ACTA VETERINARIA INDONESIANA
P-ISSN 2337-3202, E-ISSN 2337-4373

Research Vol. 13, No. 1: 87–93, Maret 2025

Anthelmintic Potential of Microherbal Formulation of Basil Leaves (*Ocimum africanum*) against *Haemonchus contortus*

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Received 12 August 2024, Approved 8 January 2025

ABSTRACT

Haemonchus contortus is a prevalent and pathogenic parasite in ruminants that is resistant to common anthelmintic treatment. This study evaluated the anthelmintic effectiveness of *Ocimum africanum* leaf on a micron scale against *H. contortus* eggs, infective stage larvae (L3s), and adult worms. The effectiveness of three microherbal concentrations (10%, 5%, and 1%) was evaluated using an egg hatch assay and larval and adult worm motility inhibition assays. The negative control group used 10% PBS, while the positive control used 10 mg/mL of Albendazole. The findings of this study demonstrated that the highest concentration of microherbal treatment produced effects comparable to those of Albendazole (p>0.05) on eggs, L3, and adult worms. Adult worms become immobilized within one to four hours of exposure to 10% microherbal. This study revealed that basil leaves in the form of microherbals were effective as an anthelmintic against various stages of *H. contortus*.
Keywords: basil leaf (*Ocimum africanum*), *Haemonchus contortus*, microherbal

ABSTRAK

Haemonchus contortus merupakan salah satu parasit ruminansia yang patogen dan telah mengembangkan kemampuan resistensi terhadap pengobatan antelmintik sintetik. Penelitian ini bertujuan menguji efektivitas antelmintik ekstrak daun kemangi pada skala mikro meter secara *in vitro* terhadap telur, larva infektif (L3), dan cacing dewasa *H. contortus*. Efektivitas tiga macam konsentrasi mikroherbal (10, 5, dan 1%) diuji melalui *egg hatch assay, larval motility inhibition test, and adult worm motility inhibition assay.* Kelompok kontrol negatif menggunakan PBS 10% sementara kontrol positif menggunakan Albendazole 10 mg/ml. Data penelitian menunjukkan bahwa perlakuan mikroherbal konsentrasi tertinggi memiliki efek yang tidak berbeda nyata dengan Albendazole (p>0,05), baik terhadap telur, L3, maupun cacing dewasa. Penghambatan motilitas seluruh cacing dewasa terjadi pada 1-4 jam pertama paparan mikroherbal 10%. Hasil penelitian menunjukkan bahwa mikroherbal daun kemangi efektif dijadikan antelmintik terhadap berbagai stadium *H. contortus.*
Kata kunci: Daun kemangi *(Ocimum africanum), Haemonchus contortus*, mikroherbal

INTRODUCTION

Haemonchosis is a gastrointestinal disease in small ruminants caused by infection with *Haemonchus contortus*. This disease is common in animals living in tropical climates such as Indonesia. Haemonchosis has a significant economic impact on farmers because it decreases productivity (including milk, meat, and fur), causes anemia, and even sudden death in previously healthy livestock (Mulyadi 2018). According to Widiarso et al. (2018), the prevalence of Haemonchosis in goats in Indonesia reaches 89.4%.

Control efforts for Haemonchosis in recent decades have predominantly relied on synthetic anthelmintic; however, these treatments have been associated with the development of resistance. The incidence of resistance can become a bigger problem when worms develop "multiple anthelmintic resistance", i.e., resistance to different classes of anthelmintic (Engstrom et al., 2016). Resistance may arise from the imprudent application of anthelmintic, including prolonged use, administration of subtherapeutic doses, and the absence of rotation of the type of anthelmintic used.

On the other hand, administering anthelmintic above effective doses also causes problems, such as an increased risk of drug residues in animal products and the environment. These factors highlight the necessity of exploring alternative solutions for managing worm infections in livestock. A potential approach is the use of herbal plants as natural anthelmintic. Plant-based anthelmintic offer several advantages, such as safer application, a variety of beneficial plant species, a lower risk of resistance, and the potential to enhance overall health (Manjusa & Pradeep 2022).

Basil leaf is a readily accessible plant, particularly its leaves, which have been documented to possess anthelmintic properties (Noviana et al., 2017). The compounds that act as anthelmintic are tannins and saponins (Widiarso et al., 2018). However, using natural ingredients in their raw form still has several limitations, such as poor solubility, low bioavailability, instability, and extensive metabolism by the body.

According to Tomiotto-Pellissier et al. (2017), the effectiveness of modified herbal medicines in smaller sizes can drastically increase the absorption and release of active substances, reducing the risk of resistance. Therefore, it is essential to evaluate herbal medicines more efficiently by minimizing their size to the micrometer scale.

MATERIALS AND METHOD

Extraction of Basil Leaf

Basil leaf herbal powder was obtained from the spice and medicinal plant research center (BALITTRO). A total of 1000 grams of powder was macerated using 96% ethanol solvent at a ratio of 1:10 at room temperature for 1 × 24 hours, followed by two remacerations. The maceration results (maserat) were collected every 1 × 24 hours. The maserat obtained was combined and then evaporated using a rotary evaporator until a thick extract of Basil leaves was produced (Abubakar and Haque, 2020).

Production of Microherbs

The thick extract obtained was homogenized into an emulsion. The homogenization process required a fat phase comprising a thick extract and palmitic acid alongside a liquid phase consisting of Tween-80, maltodextrin, and distilled water. The ratios used in sequence were 2:2:1:1:20. The two phases were stirred for 5 minutes at 75°C on a hotplate. The fat phase was then dispersed into the liquid phase while stirring for 5 minutes. The emulsion was homogenized using a homogenizer at 13,500 rpm for 5 minutes and cooled at room temperature. Ultrasonication was conducted at 30% amplitude for 20 minutes. The emulsion underwent characterization using Particle Size Analysis (PSA) as outlined by Yusaf and Juboori (2014).

Collection and Examination of Feces

Fresh feces from sheep suspected of being infected with haemonchosis were collected per rectum. The examination was carried out using the test tube flotation technique. Fresh feces were weighed at 4 grams per sample and then crushed. The feces were combined with a flotation solution until the total volume reached 60 mL. After that, it was homogenized, filtered using a tea strainer, and placed in a clean glass until a suspension was obtained (Roepstorff and Nansen 1998). The suspension was added to a test tube until it reached capacity, resulting in a convex meniscus. A glass cover was placed on the mouth of the test tube, then left for 5 minutes. The cover glass was removed and placed on the object glass, then observed under a microscope at 4×10 and 10×10 magnification. Egg identification referred to Ljungström et al. (2018) and the book Helminthology of Medicine and Veterinary (Sardjono et al., 2020).

Preparation of Eggs, Larvae, and Adult Worms of H. contortus

Eggs were obtained by isolating strongyle eggs from verified positive suspension samples. This study utilized the specific type of strongyle eggs identified as representative of *H. contortus*. The size of the strongyle eggs found was about 70 um long and 50 um wide, which was characteristic of *H. contortus* eggs. The morphology of strongyle eggs exhibits numerous similarities despite originating from various species of parasitic worms. Strongyle eggs are oval, have thin cell walls, and have a clear blastomere. A micropipette was used to dispense 10 µL of a 10% PBS solution onto the suspension attached to the object glass and cover glass. The successfully collected eggs were stored in microtubes. Each tube contained 15 to 20 eggs.

The preparation of *H. contortus* larvae were accomplished by making an egg culture. The cultured eggs were obtained from adult female *H. contortus* worms collected from sheep abomasums from slaughterhouses. Breeding was conducted in a petri dish utilizing a vermiculite medium combined with sheep feces, which have been verified as negative for parasite worm eggs, along with previously collected *H. contortus* eggs. Tap water was incorporated into the medium until adequate moisture levels were achieved. The dish was loosely covered and incubated at 27°C for 7 days to obtain infective larvae (L3). The culture medium was subsequently collected utilizing a Baermann funnel containing tap water. Several L3 from the culture medium will sink to the bottom of the glass and be collected. The L3 larvae obtained from the harvest are stored in 10% PBS solution at 4°C until used.

The adult female *H. contortus* collection was sourced directly from the abomasum of sheep. The worms were collected with tweezers, placed in a dish for washing with PBS, and immediately used for testing.

In Vitro Testing

This study conducted in vitro testing using basil leaves at a micron scale to assess their effects on the eggs, larvae, and adult worms of *H. contortus*. One of the concentrations of basil leaf microherbal used in this study was 10%, as referenced in the research conducted by Noviana et al. (2017). When prepared as a thick extract at this concentration, the anthelmintic efficacy of basil leaves is nearly comparable to that of Albendazole at 10 mL/mL. The treatment consisted of five groups: P1 negative control (10% PBS solution), P2 positive control (10 mg/mL Albendazole), P3 (10% microherbs), P4 (5% microherbs), and P5 (1% microherbs). The treatment was repeated twice.

The eggs in the microtube underwent testing for the egg hatch assay (EHA). Each tube containing 15–20 *H. contortus* eggs received five treatments of 100 µL each. Incubation was carried out for 48 hours at 27°C. Observations were conducted with a microscope at 10×10 magnification of intact eggs and L1, followed by counting to determine the percentage of eggs that did not hatch, utilizing the formula established by Davuluri et al. (2020).

A larval motility inhibition test (LMIT) was conducted on the collected L3. Approximately 100 µL of the suspension containing 15–20 L3 was placed in a microtube, followed by each treatment. The treatment was incubated at 27°C for 24 hours. Observations were made using a stereo microscope on normal (moving) larvae or those experiencing paralysis (no movement could be observed for 5 seconds). The results were presented as the percentage of inhibition of larval motility.

Adult worms were assessed using the adult worm motility inhibition (%WMI) assay method. Ten adult worms were placed in separate petri dishes and subjected to five treatments at room temperature. Worm motility was monitored at 4-hour intervals over 12 hours until the worms in the negative control exhibited a loss of motility. All motile (alive) and non-motile (dead) individuals in two replicates of each concentration and the control were counted. Worm death was confirmed by the absence of motility on observation for 5–6 seconds. The percentage of worm motility inhibition (%WMI) was calculated according to Davuluri et al. (2020).

$$\text{Inhibition Percentage (\%)} = (A - B) / A \times 100$$

Note
A: Number of hatched eggs, larvae and motile worms in the negative control
B: Number of hatched eggs, larvae and motile worms in other treatments

Data Analysis

The data were presented descriptively, focusing on the damage inflicted on eggs, larvae, or adult worms following treatment. The data included the percentage of eggs that did not hatch, larval motility inhibition and the mortality of adult worms. The percentages from the three tests served as indicators of the anthelmintic activity of basil leaf microherbs. The data collected were analyzed using one-way ANOVA with the IBM SPSS Statistics software. The results of the tests that showed significant differences were analyzed using Duncan's multiple comparison test.

RESULTS

Preparation of basil Leaf Microherbs

The main herbal anthelmintic ingredient tested was basil leaves. The leaves were selected due to their reported anthelmintic properties against *H. contortus* worms (Noviana et al., 2017). Before being used as a test treatment, the basil leaves were processed to achieve the desired size. From 1000 grams of herbal medicine, 147.6 grams of extract were obtained, resulting in a yield of 14.76%. The yield achieved is significantly high, exceeding the target of 10%. The high yield suggests that the extract contains a significant concentration of bioactive compounds (Senduk et al., 2020).

The extract then went through a homogenization and ultrasonication process. The characterization results obtained through PSA indicate that the diameter of the processed basil leaves predominantly falls within the micron range, with an average particle diameter of 1.018 µm (Figure 1). Kulkarni (2011) demonstrated that micron-scale drugs offer nearly comparable benefits to those of nanometer-scale drugs. Microparticles exhibit high solubility, stability, and bioavailability. This has been demonstrated to influence the increase in drug activity relative to drugs in extract form.

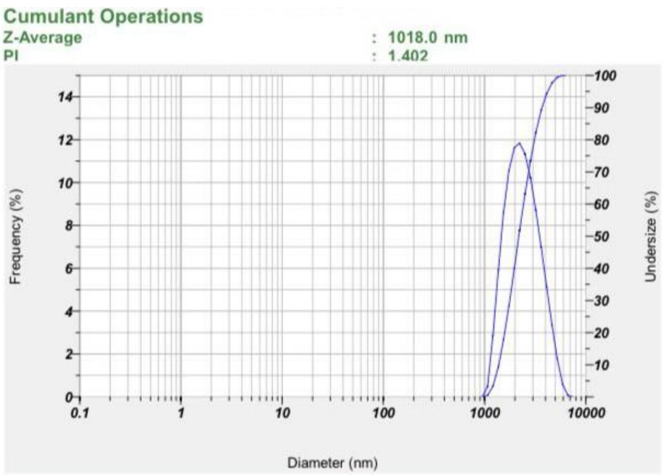

Figure 1 Basil leaves characterization results using Horiba-Sz 100 PSA

In Vitro Eflectiveness Test

The effect of basil leaf microherbs was tested in vitro on various stages of parasites, namely on eggs, larvae, and adult worms of *H. contortus*. Microherbal testing was categorized into three concentrations: 1%, 5%, and 10%. Albendazole 10 mL/mL and PBS 10% were also used in this study as a comparison. The test results are presented in Tables 1 and Table 2.

Ovicidal activity refers to the proportion of eggs that do not develop and hatch. The larvicidal activity was quantified as the percentage of L3 that remains immobile (dead or weakened) following exposure. Table 1 presents data indicating that all three concentrations of basil tested exhibited both ovicidal and larvicidal activity. The 10% microherbal treatment group exhibited the greatest ovicidal activity, effectively inhibiting the hatching and development of all eggs. Table 1 indicates that the microherbal activity at 10% was significantly greater (P<0.05) than that of the synthetic anthelmintic albendazole, which served as the positive control. The 1% micro herb treatment exhibited the lowest ovicide activity.

Table 1 Percentage of microherbal inhibition of egg hatching and larval motility of *H. contortus*

Treatments	Inhibition percentage (%)	
	EHA*	LMIT*
Microherbs 10%	100 ± 0.0^a	100 ± 0.0^a
Microherbs 5%	58.57 ± 2.02^c	71.91 ± 20.88^a
Microherbs 1%	17.15 ± 4.04^d	23.81 ± 13.46^b
PBS*	0.0 ± 0.0^e	0.0 ± 0.0^b
Albendazole (10 mg/ml)	82.85 ± 4.04^b	89.77 ± 4.38^a

Note : Superscript letters in the same column indicate a significant difference (P<0.05). *EHA: Egg Hatch Assay, LMIT: Larval Motility Inhibition Test, PBS: Phosphate-buffered saline

Table 2 Cumulative percentage of microherbal inhibition of adult worm motility of *H. contortus*

Treatments	Inhibition percentage (%)			
	1 hour	4 hours	8 hours	12 hours
Microherbs 10%	85.0 ± 21.21^a	100 ± 0.0^a	100 ± 0.0^a	100 ± 0.0^a
Microherbs 5%	25.0 ± 7.07^b	60.0 ± 0.0^b	100 ± 0.0^a	100 ± 0.0^a
Microherbs 1%	0.0 ± 0.0^b	25.0 ± 7.07^c	75.0 ± 7.07^b	100 ± 0.0^a
PBS*	0.0 ± 0.0^b	0.0 ± 0.0^d	0.0 ± 0.0^c	20.0 ± 0.0^b
Albendazole (10 mg/ml)	100 ± 0.0^a	100 ± 0.0^a	100 ± 0.0^a	100 ± 0.0^a

Note : Superscript letters in the same column indicate a significant difference (P<0.05). *PBS: Phosphate-buffered saline

Table 3 Percentage of death of adult *H. contortus* worms due to treatment with basil leaf ethanol extract

Treatments	Average	
	1st hour	4th hour
10% basil leaf ethanol extract	30.0 ± 1.6	100 ± 0.0
5% basil leaf ethanol extract	0.0 ± 0.0	90 ± 8.16

Modified from Noviana et al. 2017 J Parasite Sci. 1(2):55–58

The LMIT data in Table 1 indicate that the larvicidal activity of the albendazole group, 10% microherbs, and 5% microherbs is not statistically different. However, the effect of 10% microherbs is still higher than that of albendazole. The testing results on the eggs and larvae explain that increasing the concentration of basil microherbs can increase the percentage of eggs that fail to hatch and inhibit larval motility. The concentration of microherbs with the highest ovicidal and larvicidal activity is 10%.

In the initial hour of observation, the adult worms exhibited no significant difference in response to the 10% microherbs compared to the Albendazole group, indicating that both possess comparable anthelmintic efficacy. During the initial hour, other concentrations require a longer duration to achieve an effect comparable to Albendazole 10 mg/mL. Observations conducted at the fourth hour indicated a notable effect across all three micro herb treatment groups. The 10% microherbal treatment exhibited the most significant anthelmintic effect, resulting in the complete elimination of all worms in the dish. The 1% microherbal treatment exhibited the lowest anthelmintic effect. The test results indicate that an increased concentration of basil leaves correlates with a higher concentration of active compounds and a reduced time to worm mortality. All worms in the negative control remained alive for up to 8 hours during testing.

DISCUSSION

The anthelmintic effect in this study was related to the bioactive compounds in basil leaves. Sumiati et al. (2019) performed phytochemical tests demonstrating that the ethanol extract of basil leaves (*O. africanum*) contains flavonoids, saponins, and tannins. Several of these bioactive compounds can target *Haemonchus* during its early developmental stages, including eggs and larvae, thereby inhibiting further parasite development.

Tannins and saponins are known to work by inhibiting the larval formation and the egg- hatching process. Tannins and saponins can permeate the egg wall, halting blastomer segmentation and inhibiting the embryo's development into a larva. As a result, the egg embryo does not reach the larva form, and the egg fails to hatch (Cabardo and Portugaliza, 2017). This is also seen in the observation results shown in Figure 2. Following testing, the egg embryo was observed to be damaged; however, the egg wall remained intact.

Figure 2 Comparison of the integrity of the egg embryo after treatment. (A) Whole egg embryo. (B) Embryo damaged by Albendazole treatment. (C) Embryo damaged by microherbal treatment. Scale = 50 µm.

Larvae are considered motile when they exhibit sinusoidal mobility, as shown in Figure 3A. Observations indicate that as incubation time and micro herb concentration increase, the motility of L3 larvae decreases significantly. Additionally, it was noted that the larvae initiate coiling, as illustrated in Figure 3B, consistent with the findings of Muhamad et al. (2017). Physical changes or stress expressed as coiling are linked to a survival response to conserving energy, specifically lipid reserves. Figure 3C illustrates L3 that has succumbed, indicated by the absence of observable movement for 5 seconds. The non-motile larvae result from the ability of tannins to paralyze and disrupt the neuromuscular coordination of the larvae (Cabardo and Portugaliza, 2017).

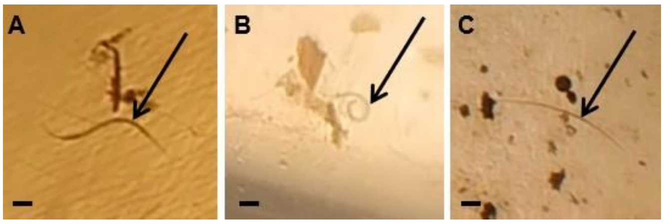

Figure 3 Infective larvae of *H. contortus*. (A) Motile L3. (B) Coiling L3. (C) Dead L3. Scale = 50 µm.

The anthelmintic effect of basil leaves at the micron scale surpasses that of the extract alone. Noviana et al. (2017) researched the anthelmintic efficacy of basil leaf ethanol extract on the motility of adult *H. contortus* worms. Table 3 presents the study's results, indicating that the percentage of adult worm deaths for the 10% extract in the first hour was 30%, whereas the 5% concentration did not result in any deaths. This value is lower than the effect of basil on a micron scale. The 10% microherbs in this study was able to cause 85% mortality in the first hour, while the 5% microherbs caused 25% mortality. The percentage increases at 4 hours of exposure due to exposure to extracts and microherbs.

The superiority of the anthelmintic effect of microherbs over thick extracts is likely due to the difference in size between the two. According to Tomiotto-Pellissier et al. (2017), the effectiveness of herbal medicines that have been modified to a smaller size can increase dramatically. Basil leaves contain bioactive compounds such as tannins and saponins. Tannins can enter the cuticle surrounding the worm's body and cause damage. Saponins work by increasing cell membrane permeability. The presence of these compounds impacts the decline of protective functions and nutrient absorption, resulting in malnutrition and motility disorders until the worms finally die (Muda et al., 2021).

The findings of this study indicate that basil leaves, at a micron scale, can be effectively utilized as an anthelmintic against different stages of *H. contortus*. The optimal effect was achieved with microherbs at a concentration of 10%.

"The authors state no conflicts of interest with the parties involved in this study".

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