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SHORT COMMUNICATION

Exploration of anthelmintic activity of *Cassia* spp. extracts on gastrointestinal nematodes of sheep

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ABSTRACT

Objective: This study aimed to explore the phytochemical constituents and anthelmintic activities of four *Cassia* spp. leaves against *Haemonchus contortus*.

Materials and Methods: The extracts were prepared from four species of *Cassia* spp. (*C. siamea*, *C. fistula*, *C. surattensis*, and *C. spectabilis*). Phytochemical screening of the extract was done based on the Harborne method. Evaluation of the anthelmintic activities against *H. contortus* was done *in vitro* using infective larvae (L_3) migration inhibition assay (LMIA). Measurement of larvae migrating was conducted through a nylon filter with a pore size of 20 µm. The doses of *Cassia* spp. extract implemented were 25, 50, 100, and 200 mg/ml.

Results: Tannins, alkaloids, phenol hydroquinone, flavonoids, steroids, triterpenoids, and saponins were present in all the extracts, whereas alkaloids were absent in *C. fistula*. No triterpenoids were found in *C. surattensis* and *C. spectabilis*. Movement of *H. contortus* larvae was significantly inhibited after exposure to *Cassia* extracts at various dosage levels ($p < 0.05$). The test results using LMIA on L_3 *H. contortus* showed the lowest inhibition in the negative control. Among the species of *Cassia*, the *C. surattensis* (at 200 mg/ml) showed the highest ($p < 0.05$) inhibition level on the larvae. The latter result corresponded to the effect of albendazole.

Conclusion: Compared to other *Cassia* spp., *C. surattensis* exhibited the highest inhibition against L_3 *H. contortus*. However, the inhibition effect of *C. surattensis* was still lower as compared to albendazole.

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Introduction

The gastrointestinal nematode is one of the animal health risks related to the use of pasture for small as well as large ruminant productions. The most dominant gastrointestinal nematodes in small ruminants in the tropics, including Indonesia, are *Haemonchus contortus* [1,2]. Control of gastrointestinal nematodes of ruminants mainly relies on the use of synthetic chemical anthelmintics. However, internal parasitic disease control using synthetic chemicals has raised big concern, such as chemical residues in the animal products, especially when the animals on the extensive livestock management system. In this respect, any alternatives substitute to the role of synthetic chemicals is, therefore, of important to be developed. Among the alternatives, tannin-rich plants seemed to be good candidates to control the internal parasitic disease in animals particularly the small ruminants on the pasture rotation system [3].

The research on anthelmintic effect of the plants containing tannin to nematodes has been carried out [4,5]. *Cassia* spp. is the plants with high condensed tannin content [6]. Kundu et al. [5] showed the broad spectrum anthelmintic effects of *C. angustifolia*, *C. alata*, and *C. occidentalis* crude ethanol extracts against helminth parasite

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of domestic fowls (*Heterakis gallinarum, Catatropis* sp., and *Raillietina tetragona*) *in vitro* at concentration levels of 10–40 mg/ml. Likewise, *Cassia tora* leaves extracts showed anthelmintic activity against *Pherethima posthuma* [7].

In respect, particularly to *C. fistula* Linn, the methanolic extract from pods, pulp, and seeds from such plant (at the concentration 100 mg/ml) showed an anthelmintic activity against *P. posthuma* [8,9]. Other *Cassia* spp., such as *C. siamea*, *C. surattensis*, and *C. spectabilis*, had reported having antibacterial activity, antibiofilm, antifungal, and antioxidant [10–13]. However, anthelmintic activities above mentioned *Cassia* spp. have never been documented. Anthelmintic activity can be affected by phytochemical compounds. Some phytochemical compounds, such as tannin, anthraquinone glycoside, naphtopyrone glycoside, phenolic compounds, flavonoids, and many others, had isolated from *Cassia* plants and suggested to the biological activity of the plants [14]. Hence, this study aimed to explore the phytochemical compound and anthelmintic activities against *H. contortus* of *C. siamea*, *C. fistula*, *C. surattensis*, and *C. spectabilis* leaves extracts.

Materials and Methods

Ethical approval

All the procedures in the study accordance were done after the approval from the Ethics Committee for Animal Use at the IPB University (approval number 44-2017 IPB).

Plants material

The plants were collected in the area of Diponegoro University, Semarang, Central Java, Indonesia. The collected plants were identified by Herbarium Bogoriense of Indonesian Institute of Sciences as *C. siamea, C. fistula, C. surattensis,* and *C. spectabilis* by the reference number 335/IPH.1.01/H.07/II/2016.

Extracts preparation

The leaves of *Cassia* spp. were separately picked and sorted to remove undesired plant parts. Drying of leaves in the room temperature was done for 7–10 days and then milled to produce homogenous flour particles. The *Cassia* leaf flours were then turned into extracts using maceration technique with n-hexane and ethanol solvent. *Cassia* spp. leaf flour (1,000 gm) was initially incubated in n-hexane (5,000 ml) and later in ethanol, 96% (5,000 ml) in the room temperature for 72 h. After incubation, extracting the extract was carried out with Whatman No. 1 filter paper. Filtering results were collected and evaporated using a vacuum rotary evaporator at 50 rpm, 40°C–50°C until the extract was obtained. Storage of the extracts before analysis was used at 4°C analysis.

Phytochemical screening

Phytochemical screening of *Cassia* spp. extract using the [15] method was done to detect tannins, saponins, alkaloids, flavonoids, steroids, triterpenoids, and hydroquinone phenolics.

Preparation H. contortus infective larvae (L_3)

Haemonchus contortus for this study was cultured from stool of a *H. contortus* purely infected sheep donor. Feces of the donor sheep was collected every morning using an apron. The feces were then mixed with vermiculite to obtain moist and airy medium *H. contortus* egg hatching. Incubation of the mixture was carried out in the room temperature for 7–8 days so that *H. contortus* eggs can hatch and develop into infective larvae (L_3). The L_3 were then harvested by a modification of the Baermann method [16]. In order to remove fecal impurities, the infective larvae harvested are screened. The collected larvae were stored at 4°C before being used for the assay.

Infective larvae assay

An *in vitro* larval migration inhibition assay (LMIA) was performed to determine anthelmintic effect of the four *Cassia* spp. Extracts according to Rabel et al. [17] method. The experiment used factorial (4 × 6) design with of *Cassia* spp. as the first factor and dose levels of the extracts as the second factor [18]. The LMIA started with the mixing of 100 L_3 (s) in each *Cassia* extracts that were prepared into 1.5-ml solution with the dosage levels of 25, 50, 100, and 200 mg/ml diluted in phosphate buffer saline (PBS).

These mixtures were then incubated at the room temperature for 3 h. After incubation, the mixtures were then washed thrice by using PBS, centrifuged at 3,500 rpm for 5 min, before the supernatant was removed. The remaining sediments (the larvae) were then sieved using 20-µm grid sieve mounted on PBS-filled microplate well. Number of the larvae migrated through the sieve to the microplate well was then counted by observing the process under a stereoscopic microscope at 20× magnification. The percentage of larval migration inhibition was then calculated using the formula of $[(A - B)/A] \times 100$ where A is the number of larvae that were prepared (100) and B is the number of larvae that migrated through the sieve at each treatment [17].

Statistical analysis

Results of the phytochemical analysis were presented descriptively, while the results of the LMIA were analyzed based on analysis of variance using SAS v 9.0 [19]. The significant effect of LMIA was further tested with DMRT. The difference in treatment was stated to be significant at $p < 0.05$.

Results

Percentage yield of extract

The highest percentage of *Cassia* spp. extract yield was found in *C. spectabilis*, followed by *C. surattensis*, *C. siamea*, and *C. fistula*. *Cassia surattensis* extract had the highest tannin content followed by the extracts *C. siamea*, *C. fistula*, and *C. spectabilis*, respectively (Table 1).

Phytochemical screening of four Cassia spp. extract

Phytochemical compounds identified in the four *Cassia* spp. extracts are shown in Table 2. Tannins, alkaloids, phenol hydroquinone, flavonoids, steroids, triterpenoids, and saponins were present in all the extracts. Alkaloids were absent in *C. fistula*, whereas no triterpenoids was found in *C. surattensis* and *C. spectabilis* extracts.

Larval migration assay of extracts Cassia spp.

Table 3 showed that all the *Cassia* spp. extracts at various doses significantly affected the percentage of inhibited L_3 *H. contortus* ($p < 0.05$). The *H. contortus* larval migration inhibition was lowest in the negative control (PBS). Among the various dose and species of *Cassia,* the *C. surattensis* at the dose level 200 mg/ml resulted in the highest inhibited of L_3 *H. contortus* ($p < 0.05$). The later result corresponded to the effect of albendazole.

Discussion

Our data showed that all *Cassia* spp. used in the present study contained most of the phytochemical compounds, i.e., alkaloids, phenol hydroquinone, flavonoid, steroid, triterpenoid, tannin, and saponin. Unlike *C. siamea*, *C. fistula* contained no alkaloids, while *C. surattensis* and *C. spectabilis* contained no triterpenoids. Corresponding to our result, Mohammed et al. [20] found that *C. siamea* contained anthraquinones, alkaloids, tannins, saponins, flavonoids, polyphenols, and glycosides. Likewise, Kamagaté et al. [21] reported that the ethanol extract of C. *siamea* leaf contained flavonoid (D-pinitol, luteolin), dihydronaphtalen one [(4-trans)-acetyl-3,6,8-trihydroxy-3-methyldihidronapthalenone], triterpenoid (lupeol), and [4-(cis)-acetyl-3,6,8-trihydroxy-3-methyldihydronaphtalenone].

Our finding showed that *C. fistula* contains phenol hydroquinone, flavonoids, steroids, triterpenoids, tannins, and saponins. However, it contained no alkaloid. In contrast, Panda et al. [22] found alkaloids, flavonoids, tannins and phenolic compound, glycosides, protein, amino acids, saponins, and triterpenoids. In Another study revealed that *C. fistula* leaves mainly contain oxalic acid, tannin, oxy anthraquinone, and anthraquinone derivative [23]. In general, the composition and concentration of secondary metabolites in particular plant is influenced by several factors, including genetic factors, climate, soil, harvest time, and solar radiation [24].

Compared to PBS group, all *Cassia* spp. were capable of increasing the percentage inhibition of *H. contortus*, of the doses, applied. Yet, the values were still lower as compared to the albendazole group. In this respect, *C. surattensis* had anthelmintic activity, especially against *H. contortus*. Several studies used else species of the genus *Cassia*, such as the *C. tora*, *C. auriculata*, *C. angustifolia*, *C, ocidentalis*, and *C. alata* which contain active compounds as tannins, flavonoids, saponins, and alkaloids that shown to be anthelmintic [5,7,25]. The anthelmintic activities may be contributed by tannins, alkaloids, flavonoids, steroids, phenol hydroquinone, triterpenoids, and saponins.

Very limited study on the mechanisms of phytochemical compounds of *Cassia* spp. to destruct *H. contortus.* However, tannin has been demonstrated to block through uncoupling the oxidative phosphorylation leading to the death of parasites. Another possible mechanism could be through binding of tannins to free proteins in the gastrointestinal tract of the animal, or glycoprotein on the cuticle of the helminth's body surface causing a paralysis, and thus death [26]. Cuticle and digestive tissue of larvae are significantly damaged due to the presence of tannins. The size of the polymer and the tannin molecule has a relationship in influencing the strength of the

Table 1. Percentage yield and tannin content from all four extracts of *Cassia* spp.

Extract	Percentage yield (W/W)	Tannin (%)
C. siamea	4.49	4.92
C. fistula	2.36	3.98
C. surattensis	5.23	7.82
C. spectabilis	11.37	2.67

Table 2. The phytochemical constituent of extracts of *Cassia* spp.

Phytochemical	Extracts			
	C. siamea	*C. fistula*	*C. surattensis*	*C. spectabilis*
Alkaloids	+	–	+	+
Phenol Hydroquinone	+	+	+	+
Flavonoids	+	+	+	+
Steroids	+	+	+	+
Triterpenoids	+	+	–	–
Tannins	+	+	+	+
Saponins	+	+	+	+

+: present; –: absent.

Table 3. Percentage L_3 *H. contortus* inhibited after exposure to 3 h on the species and dose of extract o*f Cassia* spp. different*.

Extracts	PBS (negative control)	C-25	C-50	C-100	C-200	Albendazole (positive control)
C. siamea	22.11 ± 2.84^{g}	59.89 ± 1.30^{f}	63.18 ± 1.09^{def}	63.34 ± 1.22^{def}	65.31 ± 1.25^{cd}	82.71 ± 1.19^{a}
C. fistula	24.89 ± 7.32^{g}	59.88 ± 1.54^{bc}	62.30 ± 4.42^{def}	60.90 ± 3.13^{f}	63.46 ± 1.49^{def}	81.82 ± 1.19^{a}
C. surattensis	23.81 ± 0.85^{g}	63.56 ± 2.41^{def}	65.90 ± 2.56^{cd}	67.99 ± 2.06^{c}	71.62 ± 2.49^{b}	84.94 ± 0.78^{a}
C. spectabilis	22.56 ± 2.64^{g}	59.95 ± 1.25^{f}	61.10 ± 1.71^{ef}	62.27 ± 1.71^{def}	64.98 ± 1.17^{cde}	85.36 ± 0.94^{a}

Values are mean ± SD of six replicates.
$^{a–g}$Means within a row and column with different superscripts differ ($p < 0.05$).
C- means dose of species *Cassia* extracts (mg/ml).

anthelmintic effect. In addition, there are effects of the structural units of monomeric tannins because gallocatechin and epigallocatechin monomers have significant anthelmintic activity on the contrary monomer catechin and epicatechin [27]. In addition to tannin, other bioactive compounds in *Cassia* spp. may contribute the anthelmintic activity against *H. contortus*. Indeed [28–30], suggested flavonoid, terpenoid, saponin, and alkaloids may exert an anthelmintic effect on *H. contortus*.

Previous studies in laboratory animals have demonstrated the safety of *Cassia* spp. as medicinal plants. Thus, no clinical signs of toxicity and mortality were found in mice receiving single oral doses of 2,000–5,000 mg/kg body weight extracts of *C. spectabilis* [13,31], *C. siamea* [21], and *C. surattensis* [32], respectively. However, long-term administration of *Cassia* spp. extracts may cause reversible hepatotoxicity in rats and mice [21].

Conclusion

Compared to other *Cassia* spp., *C. surattensis* exhibited the highest migration inhibition against L_3 *H. contortus*. However, the inhibition effect *C. surattensis* was still lower as compared to albendazole. Owing to the latter fact, it could be inferred that *C. surattensis* may be used to substitute the role albendazole as anthelmintic provided that dose is increased. Hence, the future study is necessary to confirm optimal doses of *C. surattensis* as substitute for albendazole.

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Conflict of Interests

The authors declare that they have no conflict of interest.

Authors' contribution

Sri Wahyuni and Fadjar Satrija designed the study, conducted the experiments, analyzed the data, and prepared the article. Sunarso Sunarso and Bambang Waluyo Hadi Eko Prasetiyono corrected the article.

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