Jurnal Sain Veteriner, Vol. 43. No. 1. April 2025, Hal. 58-68
DOI: 10.22146/jsv.70842
ISSN 0126-0421 (Print), ISSN 2407-3733 (Online)
Tersedia online di https://jurnal.ugm.ac.id/jsv

Anthelmintic Potential of *Annona muricata* seed Infusion: An In Vitro Study on *Haemonchus contortus* in Goats

Potensi Anthelmintik Infus Biji Annona muricata: Studi In Vitro pada Haemonchus contortus pada Kambing

Zein Ahmad Baihaqi[1,2]*, Ertika Fitri Lisnanti[2], Safira Tamara[2], Fitrine Ekawasti[3], Santika Anggrahini[1], Ima Fauziah[3]

[1]Research Center for Animal Husbandry, National Research and Innovation Agency (BRIN), Bogor, West Java, Indonesia
[2]Department of Animal Science, Faculty of Agriculture, Universitas Islam Kadiri, Kediri, East Java, Indonesia
[3]Research Center for Veterinary Science, Research Organization for Health, National Research and Innovation Agency (BRIN), Bogor, West Java, Indonesia.
*Email: zein001@brin.go.id

Article submitted: October 14, 2024, revised: December 19, 2024, accepted: March 30, 2025

Abstrak

Infeksi parasit, terutama yang disebabkan oleh *Haemonchus contortus*, secara signifikan mempengaruhi kesehatan dan produktivitas ruminansia kecil di seluruh dunia. Pengobatan antelmintik tradisional semakin menghadapi tantangan terkait masalah resistensi, sehingga mendorong penemuan pengobatan alternatif. Tujuan dari penelitian ini adalah untuk menilai efektivitas infus biji *Annona muricata* terhadap mortalitas *Haemonchus contortus* pada kambing secara *in vitro*. Penelitian ini dilaksanakan pada bulan November 2023 hingga Maret 2024 di Laboratorium Fakultas Pertanian, Universitas Islam Kadiri dan BRIN. Metode yang digunakan adalah ANOVA satu arah. Perlakuan yang diberikan meliputi kontrol negatif dengan NaCl 0,9%, infus konsentrasi 10%, infus konsentrasi 20%, dan albendazole. Tingkat mortalitas dinilai pada interval 0.5, 1, 2, 4, 6, 8, 10, dan 12 jam. Hasil penelitian menunjukkan bahwa infus biji *Annona muricata* mengandung senyawa seperti tanin, flavonoid, alkaloid, saponin, dan steroid yang mampu merusak kutikula dan jaringan bukal *Haemonchus contortus*. Kandungan fenolik yang terukur adalah total fenolat sebesar 9,1 mg RE/g Dw, total flavonoid 4,7 mg RE/g Dw, total tanin 7,2%, tanin terkondensasi/CT 3,4%, dan tanin terhidrolisis/HT 3,9%. Infus dengan konsentrasi 10% dan 20% mencapai 100% mortalitas *Haemonchus contortus* setelah 8 jam. *Scanning Electron Microscopy* (SEM) menunjukkan adanya perubahan struktur pada *Haemonchus contortus* pada konsentrasi infus yang berbeda, termasuk kerutan, garis yang tidak beraturan, dan adanya lubang pada daerah kutikula dan bukal. Infus biji *Annona muricata* efektif sebagai antelmintik terhadap *Haemonchus contortus*, mencapai kematian 100% pada konsentrasi 10% dan 20% dalam waktu 8 jam. Penelitian ini juga menunjukkan kerusakan struktural pada cacing sehingga menegaskan potensi infus biji *Annona muricata* sebagai pengendali parasit alami pada ruminansia kecil.

Kata kunci: *annona muricata* seed; bioanthelmintik; *haemonchus contortus*; imbuhan pakan

Abstract

Parasitic infections, particularly those caused by *Haemonchus contortus*, significantly impact the health and productivity of small ruminants worldwide. Traditional anthelmintic treatments are increasingly challenged by resistance issues, prompting the search for alternative treatments. The objective of this study was to assess the effectiveness of *Annona muricata* seed infusion on *Haemonchus contortus* mortality in goats in vitro. The research was conducted from November 2023 to March 2024 at the Laboratory of the Faculty of Agriculture, Islamic University of Kadiri, and BRIN. The method employed was one-way ANOVA. Treatments included a

negative control with 0.9% NaCl, 10% concentration infusion, 20% concentration infusion, and albendazole. Mortality rates were assessed at 0.5, 1, 2, 4, 6, 8, 10, and 12-hour intervals. The results revealed that *Annona muricata* seed infusion contains compounds such as tannins, flavonoids, alkaloids, saponins, and steroids, which are capable of damaging the cuticle and buccal tissues of *Haemonchus contortus*. Phenolic content was measured at total phenolics of 9.1 mg RE / g Dw, total flavonoids of 4.7 mg RE / g Dw, total tannins of 7.2%, condensed tannins / CT of 3.4%, and hydrolyzable tannins / HT of 3.9%. Infusions at 10% and 20% concentrations achieved 100% mortality of *Haemonchus contortus* by the 8th hour. Scanning Electron Microscopy (SEM) revealed structural changes in *Haemonchus contortus* at different infusion concentrations, including wrinkling, irregular lines, and the presence of holes in the cuticle and buccal regions. The infusion of *Annona muricata* seed infusion is effective as an anthelmintic against *Haemonchus contortus*, achieving 100% mortality at concentrations of 10% and 20% within 8 hours. The study also demonstrates structural damage to the worms, affirming the potential of *Annona muricata* seed infusion as a natural parasitic control in small ruminants.

Keywords: *annona muricata* seed; bioanthelmintic; feed additive; *haemonchus contortus*

Introduction

Tropical climates present significant challenges for livestock producers, particularly concerning animal health. The environmental conditions in these regions promote the spread of various diseases caused by pathogenic micro-organisms, including parasites. Among these, the nematode *Haemonchus contortus* poses a major threat to ruminant livestock, especially goats. This parasite thrives in the digestive systems of ruminants and is highly effective at completing its life cycle in tropical environments (Arifin *et al*., 2019; Baihaqi *et al*., 2019). *H. contortus* primarily infects the abomasum of ruminants, with goats being particularly susceptible to its detrimental effects (Basier *et al*., 2016).

As a blood-sucking gastrointestinal nematode, *H. contortus* can cause serious health issues, including anemia, due to its feeding habits within the host (Arsenopoulos, 2021). Infected animals often suffer from weight loss as a result of decreased digestive efficiency, and severe, prolonged infections can even lead to death. Consequently, *H. contortus* infections can result in substantial economic losses in the livestock industry, with prevalence rates reaching as high as 80% (Yuswandi and Rika, 2015). Infected livestock typically require prompt treatment with anthelmintic drugs, such as benzimidazole, levamisole, or ivermectin. However, the frequent use of these medications has led to increasing resistance among parasites (Ekawasti *et al*., 2022).

In response, there has been growing interest in using natural remedies as alternatives for treating *H. contortus* infections. These remedies are often perceived to have fewer side effects than conventional drugs and can be more readily accessible (Hutagalung, 2014). One promising natural treatment is *Annona muricata* seed, which contains active compounds known for their anthelmintic properties. Other plants with similar potential include pineapple leaves, Carica pubescens, and sengon bark (Baihaqi *et al*., 2020a; Baihaqi *et al*., 2020b; Baihaqi *et al*., 2022; Baihaqi *et al*., 2023). Baihaqi *et al*. (2020) reported that sengon bark is rich in tannins, alkaloids, flavonoids, saponins, and steroids, all of which have been shown to enhance mortality rates and cause structural damage to the cuticle and longitudinal reticular layer of *H. contortus*. Additionally, various active compounds derived from plant-based agro-industrial byproducts have been recognized for their effectiveness as alternative treatments, serving as bio-anthelmintics, bio-antibacterials, methane reduction agents, and productivity enhancers for livestock (Baihaqi *et al*., 2024; Sakti *et al*., 2024; Rokana *et al*., 2024; Prasetyo *et al*., 2024; Lokapirnasari *et al*., 2024; Prayudi *et al*., 2023; Lisnanti *et al*., 2023). Given this background, the aim of this study is to evaluate the in vitro effects of *Annona muricata* seed on the mortality of *H. contortus*.

Material and methods

Ethical clearance

The research implementation has received approval from the Universitas Gadjah Mada Animal Care and Utilization Committee with number 0013/EC-FKH/Int./2019.

Time and Location

This study was conducted from November 2023 to March 2024 at the laboratory of the Animal Husbandry Program, Faculty of Agriculture, Universitas Islam Kadiri (UNISKA). *Annona muricata* seed were collected from Kediri Regency. *Haemonchus contortus* images were scanned using Scanning Electron Microscopy (SEM) at BRIN Playen, Gunung Kidul.

Research Methods

The research employed an ANOVA (Analysis of Variance) to analyze differences in the mortality rates of *H. contortus* hourly over 12 hours (Baihaqi *et al*., 2020; Muda *et al*., 2021). The following treatments were used:
P0 = Negative control with 0.9% NaCl
P1 = 10% *Annona muricata* seed infusion
P2 = 20% *Annona muricata* seed infusion
P3 = Positive control (albendazole)

Implementation of the Study

Preparation of *Annona muricata* seed Infusion

Annona muricata seeds were chopped (Joyo Langgeng Chopper),and the dried samples were weighed at 10g and 12g. The sample, after being finely chopped in the chopper, was dried in an oven at 55 degrees Celsius for 3 days with a moisture content of approximately 23%. Each sample was dissolved in 100 ml of distilled water (aquades) and heated for 15 minutes at 90°C. After heating, the infusion was filtered using filter paper with 8-12 µm (Rahmiati *et al*., 2018).

Preparation of *Haemonchus contortus*

Haemonchus contortus worms were obtained from the abomasum of goats by cutting the section adjacent to the rumen and duodenum and tying it off with string to secure the worms. These sections were stored in a styrofoam box during transport from the slaughterhouse to the laboratory. Only adult female worms were used in this study. The worms were initially placed in a NaCl solution before being transferred to petri dishes containing the respective treatments for observation (Noviana *et al*., 2017).

Mortality Testing

Mortality testing of *H. contortus* was conducted with four treatments groups, each with five replicates, and each replicate consisted of two female worms. The administration of 10 ml of Annona muricata infusion for each petri dish. The worms were placed in petri dishes at room temperature and observed at intervals of half an hour, 1 hour, 2 hours, 4 hours, 6 hours, 8 hours, 10 hours, and 12 hours. Dead worms were identified by lack of movement. The worm's death was confirmed by shaking the petri dish or pressing the worm with forceps. Additionally, using warm water for 5 seconds ensured worm mortality (Karim *et al*., 2021).

Scanning Electron Microscopy

Dead worms were fixed in 2% glutaraldehyde with sodium cacodylate buffer (0.1 M) for 4 hours at 4°C. After washing twice with the same buffer (0.2 M), the worms were dehydrated in a graded ethanol series, dried with an EMSCOPE CPD 750 critical point dryer, and coated with gold-palladium for 5 minutes at a rate of 100Å/minute. The samples were then observed using a Hitachi S450 SEM at an accelerating voltage of 15 kV (Baihaqi *et al*., 2020).

Data Analysis

The data obtained were analyzed using one-way ANOVA (Analysis of Variance) followed by Tukey's test to determine significant differences ($P<0.05$) using SPSS 27.0 (Baihaqi *et al*., 2020). The ANOVA method was used to analyze worm mortality at each treatment dose, while Tukey's test was employed to identify differences between groups (Widiarso *et al*., 2021). SEM data on *H. contortus* were analyzed descriptively.

Results and Discussion

Active compound content in *Annona muricata* seeds

The part of the soursop plant used in this study is the seeds of *Annona muricata*. Qualitative phytochemical analysis of *Annona muricata* seed revealed the presence of active compounds such as tannins, flavonoids,

alkaloids, saponins, and steroids, as shown in (Table 1). Tannins are secondary metabolites belonging to the phenolic group that have astringent properties, which can constrict the intestinal mucosa and minimize fecal output due to diarrhea. According to Zhong *et al.*. (2014), tannins can affect adult worms both directly and indirectly. The direct reaction occurs when tannins adhere to the cuticle of the worms.

Tannins also have the potential to act as anthelmintics by damaging the body membranes of the worms, which can lead to loss of muscle function, commonly referred to as paralysis, resulting in the death of the worms (Siswanto *et al.*, 2020).

Table 1. Qualitative Phytochemical Analysis of Annona muricata

Secondary metabolites	*Annona muricata* seeds
Tannin	+
Flavonoid	+
Alkaloid	+
Saponin	+
Steroid	+

The study by Raipuria *et al.,* (2018) indicates that ethanol used for the qualitative analysis of active compounds in plants does not show significant differences when analyzing these compounds across various plants. Phytochemical screening can be utilized to determine the potential of active compounds within plants. Active phytochemical tests can be naturally produced by pathogens or can help suppress diseases (Amadioha, 2019).

The results of the quantitative phytochemical analysis of soursop seeds (*Annona muricata*) in Table 2 show total phenolics of 9.1 mg RE / g Dw, total flavonoids of 4.7 mg RE / g Dw, total tannins of 7.2%, condensed tannins / CT of 3.4%, and hydrolyzable tannins / HT of 3.9%. The tannin content in soursop seeds (*Annona muricata*) is 7.2%. It can be concluded that the high tannin content may affect the nerve damage and physical form of *Haemonchus contortus*.

Tannins are divided into two groups: condensed tannins and hydrolyzable tannins. Condensed tannins are polymers of flavonoid compounds with carbon bonds such as gallocatechin and catechin, while easily hydrolyzable tannins are polymers of gallic and ellagic acid that are ester-bound to a sugar molecule (Patra and Saxena, 2010). Both condensed and hydrolyzable tannins bind to proteins. The binding of tannins and proteins can affect protein digestibility (Mueller, 2006). Hydrolyzable tannins are present in higher amounts than condensed tannins, which relates to the protein capacity of tannins; hydrolyzable tannins precipitate more protein than condensed tannins (Jayanegara *et al.*, 2009). Condensed tannins have a lower toxic effect compared to hydrolyzable tannins (Beauchemin *et al.*, 2008). Condensed tannins are thought to act as bioanthelmintics by inhibiting enzymes and damaging cuticle membranes. The inhibition of these enzymes can lead to nutrient deficiencies in worms, resulting in their death. The content of these compounds is higher than those reported in the study by Baihaqi *et al.* (2023), which found that the extract of Carica pubescens contained phenolics of 9.5 mg GAE / g Dw, flavonoids of 3.1 mg RE /g Dw, tannins of 5.4%, CT of 3.8%, and HT of 2.9%.

In vitro mortality test

In this study, an in vitro experiment was conducted using female *Haemonchus contortus* worms treated with a soursop seed infusion. The first mortality occurred at hour 0, with 100% mortality recorded by hour 10. The results indicate that at a concentration of 20%, death occurred more rapidly in *Haemonchus contortus*.

Table 2. Quantitative Phytochemical Analysis

Plant Infusion	Flavonoid Content (mg RE / g Dw)	Total Phenolics (mg GAE / g Dw)	Total Tannins (%)	Condensed Tannins / CT (%)	Hydrolyzable Tannins / HT (%)
Annona Muricata seeds	4.7	9.1	7.2	3.4	3.9

Table 3. Mortality Test of *Haemonchus contortus* In Vitro with Soursop Seed Infusion at Various Incubation Times

Treatments	Mortality Percentage of *Haemonchus contortus* (%)							
	0 h	1 h	2 h	4 h	6 h	8h	10jam	12jam
P0 (NaCl)	0±0.00	0±0.00	0±0.00	20±27.38[a]	50±35.35[a]	100±0.00[a]	100±0.00[a]	100±0.00[a]
P1(10%)	0±0.00	0±0.00	20±27.38[a]	60±22.36[ab]	90±22.36[a]	100±0.00[a]	100±0.00[a]	100±0.00[a]
P2 (20%)	10±22.36[a]	20±27.38[a]	50±50.00[a]	90±22.36[ab]	90±22.36[a]	100±0.00[a]	100±0.00[a]	100±0.00[a]
	0±0.00	0±0.00	30±27.38[a]	60±22.36[ab]	80±27.38[a]	90±22.36[a]	100±0.00[a]	100±0.00[a]

The ANOVA test showed that ($P < 0.05$), indicating significance, except at hour 4 where it showed ($P > 0.05$), indicating no significance. Tannins can inhibit the activity of the enzyme acetylcholinesterase, thereby disrupting the metabolic processes of digestion in the worms. Consequently, the worms may experience nutrient deficiencies, leading to death due to lack of energy. According to research by Wildan (2021), in vitro experiments on *Haemonchus contortus* indicated that the best concentration of ketapang plant infusion was 8%, resulting in the highest mortality rate in *Haemonchus contortus*. Riefqy (2020) also reported that in vitro experiments with a 50% infusion concentration produced high mortality rates; the mechanism of the castor leaf extract involves damaging the body membranes of the worms, causing paralysis and death. The anthelmintic efficacy of the castor leaf extract is associated with the metabolite compounds contained in the leaves, particularly tannins, which affect the metabolic processes of the worms.

In this study, the NaCl solution showed a relatively slow onset of death, occurring at hour 4, with 100% mortality by hour 8. In contrast, the albendazole solution showed the first mortality at hour 2 and reached 100% mortality by hour 10. Although the average values differed, their notations were similar, indicating no significant difference at hour 8. The P0 condition may suggest variations in the worms or their strength, leading to diversity in the results of this research. Notations serve to differentiate which treatment had the most significant effect in this study.

In a study using young leaves of *Acacia nilotica* at 3.5% and young leaves of *Desmanthus virgatus* at 2.5% with a NaCl solution, the highest mortality percentage of *Haemonchus contortus* was observed after a seven-hour immersion compared to other treatment concentrations. Meanwhile, the positive control using the chemical albendazole achieved 100% mortality with just one hour of immersion (Wildan *et al.*, 2021).

Scanning Electron Microscope (SEM) Examination of *Haemonchus contortus*

The SEM analysis of *Haemonchus contortus* treated with NaCl solution is shown in Figure 4 (parts A1 and A2). The images indicate that the worms did not experience significant damage; the cuticle appears wrinkled, and the onset of death was relatively slow. The fastest mortality occurred at hour 4, with 100% mortality recorded by hour 8. According to Sambodo (2018), *Haemonchus contortus* treated with NaCl displayed a longitudinal reticular structure without significant damage, with a concave cuticle that appeared smooth, and the mortality was notably slow.

These findings suggest that while NaCl treatment affects the worms, the impact on their structural integrity is minimal, leading to a gradual decline in viability rather than immediate death.

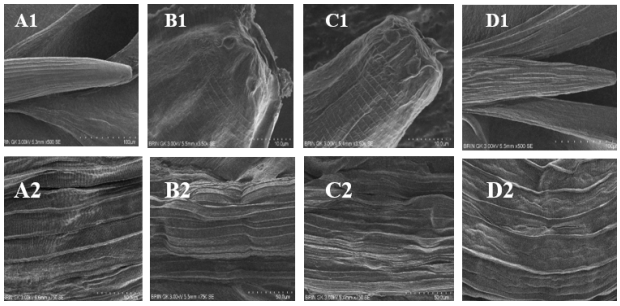

Figure 1. The SEM analysis of *Haemonchus contortus* treated with NaCl solution (A1 and A2), 10% *Annona muricata* seed infusion (B1 and B2), 20% *Annona muricata* seed infusion (C1 and C2), and the positive control, which showed signs of cuticle wrinkling (D1 and D2).

The treatment with infusion solutions at concentrations of 10% and 20% resulted in damage to *Haemonchus contortus*. Figure 4 (parts B1 and B2) shows that at the 10% treatment, the cuticle appears wrinkled, with the first mortality occurring at hour 2 and reaching 100% mortality by hour 8. In contrast, the 20% treatment (C1 and C2) shows that the buccal area exhibits many wrinkles, with the first mortality occurring at hour 0 and reaching 100% by hour 10. According to research by Ramirez *et al.* (2019), the effects of *Castena sativa* on *Haemonchus contortus* resulted in the release of components from the digestive tract, as well as damage to the copulatory bursa, anus, vulva, and oral area. Similar studies have shown that tannins can damage the cuticle of *Haemonchus contortus*, leading to transverse and longitudinal wrinkling after exposure to *Biophytum petersianum*, which contains high levels of tannins, as evaluated by Sambodo *et al.* (2018).

Changes in the cuticle structure can lead to nutritional disturbances in nematodes, resulting in malnutrition (Yoshihara *et al.*, 2015). Olivas-Aguirre *et al.* (2015) noted that the mechanism of damage to nematode body parts upon contact with plant active compounds involves the formation of collagen-tannin complexes due to damage to the nematode cuticle by secondary metabolites from plants or elevated proline levels in collagen. Plants with high tannin concentrations can accelerate the mortality of *Haemonchus contortus*, which is consistent with other studies. The soursop seed infusion (*Annona muricata*) at higher concentrations is well-suited for use as a natural anthelmintic. One health challenge in sheep that can hinder productivity is parasitism; albendazole is commonly used to address this issue. Its affordability and effectiveness in eliminating worms are notable. However, continuous use of albendazole leads to resistance in livestock.

In Figure 4 (parts D1 and D2), the cuticle shows signs of wrinkling. According to Sasongko *et al.* (2019), the risk factors for reduced effectiveness of albendazole arise because of the development of resistance genes in worms due to the use of the same class of anthelmintics. Research concerning the presence of larvae in the abomasum tissues of groups treated with albendazole indicated that this is due to low drug doses contacting the parasites when entering the host's body. The metabolism of the anthelmintic in the host affects the drug's concentration within the parasites (Lifschitz *et al.*, 2017).

Conclusion

This study concludes that soursop seeds (*Annona muricata*) contain qualitative phytochemical active compounds, including tannins, flavonoids, alkaloids, saponins, and steroids, which have been shown to induce mortality in the nematode *Haemonchus contortus*. Scanning Electron Microscope (SEM) analysis revealed significant damage in response to treatments with *Annona muricata* seeds. The optimal concentration was determined to be 20%, with the first mortality occurring at hour 0 and reaching 100% by hour 8.

Acknowledgments

This study was supported by the Program Kerjasama Penelitian UNISKA – BRIN Tahun 2023, with grant number 010/040.1.6/HK.01.03/VIII/2023.

References

Amadioha, A.C. and Chidi, K.P. (2019). Komposisi fitokimia ekstrak daun berair dan etanol *Piper guinea*, *Cassia alata*, *Tagetes erecta*. *Int. J. Pharm. Res*. 26(3): 1-8.

Amir, O.Y., Victor, S. and Bethwell, O.O. (2014). *In-vitro* anthelmintic bioactivity of Eclipa Prostrata L. adult *Haemonchus contortus* worms: A case study of Migori County, Kenya. *International Journal of Pharmacy and Biological Sciences*. 9(6):45-53.

Anderson, R.A. (2000). Nematode parasites of vertebrates: Their development and transmission 2 ed. CABI Publishing.UK.

Andhika, D.R., Atsarina, L.A., Viny, V.T. and Heni, R. (2018). Teknik Pengamatan Sampel Biologi dan Non-Konduktif Menggunakan Scanning Electron Microskopy. *Seminar Nasional*

Instrumentasi, Kontrol dan Otomasi. Bandung.

Arsenopoulos, K.V., Fthenakis, G.C., Katsarou, E.I. and Papadopoulos E. (2021). Haemonchosis: A Challenging Parasitic Infection of Sheep and Goats. *Animals*. (11) : 2-28.

Acevedo-Ramírez, P.M.D.C., Hallal-Calleros, C., Flores-Pérez, I., Alba-Hurtado, F., Mendoza-Garfías, M.B., Castro Del Campo, N. and Barajas, R. (2019). Anthelmintic effect and tissue alterations induced *in vitro* by hydrolysable tannins on the adult stage of the gastrointestinal nematode *Haemonchus contortus*. *Vet Parasitol*. 266: 1-6.

Baihaqi, Z.A., Widiyono, I. and Nurcahyo, W. (2019) Prevalence of gastrointestinal worms in Wonosobo and thin-tailed sheep on the slope of Mount Sumbing, Central Java, Indonesia. *Veterinary World*. 12(11): 1866–1871.

Baihaqi, Z.A., Widiyono, I. and Nurcahyo, W. (2020). Prevalence naturally infected gastrointestinal parasites and complete blood count condition on Wonosobo sheep at Wonosobo District, Central Java, Indonesia. *Biodiversitas*. 21(7): 3057-3061.

Baihaqi, Z.A., Widiyono, I. and Nurcahyo, W. (2020). Potential of *Carica pubescens* fruit peel as an alternative method to control *Haemonchus contortus* in small ruminants. *Livest. Res. Rural. Dev*. 32(7): 106. Retrieved October 7, 2024, from http://www.lrrd.org/lrrd32/7/zein32106.html.

Baihaqi, Z.A., Widiyono, I., Angeles, A.A., Suwignyo, B. and Nurcahyo, W. (2023). Anthelmintic activity of *Carica pubescens* aqueous seed extract and its effects on rumen fermentation and methane reduction in Indonesian thin-tailed sheep: An *in vitro* study. *Veterinary World*. 16(7): 1421-1428.

Baihaqi, Z.A., Sofyan, A., Suwignyo, B., Angeles A., Widiyono I., Nurcahyo W., Ibrahim A., Putri E. and Wulandari. (2024). *In vivo* study: The effects of *Carica pubescens* seed extract on the anthelmintic activity, feed digestibility, performance, and clinical parameters of thin-tailed sheep. *IOP Conf. Ser.: Earth Environ. Sci*. 1341: 012119. DOI : 10.1088/1755-1315/1341/1/012119.

Basier, R.B., Khan, L.P., Sargison, N.D. and Van Wyk, J.A. (2016). The pathophysiology, ecology, and epidemiology of *Haemonchus contortus* in small ruminants. *Adv. Parasitol*. 93: 95-143.

Ballweber, L. R. (2001). *Veterinary Parasitology*. Buterworth-Heinemann : USA

Beauchemin K.A., Kreuzer, M., O'Mara, F. and McAlister, T.A. (2008). Nutritional management for enteric methane abatement : a review. *Aust. J. Exp. Agric*. 48: 2127.

Browning, M.LL. (2006). *Haemonchus contortus (Barber Pole Worm) Infestation in Goats*. Extension Animal Scientist. Brazil (US): Alabama A & M University.

Chai, K.F., Adzahan, N.M., Karim, R., Rukayadi, Y. and Ghazali, H.M. (2018). Characteristics of fat, and saponin and tannin contents of 11 varieties of rambutan (*Nephelium lappaceum L.*) seed. *International Journal of Food Properties*. 21(1): 1091-1106.

Cheruiyot, K., Kutima, H., Kareru, P.G., Njonge, F., Odhiambo, R., Mutembei, J. and Madivoli, E. (2015). *In vitro* ovicidal activity of encapsulated ethanolic extracts of prosopis juliflora against *Haemonchus contortus* eggs. *Journal of Pharmacy and Biological Sciences*. 10(5): 18-22.

Clark, C.G. (1962). Measurement of Blood Loss Caused by *Haemonchus Contortus* Infectionin 177 Sheep. *Ondersstepoort J. Vet.Res*. 30(2):145-153.

Dalimartha, S. (2009). *Atlas Tumbuhan Obat Indonesia*. Penebar Swadaya. Jakarta.

Deviana, Riza. (2012). *Pengaruh Ekstrak Buah Sirsak (Annona muricata) terhadap Waktu Kematian Cacing Ascaris suum, Goeze In Vitro*. Skripsi. Fakultas Kedokteran Universitas Sebelas Maret.

Dwinata, 1. (2017). *Modul Identifikasi Parasit Cacing*. Retrieved pada October 9, 2024. https://simdos.unud.ac.id/uploads/file_pendidikan_1_dir/e3ca741312f1b844f8b0a0216935177.pdf.

Ekaswati, F., Dewi, D.A., Suhardono, Sawitri, D.H., Martindah, E., dan Wardhana, A.H. (2022). Aktivitas Ovisidal, Larsidal dan Vermisidal Ekstrak Herbal terhadap Nematoda *Haemonchus contortus* secara *in vitro*. *Jurnal Veteriner*. 23(2): 146-156.

Ferreira, L.E., Castro, P.M.N., Chagas, A.C.S., Franca, S.C. and Beleboni, R.O. (2013). *In Vitro* Anthelmintic Activity of Aqueous Leaf Extract of *Annona muricata* Against *Haemonchus Contortus* from Sheep. *Exp Parasitol*. 134(3):327-32.

Gairtua, B., Utomo, R., Hanim, C., Baihaqi, Z.A. and Wulandari. (2024). Assessing the effectiveness of ash soaking in reducing hydrogen cyanide (HCN) levels in cassava peel. *Livestock Research for Rural Development*. 36 (39). Retrieved October 11, 2024, from http://www.lrrd.org/lrrd36/4/3639wula.html.

Garedaghi, Y., Saber, R.A.P., Naghizadeh, A., and Nazeri, M. (2011). Survey on Prevalence of Sheep and Goats Lungworms In Tabriz Abattoir, Iran. *Advances in Enviromental Biology*. 5(4):773-775.

Harahap, M.A., Widodo, S., Handayani, U.F., Altandjung, R.I., Wulandari, Sakti, A.A., Atmoko, B.A., Negara, W., Dewi, Y.L., Julendra, H., Sofyan, A., Wahyono, T., Ujilestari, T., Ahmed, B., Qomariyah, N., Sholikin, M.M. and Baihaqi, Z.A. (2024). Examining performance, milk, and meat in ruminants fed with macroalgae and microalgae: A meta-analysis perspective. *Trop Anim Health Prod*. 56(7): 243.

Hasanah, A.M., Kurniawan, K. and Amal, F. (2023). Perbandingan Kadar Total Flavonoid Metode Infusa Dan Rendaman Buah Kurma Ajwa (*Phoenix Dactylifera L.*) Menggunakan Spektrofotometri Uv-Vis. *Jurnal Ilmiah Global Farmasi*. 1(1): 917.

Herdaningsih, S., and Mela, K. (2021). Uji Aktivitas Anthemintik Ekstrak Umbi Batang Rotan (*Calamus rotang L.*) terhadap Cacing Gelang Ayam (Ascaridia galli) secara *In vitro*. *Journal Pharmaceutical Science*. 1(1): 90-98.

Hutagalung. P.S.U. (2014). Efek Antelmintik Ekstrak Buah Nanas (*Ananas comosus*) terhadap *Ascaris suum* cecara *In Vitro*. Skripsi. Fakultas Kedokteran, Universitas Kristen Maranatha. pp. 30-41.

Indriyani P.L.N. 2012. The growth evaluation of two Annona spesies At seedling phase. *APRN Journal of Agricultural and Biological Science*. 7:541-544

Kamaraj, S., Ramakrishnan, G., Anandakumar, P., Jagan, S. and Devaki, T. (2009). Antioxidant and Anticancer Efficacy of Hesperidin in Benzo(a)pyrene Induced Lung Carcinogenesis in Mice. *Invest New Drugs*. 27(3): 214-222.

Karim, S.F., Nurfiddin, F., Hilmiati, W. and Musdalifa. (2021). Effectiveness Test of Ethanol Extract of Basil Leaves (*Ocimum americanum L.*) Against Roundworms (*Ascaris lumbricoides*) *In Vitro*. *Journal of Pharmaceutical Science and Clinical Research*. 6(3): 254-263.

Krisdamaiyanti, D.A., Arif, R. and Retnani, E.B. (2022). Meta-Analisis: Kuantifikasi Efektivitas Anthelmintika Herbal pada Pengujian *In Vivo*. *Acta Veterinaria Indonesiana*. 10(1): 96-102.

Lisnanti, E.F., Lokapirnasari, W.P., Hestianah, E.P., Al Arif, M.A. and Baihaqi, Z.A. (2023). The Effectiveness of Giving Marsh Fleabane (*Pluchea Indica L.*) Water Extract on Broiler Hematology and Blood Glucose. *Advances in Animal and Veterinary Sciences*. 11(8): 1348-1356.

Lisnanti, E.F., Lokapirnasari, W.P., Hestianah, E.P., Al Arif, M.A., Baihaqi, Z.A. and Yulianto, A.B. (2024). Antibacterial alternatives using the potential of the ant nest plant (*Myrmecodia* spp.). *Int. J. One Health*. 10(1): 148-152.

Lokapirnasari, W.P., Al-Arif, M.A., Hidayatik, N., Safiranisa, A., Arumdani, D.F.,

Zahirah, A.I., Yulianto, A.B., Lamid, M., Marbun, T.D., Lisnanti, E.F., Baihaqi, Z.A., Khairullah, A.R., Kurniawan, S.C., Pelawi, E.B.S. and Hasib, A. (2024). Effect of probiotics and acidifiers on feed intake, egg mass, production performance, and egg yolk chemical composition in late-laying quails. *Veterinary World*. 17(2): 462-469.

Mubarokah, W.W., Wisnu, N., Joko, P., and Kurniasih, K. (2019). Populasi Profil Protein dan Ultra struktur *Ascaridia galli* pada Ayam yang diberi Perlakuan Menggunakan Ekstrak Air Mentah Pinang (*Areca catechu*). *Journal Indonesian Tropical Animal Agriculture*. 44(4): 392-399.

Muda, I., Joko, P., Wisnu, N. and Sarmin, S. (2021). Efek Anthelmintik *Indigofera tinctoria L*. pada *Haemonchus contortus* Diperoleh dari Domba di Indonesia. *Dunia Veteriner*. 14(5): 1272-1278.

Mueller, H.I. (2006). Unrevelling the conundrum of tannins in animal nutrition and health. *J. Sci. Food Agric*. 86: 2010-2037.

Mulyawati, A.P., Hayati E.K, Nashihudin, A. and Tukimin. (2010). Uji Efektivitas dan Identifikasi Senyawa Ekstrak Biji Sirsak (*Annona muricata*) yang Bersifat Bioaktif Nabati terhadap Hama Thrips. *Alchemy*. 2.1.

Noviana, R., Anwar, C., Sunarso, A., Koesdarto, S., Mumpuni, S. and Sahrial, I.H. (2017). Daya antelmintika ekstrak etanol daun kemangi terhadap mortalitas cacing *Haemonchus contortus* secara *in vitro*. *Journal of Parasite Science*. 2(1): 55-58.

Olivas Aguirre, F., Wall-Medrano, A., Gonzalez-Aguilar, G., Lopez Diaz, J.A., Alvarez-Parrilla, E., de la Rosa, A. and Ramos-Jimenez, A. (2015). Taninos hidrozables; bioquimica, aspectos nutricionales y analiticos y efectos en la salud. *Nutr. Hosp*. 31(1): 55-66.

Patra, A.K. and Saxena, J. (2010). A new perspective on the use of plant secondary metabolites to inhibit methanogenesis in the rumen. *J. Phytochemistry*. 71(11-12): 1198-1222.

Prabowo, A. (2010). *Petunjuk Teknis Budidaya Ternak Kambing. Materi Pelatihan Agribisnis bagi KMPH*. BPTP Sumatera Selatan. Report No: 51.12.

Pradito, S.A., Muthmainah, N. and Biworo, A. (2022). Perbandingan Aktivitas Antibakteri Sediaan Infus dan Sediaan Ekstrak Daun Sungkai (*Peronema canescens Jack*) terhadap Bakteri *Staphylococcus aureus*. *Jurnal Mahasiswa Pendidikan Dokter*. 5(1): 135-144.

Prasetyo, E.N., Rokana, E., Baihaqi, Z.A. and Samudi, S. (2024). Anthelmintic effects of Podang mango (*Mangifera indica*) fruit peel waste extract through *in vivo* application on Indonesian Etawa goat production and health. *Veterinary World*. 17(6): 1291-1298.

Prayudi, S.K.A., Effendi, M.H., Lukiswanto, B.S., Az Zah-Ra, R.L., Benjamin, M.I., Kurniawan, S.C., Khairullah, A.R., Silaen, O.S.M., Lisnanti, E.F., Baihaqi, Z.A., Widodo, A. and Riwu, K.H.P. (2023). Detection of Genes on *Escherichia coli* Producing Extended Spectrum β-lactamase Isolated from the Small Intestine of Ducks in Traditional Markets Surabaya City, Indonesia. *Journal of Advanced Veterinary Research*. 13(8): 1600-1608.

Purwaningsih., Noviyanti and Sambodo, P. (2017). Infestasi Cacing Saluran Pencernaan pada Kambing Kacang Peranakan Etawa di Kelurahan Amban Kecamatan Manokwari Barat Kabupaten Manokwari Provinsi Papua Barat. *Jurnal Ilmiah Peternakan Terpadu*. 5(1): 8-12.

Puspitasari, A., Boedi, S., Setiawan, K., Kusnoto, Soeharsono and Poedji, H. (2019). Sebaran Telur Cacing Saluran Pencernaan Kambing di Kecamatan Rambon Kabupaten Nganjuk. *Journal of Parasite Science*. 3(2):47-100.

Rahmiati, N.I., Abdul, W.J. and Bone, R. (2018). Activity of Guava Leaf Infusion (*Psidium guajava L.*) Against *Haemonchus* sp. Nematodes from Bali Cattle (*Bos sondaicus*) *In Vitro*. *Agrisystem Journal*. 14(1): 27-36.

Raipuria, N., Kori, D., Saxena, H.O, Pawar, G. and Choubey, S.K. (2018). Evaluasi kualitatif dan kuantitatif metabolit sekunder pada daun, akar, dan batang *Cleome viscosal*. *Int. J. Farmasi Hijau*. 12(4): 285.

Rokana, E., Fatimah, I.R., Dianingtyas, B.D., Hasanah, N., Wulandari and Baihaqi, Z.A. (2024). Impact of various fiber sources in ration formulas on feedlot performance of sheep in Indonesia. *J. Anim. Health Prod*. 12(3): 325-330.

Sakti, A.A., Baihaqi, Z.A., Suwignyo, B., Sofyan, A., Herdian, H. and Kustantinah. (2024). Anthelmintic Activity of Red Macroalgae *Acrocystis* sp. and *Acanthophora* sp. Etanolic Extract Against *Haemonchus contortus* in Sheep *In Vitro*. *IOP Conf. Ser.: Earth Environ. Sci*. 1360. 012004.

Sambodo, P., Prastowo, J., Kurniasih, K., Mubarokah, W.W. and Indarjulianto, S. (2020). *In vivo* efficacy of *Biophytum petersianum* on *Haemonchus contortus* in goats. *Adv. Anim. Vet. Sci*. 8(3): 238-244.

Sambodo,P., Prastowo, J., Kurniasih, K. and Indarjulianto, S. (2018). *In Vitro* Potential Anthelmintic Activity of *Biophytum petersianum* on *Haemonchus contortus*. *Veterinary World*. 11(1): 1-4.

Sasongko, N.D., Adhim, F. and Zalihar, L. (2019). Efficacy of anthelmintic towards gastrointensinal nematode infection in thin-tailed sheep at smallholder farms in Sukomulyo Village, Malang Regency. *IOP Conference series: Earth and environmental science*. 247(1): 1-6.

Siswanto, R.T., Sudira, I.W., Merdana, I.M. and Dwinata, I.M. (2020). Efektifitas antelmentik larutan asam jawa terhadap cacing *Ascaris suum* secara *in vitro*. *Indonesia Medicus Veterinus*. 9(1): 21-27.

Sutama and Budiarsa. (2009). *Panduan Lengkap Kambing dan Domba*. Penebar Swadaya. Jakarta.

Soulsby, E.J.L. (1982). *Helminths, Arthropods and Protozoa of Domesticated Animal*. Lea and Febiger: Philadelphia.

Tabuni, L., Wahjuni, S. and Ratnayani, O. (2014). Identifikasi Senyawa Aktif Antimakan Biji Sirsak (*Annona muricata*) Terhadap Ulat Kubis (*Plutella Xylostella*). *Jurnal Kimia*. 8(2): 205-212.

Tenrirawe, A. (2011). *Pengaruh Ekstrak daun sirsak (Annona muricata L.) terhadap mortalitas larva Helicoverpa armigera pada jagung*. Balai Penelitian Tanaman Serelia: Maros, Sulawesi Selatan.

Tiwow, D., Widdhi, B., and Novel, S.K. (2013). Uji Efek Antelmintik Ekstrak Etanol Biji Pinang (*Areca catechu*) terhadap Cacing *Ascaris lumbricoides* dan *Ascaridia gallisecara In vitro*. *Jurnal Ilmiah Farmasi*. 2(2):76-80.

Tolistiawaty, I., Widjaja, J., Lobo, L. T. and Isnawati, R. (2016). Parasit Gastrointestinal pada Hewan Ternak di Tempat Pemotongan Hewan Kabupaten Sigi, Sulawesi Tengah. *Jurnal Litbang Pengendalian Penyakit Bersumber Binatang Banjarnegara*, 12(2): 71-78.

Vanimisetti, H.B. (2003). *Genetics of resistance to Haemonchus contortus infections in sheep*. Thesis. Virginia Polytechnic Institute, Blacksburg, Virginia.

Widiarso, B.P., Dias, A.D., Kurniati, S. and Dhestalia, E.P. (2021). *In Vitro* Potency of a Crude Aqueous Extract of *Artocarpus heterophyllus* Leaves as an Anthelmintic against *Haemonchus contortus* in Jawarandu Goats. *Advances in Animal and Veterinary Sciences*. 9(9): 14981503.

Widyastuti, Y., Yuliani, N. and Widyastini, I.G.A.M. (2019). Aktivitas Antibakteri Infusa Daun Lidah Buaya (*Aloe vera L.*) terhadap Pertumbuhan *Staphylococcus aureus* dan *Escherichia coli*. *Jurnal SainsNatural*. 6(1): 33-43

Wildan, H.P. (2021). Kajian Aktivitas Anthelmintik Tanaman Ketapang terhadap Cacing *Haemonchus contortus* secara *In Vitro*. Surakarta - Fak. Pertanian.

Winarsi, H. (2007). *Anti oksidan Alami dan Radikal Bebas Potensi dan Aplikasinya dalam Kesehatan*. Yogyakarta. Kanisius.

Winarso, A. (2018). Infeksi Parasit Gastrointestinal pada Kambing di Kupang. *ARSHI Vet Lett*. 2(2):25-26.

Wirawan, I.G.K.O., Aoetpah, A. and Jacob, J.M. (2021). Perbandingan Efektivitas antara Ekstrak Daun Muda *Acacia nilotica* dengan *Desmanthus virgatus* terhadap Daya Vermisidal *Haemonchus contortus* Secara *In-vitro*. *Jurnal Sain Veteriner*. 39(2): 168-177.

Wirawan, I.G.K.O., Ni, S.Y., Novianti, T. and Suryawati. (2022). Perkembangan Telur Cacing *Haemonchus contortus* menjadi Larva Stadium Pertama pada Media Air Secara In Situ. *Buletin Veteriner Udayana*. 14(6): 759-765.

Yoshihara, E., Minho, A.P., Tabacow, V.B.D., Cardim, S.T. and Yamamura, M.H. (2015). Ultrastructural changes in the *Haemonchus contortus* cuticle exposed to *Acacia mearnsii* extract. *Semina: Ciências Agrárias, Londrina*. 36(6): 3763-3768.

Zhong, R.Z., Sun, H.X., Liu, H.W. and Zhou, D.W. (2014). Effects of Tannin Acid on *Haemonchus contortus* Larvae Viability and Immune Responses of Sheep WhiteBlood Cells *In Vitro*. *Parasite Immunol*. 36:100-106.