increase in population, increase in income and increase in public awareness to consume highly nutritious food [1].

Livestock raised in modern and traditional ways cannot be separated from the constraints of disease due to parasitic worms. Worm infections in livestock can have a huge risk impact, where the decrease in growth and body weight reaches 38% with a mortality rate of up to 17% and a decrease in meat quality [2]. Whereas in the digestive tract, worms can damage the intestinal mucosa which can result in inhibition of the digestive system in carrying out its functions and disrupt the metabolic system [3]. Therefore, worm infections cause huge economic losses, so worm infections are often called economic diseases [4].

Worm infections can be caused by the maintenance system, and the cleanliness of the cage or cage sanitation [5]. And it can also occur through feed and drink contaminated by worm larvae then enter the body of livestock through the mouth along with the swallowing of food consumed, and the time of taking forage [6]. The persistence of worm parasites in the body of livestock is caused by uncontrolled deworming [7].

Worm infections can be treated by giving anthelmintics, usually farmers give anthelmintics derived from synthetic drugs. This can cause side effects in livestock such as the emergence of worm parasites that are resistant to anthelmintics and residues of livestock products. To avoid this, some plants that grow nearby can be used as anthelmintics. Areca nut can be a possible alternative for worm infections because it has antioxidant and antimutagenic, astringent, and anthelmintic effects. This is the background to test the anthelmintic activity of young areca nut extract (*Areca catechu L*.) against *Fasciola sp*. worm eggs in goat feces in vivo.

2. Materials and Methods

2.1. Place and Time

The research was conducted in Phytochemistry Laboratory, Faculty of Pharmacy, Universitas Sumatera Utara and Parasitology Laboratory, Veterinary Center Medan, North Sumatra. This research ran for 3 months, namely November 2023 - January 2024.

2.2. Materials and Equipment

The tools used in this study are spatulas for taking feces, mortars and pestles as stirring containers, sieves as feces filters, 50ml centrifuge tubes, slow centrifuge, scales, pipettes, glass slides, *glassbeads* size 0.59 - 0.71 mm, mess 60 filters, aspirators and microscopes to observe eggs.

The materials used in this study were areca nut as a traditional medicine, goat feces as a sample for egg collection, goats as research samples, and distilled water as an extract solvent.

2.3. Research Methods.

This research was conducted experimentally with a community field trials design that divided the community into two groups, namely the group before and the group after treatment [8].

This study consisted of 5 treatments 4 replicates, the number of replicates in this study was determined using the calculation:

$T (n -1) \geqslant 15$

$5(n-1) \geqslant 15$

$5n - 5 \geqslant 15$

$n \geqslant 4$

The treatments tested in this study were

P0 = 0mg/ml areca nut extract (Control)
P1 = 10mg/ml areca nut extract
P2 = 20mg/ml areca nut extract
P3 = 30mg/ml areca nut extract
P4 = kalbazen anthelmintic (positive control)

2.4. Research parameters

2.4.1. Identification of worm egg types

Goat feces samples were taken and carried out qualitative tests with the *glass beads* sidementation method to determine the number of worm eggs and identified using a microscope by looking at the guidebook to determine the type of worm eggs [9].

2.4.2. Reduction in the number of dead worm eggs

The formula for calculating FECRT is as follows:

$$FECRT (\%) = 100 \times [1-(T2/T1)].$$

Description:
FECRT = *Fecal Egg Count Reduction Test*
T1 = Mean *pre-treatment* FEC in the treated group
T2 = Mean *post-treatment* FEC in the treated group.

2.4.3. Lethal concentration

The lethal concentration of young areca nut extract against *Fasciola sp*. worm eggs can be known by using the calculation of $_{LC50}$ (Lethal Concentration 50) through probit regression analysis. $_{LC50}$ is used to determine at what concentration the young areca nut extract can kill 50% of *Fasciola* sp. $_{LC50}$ is obtained by calculating the mortality (% death) of the sample at each concentration. Percent mortality is obtained from the result of multiplying the ratio by 100%, which is the dead sample divided by the number of initial samples multiplied by 100% for each replicate [10]. Probit regression analysis in this study using SPSS *software*

2.4.4. Effectiveness of areca nut extract against worm eggs

Analysis of the effectiveness of areca nut extract was carried out using the paired t test, paired t test is used to determine the difference between samples before treatment and after treatment [11]. Statistical analysis in this study used the help of SPSS *software*

3. Result and discusion

3.*1 Identification of worm egg species*

In this study, a total of 20 goat feces samples were collected and examined in the laboratory, all were detected positive for worm eggs. Five worm egg species were found, belonging to the *Trematoda* and *Nematoda* worm families. The species of worm eggs found from the *Trematoda* class were *Fasciola sp*. and *Paramphistomum sp*. While the species of worm eggs from the *Nematode* class found in the examined samples were *Trichuris sp*., *Strongyloides sp.*, and *Bunostomum sp.*

Table 1. Worm egg species found in the samples

Class	Species	Infected samples (tails)	Prevalence (%)
Trematoda	*Fasciola sp*.	20	100
	Paramphistomum sp.	9	45
Nematoda	*Bunostomum sp.*	4	20
	Strongyloides sp.	3	15
	Trichuris sp.	1	5

Based on Table 1, it can be seen that the largest percentage of worm eggs is *Fasciola sp.* with a percentage of 100%, the second largest worm egg is *Paramphistomum sp.* with a percentage of 45% and both worms are from the *Trematoda* class . The smallest worm egg percentage was Trichuris sp. with a percentage of 5% or the same as infecting only 1 goat.

The highest prevalence of worm egg infection studied was *Fasciola* worms from the *Trematoda* class found in all test samples. *Fasciola* has a long life cycle and is zoonotic. Worm eggs that come out with feces will develop into embryonated eggs within 9-15 days if they find water / puddles with suitable temperatures between 23-26 ºC *Fasciola* can infect hosts through food. Hosts that eat wet grasses containing *Fasciola* eggs are carried by the snail *Lymnaea sp*. The snail carries *cercariae* from eggs ingested with food. Infection can also occur from cattle drinking water from streams that contain eggs carried by the snails. Once the cercariae find a host, they travel to the small intestine where they become mirasidiums that will develop and travel to the host's liver [12].

According to [13], *Paramphistomum sp*. worms from the trematode class require snails as intermediate hosts, then infestation of the definitive host occurs when livestock eat grass or drink water containing metacercariae of the worm. *Paramphistomum sp.* is a worm from the trematode class with the second highest percentage found in the test samples. This worm has a life cycle that requires an intermediate host to thrive. The cause of the high percentage of *Paramphistomum sp*. worms is that the worms develop in the rumen then become adults and bite the rumen mucosa and can survive for a long time.

3.2 Number of fasciola sp. eggs reduction

FECRT is a test that compares the number of eggs before and after *anthelmintic* administration. *Faecal Egg Count Reduction* (FECR) includes the calculation of FEC mean, variance, upper, and lower 95% confidence limits according to the recommendations of the WAAVP (World Association for the Advancement of Veterinary Parasitology) guidelines for evaluating the effectiveness of *antihelminths* in ruminants.

Table 1. Reduction in the number of eggs of *Fasciola sp.* (%)

Treatment	Repeat				Mean ± SD
	1	2	3	4	
P0	-1	-42	-14	-31	-21,94^{a}± 18,226
P1	33	14	54	25	31,50^{b}±16,902
P2	48	18	36	14	29,00^{b}±15,875
P3	48	0	79	100	56,75^{b}±43,446
P4	3	75	64	85	56,75^{b}±36,845

Notes: Different letters in the same column indicate significant differences (P>0.05).

Based on Table 2, it can be seen that the dosing of areca nut extract in goats is able to significantly affect the reduction in the number of *Fasciola Sp.* The provision of areca nut extract with a concentration of 30% (P3) is the provision with the largest percentage of reduction in the number of *Fasciola Sp.* eggs with an average percentage of 56.75% as large as the administration of kalbazen anthelmintic (P4). Dosing of areca nut extract with a concentration of 20% (P2) was the lowest concentration of all treatments. Giving areca nut extract with a concentration of 20% can reduce the number of *Fasciola Sp.* eggs by 29%.

The best treatment in the reduction of *Fasciola Sp*. eggs was at a concentration of 30% areca nut extract (P3). The 10% concentration (P1) reduced more *Fasciola Sp*. eggs than the 20% concentration (P2). This is because the quantity of Fasciola Sp. eggs observed in P1 is less than P2. According to [14], areca nut seeds contain alkaloid, flavonoid, tannin, saponin, and polyphenol compounds which are known to have antibacterial properties. Alkaloids and saponins can show antagonistic activity, as evidenced by testing the inhibitory activity of each fraction at 80%, which was reduced to 53% after combining the two fractions [15]. Thus, the high dose did not result in a decrease in *egg per gram* levels compared to the low dose.

3.3 Lethal concentration

The lethal concentration of young areca nut extract against *Fasciola sp*. eggs can be known by using the calculation of $_{LC50}$ (*Lethal Concentration* 50) through probit test. The average lethal concentration ($_{LC50}$) generally describes the concentration of a particular chemical that causes death in 50% of

organisms in a given population under a defined set of experimental conditions [16]. The $_{LC50}$ calculation can be seen in the following Table 3.

Table 2. Lethal Concentration 50 of Areca Nut Extract

Probability	95% Confidence Limits for konsentrasi		
	Estimate	Lower Bound	Upper Bound
0.45	24.372	16.974	45.610
0.5	26.577	19.212	52.807
0.55	28.782	21.157	60.298
0.6	31.022	22.943	68.100

Based on Table 3, it can be seen that the LC50 of young areca nut extract is 26.57% obtained from the results of probit regression analysis on the concentration and percentage of dead *Fasciola sp*. eggs. The relationship between the concentration level of the dose given with the percentage of dead worm eggs by 95% means that at a concentration of 26.57% young areca nut extract is able to kill 50% of *Fasciola sp*. worm eggs invivo. This is not much different from the results of research on areca nut extract against *Ascaris lumbricoides* and *Ascaridia galli* worms in vitro by [17] that the LC50 value of areca nut ethanol extract is 27.11%, which means that the effective concentration of areca nut can cause paralysis in 50% *Ascaridia galli.*

This anthelmintic ability is related to the content of tannin compounds from areca nut extract that can inhibit enzymes, and damage membranes [18]. The inhibition of enzyme work can cause the digestive metabolic process to be disrupted so that the worms will lack nutrients and eventually the worms will die due to lack of energy. Worm membranes that are damaged by tannins cause worms to paralyze which eventually die. Tannins are generally derived from polyphenolic compounds that have the ability to precipitate proteins by forming water-insoluble copolymers [19]. Tannins also have ovicidal activity, which can bind to worm eggs whose outer layer consists of proteins so that cell division in the egg will not take place in the end the larvae are not formed.

[20] stated that tannin extracts from L. cuneata plants can reduce the development of nematode worm larvae by 91%, reduce the number of eggs that hatch by 34% and reduce the motility of the larvae by 30%. A previous study showed that goats consuming L. cuneata containing tannins significantly reduced the number of worm eggs compared to goats consuming control feed that did not contain tannins. It was found that plants containing 5% tannin extract can reduce larval contamination and can be used as anthelmintics.

3.4 Effectiveness of areca nut extract

The effectiveness of areca nut extract was calculated using the paired t-test. The paired t-test is used to see significant differences between two conditions in the same group. In this study, it was used to see whether the administration of areca nut extract significantly reduced the number of *Fasciola sp* eggs.

Table 3. Paired t test result

	95% Confidence Limits for konsentrasi				
	Mean	Standard deviation	t	Free degree	p-value
Before - After	17,650	19,754	3,996	19	0,001

Notes: There is a significant difference between groups (P<0.05).

Table 4 shows that the p-value of 0.001 is smaller than 0.05, indicating that the difference between the conditions before and after treatment is statistically significant. This means that the administration of areca nut extract in the dose tested can have a strong enough effect to significantly reduce the number or level of *Fasciola sp.* infection in goats.

The value of P<0.05 indicates that areca nut extract has anthelmintic activity strong enough to interfere with the development or reduce the number of *Fasciola sp* . eggs significantly. The active ingredients in areca nut seeds, such as aracholine, tannins, flavonoids, and alkaloids have been known to have atelmintic potential, which works by paralyzing or killing parasites through toxic effects on parasite cells or metabolic systems [21][22].

The alkaloid and tannin content in areca nut extract is thought to inhibit essential enzymes needed by the parasite to develop, resulting in a decrease in the viability of *Fasciola sp*. Previous studies have shown that tannins can damage the parasite cell wall and cause leakage of cell contents, which can inhibit the development of eggs into larvae [23].

Several studies compared the effectiveness of herbal plants, including areca nut seeds, with synthetic anthelmintics. A meta-analysis conducted by [23] showed that although herbal anthelmintics have lower effectiveness compared to synthetic drugs, the advantage of herbal anthelmintics is in terms of safety and potential reduction of parasite resistance to drugs. This indicates that the use of areca nut as a natural anthelmintic can be a good option.

4. Conclusion and Suggestion

4.1 Conclusion

The utilization of areca nut extract as a natural anthelmintic is able to reduce the type of *Fasciola sp*. eggs in vivo with an LC^{50} value of 26, 57%. The provision of areca nut extract with a concentration of 30% is the best result by being able to reduce *Fasciola sp*. eggs by 56.75%, the same as those given kalbazen deworming treatment.

4.2 Suggestion

It is recommended to conduct further testing with various doses and combinations of other herbs, and conduct more specific analysis of active components to determine which compounds are most responsible for the anthelmintic effect.

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