Efficacy of calliandra (*Calliandra calothyrsus*) leaf extract on *Haemonchus contortus* mortality in vitro

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**Abstract.** Calliandra (*Calliandra calothyrsus*) is a feed plant that contains high tannins. Tanin in calliandra leaves can cause death in nematodes. The purpose of this study was to determine the effectiveness of calliandra leaf extract, comprising optimum concentration and time, on mortality of *Haemonchus contortus* nematode. Calliandra leaves were extracted by 70% ethanol then analyzed for the tannin content. Twenty-five adult *H. contortus* nematodes were used for mortality test in vitro with a gradual concentration of calliandra leaf extract. The study used a completely randomized design with a factorial pattern consisting of six treatments and four replications. The treatment groups were as follow: R0 as negative control group given normal saline (0.9% NaCl), R1 as positive control (albendazole 10 mg/ml), R2 (10% calliandra leaf extract), R3 (25% calliandra leaf extract), R4 (50% calliandra leaf extract), and R5 (100% calliandra leaf extract). Extraction of calliandra leaves using 70% ethanol produced a tannin content of 5%. In vitro examination showed that administration of the calliandra extract had a significant effect (P<0.01) on mortality of *H. contortus* and showed an interaction between treatment and mortality time. A higher concentration of calliandra extract caused faster mortality of *H. contortus*. The concentration of 100% calliandra extract caused 100% mortality after 3 hours, while the concentration of 10%, 25%, and 50% caused 100% mortality after 4 hours. This study showed that calliandra leaf extract had an anthelmintic efficacy on *H. contortus*.

1. **Introduction**
Nematode infestation highly affects livestock productivity by causing weight loss, reproductive disorders, and mortality. *Haemonchus contortus* is a blood-sucking nematode of ruminants recognized as the economically important parasite worldwide [1]. The infection, called haemonchosis, caused anemia since each worm can suck the blood of 0.049 ml/day [2], and lead to various other effects. The worm infests in the abomasum, [3] reported that 20,000–50,000 worms were found in hyperacute infection causing death in sheep.

Nematode infestation generally treated by anthelmintic drugs. The use of chemical anthelmintics was indeed effective, but caused side effects to the livestock and not economically efficient. Albendazole, a derivative of benzimidazole, is one of the anthelmintic drugs that widely used by farmers. This drug works by affecting the intestinal cell of the nematodes. However, the excessive use of anthelmintic was reported to cause resistance in calves and small ruminants. Nematodes which were resistant to benzimidazole had been reported as a result of repeated use of this anthelmintic [4].

Anthelmintic active compounds can be found in several plants in Indonesia. The compounds are secondary metabolites, i.e. alkaloids, saponins, tannins, phenolics and flavonoids which had...
antibacterial and antifungal activity, also an anthelmintic activity which was obtained from tannin content [5]. In vitro research carried out using ethanol extract of pare (Momordica charantia L.) leaf showed the anthelmintic effect on female Ascaris suum nematodes [6]. Study on the ethanol extract of basil (Ocimum americanum Linn.) leaf showed the anthelmintic effects on H. contortus with the optimum concentration obtained was 10% [5]. Tannin compounds worked by damaging membranes and inhibiting enzymes. Calliandra is a feed plant belongs to the family of legumes which has a high tannin content of more than 10% [7]. The high content of tannins in this plant was potential as anthelmintics. Further, [8] suggested that Tannin worked by damaging microvilli, damaging tegument [9], and having ovicidal activity [10]. Tannin extracted from L. cuneata plants reduced the development of nematode worm larvae (L3) to 91%, reduced the number of eggs hatched up to 34% and reduced motility of L3 larvae to 30% [11].

The treatment of nematode infestations by chemical anthelmintic was concerned to cause resistance and also economically inefficient for farmers. Anthelmintic active compounds from plants, including calliandra (Calliandra calothyrsus) leaf might become an alternative for helminthiasis treatment. This study examined the in vitro efficacy of the ethanol extract of calliandra leaf on mortality of H. contortus.

2. Methods

2.1. Calliandra leaf extraction

Calliandra leaves were washed, separated from the leaf bone, then sun drained. Dry simplicia was pollinated and sifted to obtained calliandra leaf powder. Further, 70% of ethanol was added in the ratio of 1:10, soaked for 2×24 hours and stirred occasionally. The mix then filtered by filter paper. The filtrate then evaporated using a rotary evaporator (40ºC dan 50 rpm) [12] to obtained final calliandra leaf extract product.

2.2. Haemonchus contortus preparation and treatments

A total of 120 adult H. contortus were used in this study. The worms in cattle abomasum were obtained from slaughter house. The study used a completely randomized design with factorial pattern consisting of six treatments and four replications. The treatment groups were as follow: R0= negative control group given normal saline (0.9% NaCl, 25 ml), R1= positive control (albendazole 10 mg/ml, 25 ml), R2=10% calliandra leaf extract (w/v, 2.5 g calliandra leaf extract + 25 ml NaCl 0.9%), R3=25% calliandra leaf extract (w/v, 6.25 g calliandra leaf extract + 25 ml NaCl 0.9%), R4=50% calliandra leaf extract (w/v, 12.5 g calliandra leaf extract + 25 ml NaCl 0.9%), and R5=100% calliandra leaf extract (w/v, 25 g calliandra leaf extract + 25 ml NaCl 0.9%).

2.3. In vitro observation

In vitro observation was carried out by placing the worms in 24 petri dishes. Each dish consisted of five worms and filled with the solution according to the treatment groups. The observation was carried out every 15 minutes for 6 hours to determine mortality. The worms were considered dead if did not show any signs of life, for instance, they did not move when given movement stimulation in the solution, and or did not respond when touched by anatomical tweezer. Each treatment dish was filled with 0.9% NaCl 25 ml. Group R0 a negative control, R1 filled with albendazole 10 mg/ml as a positive control. Group R2, R3, R4, and R5 dishes, calliandra leaf extract was added according to the prescribed concentration. Each dish then filled with five worms. Observations were carried out every 15 minutes for 6 hours to determine the death of worms. The mortality rate (%) of each treatment was calculated from each dish.
3. Results and discussion

3.1. Active compound with anthelmintic activity in calliandra leaf extract
The extraction of calliandra leaf using 70% ethanol showed that tannin was an active compound contained in the leaf extract. Quantitative analysis showed the tannin content of 5%. Thus, tannin concentration for each treatment could be calculated as presented in table 1.

Table 1. Tannin content in calliandra leaf extract

| Treatment | Calliandra leaf concentration (%) | Tannin content (%) |
|-----------|-----------------------------------|-------------------|
| R0        | 0                                 | 0                 |
| R1        | 0                                 | 0                 |
| R2        | 10                                | 0.5               |
| R3        | 25                                | 1.25              |
| R4        | 50                                | 2.5               |
| R5        | 100                               | 5                 |

R0=normal saline (0.9% NaCl, 25 ml), R1=albendazole 10 mg/ml (25 ml), R2=10% calliandra leaf extract (w/v, 2.5 g calliandra leaf extract + 25 ml NaCl 0.9%), R3=25% calliandra leaf extract (w/v, 6.25 g calliandra leaf extract + 25 ml NaCl 0.9%), R4=50% calliandra leaf extract (w/v, 12.5 g calliandra leaf extract + 25 ml NaCl 0.9%), and R5=100% calliandra leaf extract (w/v, 25 g calliandra leaf extract + 25 ml NaCl 0.9%).

Calliandra leaf extract was potential for anthelmintic for its tannin content. Tannin worked by damaging the membrane, entering the digestive tract of worms and disturbing the mechanism of protein formation for worm activity [5,8]. Further, [8] suggested that tannins were thought to contact with the outer layer of the worm's body then absorbed by microvilli through diffusion or active transport. Microvilli found in the outermost layer of the worm serves to absorb food. Microvilli needed phosphatase enzyme in the process. This enzyme was bound to tannin, thus the absorption of food by microvilli was disrupted. As a result, nutrients from outside the worm's body were not properly absorbed.

The presence of tannins in the outer layer of the worm's body also affected the tegument. The tegument is very important for nutrient absorption. Ridwan et al. [9] suggested that worm tegument consisted of glycoproteins and mucopolysaccharides, were being damaged by tannins by precipitating proteins. Tannins generally come from polyphenol compounds that have the ability to precipitate proteins. Glycoproteins will experience protein denaturation and lead to tegument damage. Lack of nutrient would cause the worms to be unable to form ATP and eventually died.

3.2. Effect of calliandra leaf extract on the time and mortality of Haemonchus contortus in vitro
The result of in vitro examination of the time and mortality during the 4 hours of observation were presented in table 2. Treatment R1 as positive control showed that the administration of 10 mg/ml albendazole caused 100% mortality within 2 hours. Albendazole inhibited the source of energy in worms. Albendazole worked by blocking glucose uptake by adult larvae and worms. The absorbed albendazole bound to the fumarate reductase enzyme so that the oxidation process of NADH to form energy (ATP) and glucose in the mitochondria becomes obstructed or decreases [13]. Administration of 10% and 25% of calliandra leaf extract for one hour were not able to cause worm death since tannin had not been absorbed by microvilli. The number of worm deaths continued to increase along with the length of time observed.

The administration of calliandra leaf extract at a concentration of 10%, 25%, and 50% caused 100% mortality to H. contortus after four hours of treatment. Worm mortality in each treatment was influenced by the high content of tannins in calliandra leaf extract. The higher concentration of extract
contained a higher content of tannin and reduced mortality time. The outer layer of worm eggs consisted of proteins. Tannin had ovicidal activity, which could bind to worm eggs, altered the cell division in the egg and the larvae were not formed eventually [10]. Some studies showed that herbs containing tannins could be used as anthelmintics, such as jatropha leaves (Jatropha curcas L.) [13], areca seeds (Areca catechu) [10], and avocado leaves (Persea americana Mill.) [12].

Table 2. *Haemonchus contortus* mortality (%) on each treatment for four hours of observation.

| Treatment | Time (hours) | 1 | 2  | 3  | 4  |
|-----------|-------------|---|----|----|----|
| R0        |             | 0 | 0  | 0  | 0  |
| R1        |             | 75 | 100 | 100 | 100 |
| R2        |             | 0 | 35 | 100 | 100 |
| R3        |             | 0 | 25 | 70  | 100 |
| R4        |             | 10 | 60 | 95  | 100 |
| R5        |             | 20 | 85 | 100 | 100 |

abcd Superscript with different notation letter on the same column showed highly significant difference (P<0.01). R0=normal saline (0.9% NaCl), R1=Albendazole 10 mg/ml, R2=10% calliandra leaf extract, R3=25% calliandra leaf extract, R4=50% calliandra leaf extract, R5=100% calliandra leaf extract.

Groups treated by albendazole resulted in 100% mortality of *H. contortus* faster than groups treated by calliandra leaf extract. Administration of 10 mg/ml albendazole took only 2 hours while the administration of calliandra leaf extract at a concentration of 10%, 25%, and 50% took 4 hours and the highest concentration of 100 % took 3 hours to cause 100% mortality. The optimal time required for calliandra leaf extract to kill *H. contortus* worms were determined from the time of minimum 50% of worms died. The results were as follow the concentration of 10% after 4 hours, 25% after 3 hours, 50% and 100% after 2 hours. These results indicated that a higher concentration of calliandra leaf extract reduced the time needed to kill *H. contortus*. A study conducted by [8] using calliandra leaf infusion showed that higher infusion concentration gave a faster anthelmintic effect on chicken tapeworm. The most effective concentration was 60.4% at 90 minutes which killed 50% of Raillietina echinobothrida.

Administration of calliandra leaf extract killed *H. contortus*, but the worm’s body remains intact, while administration of albendazole caused the worm body to contract then destroyed. According to [14], albendazole had the destruction effect on worms. Albendazole worked by blocking glucose uptake by larvae and adult worms. The absorbed albendazole bound to the fumarate reductase enzyme; thus the oxidation process of NADH to form energy (ATP) and glucose in the mitochondria was inhibited and decreased [15].

4. Conclusion
The administration of calliandra (*C. calothyrsus*) leaf extracts was effective to cause mortality of *H. contortus* worms with the optimal time was 2 hours. This study showed that calliandra leaf extract had an anthelmintic efficacy on *H. contortus*.

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