Screening the anthelmintic effects of leaves and fruits extracts from various *Acacia* species as a potential feed for small-ruminant animals

EKA HANDBAYANTA*, RENDI FATHONI HADI, FAROUQ HEIDAR BARIDO

Department of Animal Science, Faculty of Agriculture, Universitas Sebelas Maret Surakarta, Jl. Ir. Sutami 36A, Surakarta 57126, Central Java, Indonesia. Tel.: +62-271-637457, *email: ekahandayanta@staff.uns.ac.id

Manuscript received: 27 February 2023. Revision accepted: 8 June 2023.

Abstract. Handayanta E, Hadi RF, Barido FH. 2023. Screening the anthelmintic effects of leaves and fruits extracts from various *Acacia* species as a potential feed for small-ruminant animals. Biodiversitas 24: 3140-3144. This study aimed to determine the anthelmintic properties of leaves and fruits obtained from various *Acacia* species against *Haemonchus contortus* Rudolphi, 1803. Both leaf and fruit samples were allocated into five different groups: negative control (NC), 0.9% of NaCl solution; positive control (PC), albendazole solution; and solutions containing 12% concentration of each *Acacia auriculiformis* A.Cunn. ex Benth. (AA), *Acacia mangium* Willd. (AM), and *Acacia crassicarpa* A.Cunn. ex Benth. (AC) powder. The added concentration was set at 12%, and each treatment group was brought to incubation in 6 different time parameters (1, 2, 3, 4, 5, and 6 hours) against *H. contortus*. Each *Acacia* leaves extract did not significantly differ in eliminating *H. contortus*, unless for AM under 4 hours of incubation (p<0.05), wherein at the final incubation time, all *Acacia*-treated samples displayed complete mortality. Meanwhile, treatment with *Acacia* fruits during 6-hour incubations led to a removal of 76.66-83.33 % *H. contortus* population, in which Albendazole-treated groups showed complete mortality. Thus, this study suggested the leaves from *Acacia* are a potential animal feed as anthelmintic against *H. contortus* parasites. However, further studies investigating the potential mechanism by which various *Acacia* species present anthelmintic activity are necessary.

Keywords: *Acacia*, anthelmintic, feed, *Haemonchus contortus*, small ruminant

INTRODUCTION

Gastrointestinal parasites comprise the major constraint in the management of livestock. It adversely affects the productivity of animals by lowering nutrition absorption, feed intake, and conversion. The eventual consequences are the failure of various livestock management purposes, including breeding, fattening, and milk production (Hoste et al. 2016). Most gastrointestinal diseases are caused by helminths or parasitic worms that benefit from living in a host of animals' digestion systems. Therefore, they interfere with the animal metabolic homeostasis by using up nutrition intake and benefit from protection while leaving infected animals with weakness and symptoms that include indigestion, diarrhea, and even death (Hoste et al. 2015). However, the symptoms caused by helminths leave an undetectable clinical sign under a small population and appear at high loads marked with a lean appearance.

In developing countries, a digestive infection caused by parasitic worms is extremely difficult to avoid. For example, infection of small ruminants by helminths was reported in at least 80% of Indonesia (Yuswandi and Rika 2015), while 84% of digestive parasitosis was encountered in Benin, West Africa (Tchetan et al. 2022). Presumed reason is due to a rapid infection of worms via soil transmission in pasture and legume-fed animals. With the difficulties in accessing veterinary services and drugs owing to the low purchasing power, most livestock farmers in developing countries rely on ethnoveterinary medicines. However, this medication highly depends on traditional beliefs and native knowledge and practices to protect animal health and cure diseases. Thus, exploration toward proof and accurate efficacy of various traditional practices are the object of interest. Besides ethnoveterinary medications, treatment with synthetic anthelmintic drugs, such as ivermectin (ivomec) and commercial anthelmintics, are commonly applied without in-depth reasoning to counteract diseases in worm-infected animals (Hoste et al. 2015). At the same time, continuous utilization of these drugs leads to multi-resistant strains of the worm, which become another problem (Redman et al. 2012).

Evaluating the outdoor animal activity system suggests an effort to abolish the pathophysiological effect of gastrointestinal nematodes. Those supported by comprehensive modulation of helminths biology, and increased utilization of plant bioactive compounds with anthelmintics properties as an animal feed (Rodriguez-Hernández et al. 2023), approaches to explore and select particular plants with specific functionalities as animal feed are the object of studies for several decades (Hoste et al. 2015; Kusuma et al. 2022; Tchetan et al. 2022; Rodriguez-Hernández et al. 2023). Various methodological studies, including in-vitro and in-vivo approaches, revealed that major plants with abundant condensed tannins (CTs) possess potential nutraceutical properties to remedy diseases caused by gastrointestinal parasites. Tannins play an essential role in breaking the life cycle of gastrointestinal parasites by interfering with their reproductive system. They lower the excretion rate of eggs and larvae by adult worms and the impairment of larvae development into further stages of life (Rodriguez-Hernández et al. 2023). Besides, various plants containing tannins are reported to
possess antimicrobial activity by protecting proteins from degradation caused by microorganisms (Nawab et al. 2020; Cho et al. 2022; Cho et al. 2023).

_Acacia_ is reported to contain abundant bioactive compounds, including tannin. This plant species that belong to a family of evergreen trees are native to the tropical area of Indonesia, Australia, Papua New Guinea, and some parts of South China. They are widely cultivated for a broad range of functions in forestry, agroforestry, and retrieval of degraded soils (Aguiria et al. 2014). Most species of _Acacia_ are carbon sequesters. They can capture atmospheric carbon dioxide and store it for long-term savings inside the soil system. Therefore, _Acacia_ has been widely planted inclusively in subtropical areas to mitigate global warming (Forrester et al. 2015). This fast-growing plant species of the _Mimosa_ subfamily is an industrial plant mostly harvested for furniture materials, wood workings, and fuels (activated carbon and charcoal) (Mutiar et al. 2019). Besides its various ecological functionalities, its bark was mentioned to possess approximately 24.80-25.59% of tannin dissolved in water solvent (Mutiar et al. 2019). However, considering its potential as an anthelmintics remedy, a study to evaluate the efficacy of tannin derived from different species and parts of _Acacia_ plants has not been well discovered. By time- and dose-dependent manner, Zarra-Albarrán et al. (2020) mentioned that fruit from _Acacia farnesiana_ (L.) Willd. was proven to held a robust anthelmintic properties by inhibiting larval growth and leading to a complete death against infective larvae _Haemonchus contortus_ Rudolphi, 1803. Similarly, leaves of _Acacia cochliacantha_ Humb. & Bonpl. at 5% concentration from diet was recorded to lower fecal egg count in boar goat kids (Castillo-Mitre et al. 2021). The main purpose of this study is to determine and compare the anthelmintics properties of leaves and fruit obtained from various _Acacia_ species.

MATERIALS AND METHODS

Sample preparation

Fresh leaves and fruits from _Acacia auriculiformis_ A.Cunn. ex Benth (AA), _Acacia mangium_ Willd. (AM), and _Acacia crassicarpa_ A.Cunn. ex Benth (AC) were obtained from the Jatikuwung experimental farm, district of Karanganyar, Central Java, Indonesia with a soil surface of ±150 m above sea level. After the samples were cut into smaller pieces to enlarge the surface area, they were indirectly sun-dried for 10-11 days under the parano to avoid direct sunlight exposure. The average temperature of the Karanganyar district during this study (April 2021) was approximately 26°C (BPS 2021). Following sun drying, prepared samples were subjected to milling to obtain a sample size of ±2 mm. Both leaf and fruit samples were allocated into 5 different groups: the negative control (NC) using 0.9% of NaCl solution according to protocol by Sambodo et al. (2018); the positive control (PC), albendazole solution at a concentration of 112.5 mg/mL; and solutions containing 12% concentration of each _A. auriculiformis_ (AA), _A. mangium_ (AM), and _A. crassicarpa_ (AC) powders. The 12% concentration was obtained from the best result during the preliminary study (Handayanta et al. unpubl. data). Distilled water was used to dilute the lyophilized _Acacia_ powder. Each treatment group was brought to incubation in 6 different time parameters (1, 2, 3, 4, 5, and 6 hours), and the extraction solutions were subjected to anthelmintic evaluation against _H. contortus._

_Haemonchus contortus_ collection

_Haemonchosis_, particularly of _Haemonchus contortus_ (H. _contortus_), was chosen to evaluate the anthelmintic activity of various _Acacia_ species owing to its common behavior in infecting small ruminants. Clinical symptoms are marked by decreased production, anemia, and cachexia (loss of tissue mass), leading to weight loss. The _H. contortus_ is included as a gastrointestinal nematode and blood-sucking worm (Hoberg and Zarlenq 2016). In this study, _H. contortus_ was prepared from the sacrificed lamb abomasum infected by the _H. contortus_ worm, which was carefully taken and put into a Petri dish containing 0.9% NaCl. The NaCl solution aimed to allow _H. contortus_ to be alive during worm harvesting at room temperature. This study limited the utilization of female _H. contortus_ marked with a white and red twist of 18-30 mm length.

Anthelmintic activity

The anthelmintic activity of leaf and fruit extracts from various _Acacia_ species was evaluated through the larval migration inhibition assay (LMIA) according to the protocol by Rabel et al. (1994). Briefly, the harvested _H. contortus_ worms were incubated in solutions containing different extracts from various _Acacia_ species at determined concentrations. The 0.9% NaCl and albendazole solutions were determined as a negative and positive control, respectively. The incubation differed at 6 different times (1-6 hours), and the results were carefully compared; each treatment consisted of 10 replications. The larval migration inhibition rate was calculated with the formula:

$$A = \frac{T - M}{T} \times 100$$

Where: A indicates the larval migration rate, and T indicates the total number of _H. contortus_ larvae put into a petri dish. Meanwhile, the M represents the number of remaining larvae during the observation times. Finally, the inhibition rate was denoted with the mortality percentages (%).

Statistical analysis

The obtained data for the anthelmintic activity parameter were analyzed using multivariate analysis of variance (MANOVA) using a Completely Randomized Design (CRD). The statistical analysis software was R Studio version 1.0.136-©2009-2016, with the degree of confidence determined at p<0.05. If the ANOVA results reflect the effect on the treatment, it was followed by Duncan’s Multiple Range Test (DMRT) tests.
RESULTS AND DISCUSSION

The anthelmintic activity of leaves extracts from various Acacia species

Anthelmintic properties from various Acacia species in different plant parts, including leaves and fruit, were determined using larval migration inhibition assay (LMIA). It depicts the percentage of worm mortality at a given period. In this study, each Acacia leaf extract did not significantly differ in eliminating H. contortus, unless for AM under 4 hours of incubation (p<0.05). Table 1 shows it had a pathophysiological effect similar to albendazole as PC (p>0.05). While sharing a notably higher mortality rate than NC in killing H. contortus (p<0.05). At the final incubation period (6 hours), although it shared no significant differences, samples subjected to AM treatment displayed a lower mortality percentage among remaining treatments (AA, AC, and albendazole) at 93.33±11.54 %, while others displayed 100% complete mortality.

Besides, experimental study on the incubation period to eradicate gastrointestinal tract parasites is also an object of extensive research to determine the effectiveness and dose of administration of certain drugs. In this study, the anthelmintic properties of various Acacia leaf extracts were evaluated through the mortality rate of H. contortus following different incubation periods. It was revealed that H. contortus parasites completely perished after 6 hours incubation period in all treatment groups. Its mortality percentages did not significantly differ from PC under albendazole administration (p>0.05). This indicates the similar anthelmintic activity potential on Acacia leaf extracts with the albendazole ones. In addition, as displayed in Table 1, no administration of Acacia leaf extracts (NC treatment) promoted only 20% of H. contortus parasites mortality. However, a shorter incubation period of only 5 hours did not display significant differences in eradicating H. contortus parasites compared to 6 hours of incubation (p<0.05). The mortality percentages were recorded between 16.66±05.77% to 96.66±05.77%, with PC being the highest and NC having the lowest mortality rate (p<0.05). No significant differences in this study (p<0.05) concerning the different Acacia species were observed.

The anthelmintic activity of fruit extracts from various Acacia species

Extracts derived from various Acacia fruits were tested and seen to perform significant inhibition against the growth of gastrointestinal H. contortus parasites. After two hours of controlled incubation, fruit extracts from AM and AC species displayed similar potential activity with albendazole to eradicate H. contortus population (p<0.05). However, as the incubation period extended, they showed markedly lower anthelmintic activity than albendazole. In this study, complete removal of gastrointestinal H. contortus parasites was only achieved under treatment with PC, receiving incubation with albendazole for 6 hours. None of the Acacia fruit extracts showed 100% eradication against helminths as PC-treated groups did. In addition, treatment groups were recorded to have a significantly higher mortality rate than NC for 2 hours and withstand until the end of the incubation period. Besides, the result of this study revealed the potentially stronger efficacy of extracts from Acacia leaves against gastrointestinal helminths compared to that derived from fruits.

Regardless of the Acacia species, as seen in Table 2, an extended incubation period was observed to significantly increase the mortality rate of H. contortus (p<0.05). The extension of incubation up to 6 hours resulted in a removal of the H. contortus population to 100.00±00.00%; 83.33±05.77%; 80.00±00.00%; 76.66±05.77%; and 26.66±05.77% for albendazole; AM; AC; AA; and 0.90% NaCl treated groups, respectively. Further, the results of this study indicate the inferior anthelmintic activity of fruit extracts derived from A. auriculiformis against H. contortus compared to other species. Throughout the incubation period, this study recorded both A. mangium and A. crassiscarpa to display markedly higher mortality percentages against determined gastrointestinal helminths (p<0.05).

The study to investigate the anthelmintic activity from plant materials has been the focus of discussions in the last few decades (Hoste et al. 2015; Doyle and Cotton 2019; Tchetan et al. 2022). Those discussions were stimulated by the widespread resistance of small-ruminant animals against gastrointestinal parasites due to the continuous use of general synthetic medication. For example, ivermectin, benzimidazole, and numerous others (Doyle and Cotton 2019). Furthermore, anthelmintics indicate the utilization of natural and synthetic medication to cure infection from parasitic worms (Holden-Dye and Walker 2014; Hadi et al. 2021). Curing action given against roundworms (nematodes), flukes (trematodes), and tapeworms (cestodes) are included as anthelmintic properties. Therefore, they hold an essential role in the veterinary medicine sector. This is due to a major burden on economic aspects, manifested by the significant loss of weight and milk production in worm-infected animals.

| Table 1. Mortality percentage of H. contortus nematodes following treatment with various species of Acacia leaf extract |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Treatments**                  | **Incubation times** |
|                                 | 1 hour | 2 hours | 3 hour | 4 hour | 5 hour | 6 hour |
| NaCl 0.9 % (-)                  | 00.00±00.00Ab | 00.00±00.00bc | 03.33±05.77bc | 13.33±05.77cA | 16.66±05.77bA | 20.00±00.00bA |
| Acacia auriculiformis            | 06.66±05.77bA | 26.66±05.77Ad | 50.00±00.00Ab | 73.33±05.77ABb | 93.33±11.54Ab | 100.00±00.00Ab |
| Acacia mangium                  | 06.66±01.54Ad | 20.00±10.00Ac | 36.66±05.77Ac | 70.00±10.00Ab | 83.33±15.27Ab | 93.33±11.54Ab |
| Acacia crassiscarpa              | 13.33±05.77bA | 26.66±05.77Ad | 56.66±05.77Ab | 76.66±05.77ABb | 86.66±05.77Ab | 100.00±00.00Ab |
| Albenzadole (+)                 | 10.00±10.00Ad | 30.00±10.00Ac | 50.00±10.00Ab | 86.66±05.77Ab | 96.66±05.77bA | 100.00±00.00bA |

Note - Means with different superscripts within the same row indicate a significant difference among incubation periods; *Means with different superscripts within the same column indicate a significant difference among treatments.
With the potential resistance from the improper usage and dose of synthetic anthelmintic drugs, a study to carefully investigate the alternative materials originating from plants to control gastrointestinal diseases is an obvious necessity. Anthelmintic medication from plant materials is safer and more effective in eradicating digestive helminths (Soro et al. 2013). This present study screened the efficacy of extracts from fruits and leaves of diverse Acacia species against digestive parasites, H. contortus. It is representative worm strain and predominant gastrointestinal helminth with the most pathogenic properties against small ruminants in tropical countries (Das et al. 2014; Alowanou et al. 2019). This study revealed distinct effectiveness from different plant part extracts of Acacia. The leaves tended to possess more robust anthelmintic properties against H. contortus than fruit parts.

This study recorded the significant role of various Acacia species in anthelmintics activity. As stated in the results, leaves extracted from all species were reported to completely diminish the population of H. contortus worm after 6 hours of incubation. Nevertheless, although they shared no significant differences, samples subjected to AM treatment displayed a lower mortality percentage at 93.3±11.54% among the remaining treatments. On the other hand, at the final incubation storage at 6 hours, however, none of the Acacia fruit extracts could completely remove helminths. After treating Acacia fruit extracts, the highest mortality rate was only 76.66±0.577% to 83.33±0.577%, while albendazole-treated samples showed complete removal. Numerous publications suggest the functional properties, including antimicrobial, anthelmintic, and antioxidant from plants, are highly determined by their parts (Jang et al. 2018; Barido and Lee 2022; Zheng et al. 2022). Jang et al. (2018) elaborated that the content of phenolic contents is predominantly responsible for certain biological activities, and those owned by leaf extracts are comparatively stronger than fruits, which is also confirmed by this present study.

Acacia is reported to contain abundant bioactive compounds, including tannin. This plant species genus, which is a member of an evergreen tree family, is indigenous to the tropical regions of Indonesia, Australia, Papua New Guinea, and some regions of South China. Their enormous adaptation ability allows them to grow and be harvested all year long. Thus, farmers are benefitted from a broad range of Acacia regardless of the weather

(Aguiar et al. 2014). Besides, its bark was mentioned to possess approximately 24.80-25.59% of tannin dissolved in water solvent (Mutiar et al. 2019). Various studies highlighted natural substances' anthelmintic action due to the number of bioactive compounds, mainly tannins, and flavonoids. They contribute to direct action against diverse types of gastrointestinal parasites by reducing the population, lowering the excretion of eggs by adult worms, and interfering with the further growth of larvae. The pharmacological effects after tannin administration supposedly contribute to the improved immunological response from the increased by-pass protein absorption. (Hoste et al. 2015; Barido et al. 2021). The result of this study suggested the efficacy of Acacia leaves and fruit extracts as a remedy to cure gastrointestinal parasites caused by H. contortus worm.

In conclusion, this study investigated the effect of various parts from diverse Acacia species at different incubation periods. The H. contortus worm were exposed to extracts from Acacia fruits and leaves, and the mortality rate was carefully recorded. Leaf extracts from various Acacia species, unless A. mangium shared similar efficacy in completely eliminating target worm H. contortus under 6 hour incubation time. A. mangium as revealed by this study possessed a weaker anthelmintic property against H. contortus in lambs' gastrointestinal. Meanwhile, with regard to that fruit extracts, only A. mangium and A. crassicarpa shared similar efficacy in eliminating H. contortus during 2 hours of incubation. However, as incubation time extended, they displayed significantly lower anthelmintic properties compared to that of albendazole. Compared to leaf, the extracts derived from Acacia fruit tended to possess a lower anthelmintic efficacy. Therefore, this study suggested the leaves from Acacia can be utilized as anthelmintic against H. contortus parasites. Further studies investigated the potential mechanism by which anthelmintic activity presented by various Acacia species requires more exploration.

ACKNOWLEDGEMENTS

Universitas Sebelas Maret, Surakarta, Indonesia fully supported this study through the Research Implementation Non-tax State Revenue (PNBP) with grant number 452/UN27.21/PN/2020.

| Note: **Means different superscripts within the same row indicate a significant difference among incubation periods; **Means different superscripts within the same column indicate a significant difference among treatments |

Table 2. Mortality percentage of H. contortus nematodes following treatment with various species of Acacia fruit extract

<table>
<thead>
<tr>
<th>Treatments</th>
<th>1 hour</th>
<th>2 hours</th>
<th>3 hours</th>
<th>4 hours</th>
<th>5 hours</th>
<th>6 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl 0.9 % (-)</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Acacia auriculiformis</td>
<td>0.33±0.05</td>
<td>0.66±0.05</td>
<td>0.66±0.05</td>
<td>0.66±0.05</td>
<td>0.66±0.05</td>
<td>0.66±0.05</td>
</tr>
<tr>
<td>Acacia mangium</td>
<td>0.33±0.05</td>
<td>0.33±0.05</td>
<td>0.33±0.05</td>
<td>0.33±0.05</td>
<td>0.33±0.05</td>
<td>0.33±0.05</td>
</tr>
<tr>
<td>Acacia crassicarpa</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Albendazole (+)</td>
<td>10.00±10.00</td>
<td>10.00±10.00</td>
<td>10.00±10.00</td>
<td>10.00±10.00</td>
<td>10.00±10.00</td>
<td>10.00±10.00</td>
</tr>
</tbody>
</table>

| Handayanta & Hadi – Anthelmintics of Acacia leaves and fruits extracts | 3143 |
REFERENCES


Yuswandi RY. 2015. Studi biologi larva dan cacing dewasa Haemonchus contortus pada kambing. Jurnal Sains Veteriner 33: 51-52. [Indonesian]
