https://doi.org/10.32854/agrop.v14i4.1877

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

Rivera-Torrez, Diana L.[1]; Hernández-Villegas, Manuel M.[1*]; Bolio-López, Gloria I.[1]; Almenares-López, Damianys[1]; Córdova-Sánchez, Samuel[1]; De la Cruz-Burelo, Patricia[1]

[1]Universidad Popular de la Chontalpa. División Académica de Ingenierías y Ciencias Agropecuarias. Carretera Cárdenas-Huimanguillo km 2.0. Cárdenas, Tabasco, México. C. P. 86500.

***Corresponding author**: manuel.hdez@upch.mx

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 (CH_3). 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.

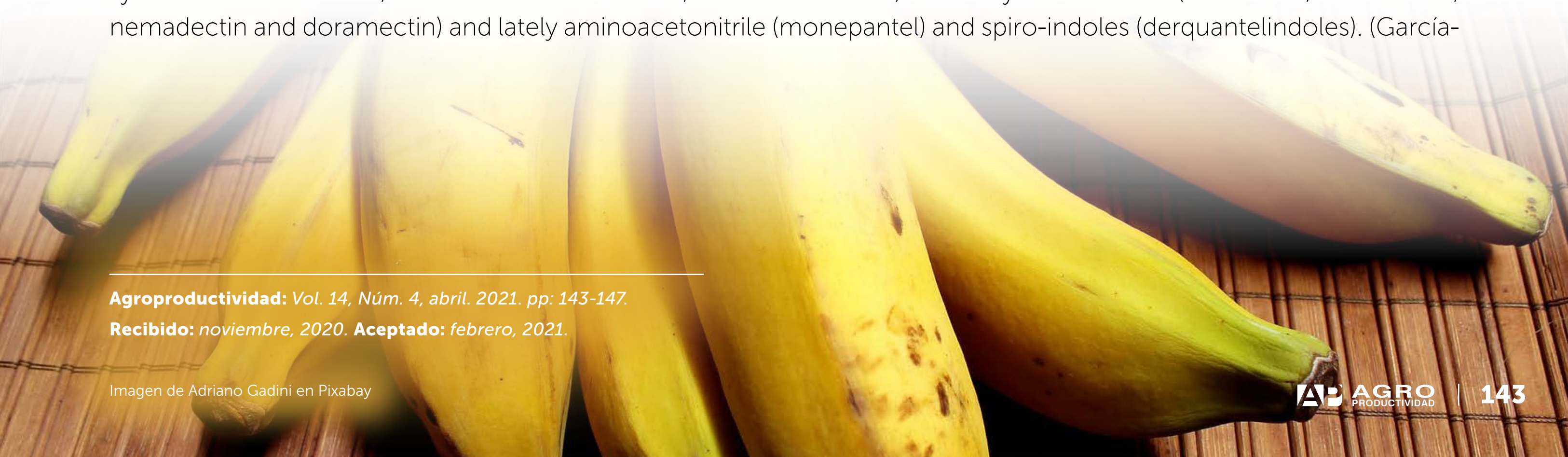
Study limitations/implications: Further studies are required in *in vivo* systems.

Findings/Conclusions: With a LC_{50} 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, nemadectin and doramectin) and lately aminoacetonitrile (monepantel) and spiro-indoles (derquantelindoles). (García-



Agroproductividad: *Vol. 14, Núm. 4, abril. 2021. pp: 143-147.*
Recibido: *noviembre, 2020.* **Aceptado:** *febrero, 2021.*

Imagen de Adriano Gadini en Pixabay

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.

Table 1. Chemical compounds found the aqueous extracts of *M. balbisiana*.

Extract	Alkaloids	Sterols	Flavonoids	Saponins	Tannins
Leaves	–	++	++	–	++
Peel	++	+++	++	–	++
Root	+	++	–	+++	–

+ = Weak presence; ++ = Moderate; +++ Abundant; + = Negative.

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 LC_{50} 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.

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.

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.

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 groups (CH_3) associated with flavonoids; 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 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.

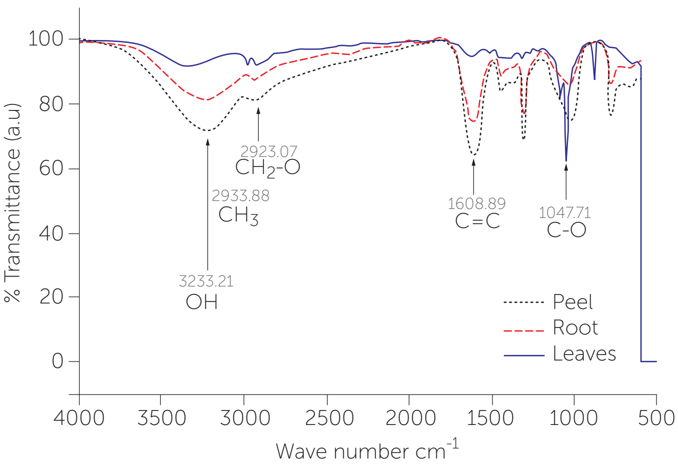


Figure 1. Interferogram of *M. balbisiana* leaves, peel and root extracts.

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.

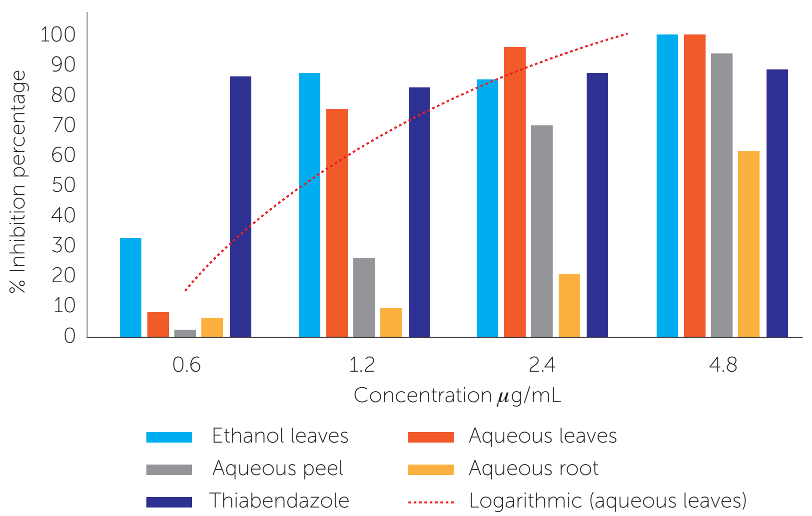


Figure 2. Mean *H. contortus* egg hatching inhibition by *M. balbisiana* extracts.

The mean lethal concentration of the extracts is shown in Table 3. The aqueous extract had the lowest CL_{50} (225 μg/mL), followed by the ethanolic extract with twice the concentration (481.7 μg/mL).

Table 3. Lethal concentration required to inhibit to 50% (LC_{50}) 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*.

Extract	LC_{50} μg mL^{-1}	Confidence limits 90% μg mL^{-1}		Confidence limits 95% μg mL^{-1}	
		Lowers	Higher	Lowers	Higher
Ethanol from leaves	481.7	320.6	603.8	276.7	627.9
Aqueous from leaves	225.0	60.8	385.7	33.0	418.4
Aqueous from peel	1500.6	1216	1794	1146	1869
Aqueous from root	2900.0	2358	3828	2256	4169

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

Aline-Kakimori, M.T., Rostirolla-Debiage, R., Ferreira-Gonçalves, F., Gonçalves-da-Silva, R.M., Yoshihara, E., Toledo-de-Mello, E.C. (2019). Anthelmintic and antioxidant potential of banana bracts (*Musa paradisiaca*) extract in ruminants. Veterinaria Brasilica 13: 18-23.

Anzora Vásquez, A. D., Fuentes Cañas, C. E. (2008): "Obtención de un colorante a partir de *Musa paradisiaca* (Plátano verde) con aplicación en la industria textil". Tesis Licenciatura, Universidad de El Salvador.

Association of Official Analytical Chemistry (AOAC). (2000). Official Methods of Analysis of the Association of Official Analytical Chemistry. 17a ed. Maryland, USA. Association Official Analytical Chemists.

Castañeda-Castillo J.G., Hernández-Almanza A.Y., Sáenz-Galindo A., Ascacio-Valdés J.A. (2017). Extracción asistida por ultrasonido de compuestos fenólicos de la cáscara de plátano maduro (*Musa cavendish*) y evaluación de su actividad antioxidante. Memorias de los congresos de la sociedad química de México 52° congreso mexicano de química y 36° congreso nacional de educación Química de Productos Naturales (QPNT). pp. 33-35.

Chan-Pérez, J.I., Torres-Acosta, J.F.J., Sandoval-Castro, C.A., Hoste, H., Castañeda-Ramírez, G.S., Vilarem, G., Mathieu, C. (2016). *In vitro* susceptibility of ten *Haemonchus contortus* isolates from different geographical origins towards acetone: - water extracts of two tannin rich plants. Veterinary Parasitology 217: 53-60.

Coles, G.C., Bauer, C., Borgsteede, F.H., Geerts, S., Klei, T.R., Taylor, M.A., Waller, P.J., (1992). (W.A.A.V.P.) Methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Veterinary Parasitology 44: 35-44.

Dominguez-Xorge-Alejandro.1979. Métodos de Investigación fitoquímica. Editorial: Limusa, S.A. México, D.F.

García-Bustos, J.F., Sleebs, E.B., Gasser B.R. (2019). An appraisal of natural products active against parasitic nematodes of animals. Parasites and Vectors 12; 306.

Jasso-Díaz, G., Torres-Hernández, G., Zamilpa, A., Becerril-Pérez, C.M., Ramírez Bribiesca, J.E., Hernández-Mendoza, O., Sánchez-Arroyo, H., González-Cortazar, M., Mendoza-de Gives, P. (2017). *In vitro* assessment of *Argemone mexicana*, *Taraxacum officinale*, Ruta chalepensis and *Tagetes filifolia* against *Haemonchus contortus* nematode eggs and infective (L3) larvae. Microbial Pathogenesis 109: 62-68.

Kang, J., Park, S.J., Park, H.C., Hossain, M.A., Kim, M.A., Son, S.W., Lim, C.M., Kim, T.W., Cho, B.H. (2017). Multiresidue screening of veterinary drugs in meat, milk, egg, and fish using liquid chromatography coupled with ion trap time-of-flight mass spectrometry. Applied Biochemestry and Biotechnology 182: 635-652.

Marie-Magdeleine, C., Udino, L., Philibert, L., Bocage, B., Archimede, H. (2014). *In vitro* effects of *Musa* × *paradisiaca* extracts on four developmental stages of *Haemonchus contortus*. Research in Veterinary Science 96: 127-132.

Makkar, H.P.S., Bluemmel M., Borowy N. K., Becker K. (1993). Gravimetric determination of tannins and their correlation with Chemical and protein precipitation methods. Journal of Science Food Agriculture 61: 161-165.

Muchiut, S.M., Fernández, A.S., Steffan, P.E., Riva, E., Fiel, C.A. (2018). Anthelmintic resistance: management of parasite refugia for *Haemonchus contortus* through the replacement of resistant with susceptible populations. Veterinary Parasitology 254: 43-48.

Nunes-Oliveira, L., Cabral-Filho, S.L.S., Castro, G.L., Robson, D.E., Luiz, A.A., (2014). Chemical composition, degradability and methane emission potential of banana crop residues for ruminants. Tropical and Subtropical Agroecosystems 17: 197-206.

Neuwirt, N., Gregory, L., Yoshihara, E., Lima, G.S. (2015). Effect of *Musa* spp. Extract on eggs and larvae of gastrointestinal nematodes from infected sheep. Semina: Ciências Agrárias 36: 3751-3757.

Pereira, A., Maraschin, M. (2015). Banana (*Musa* spp) from peel to pulp: ethnopharmacology, source of bioactive compounds and its relevance for human health. Journal of Ethnopharmacology 160: 149-163.

Rosales, Reynoso, L.O., Agama, Acevedo, E., Aguirre, Cruz, A., Bello, Pérez, L., Dufour, D., Gibert, O., (2014). Evaluación fisicoquímica de variedades de plátanos (*Musa* spp.) Cocción y postre. Agrociencia 48: 387-401.

Sarah, M.N., Singh, N.P. (2017). Traditional uses, Phytochemistry and Pharmacology of wild Banana (*Musa acuminata* Colla): A review. Journal of Ethnopharmacology 196: 124-140.

Vargas-Magaña, J.J., Torres-Acosta, J.F.J., Aguilar-Caballero, A.J., Sandoval-Castro, C.A., Hoste, H., Chan-Pérez, J.I. (2014). Anthelmintic activity of acetone-water extracts against *Haemonchus contortus* eggs: interactions between tannins and other plant secondary compounds. Veterinary Parasitology 206: 322-327.

Vilela, C., Santos, S.A.O., Villaverde, J.J., Oliveira, L., Nunes, A., Cordeiro, N., Freire, C.S.R., Sivestre, A.J.D. (2014). Lipophilic phytochemicals from banana fruits of several *Musa* species. Food Chemistry 162: 247-252.

Yingyuen, P., Sukrong, S., Phisalaphong, M. (2020). Isolation, separation and purification of rutin from Banana leaves (*Musa balbisiana*). Industrial Crops & Products 149; 112307.

Zajicková, M., Thuy, N.L., Skálová, L., Raisová, S.L., Matousková, P. (2020). Anthelmintics in the future: current trends in the discovery and development of new drugs against gastrointestinal nematodes. Drug Discovery Today 25: 430-437.