

Research Article



Phytochemical Screening and *In vitro* Anthelmintic Activity of *Cassia alata* (L) Roxb. on *Haemonchus contortus* of Small Ruminants in Burkina Faso.

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Abstract | In Burkina Faso, small ruminant livestock owners face internal parasitism, particularly gastro-intestinal strongylosis (GIS), which causes severe losses on their farms. In response to this situation, endogenous strategies are being developed through the use of medicinal plants such as *Cassia alata* to combat GIS in the field. Thus, the present study was conducted to evaluate the anthelmintic efficacy of aqueous and hydroacetic extracts of *Cassia alata* leaves on *Haemonchus contortus*, one of the most dominant strongyles of small ruminants in Burkina Faso. For this purpose, a phytochemical screening of two extracts was carried out followed by *in vitro* antiparasitic tests with five increasing concentrations (6.25, 12.5, 25, 50 and 100 mg/ml) of the extracts on two stages life of the *Haemonchus contortus* parasite. The results obtained from the phytochemical analysis revealed the presence of steroidal and triterpenic compounds, anthracenoides, saponosides, polyphenols (tannins) and reducing compounds in the aqueous and hydroacetone extracts of the leaves of *Cassia alata*. In the *in vitro* tests, the percentages of egg hatch inhibition increased significantly ($p < 0,05$) with increasing concentrations and compared to the control. All concentrations tested showed vermifugal activity on adult *Haemonchus contortus* worms after 6 hours of exposure to freeze-dried extracts. These results show that both extracts have ovicidal and vermifugal activity on the *Haemonchus contortus* parasite. Further studies could be envisaged to evaluate the toxicity of the plant extracts followed by *in vivo* anthelmintic efficacy tests to support breeders in their use.

Keywords | *Cassia alata*, Anthelmintic activity, *In vitro*, *Haemonchus contortus*.

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INTRODUCTION

The livestock sub-sector is one of the productive sectors in which Burkina Faso has a clear comparative advantage in the West African sub-region. It occupies an important social and economic place, as it is practiced by more than 80% of households and contributes more than 18% to national value added (MRA, 2010). The highest animal species are small ruminants (sheep and goats) with a na-

tional population of more than 23 169 193 animals (MRA, 2015). These small ruminants have a low zootechnical productivity, mainly due to their management method based on the use of natural pastures (Krecek and Waller, 2006). In pasture-based production systems, the major issues are nutrition and gastrointestinal parasitism (Torres-Acosta and Hoste, 2008). A large proportion of expenditure in this type of livestock farming is attributable to pathologies and their control. The control of parasitic helminths, in-

cluding nematodes, is considered an essential part of herd health management. Within nematodes, gastrointestinal strongylosis of small ruminants is a dominant and widespread disease, responsible for enormous economic losses. This fact is aggravated by the prevalence of anthelmintic resistance in the population of gastrointestinal strongylosis, which necessitates the development of alternatives to chemical anthelmintics.

Among the solutions available, attention is paid to plants rich in secondary metabolites with anthelmintic properties. Indeed, in addition to being an important source of nutrients (especially proteins) for ruminants in some cases, numerous studies have proven the anthelmintic properties of plants on gastrointestinal nematodes, notably *Haemonchus contortus* (Hoste and Torres-Acosta, 2011).

The objectives of this study were to evaluate the ovicidal and vermifugal effects of lyophilisates of *Cassia alata* leaves on *H. contortus*, the most common parasite in small ruminant farms (Belem et al., 2005a and 2005b). They thus aim to alleviate the problem of infestation of natural pastures and parasite resistance in the use of synthetic chemicals (Hoste et al., 2018), the costs of which are high and beyond the reach of Burkinabe rural livestock farmers (Kaboré et al., 2009). In addition, the choice of the plant in this study was motivated by the fact that (i) livestock owners use its leaves in the treatment of gastrointestinal parasitosis in small ruminants (Kerharo and Adams, 1974) and (ii) the plant has not been the subject of any anthelmintic studies in livestock in Burkina Faso.

MATERIALS AND METHODS

PLANT MATERIAL

The leaves of the plant *Cassia alata* (L) Roxb. were harvested at the Saria Station of the Institute Environmental and Agricultural Research (INERA) located in Koudougou, Boulkiemde province of Burkina Faso. They were then cleaned and dried in the shade for 72 hours before being crushed to obtain powders. These powders were used to prepare two extracts.

ANIMAL MATERIAL

Naturally infested goat quails were collected from butchers in the villages surrounding Saria Station to harvest adult *Haemonchus contortus* worms at the Animal Biology and Health Laboratory (LaBioSa) of Saria Station.

PLANTS EXTRACTS

For the preparation of the aqueous macerate, 100 g of *C. alata* leaf powder was extracted with 1000 ml of distilled water by maceration under mechanical agitation for 24 hours at room temperature. The extract was filtered

through a fine-mesh nylon fabric and the filtrate obtained was then centrifuged at 2000 rpm for 10 minutes. The resulting supernatant was frozen and freeze-dried. The total dry extract mass obtained was determined as well as the extraction yield.

As for the hydroacetone macerate, the same procedure was used with 80% aqueous acetone. The extract was filtered on N° 5 wattman paper and the acetone was evaporated under reduced pressure in the rotary evaporator. The aqueous extract thus obtained was frozen and freeze-dried. At the end of the preparation of the two extracts, the aqueous macerate gave a total dry extract yield of 16.12% while the hydroacetone extract yield was 26.26%.

PHYTOCHEMICAL SCREENING

5 g of each freeze-dried extract was solubilized in 50 ml of distilled water. A volume of 25 ml of each extract was hydrolyzed for the characterization of O-heteroside compounds. The organic phase of the solution of the hydrolyzed extracts, supposed to contain the total genins, was used for the detection of steroid and/or triterpenic glycosides and anthracenosides. The non-hydrolyzed portion of the extracts was used to test for polyphenolic compounds (tannins), saponosides, and reducing compounds. The main families of secondary metabolites were characterized according to the method described by Ciulei (1982).

IN VITRO ANTHELMINTIC TESTS

Preparation of extract concentrations: For the *in vitro* anthelmintic tests of the two plant extracts, five decreasing concentrations (100; 50; 25; 12.5; 6.25) of each extract were prepared by cascade from 400 mg of each extract in 4 ml of distilled water.

Adult worms of *Haemonchus contortus* and egg preparation: Adult worms of live *H. contortus* and eggs were obtained using the technique described by Jabbar et al. (2006). For this purpose, the purchased quails were incised to collect the live adult worms and washed with distilled water. Immediately a quantity of live adult females was selected and lightly crushed in a mortar with a porcelain pestle to release the eggs. The solution obtained was filtered through sieves with different mesh sizes (1mm, 100 µm and 38 µm) to collect the released eggs and clean them with distilled water after several rinses. The live adult worms and eggs thus recovered were used to immediately conduct the *in vitro* anthelmintic tests.

IN VITRO ANTHELMINTIC TESTS ON EGGS AND ADULT WORMS

Egg hatching inhibition test: Egg hatching test was carried out using approximately 100 *H. contortus* eggs in 1 ml in microtubes (2.5 ml). In each microtube thus prepared, 1

ml of extract of the five concentrations of each of the two preparations was added. A negative control prepared with distilled water was also prepared. Then, all the microtubes were closed and incubated at laboratory room temperature of 28-30°C for 48 hours, after which two to three drops of 10% formalin were deposited in each microtube to stop the evolution of the eggs. Three replicates were carried out during the test for each of the concentrations of each extract tested and the negative control. In the end, hatched eggs and L1 larvae count was carried out under the microscope. The egg hatch inhibition percentages for each of the extract and control concentrations were calculated as follows:

% inhibition = $100 * (1 - (Ct/Cc))$ with Ct the number of eggs hatched at the extract concentrations and Cc the number of eggs hatched at the control.

Test for inhibiting the mobility of adult worms: The technique described by Sharma *et al.* (1971) with slight modifications was applied. Briefly, three (3) live adult worms of *H. contortus* contained in 2 ml of PBS were exposed in each well of a 24-well PCR microplate (Becton Dickinson brand) for separate treatments at laboratory room temperature (25-30°C). The treatments consisted of 1 ml of the five increasing concentrations with the two plant extracts and an untreated negative control (distilled water). Each treatment was repeated three times. Inhibition of adult worm motility was used as the criterion for anthelmintic activity for each treatment. Worm motility was observed at intervals of 2, 4, and 6 hours.

STATISTICAL ANALYSIS

The collected data were used to calculate means (± standard deviation) before being subjected to a one-way analysis of variance to discriminate the measured parameters. The comparison of the calculated means was carried out using the Tukey-Kramer test. The Kruskal-Wallis test was used to evaluate the effect of the concentrations of the prepared extracts. All analyses were carried out with the Costat software (version 6.20.4) at 5%. Beforehand, the collected data underwent a transformation by the log formula (x + 1) to normalize them. Then, the inhibitory concentrations 50 (IC₅₀) of the extracts tested, i.e. the concentration capable of producing 50% egg inhibition of each extract, were calculated by probit-analysis with IBM SPSS software for Windows (Version 20.0.0).

RESULTS

PHYTOCHEMICAL SCREENING OF EXTRACTS

Phytochemical screening revealed the presence of steroidal and triterpenic compounds, anthracenosides, saponosides, polyphenols (tannins) and reducing compounds in both extracts of the plant *Cassia alata* (Table 1). In contrast to

the reducing compounds, both extracts gave a similar profile of phytochemical constituents.

Table 1: Groups of phytochemical compounds detected in the aqueous and hydroacetic extracts of *Cassia alata* leaves.

Chemical groups	Extracts	
	Aqueous (aq)	Hydroacetic (Ac)
Sterols and triterpenes	+	+
Anthracenosides	+	+
Saponosides	+	+
Polyphenols (tannins)	+	+
Reducing compounds	-	+

+ : presence - : absence

EGG HATCHING INHIBITION TEST

The average hatching rate of the control (distilled water) was 79.6%. The average hatching inhibition percentages of *Haemonchus contortus* eggs of the different concentrations of the tested extracts are shown in Table 2. The percentages of egg hatch inhibition according to the concentrations of the *Cassia alata* extracts ranged from 23.43% to 75.31% for the aqueous extract and from 26.35% to 76.56% for the hydroacetic extract. These inhibition rates increased significantly (p < 0.05) with the increase in the concentrations of the two freeze-dried extracts. Between the two extracts, only the concentration of 100 mg/ml did not show a significant difference (p > 0.05) unlike the other concentrations which showed a significant difference between them (p < 0.05).

Table 2: Percentage inhibition of *H. contortus* egg hatch as a function of the concentrations of plant extracts and control tested in the study

Concentrations	<i>C. alata</i> extracts	
	Aqueous	Hydroacetic
100	75.31 ± 7.13 ^{aA}	76.56 ± 4.03 ^{aA}
50	53.97 ± 7.66 ^{bcB}	65.27 ± 3.15 ^{abC}
25	40.16 ± 3.15 ^{cdD}	60.25 ± 5.66 ^{abE}
12,5	35.95 ± 5.47 ^{deE}	50.20 ± 1.91 ^{bcB}
6,25	23.43 ± 4.52 ^{ff}	26.35 ± 4.03 ^{eff}

The letters (abcdef) compare the averages between the concentrations by column and the capital letters (ABCDEF) compare the averages between the lines of each concentration. Different ones indicate significantly different values (p < 0.05).

Kruskal-Wallis tests carried out showed a significant difference between the concentrations tested with the aqueous extract of *Cassia alata* (p = 0.0114) and the hydroacetic extract (p = 0.0111). The concentration-response effect profile of egg hatch inhibition is shown in Figure 1. Depending on the concentrations tested, a decrease in

egg hatch with the lyophilized extracts in contrast to the negative control with distilled water was observed.

cant ($p < 0.05$) reduction in worm mobility compared to the negative control. Between 4 h and 6 h of exposure, nearly 50% of the worms contained in the different concentrations had lost their mobility.

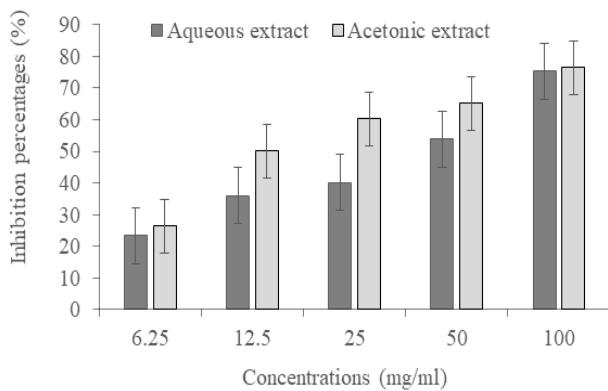


Figure 1: Dose-response profile of the inhibition of egg hatch of *H. contortus* eggs subjected to concentrations of aqueous and acetone extracts of *C. alata* during the study.

The inhibitory concentrations 50 (IC_{50}) of the two *Cassia alata* extracts calculated using the probit-analysis method are summarized in Table 3. The IC_{50} values obtained were 60.924 mg/ml for the aqueous extract and 17.651 mg/ml for the hydroacetonic extract.

Table 3: Inhibitory concentration 50 (IC_{50}) values for *H. contortus* eggs subjected to the two *Cassia alata* extracts in the study

Extracts of <i>Cassia alata</i>	IC_{50} (LCL - UCL)
Aqueous	60.924 (20.242 – 86.643)
Hydroacetonic	17.651 (0.460 – 59.785)

LCL: Lower Confidence Limit
UCL: Upper Confidence Limit

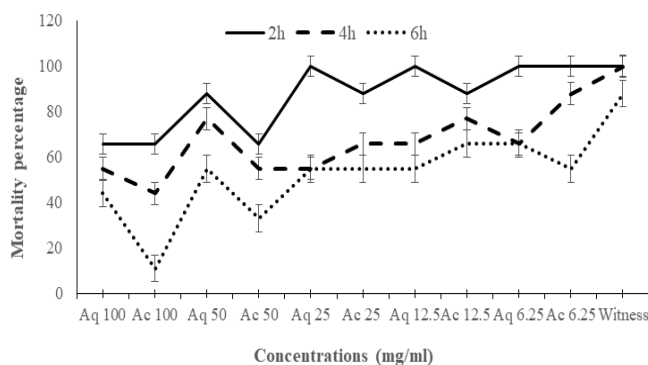


Figure 2: Percentage of live worms of *H. contortus* exposed to concentrations of plant extracts and the negative control at different exposure times.

MOBILITY INHIBITION TEST FOR ADULT WORMS

The results on the mobility of live adult worms of *H. contortus* with the two extracts of *C. alata* and the negative control are shown in Figure 2. The different concentrations of the plant extracts were associated with a signifi-

DISCUSSION

One approach that could reduce the development of resistant parasites in livestock is the use of anthelmintic medicinal plants as an alternative to synthetic chemicals (Hoste et al., 2006; Ketzis et al., 2006; Martinez-Ortiz-de-Montellano, 2010).

The results obtained in the *in vitro* tests carried out showed that practically all concentrations of the aqueous and hydroacetone extracts significantly inhibited the hatching of *H. contortus* eggs. Similar results were obtained using extracts of *Anogeissus leiocarpus* and *Daniellia oliveri* by Kaboré et al. (2009) and *Piliostigma reticulatum* by Wadré et al. (2015) on the *H. contortus* parasite. However, the hydroacetone extract of *Cassia alata* had the highest ovicidal activity in the study compared to the aqueous extract. This is certainly due to a greater presence of secondary metabolites of anthelmintic interest (tannins, glycosides and triterpenes) in the hydroacetone extract than in the aqueous extract. Marie-Magdeleine et al. (2010) obtained a greater ovicidal activity through the use of dichloromethane extract, unlike other extracts of the plant *Tabernaemontana citrifolia* on *Haemonchus contortus*.

The vermicial activity observed with both plant extracts compared to the control shows that *C. alata* also has anthelmintic properties on adult worms of *H. contortus*. Hounzangbe-Adote et al. (2005) obtained similar results with the alcoholic extracts of the plants *Zanthoxylum zanthoxyloides*, *Newbouldia laevis*, *Morinda lucida* and *Carica papaya*.

The anthelmintic effects of the two *C. alata* extracts observed in the study are certainly related to the active ingredients (secondary metabolites) contained in the plant. Indeed, both plant extracts contain sterols and triterpenes, anthracenosides, saponosides and polyphenols (tannins). These results are close to those obtained by Wadré et al. (2015) with the leaves of *Piliostigma reticulatum*. The anthelmintic properties of these secondary metabolites are reported by Paolini et al. (2003), Barrau et al. (2005) and Hoste et al. (2006). Studies have shown that these secondary metabolites, notably flavonoids and tannins, are involved in anthelmintic activity (Brunet, 2008). The latter would probably act by inhibiting the oxidative phosphorylation of the parasites (Vedha Hari et al., 2011) by binding to a glycoprotein (collagen) which plays a protective role in the parasite cuticle (Ongoka et al., 2012). This

fixation would lead to a disruption of the integrity of the cuticle and then to the death of the parasite (Athanasiadou, 2001; Vidyadhar et al., 2010). In addition, tannins, especially condensed tannins, are believed to diffuse to the surface of egg membranes to bind to free membrane proteins and thus induce inhibition of egg hatch (Min et al., 2003; Brunet et al., 2007; Koffi Yao et al., 2018).

All the results of the *in vitro* anthelmintic activity of the freeze-dried extracts of the *Cassia alata* plant obtained from the eggs and adult worms of *H. contortus* would therefore justify the use of the plant in traditional veterinary medicine. Above all, in our tropical context where parasitism causes enormous losses in small ruminant farms. The use of aqueous and hydroacetone extracts of *C. alata* could be an alternative for the control of gastro-intestinal nematodes in small ruminants in farming environments.

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CONFLICT OF INTEREST

The authors acknowledge that there is no conflict of interest regarding the content of the manuscript.

AUTHORS CONTRIBUTION

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