In vitro anthelmintic activities of shrub plants extracts for Haemonchus contortus worms

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Abstract. This study was conducted to evaluate the anthelmintic activity and effectiveness of shrubs as forage containing tannins as anti-parasites in the digestive tract of small ruminants by in vitro. The types of shrubs used in the study included anting-anting (Acalypha australis linum), bandotan (Ageratum conyzoides), som jawa (Talinum paniculatum), suruhan (Peperomia pellucid L.), and tapak liman (Elephantopus scaber). This study at P0: aquadest (negative control) P1: 10% concentration level of shrubs; P2: 20% concentration level of shrubs, and P3: albendazole 100% (positive control) after 60 minutes of time. The methods of Haemonchus contortus adult worm mortality test were used to investigate the inhibiting effect of in vitro worm mortality from shrub plant extracts. The results of this study on in vitro screening of adult worms, at an increasing concentration, the number of worms that died was significantly more (P <0.05). The highest worm mortality was seen at the concentration level of tapak liman leaf extract (Elephantopus scaber) as much as 20% with a tannin content of 8.89% so that the anthelmintic potential was seen in adult worm mortality. From this study, it can be concluded that the extract of shrubs shows significant anthelmintic activity against the death of adult female Haemonchus contortus worms so that it has the potential to play a role in parasite control in the digestive tract of small ruminants.

1. Introduction
Forage is an important factor in ruminant farming. Forage is all fed ingredients derived from plants, can be in the form of leaves, or sometimes still mixed with stems, twigs, and flowers, which generally come from grass-country plants (gramineae), legumes, and legumes. Forage from other plants. Forage groups as animal feed ingredients can be given in two forms, namely fresh forage and dry forage [1]. Several types of forage that are often used are shrubs. Bush plants are plants that have secondary metabolic content in them. Examples contain lots of tannins, saponins, flavonoids, alkaloids, essential oils, citrates, eugenols, polyphenols, fatty acids, and others. These plants provide abundant natural compounds used in various disease treatments, including the treatment of parasites. In addition to modern approaches, plant bioactive compounds, particularly secondary metabolites, have been identified as promising ways to modulate the biological aspects of parasites. Pharmacologically, condensed tannins present ovicidal, larvicidal, and vermicidal properties [2]. Various of the tropical plants such as Carica papaya, Manihot esculenta Crantz, Coriandrum sativum, Gliricidia sepium, Calliandra calothyrsus, and Artocarpus heterophyllus have been shown to have anti-parasitic/anthelmintic effects [3].

Haemonchus contortus is a major problem in small ruminants in Indonesia. Haemonchus contortus is a nematode class type of gastrointestinal parasite that causes haemonchosis. Haemonchus contortus is large and most pathogenic worms are found in the abomasums of goat and sheep [4]. This disease is caused by intestinal worms/nematoda class [5]. The frequent use of the anthelmintic drugs has given rise to drug-resistant populations which increase the need for new anthelmintic compounds, particularly from endemic plants [6]. The high percentage of Haemonchus contortus larvae compared to other worm larvae can endanger livestock productivity so it is necessary to have an appropriate control strategy against haemonchosis [7].

The use of natural anti-parasitic compounds is considered to be an appropriate treatment for controlling parasites. There is concern that the use of commercial anthelmintic drugs such as albendazole and avermectin, which have a broad spectrum of antiparasitic agents, will result in resistant parasites due to
the inefficiency of the treatment itself [8,9]. Tannins have a beneficial effect on reducing mucosal cells and permeability and act as anti-parasitic compounds in livestock intestines [10]. Naturally, tannins protect plants from predators. However, tannins have two side effects, both positive and negative impacts on animal health. Beneficial effects include the reduction of anti-parasitic, anti-tick, and methane compounds [11]. This study aims to determine the anthelmintic activity of several shrubs against the mortality rate of *Haemonchus contortus* worms in vitro.

2. Materials and methods

2.1. Forage sample preparation and edible portion

Plant samples were taken from bushland in the Karanganyar and Sragen areas. The plant samples used included earrings anting-anting (*Acalypha australis* linn), bandotan (*Ageratum conyzoides*), som jawa (*Talinum paniculatum*), suruhan (*Peperomia pellicid L*), and tapak liman (*Elephantopus scaber*). The manufacture of shrub plant infusions was prepared according to the procedure used by [3]. Infuse is made from shrubs in the edible portion by drying it in the air for 5-7 days until the weight is constant. It was ground using a disk mill with a screen diameter of 2 mm, then weighed as much as 10 g, and 20 g, beaker glass was added separately for the stock solution. The beaker glass was filled with a sample of shrub plants and then filled with 100 ml of aquadest. The beaker glass is then put in the oven at 90°C for 15 minutes. The remaining liquid in the beaker glass is taken and filtered to obtain 10% and 20% bush plant infusion.

2.2. Collection of *Haemonchus contortus* worm

*Haemonchus contortus* adult female worms are obtained directly from the abomasum of naturally infected sheep slaughtered in slaughterhouses. The abomasum is selected from the part between the rumen and duodenum. *Haemonchus contortus* worms are obtained by carefully separating the feed particles in the abomasum and parasites that appear to be collected using pinset. *Haemonchus contortus* worms obtained were collected in a dish containing 0.9% physiological NaCl and immediately used for testing.

2.3. Analysis of worm mortality

The worm mortality test was carried out according to [12]. A total of 160 adult female worms were divided into 4 treatment groups with each petri dish consisting of 10 worms with 4 replications per shrub plants sample. Each group was immersed in a bush infusion with various doses as follows: P0: aquadest (negative control); P1: 10% concentration level of shrubs; P2: 20% concentration level of shrubs, and P3: albendazole 100% (positive control) after 60 minutes of time.

2.4. Analyze the total tannin content of the sample

The total tannin content was determined by the method [13] with slight modifications. Tannin content contained in the bush plant samples was obtained and measured by UV-Visible Spectrophotometer with a maximum wavelength (λ) of 725 nm using tannic acid as the standard. Then the total tannin content (%) was obtained in the sample.

2.5. Data analysis

Data analysis on tannin content was carried out using descriptive methods and the mortality rates of adult female worms at different doses were observed using one-way analysis of variance (ANOVA) and differences between treatment means were further analyzed using Duncan’s New Multiple Range Test (DMRT) with significance level of p<0.05.

3. Results and Discussions

3.1. Tannin content of shrubs

Total tannin contents of the five shrub plants ranged from 2.25 - 8.89% of dry matter basis (DM) (Table 1). Shrub plants with a high enough tannin content can be used as a source of anthelmintics in ruminant feed. Tannins are a secondary metabolic compound contained in plants, where metabolic compounds such as tannins can be used for anti-parasitic drugs.
Table 1. Total tannins content in the different species of shrub plants (%).

| No. | Shrub Plants | % Tannins |
|-----|--------------|-----------|
| 1.  | Anting-anting (Acalypha australis linn) | 5.21 % |
| 2.  | Bandotan (Ageratum conyzoides) | 6.85 % |
| 3.  | Som jawa (Talinum paniculatum) | 2.25 % |
| 4.  | Suruhan (Peperomia pellucid L.) | 3.66 % |
| 5.  | Tapak liman (Elephantopus scaber) | 8.89 % |

Forage plants provide abundant natural compounds used in various disease treatments, including the treatment of parasites. Plant bioactive compounds, particularly secondary metabolites, have been identified as promising ways of modulating the biological aspects of parasites. Pharmacologically, condensed tannins present ovicidal, larvicidal, and vermicidal properties [2][10]. Tannins have a beneficial effect on reducing mucosal cells and permeability and act as anti-parasitic compounds in livestock intestines [10]. Naturally, tannins protect plants from predators. However, tannins have two side effects, both positive and negative impacts on animal health [11]. The use of new anthelmintics shows that tanninferous plants can be considered as a potential alternative for controlling nematode infestations in small ruminants [15].

3.2. Mortality Rate of Worms

Shrub plant infusion was capable of reducing Haemonchus contortus adult worms after 60 minutes of immersion in vitro. Based on the results of the observation of the screening test of 5 types of shrubs, it was shown that those that had the most effective potency with a concentration level of 10% and 20% as an anthelmintic for 60 minutes of observation, namely tapak liman (Elephantopus scaber), bandotan (Ageratum conyzoides), suruhan (Peperomia pellucid L.), anting-anting (Acalypha australis linn), and som jawa (Talinum paniculatum) (Table 2). This is supported by the data on the total tannin content found in shrubs (Table 1.).

Table 2. Anthelmintic activity of shrub plants infusion on mortality rate of adult female worms after 60 minutes of time (%).

| Treatment | Acalypha australis linn | Ageratum conyzoides | Talinum paniculatum | Peperomia pellucid L. | Elephantopus scaber |
|-----------|-------------------------|---------------------|---------------------|-----------------------|---------------------|
| P0        | 0.00±0.0                | 0.00±0.0            | 0.00±0.00          | 0.00±0.00            | 0.00±0.0           |
| P1        | 10.00±0.0, 0.00±0.0      | 15.00±0.00         | 10.00±0.00         | 15.00±0.00           | 20.00±0.00         |
| P2        | 15.00±0.0, 0.00±0.0      | 25.00±0.00         | 13.33±5.77         | 15.00±0.00           | 33.33±5.77         |
| P3        | 75.00±0.0, 0.00±0.0      | 76.67±5.77         | 80.00±0.00         | 73.33±5.77           | 81.67±5.77         |

P0: aquadest (negative control) P1: 10% concentration infuse of shrubs; P2: 20% concentration infuse of shrubs, and P3: albendazole 100% (positive control) after 60 minutes of time.

Different superscripts within the same row indicates significant differences (P<0.05).

The higher the tannin content in shrubs, the higher the potential as an anthelmintic. In the testing process, the worms were immersed at a concentration of 10% and 20%. High concentrations and immersion can result in direct contact between bush plant infusions containing tannins and worms, causing a paralysis reaction and then death. This is in accordance with the opinion [16], where the paralysis time is stated if the worm does not move unless disturbed by using a spatula. The time of death is stated if the worms...
remain immobile even if immersed in the warm water of 40–50 °C and the worms slowly lose their color. The high amount of tannins can play an important role plays role in binding proteins and rotating the walls of the nematodes to become inactive and kill them [2]. Tannins can be divided into 2, namely hydrolyzed tannins and condensed tannins. Tannins are commonly found in plants (legumes) are condensed tannins [17]. Condensed tannins are effective against gastrointestinal parasites. The effect of condensed tannins on gastrointestinal parasites is dole out directly or indirectly. Tannins directly through viscous tannin-nematode interaction affect hatching and influence the expansion of infective larvae. The condensed tannins even have the flexibility to bind to proteins, inactivating the walls of the nematodes, and killing them. Indirectly, tannins can bind to the plant proteins within the rumen to stop microbial degradation. In turn, this can increase the flow of plant proteins to the duodenum [18].

Figure 1. Mortality rate of adults female worms with different types of shrub plants.

Figure 1 illustrates the increased mortality of worms immersed in the infusion of five types of shrubs with increasing doses ranging from 0%, 10%, and 20%. Long immersion for 60 minutes in a shrub infusion was observed to increase the worm mortality rate. In Figure 1, the best dose to increase the mortality of adult female Haemonchus contortus worms is 20% tapak liman (Elephantopus scaber) plant, based on its ability to kill 33.33% of worms after 60 minutes. This finding is related to the tannin content in tapak liman which is 8.89% which has the highest content among other shrubs. [19] states that the selection of natural medicine as an anthelmintic must contain active ingredients such as saponins, tannins, flavonoids, and alkaloids. These chemical compounds have ovicidal activity, which can bind worm eggs whose outer layer consists of protein so that cell division in the eggs will not take place eventually larvae are not formed, killing and breaking the life cycle of adult worms, larvae, and worm eggs [20]. This anthelmintic ability is thought to be due to the presence of tannin and saponin compounds [21]. The contents of 5% tannins from plants can increase worm mortality so that tannins in plants can be used as a natural anthelmintic [17].

Shrubs as forage can be a natural anthelmintic medicine so that it can replace the use of commercial worm medicine. The mechanism of anthelmintic action in eradicating worms is to kill larvae, adult worms and can inhibit the development of worm eggs [22]. Nematode worm medicine extracts of herbal plants are tested for their effectiveness against eggs, larvae, and adult worms using the parameters of intestinal cell degeneration which inhibit egg hatchability (bad embryos), kill larvae (dead/undeveloped larvae), and kill adult worms. The content of tannins contained in forage, potential as animal feeds, supports their use by farmers in traditional animal health care. Further controlled in vivo experimental studies are required to identify possible negative effects on the performance of the animals before any plant can be recommended for save use [3].

4. Conclusion
The highest worm mortality was seen at the concentration level of tapak liman leaf extract (Elephantopus scaber) as much as 20% with a tannin content of 8.89% so that the anthelmintic potential was seen in adult worm mortality. The extract of shrubs shows significant anthelmintic activity against the death of adult female Haemonchus contortus worms so that it has the potential to play a role in parasite control in the digestive tract of small ruminants.
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