Journal of Animal & Plant Sciences, 36(1): 2026, Page: 248-261
ISSN (print): 1018-7081; ISSN (online): 2309-8694
https://doi.org/10.36899/JAPS.2026.1.0021

AN *IN VITRO* EXPERIMENTAL INVESTIGATION OF THE ANTIBACTERIAL, ANTIOXIDANT, AND THERAPEUTIC EFFICACY OF *ACACIA MANGIUM* BARK EXTRACTION AGAINST *Haemonchus contortus* INFECTION

T. U. P. Sujarnoko[1], D. Budiono[1], N. A. Sholeha[1], R. Siskandar[1], T. M. Syahniar[2], M. Andriani[2], A. U. Prastujati[3], A. Fiveriati[4] and M. M. Sholikin[5*]

[1] College of Vocational Studies, IPB University, Bogor, Indonesia; [2] Department of Animal Science, Politeknik Negeri Jember, Jember, Indonesia; [3] Department of Livestock Product Processing Technology, Politeknik Negeri Banyuwangi, Banyuwangi, Indonesia; [4]Department of Mechanical Engineering, Politeknik Negeri Banyuwangi, Banyuwangi, Indonesia; [5]Reserach Center for Animal Husbandry, National Research and Innovation Agency (BRIN), Bogor, Indonesia

Sujarnoko {0000-0001-8088-4851}; Budiono {0000-0002-6003-2173}; Sholeha {0000-0003-2912-2338}; Siskandar {0000-0003-2911-3108}; Syahniar {0000-0002-3464-2007}; Andriani {0000-0001-6996-8640}; Prastujati {0000-0003-3183-2064}; Fiveriati {0000-0003-3105-2922}; Sholikin {0000-0001-8677-6982}

*Corresponding author's email: mohammad.miftakhus.sholikin@brin.go.id

ABSTRACT

The significant economic losses in the small ruminant sector caused by *Haemonchus contortus* highlight the urgent need for alternative therapeutic solutions. These alternatives should not only be environmentally sustainable but also prevent the development of resistance, with *Acacia mangium* emerging as a potential option. The study aimed to evaluate the efficiency of *A. mangium* bark extract against adult *H. contortus* and to assess its antibacterial and antioxidant properties using hot water extraction method. The extraction process used a factorial completely randomized design with three temperatures (40, 60, and 80°C) and two durations (45 and 60 minutes). The concentrations of phenols, tannins, and condensed tannins were measured, along with antioxidant (DPPH scavenging) and antibacterial activities. The anthelmintic efficacy was compared to albendazole over a seven-hour period.

Result: Extraction at 80°C significantly increased the concentration of phenols, tannins, tannin-to-phenol ratio, and condensed tannins compared to lower temperatures ($P \leq 0.01$). A 60-minute duration yielded higher levels of bioactive compounds than 45 minutes ($P \leq 0.01$). The strongest DPPH activity occurred at 40°C for 60 minutes ($P \leq 0.05$). The 100% and 75% extract concentrations showed comparable anthelmintic activity to albendazole after seven hours. The extract also demonstrated significant antibacterial activity, producing larger inhibition zones than the control ($P \leq 0.01$).

Conclusion: Hot water extract of *A. mangium* bark exhibits potent anthelmintic, antioxidant, and antibacterial activities, support its potential as a natural agent for parasite control in small ruminants.

Recommendation: Further *in vivo* studies are recommended to confirm the efficacy, safety, and potential integrity of the extract in small ruminant health practices.

Keywords: Antioxidant compounds, condensed tannins, gastrointestinal nematodes, high-temperature plant extraction, phenolic compounds, small ruminants.

Published first online December 05, 2025 Published final January 20, 2026

INTRODUCTION

Parasitic worm infections, particularly those caused by *Haemonchus contortus*, posed a significant challenge for small ruminants in developing countries (Rodríguez-Hernández *et al.*, 2023; Solomon *et al.*, 2024). These infections occurred frequently among pasture-raised ruminants (Esteban-Ballesteros *et al.*, 2019; Hoste *et al.*, 2022) and resulted in substantial economic losses for farmers (Gazzonis *et al.*, 2023; Werszko *et al.*, 2024; Zafari *et al.*, 2022).

The *H. contortus* measured approximately 1.5 to 2.5 cm in length (Arsenopoulos *et al*., 2021). The anterior end featured a blood-sucking mouthpart shaped like an axe, which attached to and extracted blood from the host's abomasum (Nath *et al*., 2021). This parasite followed a direct life cycle without an intermediate host, and its third larval stage (L3) served as the infective form (Arsenopoulos *et al*., 2021; Gareh *et al*., 2021). Under

warm environmental conditions, the larvae developed and were ingested by grazing ruminants (Nath *et al*., 2021). In general, the presence and activity of female *H. contortus* significantly influenced parasite load and disease severity, as females produced new larvae capable of infecting other hosts or different organs within the same host (Arsenopoulos *et al*., 2021; Liu *et al*., 2023). Infected ruminants commonly suffered from diarrhea, anemia, anorexia, and, in severe cases, mortality (Julienne *et al.*, 2021). The excessive use of anthelmintics led to drug resistance, residue accumulation in animal products, and environmental risks (Ahmed *et al.*, 2024; Cunha *et al.*, 2024; Esteban-Ballesteros *et al.*, 2019). As a result, alternative parasite control strategies became necessary. One promising approach involved the use of plant-derived tannins with natural anthelmintic properties. *Acacia mangium* emerged as a potential source due to its high concentration of bioactive tannins (Sujarnoko, Ridwan, *et al.*, 2020).

Acacia bark, commonly known as Mangium bark, served as an underutilized by-product of the paper industry. Residual bark from the production process potentially contributed to environmental pollution when exposed to water (Margina *et al*., 2023). The bark contains high concentrations of phenolic compounds and tannins (Ćućuz *et al*., 2022; Ruiz-Aquino *et al*., 2023; Sujarnoko, Ridwan, *et al*., 2020), making A. mangium a promising candidate for natural parasite control (Sujarnoko, Ridwan, *et al*., 2020). Studies have indicated that heated water extraction enhances tannin yield from plant materials such as durian and rubber tree bark (Sujarnoko, Jayanegara, *et al*., 2020). This water-based extraction technique is considered environmentally sustainable and safer for livestock applications (Harahap *et al*., 2024; Kļaviņa *et al*., 2023; Ujilestari *et al*., 2025).

Tannins represented a class of secondary metabolites with extensively documented anthelmintic properties (Chylinski *et al.*, 2023; Motta *et al.*, 2020). Their capacity to disrupt parasite metabolism, reduce worm burden, and suppress gastrointestinal nematode development had been well established, particularly in *H. contortus* (Costa *et al*., 2023; Teng *et al*., 2023). Specifically, tannins extracted from conifer sources inhibited 100% of egg hatching and significantly reduced L3 larval motility to levels indicative of mortality (Chylinski *et al*., 2023; Greiffer *et al*., 2022). Significant negative correlations were also observed between condensed tannin (CT) concentration and egg hatching rates of *Trichostrongylus colubriformis*, indicating that higher CT content enhanced anthelmintic efficacy against *T. colubriformis* (Chylinski *et al*., 2023; Hoste *et al*., 2022). Further evidence demonstrated damage to the cuticle surface and alterations in its texture following tannin treatment. This finding suggested that tannins directly interacted with cuticular components, compromising the structural integrity and physiological function of the larval L3 protective layer (Greiffer *et al*., 2022).

Tannins also provided desirable nutritional benefits. They decreased methane emissions in ruminant animals (Engström *et al.*, 2022), acted as bypass proteins (Sujarnoko, Ridwan, *et al.*, 2020), and contributed to the preservation of silage quality (Jayanegara *et al.*, 2019).

Based on previous research evidence, the null hypothesis stated that variations in temperature and extraction time did not affect the anthelmintic activity of tannins extracted from *A. mangium* against *H. contortus*. In contrast, the alternative hypothesis stated that such variations influenced the anthelmintic activity of the extracted tannins. The aim of this study was to determine the optimum temperature and extraction time in order to obtain *A. mangium* tannins with enhanced anthelmintic activity. The extracted tannins were screened against *H. contortus* to determine their potential as a natural parasite control option.

MATERIALS AND METHODS

The study followed two main methodological stages. The first stage focused on extracting and characterizing the phytochemical profile of *A. mangium* bark using a hot water extraction method under varying temperature and time conditions. It then assessed total phenols, total tannins, CT, hydrolysable tannins (HT), antioxidant activity, and antibacterial properties. The second stage involved an *in vitro* evaluation of the anthelmintic efficacy of the extract against adult gastrointestinal nematodes, with larval motility used as the primary endpoint.

Extraction by hot water: The bark of *A. mangium* was uniformly cut into pieces measuring 0.5 cm × 1 cm and placed in a hot water extraction system. The extraction process was conducted at three different temperatures (40°C, 60°C, and 80°C) and for two durations (45 and 60 minutes), with each treatment replicated five times. The extracted bark was analyzed for its phytochemical composition, while tannins were removed and leached into the water. The resulting extract was then assessed for total phenol, total tannin, CT, and HT (Engström *et al.*, 2022; Palacios *et al.*, 2021). The temperature and extraction time that resulted in the highest tannin recovery were selected for further procedures.

Antioxidant activity of Acacia extract: The antioxidant activity was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and the ferric reducing antioxidant power (FRAP) method to determine the extent to which the Acacia extract inhibited oxidative reactions. A 50 ppm DPPH solution was prepared by dissolving 5 mg of DPPH in 100 mL of analytical-grade methanol. Similarly, a 50-ppm standard solution of

ascorbic acid was prepared by dissolving 5 mg of ascorbic acid in 100 mL of distilled water. The solution was then measured in volumes of 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mL to obtain final concentrations of 1, 2, 3, 4, 5, and 6 ppm in a 25 mL volumetric flask. A control solution was prepared using 2 mL of the 50 ppm DPPH solution.

The antioxidant activity assessment was performed as follows. A 2 mL aliquot of Acacia bark extract and a 2 mL aliquot of the standard solution were separately transferred into dark test tubes. Subsequently, 2 mL of the 50 ppm DPPH solution was added to each tube. The mixtures were then agitated using a shaker for 30 minutes. Absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm (Liyana-Pathirana & Shahidi, 2005). The antioxidant activity determined using the DPPH method was expressed as percent inhibition, as calculated by Equation 1.

$$Inhibition\ (\%) = \frac{(Absorbance\ of\ control - Absorbance\ of\ sample)}{Absorbance\ of\ control} \times 100\% \quad (1)$$

One milliliter of Acacia bark extract and one milliliter of standard ascorbic acid solution were separately transferred into test tubes. Each test tube was then supplemented with 1 mL of 0.2 M phosphate buffer (pH 6.6) and 1 mL of 1% potassium ferricyanide solution. The mixtures were incubated at 50°C for 20 minutes. After incubation, 1 mL of 10% trichloroacetic acid (TCA) solution was added to each test tube, followed by centrifugation at 3000 rpm for 10 minutes.

After centrifugation, 1 mL of the supernatant was carefully collected and mixed with 1 mL of distilled water and 0.5 mL of 0.1% ferric chloride ($FeCl_3$) solution. The mixture was left to stand for 10 minutes before its absorbance was measured using a UV-Vis spectrophotometer at a maximum wavelength within the range of 400–800 nm. An oxalate solution was used as the blank, and the results were expressed in milligram equivalents of ascorbic acid per gram of sample.

Antibacterial assay of Acacia extract: The disc diffusion method was employed using nutrient agar (NA) as the bacterial growth medium. The NA was prepared by heating and then aseptically pouring it into sterile Petri dishes. The *Escherichia coli* culture was inoculated onto the NA medium by adding 1 mL of the diluted bacterial suspension and stirring until evenly distributed. Paper discs were immersed in tannin extract at varying concentrations (50%, 75%, and 100%), an anti-diarrheal drug (as a positive control), and distilled water (as a negative control) for one hour to facilitate absorption. The discs were then air-dried before being placed on the NA medium inoculated with bacteria. The Petri dishes were incubated, and the inhibition zones surrounding the paper discs were measured on the first and fifth days. The area of inhibition, or clear zone, was subsequently calculated.

Ethical approval: The study received ethical approval from the National Research and Innovation Agency (BRIN) under ID: 078/KE.02/SK/10/2022.

Anthelmintic activity of Acacia extract: The worm samples were collected from rumen waste at a slaughterhouse, specifically from the greater curvature region. Before the larval motility assay, the worms were incubated in phosphate-buffered saline (PBS) to maintain viability. Adult female worms were subsequently selected as test subjects. A motility test was then performed on the worms using Acacia extract dilutions ranging from 25% to 100%.

The anthelmintic efficacy test lasted seven hours at ambient temperature (28–30°C). This exposure period fell within the commonly accepted range of 6 to 12 hours reported in previous *in vitro* studies, during which the peak reduction in motility typically occurred between 7- and 8-hours post-exposure (A. Al-Saeed, 2023; Josiah *et al*., 2023). After the exposure period, the worms were transferred into phosphate-buffered saline (PBS) for 10 minutes. Specimens that displayed visible movement were classified as alive, whereas those that showed no response were recorded as dead. The anterior and posterior regions of the worms were examined under a compound light microscope at 10×10 magnification. Morphological changes in both ends of the worms were documented using a calibrated camera attached to the microscope.

The experiment consisted of seven treatment groups: albendazole at 19 mg/mL as a positive control (T1); PBS as a negative control (T2); 100% Acacia extract (T3); 75% Acacia extract diluted with 25% distilled water (T4); 50% Acacia extract with 50% distilled water (T5); 25% Acacia extract with 75% distilled water (T6); and 100% distilled water as a diluent control (T7).

Each treatment underwent three replications (n = 3). In each replicate, researchers randomly assigned 10 adult worms, yielding a total of 30 worms per treatment group. The replication scheme adhered to the minimum accepted standard for *in vitro* anthelmintic testing, enabling reliable statistical comparisons and reducing biological variation. The sample size of 30 was determined using Equation 2 (Ko & Lim, 2021), assuming a significance level of 0.05, a statistical power of 80%, a standard deviation (σ), and a minimum difference (Δ) considered biologically meaningful.

$$n = \frac{2(Z_{\frac{\alpha}{2}} + Z_{\beta})^2 \cdot \sigma^2}{\Delta^2} \quad (2)$$

Worms were considered dead if they showed no movement when touched. Mortality data were then calculated as a percentage using Equation 3.

$$Mortality\ index\ (\%) = \frac{\text{Number of dead worm}}{\text{Total number of worms per replicate}} \times 100 \quad (3)$$

Statistical analysis: The study examined the effects of temperature and extraction time on the quality of Acacia extract under a completely randomized design with two factors. Temperature levels were set at 40, 60, and 80°C, and extraction times were 45 and 60 minutes. Each treatment combination was replicated four times. The measured parameters included total phenol, total tannin, the tannin-to-phenol ratio (TPR), CT, HT, and free radical inhibition (DPPH and FRAP). Antibacterial activity was assessed by measuring the diameter of the inhibition zone. All data for each parameter were analyzed using ANOVA (SPSS version 25). When significant interactions between factors were detected, the Duncan post hoc test was used to determine specific differences among treatments. Furthermore, Bonferroni contrasts were conducted to evaluate each pairwise treatment difference individually. The estimates were considered substantial when the absolute value exceeded 0.8 and the significance level was below 0.05 (Amirul *et al*., 2025; Groenwold *et al*., 2021).

The mortality index included seven treatments; each replicated three times with ten worms per replicate. Data were analyzed using ANOVA (SPSS version 25), followed by the Duncan post hoc test to identify significant differences among treatments.

RESULTS

The study evaluated the effects of temperature and extraction time. Higher temperatures significantly increased the levels of phenol, tannin, TPR, CT, and HT ($P \leq 0.01$; Table 1). Extraction time also exerted a positive influence; however, it did not significantly affect free radical inhibition. The interaction between temperature and time further elevated the concentrations of these phytochemicals ($P \leq 0.01$), warranting post hoc analysis using Duncan's test (Table 2). Optimal conditions were identified at 80°C for 60 minutes, serving as the comparative reference point for individual treatment contrasts (Table 6).

A clear trend emerged, showing that an increase in temperature combined with longer extraction time correlated with higher phytochemical yields (Figure 1). The most pronounced effects occurred at 60 minutes compared to 45 minutes of extraction, particularly at the highest temperature of 80°C, where the phytochemical content peaked. The interaction between temperature and time began to manifest clearly at 80°C (Figure 1). Nonetheless, statistical analysis using partial eta squared (η^2_p) revealed minimal interaction effects, as the interaction term between temperature and time produced a low η^2_p value. Temperature alone accounted for the dominant source of variation (Table 1).

The DPPH assay demonstrates that temperature and time do not significantly influence the percentage of inhibition. However, their interaction is statistically significant ($P \leq 0.05$; Table 4). The optimal condition is achieved at 40°C for 60 minutes, resulting in an average inhibition of 72.9 ± 0.77%. Antioxidant activity can also be assessed using the FRAP method, which evaluates the reduction of Fe^{3+} to Fe^{2+}. Unlike DPPH, FRAP results indicate that higher temperatures significantly enhance inhibition capacity ($P \leq 0.01$), while extraction time has no direct effect. Nevertheless, the interaction between time and temperature significantly affects the inhibition percentage. The lowest inhibition is recorded at 40°C for 60 minutes, whereas the highest occurs at 80°C for both 45 and 60 minutes, with comparable values (Table 5). The Bonferroni comparison (Table 6) supported the finding. The combination of 80°C with extended durations (45 and 60 minutes) resulted in significantly higher FRAP values ($P \leq 0.05$) compared to other temperature–time combinations. However, no such pattern was observed for DPPH. Figure 1 illustrates the interaction patterns for both DPPH and FRAP data. An interaction effect appeared for DPPH at 60°C, indicating a temperature-specific response. Although the time factor showed overlapping trends across temperatures, a crossover interaction occurred between 60 and 80°C, suggesting that the effect of time on antioxidant activity varied depending on the extraction temperature.

The results demonstrate that Acacia extract has antibiotic potential against *E. coli*. Higher concentrations produce larger clear zones ($P \leq 0.01$; Table 3). The 100% extract yields the widest zone (10.9 ± 2.2 cm), exceeding the 75% and 50% concentrations. The optimal extraction temperature is 80°C, significantly outperforming 60 and 40°C ($P \leq 0.01$). Extraction time also affects efficacy, with 60 minutes producing significantly larger clear zones than 45 minutes ($P \leq 0.01$). Tests showed that 100% Acacia extract matched the effectiveness of albendazole after seven hours. In contrast, the control treatment with saline buffer and distilled water caused no worm mortality. Extracts at 25%, 50%, and 75% induced death but were less potent than albendazole and the full-strength extract ($P \leq 0.01$). Albendazole acted rapidly, eliminating all worms by the fifth hour, as shown in Figure 2. The anterior and posterior cross-sections of *H.*

contortus treated with albendazole and Acacia extract are presented in Figure 3. The cross-sections appeared similar across treatments at a glance. However, albendazole and Acacia extract caused noticeable wrinkling of tissues surrounding the anterior and posterior regions.

Table 1. ANOVA of phenolic and tannin characteristics affected by temperature, time, and their interaction in Acacia bark extract.

Treatment		Total phenol (mg/L GAE)	Total tannin (mg/L GAE)	TPR (%)	CT (mg/L GAE)	HT (mg/L GAE)
Temperature	40℃	7.20 ± 0.10[a]	4.29 ± 0.10[a]	60.03 ± 0.32[a]	1.01 ± 0.04[a]	3.22 ± 0.07[a]
	60℃	7.20 ± 0.39[a]	4.28 ± 0.35[a]	60.63 ± 1.33[a]	1.00 ± 0.07[a]	3.38 ± 0.24[a]
	80℃	9.10 ± 0.06[b]	5.94 ± 0.07[b]	65.32 ± 0.44[b]	1.33 ± 0.05[b]	4.61 ± 0.04[b]
Time	45 min	7.38 ± 0.36	4.46 ± 0.30	60.67 ± 0.88	0.99 ± 0.05	3.53 ± 0.23
	60 min	8.27 ± 0.23	5.22 ± 0.23	63.31 ± 0.89	1.24 ± 0.05	3.94 ± 0.19
P value						
Temperature		≤0.001	≤0.001	≤0.001	0.025	≤0.001
Time		0.188	0.174	0.149	0.209	0.437
Temp.× Time		0.073	0.078	0.06	0.584	0.168
η^2_p						
Temp.		0.819	0.822	0.756	0.508	0.711
Time		0.408	0.420	0.420	0.437	0.168
Temp.× Time		0.252	0.247	0.268	0.058	0.180
Adj. R^2		0.805	0.806	0.754	0.555	0.671

Values in the same column, nested within each factor (temperature or time), that have different superscripts showed significant differences at $P \leq 0.05$. Adj. R^2=adjusted coefficient of determination, CT=condensed tannins, GAE=gallic acid equivalents, HT=hydrolysable tannins, η^2_p=partial eta square, TPR=tannin-to-phenol ratio.

Table 2. Duncan test of phenolic and tannin characteristics affected by temperature–time combinations in Acacia bark extract.

Treatment	Total phenol (mg/L GAE)	Total tannin (mg/L GAE)	TPR (%)	CT (mg/L GAE)	HT (mg/L GAE)
40℃ × 45 min	7.00 ± 0.13[b]	4.19 ± 0.14[b]	60.32 ± 0.54[b]	1.02 ± 0.19[a]	13.22 ± 0.22[a]
40℃ × 60 min	7.40 ± 0.28[c]	4.40 ± 0.35[b]	59.73 ± 1.16[b]	1.19 ± 0.26[ab]	13.40 ± 0.19[a]
60℃ × 45 min	6.17 ± 0.11[a]	3.41 ± 0.28[a]	57.34 ± 0.69[a]	0.83 ± 0.06[ab]	13.55 ± 0.15[a]
60℃ × 60 min	8.20 ± 0.17[d]	5.15 ± 0.37[c]	63.92 ± 1.89[c]	1.17 ± 0.18[b]	14.63 ± 0.34[ab]
80℃ × 45 min	8.97 ± 0.07[e]	5.77 ± 0.08[d]	64.35 ± 0.42[d]	1.22 ± 0.08[ab]	15.36 ± 0.11[b]
80℃ × 60 min	9.22 ± 0.12[f]	6.11 ± 0.12[d]	66.29 ± 0.54[d]	1.54 ± 0.22[a]	15.78 ± 0.10[b]

Values with different superscripts in the same column indicate significant differences at $P \leq 0.05$. CT=condensed tannins, GAE=gallic acid equivalents, HT=hydrolysable tannin, TPR=the tannin-to-phenol percentage.

Table 3. ANOVA of the interaction among time, temperature, and Acacia bark extract concentration as an antibacterial agent against *E. coli*.

Treatment		Clearing zone (mm)	*P* value
Temperature	40℃	7.75 ± 1.57	0.078
	60℃	9.42 ± 1.61	
	80℃	11.25 ± 1.89	
Time	45 min	9.06 ± 2.15[a]	0.001
	60 min	9.89 ± 2.2[b]	
Extract concentration (%)	50	8.17 ± 1.76[a]	0.032
	75	9.33 ± 1.79[b]	
	100	10.92 ± 2.18[c]	
P value			
Temperature × Time		0.722	
Temperature × Extract concentration		0.460	

Time × Extract concentration	0.703
Temperature × Time × Extract concentration	0.785
η^2_p	
Temperature × Time	0.002
Temperature × Extract concentration	0.101
Time × Extract concentration	0.001
Temperature × Time × Extract concentration	0.031
Adj. R^2	0.686

Values in the same column, nested within each factor (temperature, time, extract concentration), that have different superscripts showed significant differences at $P \leq 0.05$. Adj. R2=adjusted coefficient of determination, η^2_p=partial eta square.

Table 4. ANOVA of the interaction between time and temperature on the antioxidant activity (DPPH activity and FRAP) of Acacia bark extract.

Treatment		**DPPH (%)**	**FRAP (mMCE)**
Temperature	40℃	71.91 ± 1.59	2.54 ± 0.32[a]
	60℃	71.20 ± 1.47	3.38 ± 0.31[a]
	80℃	70.43 ± 1.65	4.66 ± 0.31[b]
Time	45 min	71.25 ± 1.35[b]	3.63 ± 0.95
	60 min	71.11 ± 1.91[a]	3.42 ± 0.97
P value			
Temperature		0.916	≤0.01
Time		0.016	0.657
Temperature × Time		0.027	0.877
η^2_p			
Temperature		0.241	0.713
Time		0.020	0.015
Temperature × Time		0.331	0.015
Adj. R^2		0.393	0.637

Values in the same column, nested within each factor (temperature or time), that have different superscripts showed significant differences at $P \leq 0.05$. Adj. R^2=adjusted coefficient of determination, DPPH activity=test of 2,2-diphenyl-1-picrylhydrazyl activity, FRAP=ferric reducing antioxidant power, mMCE=millimolar catechin equivalent, η^2_p=partial eta square.

Table 5. Duncan test of antioxidant activity (DPPH activity and FRAP) affected by temperature–time combinations in Acacia bark extract.

Treatment	DPPH (%)	FRAP (mMCE)
40℃ × 45 min	70.91 ± 0.45[ab]	2.79 ± 0.11[b]
40℃ × 60 min	72.92 ± 0.77[b]	2.29 ± 010[a]
60℃ × 45 min	71.33 ± 1.08[ab]	3.27 ± 0.15[c]
60℃ × 60 min	71.08 ± 0.29[ab]	3.49 ± 0.16[c]
80℃ × 45 min	71.50 ± 0.05[ab]	4.84 ± 0.12[d]
80℃ × 60 min	69.36 ± 0.76[a]	4.48 ± 0.14[d]

Values with different superscripts in the same column indicate significant differences at $P \leq 0.05$. DPPH activity=test of 2,2-diphenyl-1-picrylhydrazyl activity, FRAP=ferric reducing antioxidant power, mMCE=millimolar catechin equivalent.

Table 6. Bonferroni test for phenolics, tannins, and antioxidant activity under temperature–time contrast in Acacia bark extract.

Contrast	Total phenol (mg/L GAE)		Total tannin (mg/L GAE)		TPR (%)		CT (mg/L GAE)		HT (mg/L GAE)		DPPH (%)		FRAP (mMCE)	
	Estimate	*P* value	Estimate	*P* value	Estimate	*P* value	Estimate	*P* value	Estimate	*P* value	Estimate	*P* value	Estimate	*P* value
40°C × 45 min vs 60°C × 45 min	0.145	1.000	0.137	1.000	1.093	1.000	0.193	1.000	-0.056	1.000	-0.427	1.000	-0.850	1.000
40°C × 45 min vs 80°C × 45 min	-2.158	≤0.001	-2.127	≤0.001	-8.403	0.001	-0.194	1.000	-1.933	0.002	-0.597	1.000	-2.178	0.001
40°C × 45 min vs 40°C × 60 min	-0.473	1.000	-0.476	1.000	-2.397	1.000	-0.167	1.000	-0.309	1.000	-3.925	0.234	-0.197	1.000
40°C × 45 min vs 60°C × 60 min	-1.240	0.031	-1.229	0.029	-5.449	0.045	-0.147	1.000	-1.081	0.187	-0.171	1.000	-1.096	0.325
40°C × 45 min vs 80°C × 60 min	-2.408	≤0.001	-2.400	≤0.001	-9.400	≤0.001	-0.516	0.012	-1.884	0.002	1.536	1.000	-2.128	0.002
60°C × 45 min vs 80°C × 45 min	-2.303	≤0.001	-2.265	≤0.001	-9.496	≤0.001	-0.387	0.109	-1.877	0.002	-0.171	1.000	-1.328	0.104
60°C × 45 min vs 40°C × 60 min	-0.618	1.000	-0.614	1.000	-3.491	0.620	-0.360	0.173	-0.254	1.000	-3.498	0.429	0.653	1.000
60°C × 45 min vs 60°C × 60 min	-1.385	0.012	-1.366	0.012	-6.542	0.010	-0.340	0.239	-1.026	0.253	0.256	1.000	-0.246	1.000
60°C × 45 min vs 80°C × 60 min	-2.553	≤0.001	-2.537	≤0.001	-10.494	≤0.001	-0.709	≤0.001	-1.828	0.003	1.962	1.000	-1.278	0.134
80°C × 45 min vs 40°C × 60 min	1.685	0.002	1.651	0.002	6.005	0.021	0.027	1.000	1.624	0.009	-3.328	0.543	1.981	0.004
80°C × 45 min vs 60°C × 60 min	0.918	0.240	0.899	0.242	2.954	1.000	0.047	1.000	0.852	0.633	0.427	1.000	1.082	0.348
80°C × 45 min vs 80°C × 60 min	-0.250	1.000	-0.273	1.000	-0.998	1.000	-0.322	0.324	0.049	1.000	2.133	1.000	0.050	1.000
40°C × 60 min vs 60°C × 60 min	-0.768	0.588	-0.752	0.589	-3.051	1.000	0.020	1.000	-0.772	0.944	3.754	0.299	-0.899	0.810
40°C × 60 min vs 80°C × 60 min	-1.935	≤0.001	-1.924	≤0.001	-7.003	0.005	-0.349	0.207	-1.575	0.011	5.461	0.024	-1.931	0.005
60°C × 60 min vs 80°C × 60 min	-1.168	0.050	-1.171	0.042	-3.952	0.343	-0.369	0.149	-0.803	0.811	1.706	1.000	-1.032	0.440

CT=condensed tannins, DPPH activity=test of 2,2-diphenyl-1-picrylhydrazyl activity, Estimate= the analysis revealed differences in effect size based on two contrast comparisons, FRAP=ferric reducing antioxidant power, GAE=gallic acid equivalents, HT=hydrolysable tannin, mMCE=millimolar catechin equivalent, TPR=the tannin-to-phenol percentage.

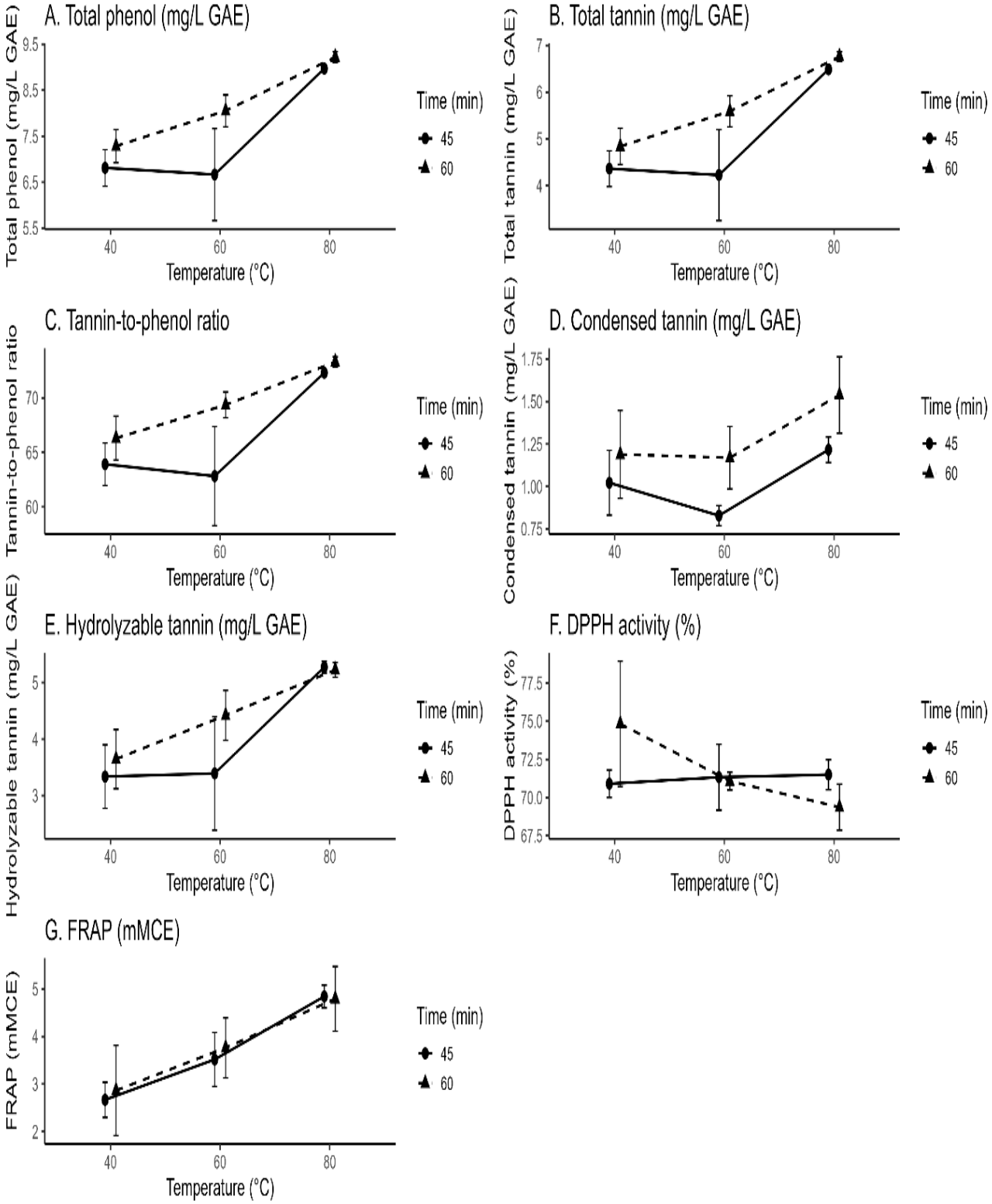


Figure 1. Interaction between temperature and time factors in the acacia extract affected phytochemical and antioxidant characteristics. A significant interaction occurred when the time factor intersected at approximately 45 and 60 minutes in combination with temperature levels of 40, 60, and 80°C.

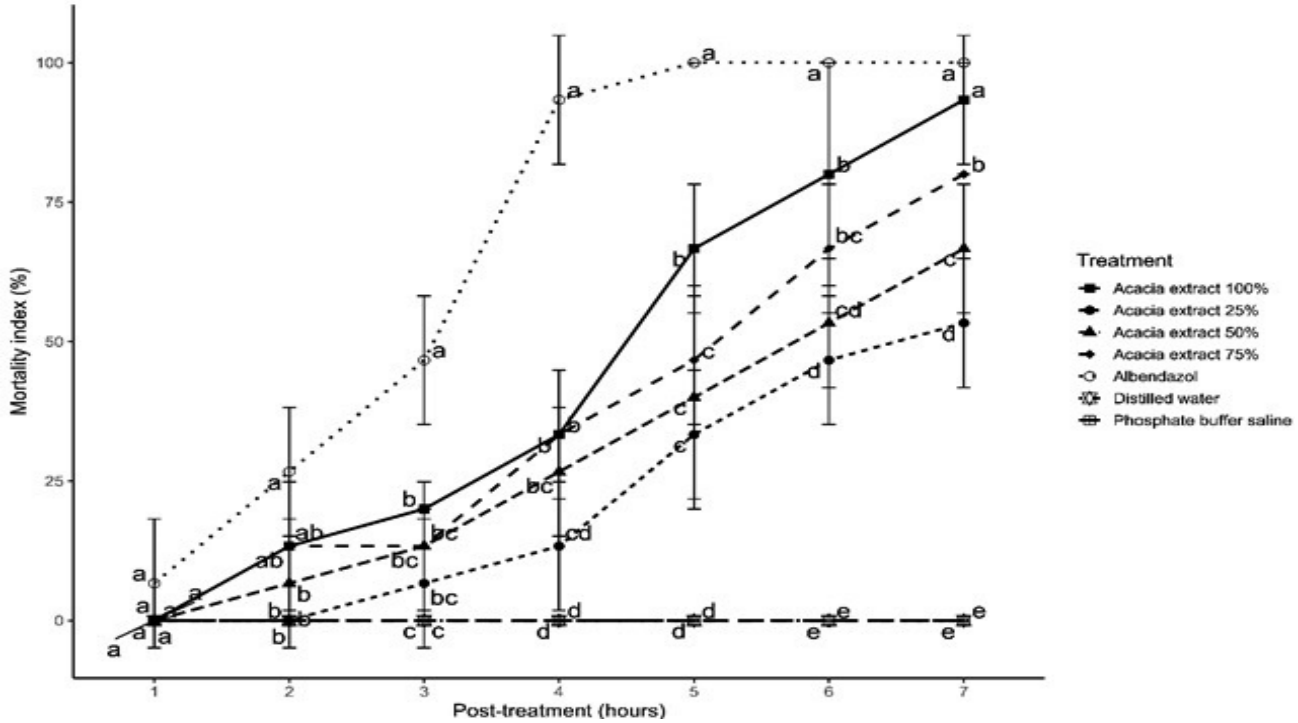
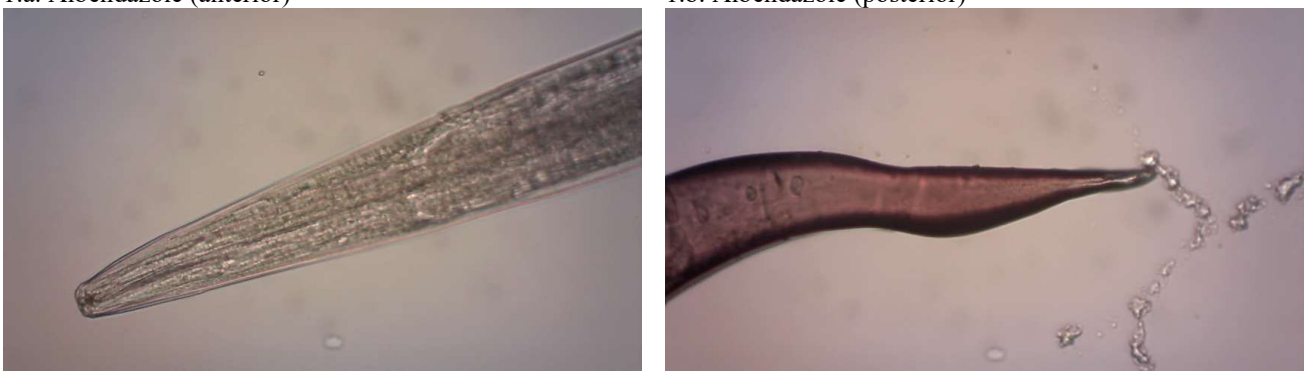


Figure 2. Presents the mortality index of *Haemonchus contortus*. The treatment groups include positive control at a concentration of 19 mg/mL (albendazole), acacia extract 100%, acacia extract 75%, acacia extract 50%, acacia extract 25%, phosphate buffer saline, and distilled water. Each replication involved 10 worms, and each treatment contained 3 replications.

1.a. Albendazole (anterior) 1.b. Albendazole (posterior)



2.a. Acacia extract (anterior) 2.b. Acacia extract (posterior)

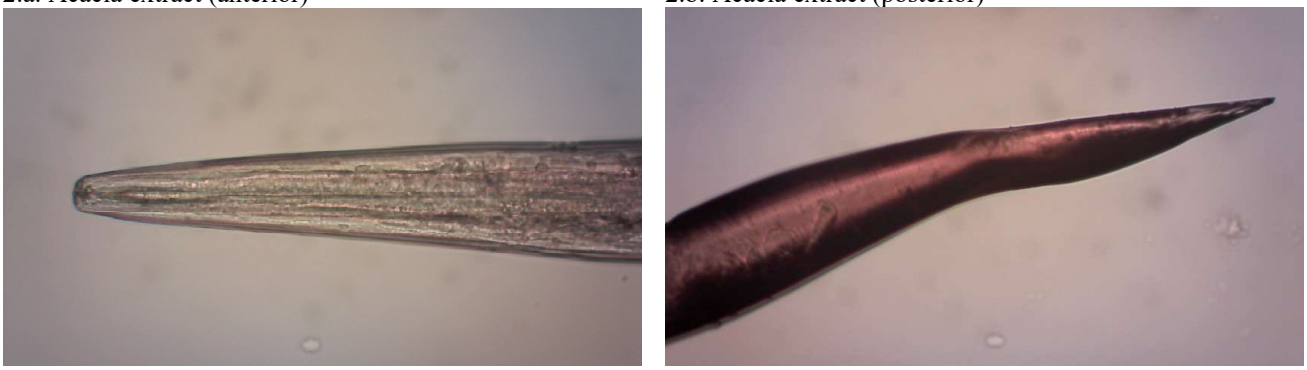


Figure 3. Anterior and posterior conditions of *Haemonchus contortus* following treatment. Treatments included a positive control (albendazole) and Acacia extract (100% extract) after 7 hours at a magnification of 10×10.

DISCUSSION

Extraction at high temperatures significantly increased the content of secondary metabolites, particularly phenolic compounds and tannins. Osman *et al*. (2024) reported that tannin concentration reached its highest level when extracted at 70 °C, compared to extractions at 30 and 50°C. This increase resulted from the reduced polarity of water at elevated temperatures (Cheng *et al.*, 2021). The η^2_p test results indicated that extraction time also played an important role in increasing phenol and tannin concentrations. However, this observation differed from the findings of Wonggo *et al*. (2024), who reported that tannin levels in mangrove seed extracts obtained through subcritical water extraction decreased as extraction time increased. Extraction time appeared less effective when the interval between processes was too short (15-mintute difference). Nonetheless, the graphical visualization still showed differences between extractions performed for 45 and 60 minutes, particularly for CT and HT concentrations, which were slightly higher at 60 minutes. Further investigation is likely required to determine the optimal extraction time for maximizing phytochemical yields, for example, by applying a response surface model (Sholikin *et al*., 2019).

Acacia bark contained a high concentration of tannins (Sujarnoko, Ridwan, *et al.*, 2020), which were widely recognized for their anthelmintic properties (Caradus *et al*., 2022; Greiffer *et al*., 2022). These compounds bound to proteins, disrupting the structural integrity of nematodes and eventually causing their death (Caradus *et al*., 2022; Engström *et al*., 2022). Visual analysis supported this mechanism, as both the anterior and posterior parts of *H. contortus* exhibited noticeable shrinkage following exposure to Acacia extract. Further evidence emerged from the mortality index data (Figure 2), which demonstrated that Acacia extract exhibited clear anthelmintic activity across dilution levels from 35% to 100%. Despite this, extracts diluted to concentrations between 25% and 75% showed lower efficacy compared to albendazole. Only the undiluted 100% extract achieved a mortality rate that closely resembled that of the commercial anthelmintic. The observed bioactivity likely resulted from the tannins' ability to bind to the mucosal and cuticular proteins of *H. contortus*, leading to cuticle lysis and, ultimately, death (Hoste *et al*., 2022). The combined visual and quantitative evidence suggested that the anthelmintic effects of Acacia tannins operated through structural degradation of the nematode's outer layers, reinforcing their potential as a phytogenic alternative to synthetic drugs. In addition to their anthelmintic capacity, tannins also exhibited antibacterial and antioxidant activities. The damage caused by *H. contortus* could lead to systemic failure; therefore, tannins offered a preventive effect by reducing bacterial infection risk during infestation and by enhancing the body's oxidative status to combat disease or modulate rumen microbiota (Makmur *et al*., 2022; Tong *et al*., 2022).

Apart from their biological role as anthelmintics, tannins demonstrated potent antibacterial activity against pathogens (Jayanegara *et al.*, 2019). Tannins in Acacia bind proteins, restricting bacterial access during silage production (Sadarman *et al.*, 2019). *E. coli*, a spoilage bacterium in silage storage, also causes diarrhea in calves (Bergholm *et al.*, 2024; Jia *et al.*, 2024). Its reduction may result from CTs, which exhibit anti-biofilm and anti-motility effects (Dakheel *et al.*, 2020). Tannins inhibit bacterial growth by binding to iron, disrupting cell walls, damaging membranes, inhibiting fatty acid biosynthesis, and interfering with quorum sensing. They also suppress gene expression, enzyme activity, motility, and the formation of biofilms, adhesins, and toxins. These statements support the findings from the acacia extract results in the present study, confirming that tannin extract from acacia had a strong effect on the inhibition zone of E. coli, particularly at 100% concentration. However, the effect of temperature was not evident, while the effect of extraction time remained significant, although the numerical difference was minimal. When compared with other antibacterial agents such as propolis and *Piper betle* leaf extract, the results were relatively favorable (Ngamsurach & Praipipat, 2022; Vică *et al*., 2021).

Tannins also exhibited antioxidant properties (Motta *et al.*, 2020). Antioxidants enhanced immunity, improved sheep productivity (Dey *et al.*, 2015), and aided in the treatment of helminth infections (Chylinski *et al.*, 2023). They protected liver cells from damage caused by free radicals during Fasciola hepatica development (Cwiklinski *et al.*, 2021). By neutralizing these radicals, antioxidants reduced liver tissue damage. They also strengthened immunity (Orzuna-Orzuna *et al.*, 2021) and alleviated physiological stress (Longobardi *et al.*, 2021). The findings aligned with the observation that higher concentrations of both CT and HT coincided with an upward trend in antioxidant activity measured by FRAP and DPPH (Figure 1). However, the DPPH results at 60°C displayed an opposite trend. Nevertheless, antioxidants were indeed influenced, in part, by tannin composition (Moccia *et al*., 2020).

Although this study served as a model to evaluate the efficacy of Acacia bark extract against *H. contortus* under *in vitro* conditions, the fully controlled environment may have reduced ecological variation that could influence the extract's effectiveness. Therefore, *in vivo* studies are essential to further assess the extract's potential as an anthelmintic adjuvant. Additionally, this study did not include toxicity testing or an evaluation of tannin–protein binding capacity, which is important for understanding potential adverse effects associated with high tannin concentrations.

Conclusion: Hot water extraction of Acacia bark demonstrates significant potential in controlling *Haemonchus contortus*, with optimal conditions of 80℃ for 60 minutes yielding the highest concentrations of total phenols, total tannins, condensed tannins, and hydrolysable tannins. Under these conditions, Acacia extract exhibits strong anthelmintic activity, particularly at a 100% concentration. Additionally, it shows antibacterial properties against *Escherichia coli* and notable antioxidant activity, with DPPH inhibition being most effective at 40℃ and FRAP activity peaking at 80℃. These findings highlight the multifunctional bioactive potential of Acacia extract, offering valuable applications in pharmaceutical, veterinary, and food industries.

Acknowledgment: None to declare.

Funding Statement: The authors acknowledge the financial support from the "Penelitian Terapan Penugasan (PTP)" Programme, funded by the Directorate General of Vocational Education, Indonesia, under contract numbers 47/SPK/D.D4/PPK.01.APTV/III/2024 and 11399/IT3.D10/PT.01.03/P/B/2024, granted to Tekad Urip Pambudi Sujarnoko.

Author contributions: TUPS: Conceptualization, Supervision, Funding Acquisition; DB: Project Administration, Methodology, Writing - original draft; NAS: Resources, Visualization, Writing - original draft; RS: Data Curation, Formal Analysis, Writing - original draft; TMS: Resources, Investigation, Validation; MA: Resources, Investigation, Validation; AUP: Data Curation, Resources, Validation; AF: Data Curation, Resources, Validation; MMS: Investigation, Validation, Writing - review & editing.

Data availability statement: None applicable.

Ethical approval: None applicable.

Conflict of interests: The manuscript has not been published or submitted to any other journal. Each author has made significant contributions and agrees with the content of the manuscript.

REFERENCES

A. Al-Saeed, F. (2023). *In vitro* anthelmintic efficacy of Haloxylon salicornicum leaves extract using adult *Heamonchus contortus* worms. The Pakistan Veterinary J., 43(1), 91-96. https://doi.org/10.29261/pakvetj/2022.091.

Ahmed, W.I., A.N. Mohammed and A.-S.A. Sleim. (2024). Efficacy evaluation of hydrogen peroxide disinfectant-based zinc oxide nanoparticles against diarrhea causing Escherichia coli in ruminant animals and broiler chickens. Scientific Reports, 14(1), 9159. https://doi.org/10.1038/s41598-024-59280-4.

Amirul, F.M.A., N.A. Mazlan, S. Sadarman, N.D. Rusli, M.M. Sholikin, A. Irawan, D. Febrina, N. Qomariyah, R.A. Nurfitriani, S. Aditya, A. Jayanegara, M.Z. Saad, D.N. Adli and H.A. Hassim. (2025). A comprehensive meta-analysis of cassava addition in a buffalo diet: *In vivo* investigations on performance and rumen health. Tropical Animal Science J., 48(4), 298–314. https://doi.org/10.5398/tasj.2025.48.4.298.

Arsenopoulos, K. V., G.C. Fthenakis, E.I. Katsarou and E. Papadopoulos. (2021). Haemonchosis: A challenging parasitic infection of sheep and goats. Animals, 11(2), 363. https://doi.org/10.3390/ani11020363.

Bergholm, J., T.S. Tessema, A.-L. Blomström and M. Berg. (2024). Detection and molecular characterization of major enteric pathogens in calves in central Ethiopia. BMC Veterinary Research, 20(1), 389. https://doi.org/10.1186/s12917-024-04258-7.

Caradus, J.R., C.R. Voisey, G.R. Cousin, R. Kaur, D.R. Woodfield, A. Blanc and M.B. Roldan. (2022). The hunt for the "holy grail": Condensed tannins in the perennial forage legume white clover (*Trifolium repens* L.). Grass and Forage Science, 77(2), 111–123. https://doi.org/10.1111/gfs.12567.

Cheng, Y., F. Xue, S. Yu, S. Du and Y. Yang. (2021). Subcritical water extraction of natural products. Molecules, 26(13), 4004. https://doi.org/10.3390/molecules26134004.

Chylinski, C., K.F. Degnes, I.M. Aasen, S. Ptochos, B.M. Blomstrand, K.-C. Mahnert, H.L. Enemark, S.M. Thamsborg, H. Steinshamn and S. Athanasiadou. (2023). Condensed tannins, novel compounds and sources of variation determine the antiparasitic activity of Nordic conifer bark against gastrointestinal nematodes. Scientific Reports, 13(1), 13498. https://doi.org/10.1038/s41598-023-38476-0.

Costa, S.N. de O., M.V.T. e Silva, J.M. Ribeiro, J.M. da C. e Castro, M.F. Muzitano, R.G. da Costa, A.E.A. Oliveira and K.V.S. Fernandes. (2023). Secondary metabolites related to the resistance of *Psidium* spp. against the nematode *Meloidogyne enterolobii*. Heliyon, 9(7), e17778. https://doi.org/10.1016/j.heliyon.2023.e17778.

Ćućuz, V., J. Cvejić, L. Torović, L. Gojković-Bukarica, J. Acevska, A. Dimitrovska, T.M.S. Aldawoud and C.M. Galanakis. (2022). Design of experiments (DoE) to model phenolic compounds recovery from grape pomace using ultrasounds. J. Food Science and Technology, 59(7), 2913–2924. https://doi.org/10.1007/s13197-021-05317-9.

Cunha, S.M.F., S. Lam, B. Mallard, N.A. Karrow and Á. Cánovas. (2024). Genomic regions associated with resistance to gastrointestinal nematode parasites in sheep—A review. Genes, 15(2), 187. https://doi.org/10.3390/genes15020187.

Cwiklinski, K., M.W. Robinson, S. Donnelly and J.P. Dalton. (2021). Complementary transcriptomic and proteomic analyses reveal the cellular and molecular processes that drive growth and development of *Fasciola hepatica* in the host liver. BMC Genomics, 22(1), 46. https://doi.org/10.1186/s12864-020-07326-y.

Dakheel, M.M., F.A.H. Alkandari, I. Mueller-Harvey, M.J. Woodward and C. Rymer. (2020). Antimicrobial *in vitro* activities of condensed tannin extracts on avian pathogenic *Escherichia coli*. Letters in Applied Microbiology, 70(3), 165–172. https://doi.org/10.1111/lam.13253.

Engström, M.T., V. Virtanen and J.-P. Salminen. (2022). Influence of the hydrolyzable tannin structure on the characteristics of insoluble hydrolyzable tannin–protein complexes. J. Agricultural and Food Chemistry, 70(41), 13036–13048. https://doi.org/10.1021/acs.jafc.2c01765.

Esteban-Ballesteros, M., J. Sanchis, C. Gutiérrez-Corbo, R. Balaña-Fouce, F.A. Rojo-Vázquez, C. González-Lanza and M. Martínez-Valladares. (2019). *In vitro* anthelmintic activity and safety of different plant species against the ovine gastrointestinal nematode *Teladorsagia circumcincta*. Research in Veterinary Science, 123, 153–158. https://doi.org/10.1016/j.rvsc.2019.01.004.

Gareh, A., N.M. Elhawary, A. Tahoun, A.M. Ramez, D.M.M. EL-shewehy, E. Elbaz, M.I. Khalifa, K.F. Alsharif, R.M.A. Khalifa, A.K. Dyab, M.E.M. Monib, M.I. Arafa and E.K. Elmahallawy. (2021). Epidemiological, morphological, and morphometric study on *Haemonchus* spp. recovered from goats in egypt. Frontiers in Veterinary Science, 8, 1–10. https://doi.org/10.3389/fvets.2021.705619.

Gazzonis, A.L., S. Panseri, R. Pavlovic, S.A. Zanzani, L. Chiesa, L. Rapetti, M. Battelli, L. Villa and M.T. Manfredi. (2023). *In vitro* evaluations and comparison of the efficacy of two commercial products containing condensed tannins and of saifoin (*Onobrychis viciifolia* scop.) hay against gastrointestinal nematodes of goats. Animals, 13(3), 547. https://doi.org/10.3390/ani13030547.

Greiffer, L., E. Liebau, F.C. Herrmann and V. Spiegler. (2022). Condensed tannins act as anthelmintics by increasing the rigidity of the nematode cuticle. Scientific Reports, 12(1), 18850. https://doi.org/10.1038/s41598-022-23566-2.

Groenwold, R.H.H., J.J. Goeman, S. Le Cessie and O.M. Dekkers. (2021). Multiple testing: when is many too much? European J. Endocrinology, 184(3), E11–E14. https://doi.org/10.1530/EJE-20-1375.

Harahap, M.A., S. Widodo, U.F. Handayani, R.I. Altandjung, Wulandari, A.A. Sakti, B.A. Atmoko, W. Negara, Y.L. Dewi, H. Julendra, A. Sofyan, T. Wahyono, T. Ujilestari, B. Ahmed, N. Qomariyah, M.M. Sholikin and Z.A. Baihaqi. (2024). Examining performance, milk, and meat in ruminants fed with macroalgae and microalgae: A meta-analysis perspective. Tropical Animal Health and Production, 56(7), 243. https://doi.org/10.1007/s11250-024-04080-1.

Hoste, H., G. Meza-OCampos, S. Marchand, S. Sotiraki, K. Sarasti, B.M. Blomstrand, A.R. Williams, S.M. Thamsborg, S. Athanasiadou, H.L. Enemark, J.F. Torres Acosta, G. Mancilla-Montelongo, C.S. Castro, L.M. Costa-Junior, H. Louvandini, D.M. Sousa, J.-P. Salminen, M. Karonen, M. Engstrom, … E.R. Morgan. (2022). Use of agro-industrial by-products containing tannins for the integrated control of gastrointestinal nematodes in ruminants. Parasite, 29, 10. https://doi.org/10.1051/parasite/2022010.

Jayanegara, A., T.U.P. Sujarnoko, M. Ridla, M. Kondo and M. Kreuzer. (2019). Silage quality as influenced by concentration and type of tannins present in the material ensiled: A meta-analysis. J. Animal Physiology and Animal Nutrition, 103(2), 456–465. https://doi.org/10.1111/jpn.13050.

Jia, Y., K. Zhang, J. Cao and W. Mao. (2024). Correlation analysis of whole genome sequencing of a pathogenic *Escherichia coli* strain of Inner Mongolian origin. Scientific Reports, 14(1), 15494. https://doi.org/10.1038/s41598-024-64256-5.

Josiah, J.G., J.Y. Adama, Z. Jiya, O.M. Abah and C. Imoisi. (2023). *In vitro* anthelmintic activities of stem and root barks extracts of *Parkia biglobosa* on infective larvae and adult of *Haemonchus contortus*. African J. Biotechnology, 22(1), 26–38. https://doi.org/10.5897/AJB2022.17528.

Julienne, K., T.A.Z. Fréjus, A.O. Pascal, G.A. Géorcelin, D.A. Adam, C.D. Christian, H.-A. Sylvie, J.B. Olaniyi and A.E. Patrick. (2021). Prevalence, effects and alternative control methods of Haemonchus contortus in small ruminants: A review. J. Veterinary Medicine and Animal Health, 13(2), 84–97. https://doi.org/10.5897/JVMAH2020.0868.

Kļaviņa, A., D. Keidāne, K. Ganola, I. Lūsis, R. Šukele, D. Bandere and L. Kovalcuka. (2023). Anthelmintic activity of *Tanacetum vulgare* L. (Leaf and flower) extracts against

trichostrongylidae nematodes in sheep *in vitro*. Animals, 13(13), 2176. https://doi.org/10.3390/ani13132176.

Ko, M.J. and C.-Y. Lim. (2021). General considerations for sample size estimation in animal study. Korean J. Anesthesiology, 74(1), 23–29. https://doi.org/10.4097/kja.20662.

Liu, L., Z. Zhang, H. Liu, S. Zhu, T. Zhou, C. Wang and M. Hu. (2023). Identification and characterisation of the haemozoin of Haemonchus contortus. Parasites & Vectors, 16(1), 88. https://doi.org/10.1186/s13071-023-05714-3.

Liyana-Pathirana, C.M. and F. Shahidi. (2005). Antioxidant activity of commercial soft and hard wheat (*Triticum aestivum* L.) as affected by gastric pH conditions. J. Agricultural and Food Chemistry, 53(7), 2433–2440. https://doi.org/10.1021/jf049320i.

Longobardi, C., S. Damiano, E. Andretta, F. Prisco, V. Russo, F. Pagnini, S. Florio and R. Ciarcia. (2021). Curcumin modulates nitrosative stress, inflammation, and dna damage and protects against ochratoxin a-Induced hepatotoxicity and nephrotoxicity in rats. Antioxidants, 10(8), 1239. https://doi.org/10.3390/antiox10081239.

Makmur, M., M. Zain, M.M. Sholikin, Suharlina and A. Jayanegara. (2022). Modulatory effects of dietary tannins on polyunsaturated fatty acid biohydrogenation in the rumen: A meta-analysis. Heliyon, 8(7), e09828. https://doi.org/10.1016/j.heliyon.2022.e09828.

Margina, Y., A. Troegubov, Y. Kulikova and N. Sliusar. (2023). Composting old bark and wood waste in cold weather conditions. Sustainability, 15(14), 10768. https://doi.org/10.3390/su151410768.

Moccia, F., A. Piscitelli, S. Giovando, P. Giardina, L. Panzella, M. D'Ischia and A. Napolitano. (2020). Hydrolyzable vs. Condensed wood tannins for bio-based antioxidant coatings: Superior properties of quebracho tannins. Antioxidants, 9(9), 804. https://doi.org/10.3390/antiox9090804.

Motta, S., M. Guaita, C. Cassino and A. Bosso. (2020). Relationship between polyphenolic content, antioxidant properties and oxygen consumption rate of different tannins in a model wine solution. Food Chemistry, 313, 126045. https://doi.org/10.1016/j.foodchem.2019.126045.

Nath, T.C., D. Lee, H. Park, S. Choe, B.A. Ndosi, Y. Kang, M.M. Bia, C. Eamudomkarn, U.K. Mohanta, K.M. Islam, J.U. Bhuiyan, H.-K. Jeon and K.S. Eom. (2021). Morphometrical and molecular characterization of *Oesophagostomum columbianum* (Chabertiidae: Oesophagostominae) and *Haemonchus contortus* (Trichostrongylidae: Haemonchinae) isolated from goat (*Capra hircus*) in Sylhet, Bangladesh. J. Parasitology Research, 2021, 1–9. https://doi.org/10.1155/2021/8863283.

Ngamsurach, P. and P. Praipipat. (2022). Antibacterial activities against *Staphylococcus aureus* and *Escherichia coli* of extracted Piper betle leaf materials by disc diffusion assay and batch experiments. RSC Advances, 12(40), 26435–26454. https://doi.org/10.1039/D2RA04611C.

Orzuna-Orzuna, J.F., G. Dorantes-Iturbide, A. Lara-Bueno, G.D. Mendoza-Martínez, L.A. Miranda-Romero and H.A. Lee-Rangel. (2021). Growth performance, meat quality and antioxidant status of sheep supplemented with tannins: A meta-analysis. Animals, 11(11), 3184. https://doi.org/10.3390/ani11113184.

Osman, Z., A. Pizzi, M.E. Elbadawi, J. Mehats, W. Mohammed and B. Charrier. (2024). Effect of technological factors on the extraction of polymeric condensed tannins from acacia species. Polymers, 16(11), 1550. https://doi.org/10.3390/polym16111550.

Palacios, C.E., A. Nagai, P. Torres, J.A. Rodrigues and A. Salatino. (2021). Contents of tannins of cultivars of sorghum cultivated in Brazil, as determined by four quantification methods. Food Chemistry, 337, 127970. https://doi.org/ 10.1016/ j.foodchem.2020.127970.

Rodríguez-Hernández, P., C. Reyes-Palomo, S. Sanz-Fernández, P.J. Rufino-Moya, R. Zafra, F.J. Martínez-Moreno, V. Rodríguez-Estévez and C. Díaz-Gaona. (2023). Antiparasitic tannin-rich plants from the south of europe for grazing livestock: A review. Animals, 13(2), 201. https://doi.org/10.3390/ani13020201.

Ruiz-Aquino, F., R. Feria-Reyes, J.G. Rutiaga-Quiñones, L.H. Robledo-Taboada and R. Gabriel-Parra. (2023). Characterization of tannin extracts derived from the bark of four tree species by HPLC and FTIR. Forest Science and Technology, 19(1), 38–46. https://doi.org/10.1080/21580103.2023.2166593.

Sadarman, M. Ridla, Nahrowi, T.U.P. Sujarnoko, R. Ridwan and A. Jayanegara. (2019). Evaluation of ration based on soy sauce by-product on addition of acacia and chestnut tannin: An *in vitro* study. IOP Conference Series: Materials Science and Engineering, 546(2), 022020. https://doi.org/10.1088/1757-899X/546/2/022020.

Sholikin, M.M., M.D. Alifian, A. Jayanegara and Nahrowi. (2019). Optimization of the *Hermetia illucens* larvae extraction process with response surface modelling and its amino acid profile and antibacterial activity. IOP Conference Series: Materials Science and Engineering, 546(6),

062030. https:// doi.org/ 10.1088/1757-899X/546/6/062030.

Solomon, L., G. Haile, N.A. Ahmed, D. Abdeta, W. Galalcha and Y. Hailu. (2024). Epidemiology and field efficacy of anthelmintic drugs associated with gastrointestinal nematodes of sheep in Nejo district, Oromia, Ethiopia. Scientific Reports, 14(1), 6841. https://doi.org/10.1038/s41598-024-55611-7.

Sujarnoko, T.U.P., A. Jayanegara, R. Ridwan and Nahrowi. (2020). Tannin characteristic from Hevea brasiliensis and *Durio zibethinus* with pressure and hot water extraction. IOP Conference Series: Earth and Environmental Science, 462(1), 012010. https://doi.org/ 10.1088/1755-1315/462/1/012010.

Sujarnoko, T.U.P., R. Ridwan, N. Nahrowi and A. Jayanegara. (2020). Extraction of tannin from Acacia (*Acacia mangium*) bark and its use as a feed additive for protecting *in vitro* ruminal degradation of tofu dregs. Advances in Animal and Veterinary Sciences, 8(7), 761-765. https://doi.org/10.17582/journal.aavs/2020/8.7.761.765.

Teng, P., H. Shao, B. Huang, J. Xie, S. Cui, K. Wang and J. Cai. (2023). Small molecular mimetics of antimicrobial peptides as a promising therapy to combat bacterial resistance. J. Medicinal Chemistry, 66(4), 2211–2234. https://doi.org/10.1021/acs.jmedchem.2c00757.

Tong, Z., W. He, X. Fan and A. Guo. (2022). Biological function of plant tannin and its application in animal health. Frontiers in Veterinary Science, 8, 803657. https://doi.org/10.3389/ fvets. 2021.803657.

Ujilestari, T., M.D. Alifian, R.A. Nurfitriani, A.F. Mohd Azmi, Nurkholis, S. Nusantoro, A.N. Respati, S. Sadarman, W.T. Sasongko, E.S. Rohaeni and M.M. Sholikin. (2025). A meta-analysis of the effects of sugarcane green waste on productivity, carcass characteristics, meat quality, and milk production in sheep. Tropical Animal Health and Production, 57(7), 303. https://doi.org/10.1007/s11250-025-04567-5.

Vică, M.L., M. Glevitzky, D.M. Tit, T. Behl, R.C. Heghedűş-Mîndru, D.C. Zaha, F. Ursu, M. Popa, I. Glevitzky and S. Bungău. (2021). The antimicrobial activity of honey and propolis extracts from the central region of Romania. Food Bioscience, 41, 101014. https://doi.org/10.1016/j.fbio.2021.101014.

Werszko, J., K. Wilamowski, O. Kraszewska, S. Bakier and A.M. Pyziel. (2024). First molecular identification of *Haemonchus contortus* (Nematoda: Trichostrongylidae), a blood-sucking gastric nematode of artiodactyles, in the ground beetle *Carabus granulatus* (Coleoptera: Carabidae). Medical and Veterinary Entomology, 38(3), 361–365. https://doi.org/10.1111/mve.12715.

Wonggo, D., C. Anwar, V. Dotulong, A. Reo, N. Taher, R.A. Syahputra, F. Nurkolis, T.E. Tallei, B. Kim and A. Tsopmo. (2024). Subcritical water extraction of mangrove fruit extract (*Sonneratia alba*) and its antioxidant activity, network pharmacology, and molecular connectivity studies. J. Agriculture and Food Research, 18, 101334. https://doi.org/10.1016/ j.jafr. 2024. 101334.

Zafari, S., S. Mohtasebi, A. Sazmand, A. Bahari, N.D. Sargison and G.G. Verocai. (2022). The prevalence and control of lungworms of pastoral ruminants in Iran. Pathogens, 11(12), 1392. https://doi.org/10.3390/pathogens11121392.