**In vitro** anthelmintic activity of *Musa balbisiana* Colla (square banana) against *Haemonchus contortus* eggs

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**ABSTRACT**

Objective: To evaluate the anthelmintic activity of aqueous and ethanolic extracts of *Musa balbisiana* Colla, against *H. contortus* eggs.

Design/methodology/approach: The anthelmintic activity was evaluated using an egg hatching inhibition test. The aqueous extracts from leaves, peels and roots were obtained by infusion and subsequently lyophilized. Ethanolic extracts were obtained by maceration and later concentrated in a rotary evaporator. Spectroscopic, phytochemical, chemical and total polyphenol content analyzes were performed. The 50% lethal concentration to inhibit *H. contortus* eggs from hatching was calculated following a Probit analysis.

Results: The identified functional groups in the FT-IR analysis were hydroxyl (−OH) and methyl groups (CH3). The proximal analysis revealed significant differences in the dry matter percentage (P<0.05). No significant differences were found in the protein content (P>0.05). The egg hatching inhibition rates at the highest concentration 4.8 mg/mL were 100% for the aqueous and ethanolic extracts from leaves, and 93.7 and 62% for the peel and roots, respectively.

Study limitations/implications: Further studies are required in **in vivo** systems.

Findings/Conclusions: With a LC50 of 225 μg/mL and a 95% confidence interval, with a range between 33 and 418.4 μg/mL, the aqueous extract from the leaves was the most active.

Key words: Anthelmintics, *Haemonchus contortus*, *Musa balbisiana*, gastrointestinal nematodes, bioactive plants.

**INTRODUCTION**

*Haemonchus contortus* represents the most frequent nematode in both temperate and tropical regions, induces large economic losses and has shown resistance to available anthelmintics. This nematode is considered one of the most pathogenic parasites due to its hematophagous habits, high prolificacy and high prevalence (Jasso Díaz et al., 2017). The treatment of hemoncosis depends on repeated applications of commercial synthetic anthelmintics, such as benzimidazoles, imidazothiazoles, macrocyclic lactones (ivermectin, moxidectin, nemaeductin and doramectin) and lately aminoacetonitrile (monepantel) and spiro-indoles (derquantelindoles). (García-
Bustos et al., 2019). Its indiscriminate and inappropriate use has led to emerging resistant populations of gastrointestinal nematodes (GIN) (Muchiut et al., 2018). Another important issue related to treatments with synthetic chemicals is that their residues can be found in animal products such as meat and milk (Kang et al., 2017).

Given the impact of GIN infections in small ruminants and the increasing anthelmintic resistance, there is an urgency to develop strategies to identify new compounds for the sustainable and effective control of GIN. One of those strategies is the treatment of bioactive plants with anthelmintic activity. There are two mechanisms responsible for the anthelmintic effects of bioactive plants. One is the direct interaction of the active compounds of the plant with the parasite. The second is through interaction with the host’s immune system (Zajícková et al., 2020). Musa balbisiana Colla (genome B), belongs to the Musaceae family, which has three genera, Musa, Ensete and Musella (Mathew and Singh, 2016). Musa spp. has been used in traditional medicine in America, Asia, Oceania, India and Africa (Pereira and Maraschin, 2014). All parts of the plant including its roots, pseudostem, stems, leaves, and flowers have long been used to treat various ailments.

Although there is not enough information on M. balbisiana on its usage, biological and pharmacological activity. Nonetheless, there is information on M. acuminata. Among the reported applications for this species is are antioxidant, antidiabetic, hypolipidemic, anticancer, antimicrobial, especially anti-HIV and antiparasitic (Sarah and Singh, 2016).

The secondary compounds identified in the Musa species include alkaloids, dopamine, steroids, phenols, flavonoids, saponins, tannins and terpenes (Vilela et al., 2014; Pereira and Maraschin, 2015). Some of these secondary compounds have been tested and shown anthelmintic effects. Therefore, the objective of this study was to evaluate the anthelmintic activity of aqueous and ethanolic extracts from Musa balbisiana Colla against H. contortus eggs.

**MATERIALS AND METHODS**

**Collection of plant material**

The studied M. balbisiana plant material was collected at the Ranchería Habanero 1st section of Cárdenas municipality, estate of Tabasco. The site is located between the coordinates 17° 97’ 08” north latitude and 93° 32’ 05” west longitude. A total of 3 kg of material were collected, corresponding to 1 kg for each organ of the plant (leaves, root and peel) of young plants.

**Extracts obtention**

Five g of dry and ground material from each of the M. balbisiana organs were placed in a beaker, to which 100 mL of distilled water were added and boiled for 5 min. Subsequently, the resulting solution was filtered and then placed in flasks for deep-freezing at −18 °C and lyophilization (LABCONCO® model 117). The ethanolic extract of leaves was obtained using 50 g of ground plant material and 500 mL of ethyl alcohol (98% purity) and macerated for 24 h. Subsequently, the solution was filtered and concentrated on a BUCHI® brand rotary evaporator; this process was repeated three times to extract the greatest number of compounds. The extract was then stored at 4 °C until its use.

**Phytochemical and chemical analysis**

For the detection of secondary metabolites (alkaloids, sterols, flavonoids, saponins, tannins) from M. balbisiana, the following qualitative tests were used: foam test (saponins), Stiasny reaction (tannins), Liebermann-Burchard test and Salkowski (sterols), Wagner test (alkaloids) and hydrochlorination reaction (flavonoids). These tests are based on the visual observation of color change and/or the formation of precipitates after adding a specific reagent. The chemical composition such as moisture content, crude protein and ashes were determined following the standard methods described by the A.O.A.C. (2000).

**Extractable polyphenols (EP) determination**

The total phenols content in the extract was quantified following the Folin-Ciocalteu spectrophotometric method with some modifications. The results were expressed as gallic acid equivalents (GAE) (mg/GAE g⁻¹ BS) (Makkar et al., 1993).

**Infrared analysis**

To assess the presence of some functional groups present in these extracts, an infrared analysis with a Fourier transformation (FTIR) THERMO SCIENTIFIC® was carried out.

**GIN egg hatch test**

To obtain eggs, a sheep artificially infected with an H. contortus resistant strain was used. The eggs recollection
was carried out according to the method by Coles et al. (1992). Four *M. balbisiana* extracts concentrations (4.8, 2.4, 1.2, 0.6 mg/mL) were used, as well as a positive control (Tiabendazole 10 μg/mL) and negative control (water), distributed in plates of 24 wells, four replicates were made per concentration and the control; each plate was incubated at 27 °C for 48 h. Subsequently, a Lugol drop was added to stop the hatching and then proceed to count the eggs and larvae with a VELAB® brand microscope.

**Statistical analysis**
To know the difference between the inhibition percentages means of the treated groups and the positive control, an analysis of variance was performed and subsequently, a Tukey’s multiple means comparisons test (5%) were performed, with the SPSS version 15.0 statistical software. The LC50 was determined through a Probit analysis using the PoloPlus 2003 statistical package.

**RESULTS AND DISCUSSION**

**Phytochemical and chemical analysis**
The phytochemical screening found a moderate presence of sterols, flavonoids and tannins in leaves. These findings coincide with those from Yingyuen et al. (2020) who report flavonoids presence in ethanolic extract of *M. balbisiana* leaves, even isolated a flavonoid called Rutin. Other compounds found in the peel are sterols and alkaloids and tannins in moderate presence of saponins and sterols were detected in the roots (Table 1). The studies by Marie-Magdeleine et al. (2014) coincides with the compounds found in this study and those observed in the leaves and stems extracts of *M. paradisiaca*. Vilela et al. (2014) evaluated the chemical composition from extracts of 10 banana cultivars, finding that they were mainly composed of free fatty acids (C12 – C30) and sterols, followed by lower quantities of long-chain aliphatic alcohols (C16 – C30), among others.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Alkaloids</th>
<th>Sterols</th>
<th>Flavonoids</th>
<th>Saponins</th>
<th>Tannins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>–</td>
<td>++</td>
<td>++</td>
<td>–</td>
<td>++</td>
</tr>
<tr>
<td>Peel</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>–</td>
<td>+++</td>
</tr>
<tr>
<td>Root</td>
<td>+</td>
<td>++</td>
<td>–</td>
<td>+++++</td>
<td>–</td>
</tr>
</tbody>
</table>
+ = Weak presence; ++ = Moderate; +++ = Abundant; + = Negative.

 REGARD the DM content of *M. balbisiana*, the highest content corresponded to the leaves (31.87%), followed by the peels (12.79%) and the lowest value was for the roots (5.72%). In relation to the protein, the peels showed the highest content (2.35%), the lowest corresponded to the roots with 0.24% (Table 2). Nunes-Oliveira et al. (2014) reported 3.5% protein content in the pseudostem, higher than that found in the peels in this study. The protein content varies depending on the plant organ, genome type, variety, altitude, climate and can increase in the fruits during the ripening process. The extractable polyphenol content was lower than that reported by Rosales et al. (2014) (1.59 - 0.23 mg GAE/g) in plantain.

**Infrared analysis**
The analysis of the leaves interferogram (Figure 1), peels and roots of *M. balbisiana*, shows functional group bands for the identified compounds in the phytochemical analysis (Table 1). The intense band observed at 3235 cm\(^{-1}\) is characteristic of hydroxyl groups (–OH) commonly present in phenolic compounds, such as tannins, flavonoids and alkaloids in the three assessed plant organs (Domínguez, 1979; Castañeda et al., 2017). The wavenumber at 2956 cm\(^{-1}\) corresponding to the leaf (solid line), is assigned to the C-H bond of methyl (CH\(_3\)) and methylene (CH\(_2\)) groups present in alkaloids, identified in the peels and roots. The band at 2923 cm\(^{-1}\) both in leaves, peels and roots is associated with C-H stretching of methylenes (CH\(_2\)) linked to sterols (Castañeda et al., 2017). In the 1609 cm\(^{-1}\) region, the elongated bands are characteristic of C=C functional groups present in alkaloids, identified in the peels and roots. In the 1048 cm\(^{-1}\) C-O region (single elongation links) associated with saponins present in the roots (Anzora and Fuentes, 2008; Castañeda et al., 2017).

**Egg hatching test**
The *in vitro* evaluation of *M. balbisiana* showed that the aqueous and ethanolic extracts of the leaves have strong *in vitro* anthelmintic activity on *H. contortus* eggs hatching. All organs and the different

| **Table 1** Chemical compounds found the aqueous extracts of *M. balbisiana*. |
|-----------------|---------|--------|----------|----------|----------|
| Extract         | Alkaloids | Sterols | Flavonoids | Saponins | Tannins  |
| Leaves          | –        | ++      | ++        | –        | ++       |
| Peel            | ++       | +++     | +         | –        | +++      |
| Root            | +        | ++      | –         | +++++    | –        |

| **Table 2** Proximal chemical composition and extractable polyphenol content of *M. balbisiana*. |
|-----------------|---------|--------|----------|----------|
| *M. balbisiana* | DM (%)  | A (%)  | CP (%)   | EP (mg GAE/g\(^{-1}\)) |
| Leaves          | 31.87c  | 10.86b | .90a     | .0616b    |
| Peel            | 12.79b  | 8.81a  | 2.35a    | .0498a    |
| Root            | 5.72a   | 12.45c | 0.24a    | .0685b    |

DM: dry matter; C: ashes; CP: crude protein; EP: extractable polyphenols; GAE: gallic acid equivalents. Different letters in the same column significantly differ P<0.05.
tested concentrations showed some effect, from 32.6% for the ethanolic extract of the leaves at a 1.2 mg/mL concentration to 100% efficacy of the same extract at a 4.8 mg/mL concentration (Figure 2). These can be seen that at the highest evaluated concentration, where 100% efficiency was obtained, both from the aqueous extract and the ethanolic extract of leaves. The aqueous extracts of leaves, peels and roots showed a dose-dependent effect.

Studies in different *Musa* species report different egg hatching inhibition percentages. Aline et al. (2019) evaluated a hydroalcoholic extract of *M. paradisiaca* flowers on gastrointestinal parasites, reporting a 78.48% inhibition at a 5 mg/mL concentration. In another study by Marie-Magdeleine et al. (2014) evaluated aqueous, methanolic and dichloromethane extracts of leaves and stems of *M. paradisiaca*, against *H. contortus* eggs. They found a mean inhibition of 48.5%. Neuwirt et al. (2015) reported a 100% inhibition in GIN eggs, with alcoholic extracts of *Musa* spp., using a 180 mg/mL concentration, a considerably higher quantity than that used in this study.

The observed anthelmintic effect of the aqueous extract of *M. balbisiana* leaves reveals that the responsible active compounds of the anthelmintic activity are relatively polar. The hatching inhibition by polar extracts of tropical plants has been associated with two action mechanisms (Vargas-Magaña et al., 2014; Chan-Pérez et al., 2016): a) true ovicidal activity that prevents the eggs from developing beyond the morula stage, similar to that observed when benzimidazole is used, which causes shrinkage and morula damage compared to negative controls; b) unhatched larvae, a fully developed larva cannot hatch from the egg. Both modes of action ultimately result in a reduction in the hatched larvae number from the eggs. Both must be evaluated to understand the anthelmintic mechanism associated with the secondary compounds of these plant extracts.

The mean lethal concentration of the extracts is shown in Table 3. The aqueous extract had the lowest CL50 (225 μg/mL), followed by the ethanolic extract with twice the concentration (481.7 μg/mL).

![Figure 1](image1.jpg)

**Figure 1.** Interferogram of *M. balbisiana* leaves, peel and root extracts.

![Figure 2](image2.jpg)

**Figure 2.** Mean *H. contortus* egg hatching inhibition by *M. balbisiana* extracts.

**Table 3.** Lethal concentration required to inhibit to 50% (LC50) of the hatching of eggs of *H. contortus* and lowers and higher confidence limits than 90% and 95% of the extracts of leaves, skin and roots of *M. balbisiana*.

<table>
<thead>
<tr>
<th>Extract</th>
<th>LC50 μg mL⁻¹</th>
<th>Confidence limits 90% μg mL⁻¹</th>
<th>Confidence limits 95% μg mL⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lowers</td>
<td>Higher</td>
</tr>
<tr>
<td>Ethanol from leaves</td>
<td>481.7</td>
<td>320.6</td>
<td>603.8</td>
</tr>
<tr>
<td>Aqueous from leaves</td>
<td>225.0</td>
<td>60.8</td>
<td>385.7</td>
</tr>
<tr>
<td>Aqueous from peel</td>
<td>1500.6</td>
<td>1216</td>
<td>1794</td>
</tr>
<tr>
<td>Aqueous from root</td>
<td>2900.0</td>
<td>2358</td>
<td>3828</td>
</tr>
</tbody>
</table>
CONCLUSIONS
The aqueous and ethanolic extracts of M. balbisiana leaves showed 100% efficacy on H. contortus eggs. The compounds that could be involved in its anthelmintic activity are sterols, flavonoids and tannins.

REFERENCES


