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Original Article

Anthelmintic and antioxidant potential of banana bracts (*Musa paradisiaca*) extract in ruminants

Potencial anti-helmíntico e antioxidante do extrato de botão floral de bananeira (*Musa paradisíaca)* em ruminantes

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A B S T R A C T

Pharmacological resistance to synthetic anthelmintic drugs is an important barrier to the control of animal parasites. Thus, this study aimed to evaluate the anthelmintic action of the hydroalcoholic extract of banana bracts (HEB) at 10%. Hatch test and larval migration inhibition test (LMI) were performed. Additionally, the total content of polyphenols, condensed tannins, and flavonoids was determined, along with the antioxidant activity of HEB. In relation to bovine species, HEB at a concentration of 2.5 mg mL^{-1} inhibited hatchability of nematode larvae by 88%. The LMI indicated 67.56% inhibition with 5 mg mL^{-1} HEB. However, in sheep, HEB did not show an influence in either tests. The HEB (10 mg mL^{-1}) had 0.38 mg EAG g^{-1} of total polyphenols, 372.70 mg EAT g^{-1} tannins, 0.42 mg RE g^{-1} flavonoids, and presented antioxidant activity at 43.03% with IC_{50} corresponding to 0.2765 mg mL^{-1}. Thus, HEB presents anthelmintic potential *in vitro*, for the parasitologic control in cattle, in addition to demonstrating antioxidant activity. These results are particularly important for agroecological, organic, and biodynamic systems of animal production, considering that the use of synthetic parasiticides is not allowed in these systems.

Palavras-chave:
Plantas Medicinais
Taninos
Vermes

R E S U M O

Resistência farmacológica a drogas anti-helmínticas sintéticas representa importante barreira para o controle parasitário animail. Assim, este estudo objetivou avaliar a ação anti-helmíntica do extrato hidroalcoólico de brácteas da banana (HEB) a 10%. Foram realizados testes de eclodibilidade e inibição da migração larval (TIML). Adicionalmente, foram determinados os teores de polifenóis, taninos condensados e flavonoides totais, além da atividade antioxidante do HEB. Em relação à espécie bovina, o HEB na concentração de 2,5 mg mL^{-1} inibiu a eclodibilidade das larvas dos nematódeos gastrointestinais em 88%. O TIML indicou 67,56% de inibição com 5 mg mL^{-1} de HEB. No entanto, em ovinos, a HEB não demonstrou influência em nenhum dos testes. O HEB (10 mg mL^{-1}) apresentou 0,38 mg EAG g^{-1} de polifenóis totais, 372,70 mg EAT g^{-1} de taninos, 0,42 mg RE g^{-1} para flavonoides totais, e 43,03% de atividade antioxidante com IC_{50} correspondente a 0,2765 mg. mL^{-1}. Assim, HEB apresenta potencial anti-helmíntico *in vitro*, para o controle parasitológico em bovinos, além de demonstrar atividade antioxidante. Estes resultados são particularmente importantes para sistemas agroecológicos, orgânicos e biodinâmicos de produção animal, considerando que o uso de parasiticidas sintéticos não é permitido nestes sistemas.

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INTRODUCTION

Gastrointestinal nematodes are one of the main economic barriers in sheep and cattle production. Verminosis can lead to losses via decrease in weight gain, increase in mortality rate, lower carcass yield, lower meat, and milk and wool production, in addition to expenditure on medicines (MORGAN et al., 2013).

These parasites are frequently controlled by the use of synthetic parasiticides. However, due to drug resistance of these parasites (BABJÁK et al., 2018; TARIQ, 2017), use of multiple and regular doses of anthelmintics is increasingly observed, causing negative effects on the development of natural immunity in the animals, and consequently promoting even more parasitic resistance to various chemical groups (CROOK et al., 2016; SANTOS et al., 2017). This resistance has been observed worldwide (KANETO et al., 2016; MUCHIUT et al., 2018), and the widespread use of parasiticides has resulted in the presence of medicinal residues in foodstuffs of animal origin, as well as in the environment (SMITH et al., 2013). However, the resistance may vary across farms and products, and therefore, specific tests are necessary (TSUKAHARA et al., 2017).

The development of the organic world market has evidenced the tendency to limit the use of synthetic drugs in order to reduce their harmful environmental effects and to promote consumer health (SMITH et al., 2013). Thus, natural products for animal health control have been explored. Additional advantages include the possibility of reducing the exposure to synthetic drugs, thus reducing the selection pressure on resistant parasites.

Medicinal plants have shown promising results in the control of gastrointestinal nematodes of ruminants (ANJOS et al., 2016; MEENAKSHISUNDARAM; HARIKRISHNAN; ANNA, 2016; MUKHERJEE et al., 2016; RAZA, 2015, ROMERO-BENAVIDES et al., 2017; SPIEGLER; LIEBAU; HENSEL, 2017). The antiparasitic properties of some tanniniferous plants have been recorded by different research groups (GONÇALVES et al., 2016; OLOUNLADÉ et al., 2017; QUIJADA et al., 2015; QUIJADA et al., 2018), specifically including *Musa* spp. (GREGORY et al., 2017; MARIE-MAGDELEINE et al., 2014; NEUWIRT et al., 2015; NOGUEIRA et al., 2012).

The banana plant (*Musa paradisiaca),* belongs to the family Musaceae*,* and is distributed abundantly in tropical countries. It is grown natively or cultivated for food purposes and its fruit is widely consumed for its sweet taste and nutritional value. Thus, the banana plant presents economic importance in many developing countries (FAO, 2016). Banana bracts are rich in carbohydrates and proteins and are an important source of minerals and fibers (FINGOLO et al., 2012). It can be used as an alternative food due to its high nutrient content and the large amount of antioxidant compounds (JOSEPH et al., 2014; SILVA; SARTORI; OLIVEIRA, 2014). Moreover, the possibility of the use of an agricultural residue (banana bracts) as an inexpensive livestock feed, could contribute to the livestock economic sustainability.

The main chemical compounds present in *M. paradisiaca* include polyphenols, tannins, eugenol, tyramine, phenolic compounds, anthocyanins, alkaloids, minerals (iron, zinc, selenium, magnesium, calcium, phosphorus, and potassium), vitamins A, C, B1, B2, and B6, and antioxidants. Furthermore, serotonin, trazterenol, dopamine (ripe fruit and peel), and steroids are also found (JUAREZ-GARCIA et al., 2006).

Among the compounds in banana bracts, it is important to highlight the condensed tannins (PEREIRA, 2015), can act on gastrointestinal nematodes in both direct and indirect manners. Tannins attach to the outer cuticle of the nematode larvae, and to their digestive and reproductive tracts, and are associated with inhibition of larval unsheathing and migration. Effects such as reduction of hatchability, inhibition of development, and reduced motility of larvae and adults were observed *in vitro* (HOSTE et al., 2012). *In vivo*, they impair the oviposition of adult females in the digestive tract of ruminants, reducing the amount of eggs per gram of feces, which may favor reduction of contamination in the pasture (SHALDERS et al., 2014). Indirect anthelmintic action of condensed tannins is associated with immune stimulation consequent to better use of the dietetic protein content. Tannins can bind to proteins by protecting them from excessive ruminal degradation, thus increasing the protein availability for intestinal absorption (HOSTE et al., 2012). Tannins have thus been associated with reduction in the number of infectious helminths.

Thus, considering that the banana plant may represent an important alternative to synthetic anthelmintic control, this study aimed to evaluate, *in vitro*, the anthelmintic action of hydroalcoholic banana bracts extract (HEB).

MATERIALS AND METHODS

The present study was duly evaluated and approved by the ethics committee for the use of animals of the State University of North Paraná, certified by the number 14/016.

Collection and processing of plant material

The bracts of banana plant, cultivar Prata anã, were collected at the State University of North Paraná, Bandeirantes city. After selection by absence of macroscopic alterations and packing in polyethylene bags, this material was sanitized with running water, weighed, dried in an oven with forced air ventilation at 40°C for 96 hours, and milled with a knife mill. For preparation of the 10% hydroalcoholic extract, 30 g of the vegetal material was added to 189 mL of 99.5% absolute ethyl alcohol and 81 mL of distilled water. The extraction was performed by mechanical stirring with a magnetic stirrer, over 24 hours, at room temperature. The

material was then subjected to vacuum filtration using a filter paper (Whatman® n. 9.0 cm). The resulting plant material was extracted twice more by adding the hydroalcoholic solution in the same proportions as described above. The extract obtained was concentrated in a rotary evaporator at a temperature of 60°C and a pressure of -400 to -500 mm Hg for 60 minutes, and was then lyophilized at -50°C at -150 mm Hg.

Hatch test (HT)

Fecal samples were obtained directly from the rectum of naturally parasitized animals, originating from commercial herds, and without antiparasitic treatment for at least 60 days. These animals were tested for egg count per gram of feces (EPG) according to the method described by Gordon; Whitlock (1939) and the fecal samples that present at least 2000 EPG, were select. The nematodes were identified according to Keith (1953) by larval culture (ROBERTS; O'SULLIVAN, 1950).

In order to obtain the nematode eggs, the fecal samples were homogenized in warm water (± 40°C) and filtered through a set of sieves with cross-sections of 750, 250, 75, and 25 µm, in the stated order.

The treatments were evaluated in quadruplicate by means of the hatch test (HT) according to Coles et al. (1992) and adapted by Bizimenyera et al. (2006). Using a cell culture plate, approximately 110 eggs were allocated to each well and subjected to HEB treatment at 0.625, 1.25, 2.5, and 5 mg mL^{-1}. Regarding fecal samples from sheep, in addition to the concentrations above mentioned it was necessary to evaluate higher concentrations such as 10, 15, and 20 mg mL^{-1}. Control treatments included a negative control with distilled water (NC) and a positive control with 0.1 mg mL^{-1} of Albendazole Suffox (PC). After incubation in a biological oxygen demand (BOD) incubator at 27°C, for 48 hours, the eggs and hatched larvae (L1) were quantified. The results were evaluated according to the percentage of larval reduction (% LR) using the formula 1:

$$\text{Effectiveness} = \left(\frac{\text{MO}}{\text{ML} + \text{MO}}\right) \text{x } 100$$

MO and ML represent the egg and larval counts, respectively.

Larval migration inhibition assay (LMI)

To verify the larvicidal effect of HEB, LMI was performed (FORTES; MOLENTO, 2013). Fresh third stage larvae (L_3) were selected and unsheathed with 2% sodium hypochlorite (150 µL mL^{-1} for 5 min). After washing for removal of sodium hypochlorite residues, the larvae were quantified and larval solution prepared such that approximately 200 larvae were present in 50 µL of the solution. Following this, 50 µL of the larval solution and 950 µL of the respective treatments were added to each well.

After incubation in a BOD incubator for 16 hours at 27°C, the respective treatments were subjected to a larval migration test using a 20 µm mesh. After an additional 24-hour incubation under the conditions described above, the larvae were counted by optical microscopy at 100 x magnification. The arithmetic mean of the number of larvae that migrated was transformed into percent migration according to the following formula 2:

$$\text{Efficacy (\%)} = \left(\frac{\text{Br} - \text{M}}{\text{Br}}\right) \text{x } 100$$

Br represents the mean of the larvae that migrated corresponding to the negative control group
M represents the mean of the larvae that migrated from the other groups.

The results were evaluated using Tukey's test at 5% probability using the Statistica software.

Extract Analysis

The contents of total polyphenols, flavonoids, tannins, and the antioxidant activity of HEB was evaluated in triplicate for the concentrations of 2.5, 4, 5.5, 7, 8.5, and 10 mg mL^{-1}.

Total polyphenols were determined by the modified *Folin-Ciocalteu* method described by Singleton; Rossi (1965), using gallic acid as the standard for comparison. The results were expressed as milligrams of gallic acid per gram of extract (mg EAG g^{-1}). Gallic acid was used as a standard as it is a precursor of several types of phenolic compounds by the fact that it has a simple structure.

The total dosage of flavonoids was determined using a UV-Vis spectrophotometer based on the complexation of flavonoids with $AlCl_3$ (ZHISHEN, 1999). The results were expressed as milligrams of rutin per gram of extract (mg RE g^{-1}). Rutin and quercetin have the basic structure of flavonoids and can, therefore, be used as indirect indicators of flavonoids.

The tannin content was determined according to the methodology of Makkar; Becker (1994) adapted by Fagbemi; Oshodi; Ipinmoroti (2005), and the results were expressed as milligrams of tannic acid per gram of dry extract (mg EAT g^{-1}).

The antioxidant activity of the extract was determined by the ability of the H+ donor to stabilize the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), according to Blios (1958). The calculation of antioxidant activity was performed according to the formula 3:

$$\text{Antioxidant activity} = \left(\frac{\text{Acontrol - Asample}}{\text{Acontrol}}\right) \text{x } 100$$

Asample represents the absorbance of samples after 30 min
Acontrol represents the absorbance of DPPH; both at 517 nm.

The ability of the extract to inhibit oxidation of the DPPH radical by 50% (IC_{50}) was also determined.

RESULTS AND DISCUSSION

The species of nematodes identified in cattle were *Strongyloides* spp. (23%), *Trichostrongylus* spp. (29%), *Haemonchus* spp. (17%), and *Cooperia* spp. (23). In samples from sheep, the identified larvae corresponded to *Haemonchus* spp. (89%), *Trichostrongylus* spp. (9.5%), and *Cooperia* spp. (2%). It is important to highlight the presence of *Haemonchus* spp. in both cattle and sheep, which demonstrates that *Haemonchus* infection continues to represent an important problem for livestock (KANETO et al., 2016; SOLDÁ et al., 2016, VERMA et al., 2018). Particularly in relation to sheep, a high infection rate (89%) was observed in this study.

HEB at 2.5 mg mL^{-1} inhibited hatchability of nematode larvae, from calf samples, by 88.05% (Table 1). This value of inhibition is considered moderately effective (GMC, 1996).

Table 1 – Arithmetic mean and standard deviation of the percentages of hatching inhibition of gastrointestinal nematode larvae in bovine by the following treatments: hydroalcoholic extract of banana bracts 10% (HEB) at concentrations of 0.625, 1.25, 2.5, and 5 mg mL^{-1}, negative control (NC), and positive control (PC).

Treatments (mg mL^{-1})	Hatching inhibition (%)
NC	36.94 ^{a} ± 3.23
HEB 0.625	63.21 ^{ab} ± 14.55
HEB 1.25	66.13 ^{ab} ± 12.70
HEB 2.5	88.05 ^{bc} ± 19.21
HEB 5	78.48 ^{bc} ± 4.97
PC	100 ^{c} ± 0
Coefficient of variation (%)	31.23

* Means followed by the same letter in the column do not differ significantly from each other by Tukey's test at 5% probability.

Larval migration was inhibited by 55.48% and 67.56% at the HEB concentration of 0.625 mg mL^{-1}, and 5 mg mL^{-1}, respectively, as shown in Table 2.

Table 2 – Arithmetic mean of the percentage of migration inhibition in gastrointestinal nematodes larvae from bovine ± standard deviation in treatments: hydroalcoholic extract of banana bracts 10% (HEB) at concentrations 0.626, 1.25, 2.5, and 5 mg mL^{-1} and negative control (NC).

Treatments (mg mL^{-1})	Larval migration inhibition (%)
NC	14.33 ^{a} ± 8.49
HEB 0.625	55.48 ^{b} ± 6.92
HEB 1.25	54.53 ^{b} ± 5.48
HEB 2.5	64.06 ^{b} ± 7.38
HEB 5	67.56 ^{b} ± 4.56
Coefficient of variation (%)	19.35

* Means followed by the same letter in the column do not differ significantly from each other by the Tukey test at 5% probability.

Although 67.56% larval migration inhibition is considered insufficiently effective (GMC, 1996), this result is important because it revealed the dose-dependent effect of HEB. Therefore, evaluating higher dosages in future studies may present an important perspective for parasite control.

However, for ovines, it was not possible to observe the influence on larval hatchability and migration, at any of the concentrations evaluated in the present study. However, despite these results, continuation of the study for this species is justified, mainly by evaluating higher doses and performing complementary tests, as anthelmintic activity was verified in previous researches (MARIE-MAGDELEINE et al., 2014; NEUWIRT et al., 2015; NOGUEIRA et al., 2012). The bracts of the heart of *Musa* spp. showed inhibition of egg hatchability in ovine nematodes (NOGUEIRA et al., 2012), although, these authors had evaluated an aqueous extract.

Marie-Magdeleine et al. (2014) evaluated the extracts (aqueous, methanolic and dichloromethane) of the stem and leaf of *M. paradisiaca*. Similar to the present study, the extracts showed no significant effects on the hatching test and the larval migration inhibition test but exhibited larval development inhibition with all the extraction methods examined.

Extracts of the leaves and stem of *Musa* spp. showed inhibition of ovine nematodes (NEUWIRT et al., 2015). However, these authors used banana leaves and higher doses than those used in this study, i.e 160–180 mg mL^{-1} to evaluate the hatching test and 800–1000 mg mL^{-1} to evaluate the larval migration inhibition test.

Another important consideration that has often been observed in sheep concerns the high drug resistance to synthetic anthelmintics. This problem is increasing, presents worldwide occurrence, and has been reported in various chemical principles (BABJÁK et al., 2018; CROOK et al., 2016; MUKHERJEE et al., 2016; SANTOS et al., 2017; TSUKAHARA et al., 2017). Thus, this problem may also have influenced the results of the present study for ovine species.

HEB presented higher levels of total polyphenols at the dosage of 10 mg mL^{-1}, which corresponded to 0.38 mg of gallic acid per gram of extract. The contents of condensed tannins were 54.25 mg tannic acid equivalent per gram of extract in the dosage of 2.5 mg mL^{-1}, reaching 372.70 mg tannic acid equivalent per gram of extract in the dosage of 10 mg mL^{-1}. As for flavonoid contents, at the concentration of 10 mg mL^{-1}, 0.42 mg equivalent of rutin per gram of extract was observed.

The HEB (10 mg mL^{-1}) presented antioxidant activity corresponding to 43.03%, with an IC_{50} corresponding to 0.2765 mg mL^{-1}. The results for antioxidant activity, total polyphenol content, flavonoids, and tannins are presented in Table 3.

Table 3 – Mean values for total polyphenols, condensed tannins, flavonoids, and antioxidant activity for different concentrations (mg mL^{-1}) of the hydroalcoholic extract of banana bracts 10% (HEB).

Extract Concentration (mg mL^{-1})	Total polyphenols (mg EAG g^{-1})*	Tannins (mg EAT g^{-1})*	Flavonoids (mg RE g^{-1})*	Antioxidant activity (%)
2.5	0.11	54.25	0.04	10.13
4	0.15	116.04	0.07	16.20
5.5	0.20	195.08	0.13	22.35
7	0.27	272.94	0.22	28.73
8.5	0.30	332.70	0.34	35.05
10	0.38	372.70	0.42	43.03
Coefficient of variation (%)	38.72	50.79	67.65	42.86

* Values expressed per mg of dry extract.

The metabolic activity of organisms constantly produces free radicals. These molecules react with proteins and other oxidizable substances, thus promoting oxidative stress and other cellular damages associated with aging and cancer among other degenerative diseases (SARRAFCHI, 2016). The antioxidant effect of some plants has been attributed to the presence of phenolic compounds. These compounds are secondary products of plant metabolism that have an aromatic ring with one or more hydroxyl groups, which allows them to act as reducing agents against oxidative stress. The antioxidant activity of HEB was 17 times higher than that found in ascorbic acid (IC50 = 4900 µg mL^{-1}) (ILHA et al., 2008), a known and potent antioxidant used as a reference in several studies (BLOIS, 1958; FAGBEMI; OSHODI; IPINMOROTI, 2005). Likewise, when Pereira (2015) compared the antioxidant activity of extracts from the bark (IC 50 393.44 µg mL^{-1}) as well as from banana pulp (690.97 µg mL^{-1}), he observed verify higher the antioxidant activity of the prepared HEB from the bracts, as a lower concentration was required to inhibit the DPPH radical.

Considering that condensed tannins are identified as promising substances for the control of helminths (HOSTE ET al., 2012; OLOUNLADÉ et al., 2017; QUIJADA et al., 2015; QUIJADA et al., 2018; SHALDERS et al., 2014; SPIEGLER; LIEBAU; HENSEL, 2017; YOSHIHARA et al., 2013), the levels of condensed tannins present in 10 mg/mL HEB, may represent another important factor to justify the continuation of the present study.

CONCLUSION

In conclusion, HEB presented antihelminthic potential for bovines, in addition to significant antioxidant activity. These properties may be used in association with other strategies for helminth infection control, and these results are particularly important for agroecological, organic, and biodynamic systems of animal production, considering that the use of synthetic parasiticides is not allowed in these systems.

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