Journal of Animal Production, *2*8 (2), pages 51-54, 2016

In Vitro Ovicidal Effect of Crude Hydro-Alcoholic Extract on *Pouteria sapota* (Mamey sapote) Seeds Against *Haemonchus contortus* Eggs. *First Report*

*Anay Delgado**, Olimpia Núñez**, Lester Aguilera-Valle**, Danays Palacio*, Josmel Salas-Romero*, Gisell Bebert**, Misleidi González*, Noemí Fernández**

*** Veterinary Department, University of Camagüey, Cuba**

**** Morphophysiology Department, University of Camagüey, Cuba**

lester.aguilera@reduc.edu.cu

ABSTRACT

The crude alcoholic extract of *Pouteria sapota* seeds is traditionally used as a pediculicide in Cuba. This study was made in order to evaluate the *in vitro* anthelmintic effect of crude hydro-alcoholic extract of *Pouteria sapota* seeds on *Haemonchus contortus* eggs. A hatching test was performed, in which several concentrations of the extract were tested for concentrations of 0.08-5.32 mg/mL. In comparison with the negative control group, a significant ovicidal effect was observed ($P < 0.05$), with 100% hatching inhibition to the greatest concentration used (5.32 mg/mL). These results suggest that *P. sapota* has an anthelmintic effect against *H. contortus*.

Key words: *anthelmintic, egg hatching test, Pouteria sapota, Haemonchus contortus, plant therapy*

INTRODUCTION

Parasitism caused by gastrointestinal nematodes is still one of the main threats to breeding of small ruminants in tropical countries. *Haemonchus contortus* is one of the most significant species, because of high prevalence and pathogenicity (Ferreira, Castro, Chagas, França, and Beleboni, 2013). The control of these parasites has relied on synthetic anthelmintics. However, the emergence of populations resistant to most medications has stimulated the search for alternative control strategies, like the use of bioactive plants abundant in secondary metabolites, with antiparasitic activity (Molento *et al*., 2011).

The embryo of mamey sapote seed (*Pouteria sapota*) is frequently used by Cubans in an alcoholic solution with pediculicidal properties (Roig, 1988); its empirical use without formulation has produced positive results. Accordingly, the goal of this work is to evaluate the ovicidal activity of a hydro-alcoholic extract from *Pouteria sapota* seeds, against *Haemonchus contortus* eggs.

MATERIALS AND METHODS

Extract preparation

Twenty grams of previously ground seed embryos were dried in the sun for 8h, and were mixed in Erlenmeyer with 100 mL of water and 100 mL of ethanol. The solution was shaken overnight for dilution of the active ingredients. Later, the solution was filtered (Whatman 0.45 μm). The filtrate was used in the experiments. The concentration was calculated after drying 100 μL in the oven.

Egg hatching test

In vitro egg hatching test was performed according to the methodology described by Coles *et al.* (1992). The eggs were collected from sheep infected with *Haemonchus contortus* only. The egg suspension was poured on a 24-well plate (0.5 mL per well), and mixed with the same volume of the plant extract. Each concentration evaluated, and the negative (distilled water), and positive (Albendazole) controls had five replicas.

After 48 h of incubation at 27 ºC, hatching was stopped with the addition of Lugol at 3%. The hatching inhibition percent was determined by the following equation: (h/h+L1) x 100, where h is the number of eggs, and L1, the number of larvae.

Statistical analysis

The percent mean of the egg hatching inhibition of the treated groups and the control, was analyzed through single-factor ANOVA, and compared using the Tukey´s multiple comparison test (5%). GraphPadPrism 5.00 was used in all the analyses performed.

RESULTS AND DISCUSSION

The results of the hatching test indicated significant ovicidal activity of the crude hydro-alcoholic extract from *P. sapota* seeds (Table 1). The first five dilutions (5.32; 3.04; 1.52; 0.38 and 0.23 mg/mL) inhibited *Haemonchus contortus* egg hatching significantly. The positive control

(albendazole 0.032 mg/mL) allowed 100% hatching, whereas it was just 9.42 % in the control group.

Non-linear regression analysis indicated the existence of dosis-dependent response (Fig. 2), with R2 = 0.91; IC50 = 0.5 mg/mL, 95 % confidence intervals of 0.26-1.03.

This is the first report of *in vitro* ovicidal effect of *Pouteria sapota* seed extract on *Haemonchus contortus*. It was also evident that at low concentrations (0.23 mg/mL) there was a significant ovicidal effect when compared to other studies of medicinal plants, in which, for example, 7.1 mg/mL of aqueous extract of *Annona senegalensis*, only inhibited 11.5% of eggs (Alawa *et al.*, 2003); and the methanolic extract of *Spigelia anthelmia* induced 97.4% inhibition, at concentrations of 50 mg/mL (Assis *et al*., 2003); whereas the extract of *P. sapota* produced inhibition of 99%, at concentrations of 3.04 mg/mL.

The egg hatching test is commonly used in *in vitro* assays (Hernández-Villegas *et al*., 2011; Botura *et al*., 2013; Ferreira *et al*., 2013), to evaluate the potential anthelmintic activity of natural products. The results of egg hatching inhibition are positively associated to *in vivo* anthelmintic effects, though some substances and compounds that are effective *in vitro* not necessarily have the same *in vivo* effects. These divergences in the results may be attributed to key factors related to bioavailability and the pharmacology of such compounds and substances in the body, and the destruction of active compounds by microorganisms from the rumen or the intestine (Peneluc *et al*., 2009).

The literature says that the plant has chemicals like coumarins, flavonoids and cyanogenic glycosides (Silva, Simeoni and Silveria, 2009; Carriço et al., 2014), which are responsible for the antiparasitic effects of other plants (Hernández-Villegas *et al*., 2011 and Botura *et al*., 2013).

Nematode egg hatching is initiated by environmental stimuli that cause the larvae to release certain enzymes, like proteases, lipases, and chitinases, whose role is to degrade the egg membrane (Mansfield *et al*., 1992). Flavonoids may act by inhibiting the activity of those enzymes. The antiparasitic effect of flavonoids is attributed to changes of enzymatic activity and/or parasite metabolic effects (Kerboeuf *et al*., 2008).

Coumarins are known for their broad range of biological activities. Wang *et al*., (2008) showed evidence of nematicidal activity of coumarins against two kinds of plant parasitic nematodes (*Bursaphelenchus xylophilus* and *Panagrellus redivirus*).

Powers *et al*. (1982) designed guidelines for in vitro evaluation of the anthelmintic effects of medications. According to the authors, effective anthelmintic agents must inhibit more than 90% of hatching; when inhibition is between 80 and 90%, they are classified as moderately effective. Consequently, the results accomplished with *P. sapota* against *H. sapota contortus* places it within the classification of effective.

Finally, the issue of traditional nematode infection treatments, using conventional anthelmintic medication goes beyond anthelmintic resistance and cost increase, because there is no clear evidence saying that synthetic anthelmintics leave no traces in the meat, thus posing a public health threat (Rodríguez, Athayde, Rodríguez, Silva and Faria, 2007).

So, identifying new plant extracts, like *P. sapota*, may help develop other safe and accessible therapeutical products from plants, with lower resistance risks than the therapeutic arsenal used today.

CONCLUSIONS

The extract had *in vitro* ovicidal activity, which is an early step to further *in vivo* studies.

REFERENCES

ALAWA, C. B.; ADAMU, A. M.; GEFU, J. O.; AJANSUI, O. J.; ABDU, P. A.; CHIEZEY, N. P. y BOWMAN, D. D. (2003). *In vitro* Screening of Two Nigerian Medicinal Plants (*Vernonia amiygdalina* and *Annona senegalensis*) for Anthelmintic Activity. *Veterinary Parasitology, 113* (1), 73-81.

ASSIS, L. M.; BEVILAQUA, M. L.; MORAIS, S. M.; VIEIRA, L. S.; COSTA, T. C.; SOUZA, A. L. (2003). Ovicidal and Larvicidal Activity *In Vitro* of *Spigelia Anthelmia* Linn. Extracts on *Haemonchus Contortus.Veterinary Parasitology, 117* (1), 43-49.

BOTURA, M.; DOS SANTOS, J. D.; DA SILVA, G. D.; DE LIMA, H. G.; DE OLIVEIRA, J. V. y DE ALMEIDA, M. A. (2013). *In Vitro* Ovicidal and Larvicidal Activity of Agave Sisalana Perr. (Sisal) on Gastrointestinal Nematodes of Goats. *Veterinary Parasitology, 192* (1), 211-217.

CARRIÇO, C.; PINTO, Z.; DUTOK, C.; CAETANO, R.; PESSANHA, R.; CHIL-NUÑEZ, I. y QUEIROZ, M. (2014). Biological Activity of Pouteria Sapota Leaf Extract on Post-Embryonic Development of Blowfly *Chrysomya Putoria* (Wiedemann, 1818) (Calliphoridae). *Brazilian Journal of Pharmacognosy, 24* (3), 304-308.

COLES, G. C.; BAUER, C.; BORGSTEEDE, H. M.; GREETS, S.; KLEI, T. R. y TAYLOR, M. A. (1992). World Association for the Advancement of Veterinary Parasitology (WAAVP) Methods for the Detection of Anthelmintic Resistance in Nematodes of Veterinary Importance. *Veterinary Parasitology*, *44* (1), 35-44.

FERREIRA, L. E.; CASTRO, M. N.; CHAGAS, C. S.; FRANÇA, S. C y BELEBONI, R. O. (2013). *In Vitro* Anthelmintic Activity of Aqueous Leaf Extract of *Annona Muricata* L. (*Annonaceae*) Against *Haemonchus Contortus* from Sheep. *Experimental Parasitology*, *134* (3), 327-332.

HERNÁNDEZ-VILLEGAS, M. M.; BORGES-ARGÁEZ, R.; RODRÍGUEZ-VIVAS, R. I.; TORRES-ACOSTA, F. J.; MÉNDEZ-GONZÁLEZ, M. y CÁCERES-FARFAN, M. (2011). Ovicidal and Larvicidal Activity of the Crude Extracts from *Phytolaccai Cosandra* Against *Haemonchus Contortus*. *Veterinary Parasitology*, *179* (1), 100-106.

KERBOEUF, D.; RIOU, M. y GUÉGNARD, F. (2008). Flavonoids and Related Compounds in Parasitic Disease Control. *Mini Rev. Med. Chem.*, *8* (2), 116-128.

MANSFIELD, L. S.; GAMBLE, H. R. y FETTERER, R. H. (1992). Characterization of the Eggshell of *Haemonchus Contortus*–l. Structural Components. *Comp. Biochem.*, *103* (3), 681-686.

MOLENTO, M.; FORTES, F.; PONDELEK, D.; BORGES, F.; CHAGAS, A. y TORRES-ACOSTA, J. F. (2011). Challenges of nematode control in ruminants: Focus on Latin America. *Veterinary Parasitology*, *180* (1), 126-132.

PENELUC, T.; DOMINGUES, L. F.; ALMEIDA, G. N.; Ayres, M. C.; Moreira, E. L. y Cruz, A. C (2009). Anthelmintic Activity of Aqueous Extract of Zanthoxylum Rhoifolium Lam. Leaves (Rutaceae). *Revista Brasileira Parasitologia Veterinaria*, *18* (1), 43-48.

POWERS, K. G.; WOOD, I. B.; ECKERT, J.; GIBSON, T. y SMITH, H. J. (1982). World Associations of the Advancement of Veterinary Parasitology (W.A.A.V.P.) Guidelines for Evaluating the Efficacy of Anthelmintics in Ruminants (Bovine and Ovine). *Veterinary Parasitology*, *10* (4), 265-284.

RODRÍGUEZ, A. B.; ATHAYDE, A. C.; RODRÍGUEZ, O. G.; SILVA, W. W. y FARIA, E. B. (2007). Evaluation of the Efficacy of Anthelmintics to Control Gastrointestinal Nematodes in Goats Raised in the State of Paraiba. *Pesquisa Veterinaria Brasileira*, *27* (4), 162-166.

ROIG, J. T. (1988). *Plantas Medicinales, aromáticas o venenosas de Cuba*. La Habana, Cuba: Editorial Científico Técnica.

SILVA, C.; SIMEONI, L. y SILVEIRA, D. (2009). *Genus Pouteria*: Chemistry and Biological Activity. *Brazilian Journal of Pharmacognosy*, *19* (2A), 501-509.

WANG, X. B.; LI, G. H.; LI, L.; ZHENG, L. J.; HUANG, R. y ZHANG, K. Q. (2008). Nematicidal Coumarins from *Heracleum candicans* Wall. *Nat. Prod. Res.*, *22* (8), 66-671.

Received: 9-22-2015
Accepted: 10-1-2015

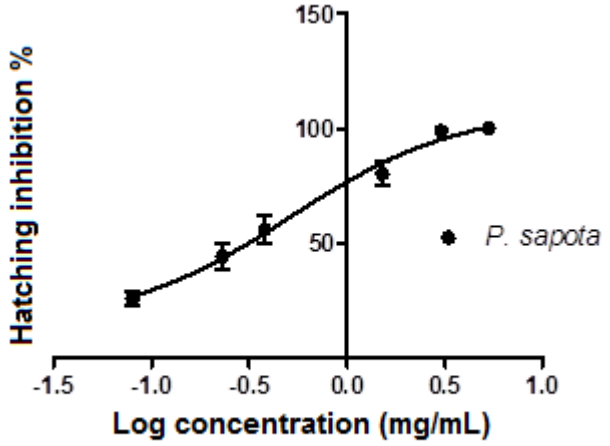

Fig. 1 Curve of dosis-dependent response of the inhibition test to *Haemonchus contortus* egg hatching, using the crude hydro-alcoholic extract from P. *sapota*

Table 1. Ovicidal effect of P. *sapota against H. contortus.*

Treatment	Concentration (mg/mL)	Egg hatching inhibition percent (mean ± D.T)
Control (-)	-	9.4240 ± 3.27
Control (+)	0.032	100 a
P. sapota sapota	5.32	100 a
P. sapota sapota	3.04	99.02 ± 0.69*****
P. sapota sapota	1.52	80.11 ± 12.04 *****
P. sapota sapota	0.38	55.84 ± 13.64 *****
P. sapota sapota	0.23	44.29 ± 12.59*****
P. sapota sapota	0.08	25.81 ± 7.36

*P < 0.05