Scientific Paper

In vitro ovicidal activity of the glycerol-rich fraction of *Jatropha curcas* L. in gastrointestinal nematodes of sheep

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Abstract

Objective: To evaluate the effect exerted by the glycerol-rich fraction of *Jatropha curcas* L. oil, on the hatching of eggs from nematodes that parasitize sheep.

Materials and Methods: A trial was conducted based on a complete randomized design. The inhibition of the hatching of eggs from sheep gastrointestinal nematodes in different concentrations of the glycerol-rich fraction (seriated dilutions from 100 to 156 mg/mL) was evaluated *in vitro*. Glycerin (10 v/v) was used as negative control, with analytical quality and distilled water. In addition, the components of the glycerol-rich fraction were identified through gas chromatography and the presence of phorbol esters was studied by mass spectrometry, coupled to a time-of-flight detector.

Results: The glycerol-rich fraction recorded a strong ovicidal activity with dose-dependent effect, with mean lethal concentration of $3{,}60 \times 10^{-4}$ mg/mL. No phorbol esters were found in the glycerol-rich fraction; while glycerin constituted the main compound (74,80 %). It was followed by palmitic, stearic, oleic and linoleic acids.

Conclusions: The glycerol-rich fraction inhibits the hatching of eggs from parasite gastrointestinal nematodes in sheep, with dose-dependent effect. No phorbol esters were detected and the main component was glycerin.

Keywords: anthelmintics, Nematoda, sheep

Introduction

Gastrointestinal parasitism constitutes an important threat for ruminant production in the tropic. For decades, parasite control in small ruminants has been developed based on arbitrary conventional antiparasitic treatments which, very often, are applied with monthly frequency, leading to the emergence of parasite genotypes resistant to most chemical products (Arece-García *et al.,* 2017).

In this context, the search for control alternatives constitutes a challenge for researchers. In recent years, the application of green medicine has gained space, which has allowed to value plant resources (Romero-Benavides *et al*., 2017; French, 2018). Anthelmintic activity has been reported in different plants and their parts, with variability in the results, depending on the plant family and the chemical composition of bioactive compounds (Borges and Borges, 2016). The United States Department of Agriculture and the Germplasm Resource Information Network have identified 1 030 plants with chemical compounds that have anthelmintic activity. Due to its high oil content, *Jatropha curcas* L. is considered an oil plant. The harvested fruits can produce up to 25 % in oil weight, after being shelled and pressed (Alherbawi *et al*., 2021). The main composition of this oil is 64 % triacylglycerols, 12 % hydrocarbons and 9 % free fatty acids, including oleic, linoleic, palmitic and stearic acids (Neupane *et al*., 2021). In turn, it shows a similar composition to that of many edible oils. However, it has not been commercialized for human consumption due to its toxic effects, ascribed to phorbol esters, curcin, among others (Phulia *et al*., 2018). Hence the *J. curcas* oil is mainly used for biodiesel production (Yaqoob *et al*., 2021).

In spite of having these toxic substances, antimicrobial, as well as molluscicidal and anticarcinogenic activity has been detected, among other biological applications (Bosou *et al*., 2020; Rahu *et al*., 2021). In the last decade, a study was conducted that proved the anthelmintic effect of the ethanol

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extract of *J. curcas* seeds on the inhibition of egg hatching and the migration of *Haemonchus contortus* larvae *in vitro* (Monteiro *et al.*, 2011).

From the biodiesel extraction process from the oil of *J. curcas* seeds the glycerol-rich fraction (GRF) is obtained, as result of its transesterification (Salaheldeen *et al.*, 2021). This product can be included in ruminant nutrition due to its glucogenic properties (Khattab, 2015) and energy values higher than corn have been obtained (Mach *et al.*, 2009). The objective of this study was to evaluate the effect exerted by the glycerol-rich fraction of *J. curcas* oil, on the hatching of eggs from nematodes that parasitize sheep.

Materials and Methods

Obtainment of the oil and glycerol-rich fraction from J. curcas. Ripe fruits of *J. curcas*, native from Cuba, harvested in the Guantánamo province, were used. They were dried under sunlight and were shelled for obtaining the seeds. These were pressed using a expeller machine, with power of 7,5 kW, rate of 1 400 r.p.m. and capacity of 200 kg seeds/hour. The obtained raw oil was filtered through a press filter, in order to guarantee a 25-micron filtrate. Afterwards, the oil was subject to a heating process at 105 ºC for the extraction of all the soluble and volatile impurities, including water (Rizo, 2019). The GRF was obtained from the biodiesel process by transesterification of oil with methanol and sodium hydroxide as catalyst (Che Hamzah *et al.*, 2020).

The GRF density was determined and work aqueous solutions were prepared in concentrations of 100; 50; 25; 12,5; 6,25; 3,125 and 1,56 mg GRF/mL of solvent.

Egg hatching inhibition test. The eggs were collected from strongyles through the technique proposed by Hubert and Kerboeuf (1992) and were deposited on flat-bottom 24-well cell culture plates (Corning™) to face them with each of the above-mentioned GRF concentrations, based on a complete randomized design with six replicas per treatment. A PA glycerin solution (10 %, v/v) (AppliChen PanReac) was used as control.

They were incubated during 48 h and after that time hatching was stopped with 10 µL of Lugol solution. The total larvae and eggs were counted and the hatching percentage was determined (Marie-Magdeleine *et al.*, 2010; Busari *et al.*, 2021).

Analysis of the chemical composition of the GRF. For determining the profile of chemical compounds, the gas chromatography technique was used (Thermo Scientific gas chromatograph), coupled to a mass spectrometer (GC-MS). A temperature of the injector and FID detector of 250 ºC was used, with flow of 1 mL/min. the acids were identified by comparison of their retention times and their mas specters, with a library of mass specter data of known compounds.

Quantification of the phorbol esters in GRF. The GRF of *J. curcas* was mixed with methanol (1:1, v:v) and was analyzed through reversed-phase chromatography, with the utilization of C-18 columns, in HPLC systems coupled with a mass spectrometer with time-of-flight analyzer (MS-TOF-Agilent Technologies 6230 TOF LC/MC, USA). The sample was analyzed in triple and methanol was used as control to determine the clean state of the column. The phorbol-12-myristate-13-acetate was used as standard of phorbol esters (PMA, SIGMA-Neu *et al.*, 2018).

Statistical analysis. The hatching percentages, according to the GRF concentrations, were compared through a simple variance analysis. Before that, the values were transformed through arcsin of the value root, and the fulfillment of the variance normality and homogeneity assumptions was verified. A significance level of 0,05 was used and the means were compared through Tukey's test. The statistical package SAS, version 9.0 was applied.

A Probit regression analysis was carried out through the statistical package SAS, version 9.0, in order to determine the mean lethal concentration (CL_{50}) for egg hatching. The values of the positive control group were applied for correcting the results, following the model proposed by González-Garduño *et al.* (2014), where:

$$Pr\,(^{response}) = C + (1\text{-}C)\ F\ (X'\beta) = C + (1\text{-}C)\ \Phi\ (b0 + b1x\ \log10\ (dose))$$

Where:

β - vector of estimated parameters
F – function of cumulative distribution (normal)
X - vector of explicative variables
Pr – probability of a response
C – natural response rate (proportion of individuals that respond to dose zero)

Results and Discussion

The results of egg hatching, according to the GRF of *J. curcas*, are shown in table 1. The GRF considerably reduced ($p < 0,05$) egg hatching, with a dose-dependent effect and constitutes the first known report of ovicidal activity of the GRF of *J. curcas* in parasite nematodes.

Table 1. Average egg hatching percentage and mean lethal concentration (LC_{50}) in different concentrations of the glycerol-rich fraction of *J. curcas*.

Indicator	Concentration, mg/mL	Hatching, %	SE ±	LC_{50}
Glycerol-rich fraction	0	97,9[1a]	0,05	3,60 ×10^{-4} mg/mL
	1,56	7,25[e]		
	3,12	6,35[e]		
	6,25	5,29[b]		
	12,5	4,07[bc]		
	25	3,26[bc]		
	50	1,88[cd]		
	100	1,00[e]		
Glycerin	10	99.90[a]		

LC: mean lethal concentration
Letras desiguales en una misma columna difieren a $p < 0,05$

Similar results to this study have been reported, but with plant parts. In this sense, Monteiro *et al.* (2011) evaluated the ethanol extract of *J. curcas* seed and obtained a hatching inhibition of 99,8 % in a concentration of 50 mg/mL. In egg hatching inhibition, Eguale and Giday (2009) reported in the aqueous and hydroalcoholic extract of this plant part LC_{50} of 0,1 and 0,23 mg/mL, respectively, higher values than the ones recorded in this study, which were 3,60 ×10^{-4} mg/mL (table 1).

Salles *et al.* (2014) state that this plant has molecules of high and low molecular weight (cut of 12 kDa) and sustain that trypsin inhibitors are the main candidate in the interference of the embryo development of eggs. Although the presence of trypsin inhibitors in the glycerol was not determined, their presence in this coproduct has not been reported, maybe due to the transesterification process itself, which leads to the denaturation of proteins.

It is possible that there are other substances in the GRF, such as salts, which have influenced this result, especially because in the transesterification of oil for obtaining biodiesel the ester bond of phorbol esters is broken, and oxidation, transesterification and epiremization reactions occur (Goel *et al.*, 2007). This coincides with the results in this study, where the phorbol esters were not found in the GRF (fig. 1). It is also in correspondence with the report by Herath *et al.* (2017), who did not find phorbol esters in the GRF of *J. curcas* either.

In the analysis of the GRF through gas chromatography no methanol remains that could be potentially toxic for the eggs were recorded (table 2). Glycerol (74,80 %) was found as larger constituent and long-chain carboxylic acids, unlike a study conducted by Pradhan *et al.* (2012). These authors found non-saponified matter, mono and diglycerides, as well as traces of methyl esters, in the glycerol of *J. curcas*.

Conclusions

The glycerol-rich fraction inhibited the egg-hatching of nematodes, gastrointestinal parasites of sheep, with dose-dependent effect. No phorbol esters were found and the main component was glycerin.

Conflicts of interests

The authors declare that there is no conflict of interests among them.

Authors' contribution

- Javier Arece-García. Generated the idea and executed the research, searched for bibliographic information and wrote the manuscript.
- Mildrey Soca-Pérez. Generated the research idea, searched for bibliographic information and reviewed the manuscript.
- Dayron Martín-Prieto. Generated ideas and contributed to the manuscript writing.
- Rosa María Rodríguez-Calle. Contributed to the manuscript writing.
- Yoel López-Leyva. Generated the research idea, searched for bibliographic information and reviewed the manuscript.
- Ramón Luck-Montero. Generated the research idea, searched for bibliographic information and reviewed the manuscript.
- José Ángel Sotolongo-Pérez. Contributed in the acquisition of oil and glycerol-rich fraction and reviewed the manuscript.

Bibliographic references

Alherbawi, M.; AlNouss, A.; McKay, G. & Al-Ansari, T. Optimum sustainable utilisation of the whole fruit of *Jatropha curcas*: An energy, water and food nexus approach. *Renew. Sust. Energ. Rev.*

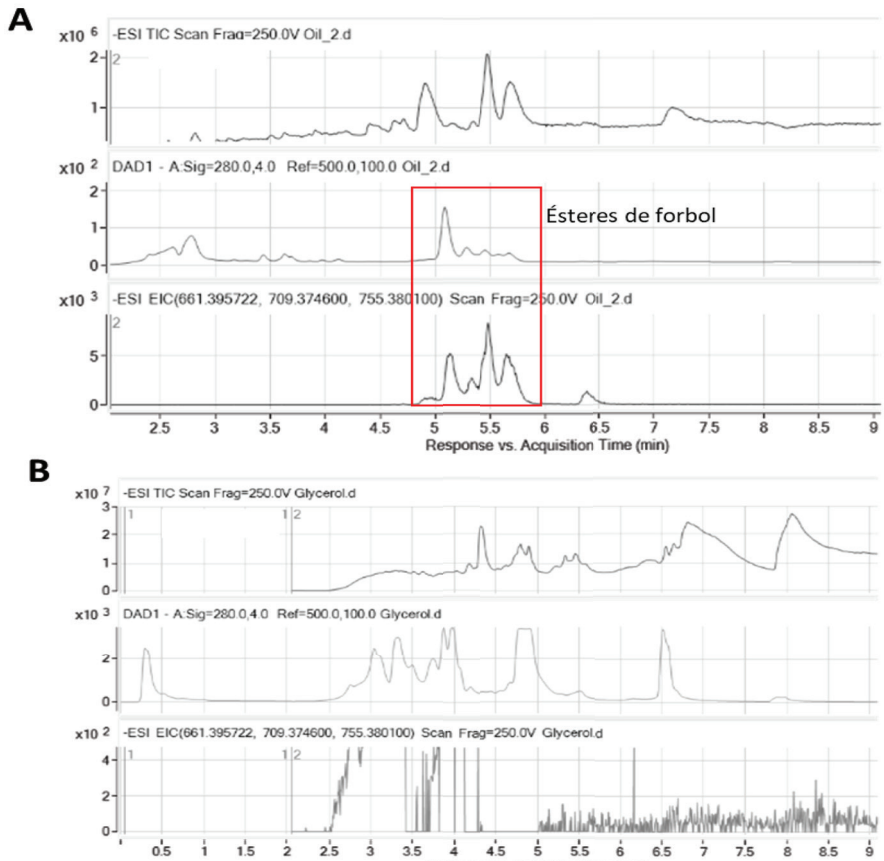

Figure 1. Detection of phorbol esters through MS-TOF in the glycerol-rich fraction. Analysis of a sample of *J. curcas* oil.

A. Analysis of a sample of the glycerol-rich fraction. In the red square the positive signs to phorbol esters are indicated

Table 2. Chemical composition for GC-MS of the GRF of *J. curcas*.

Compound	Molar mass, g/moL	%
Glycerol	92,1	74,8
Palmitic acid	256,4	5,6
Stearic acid	284,5	1,0
Oleic acid	282,0	9,0
Linoleic acid	280,0	9,0

137:110605, 2021. DOI: https://doi.org/10.1016/j.rser.2020.110605.

Arece-García, J.; López-Leyva, Y.; Olmedo-Juárez, A.; Ramírez-Vargas, G.; Reyes-Guerrero, D.; López Arellano, Ma. E. *et al.* First report of multiple anthelmintic resistance in goat farm in Cuba. *Helminthologia*. 54 (4):358-362., 2017. DOI: https://doi.org/10.1515/helm-2017-0037.

Borges, D. G. L. & Borges, F. de A. Plants and their medicinal potential for controling gastrointestinal nematodes in ruminants. *Nematoda*. 3:e92016, 2016. DOI: http://dx.doi.org/10.4322/nematoda.00916.

Bossou, Annick F. A. D.; Olaye, T.; Bogninou, Gbedossou S. R.; Koudoro, Y. A.; Agbangnan, D. C. P.; Bothon F. T. Diane *et al.* Medical benefit, pharmacology and toxicity of *Jatropha curcas* L. (Euphorbiaceae): A Review. *Int. J. of Adv. Res.* 8 (1):856-864, 2020. DOI: http://dx.doi.org/10.21474/IJAR01/10374.

Busari, O.; Soetan, K. O.; Aiyelaagbe, O. O. & Babayemi, O. J. Phytochemical screening and *in vitro* anthelmintic activity of methanolic extract of *Terminalia glaucescens* leaf on *Haemonchus contortus* eggs. *Acta Trop.* 223:106091, 2021. DOI: https://doi.org/10.1016/j.actatropica.2021.106091.

Che Hamzah, N. H.; Khairuddin, N.; Siddique, B. M. & Hassan, M. A. Potential of *Jatropha curcas* L. as biodiesel feedstock in Malaysia: A concise review. *Processes*. 8 (7):786, 2020. DOI: https://doi.org/10.3390/pr8070786.

Eguale, T. & Giday, M. *In vitro* anthelmintic activity of three medicinal plants against *Haemonchus contortus*. *Int. J. Green Pharm*. 3 (1):29-34. https://cyberleninka.org/article/n/280673.pdf, 2009.

French, Katherine E. Plant-based solutions to global livestock anthelmintic resistance. *Ethnobiol. Lett*. 9 (2):110-123, 2018. DOI: https://doi.org/10.14237/ebl.9.2.2018.980.

Goel, G.; Makkar, H. P. S.; Francis, G. & Becker, K. Phorbol esters: structure, biological activity, and toxicity in animals. *Int J Toxicol*. 26 (4):279-288, 2007. DOI: https://doi.org/10.1080/10915810701464641.

González-Garduño, R.; López-Arellano, M. E.; Ojeda-Robertos, N.; Liébano-Hernández, E. & Mendoza-de Gives, P. Diagnóstico *in vitro* y en campo de resistencia antihelmíntica en nematodos gastrointestinales de pequeños rumiantes. *Arch. med. vet*. 46 (3):399-405, 2014. DOI: https://dx.doi.org/10.4067/S0301-732X2014000300008.

Herath, K.; Girard, Lauren; Reimschuessel, Renate & Jayasuriya, Hiranthi. Application of time-of-flight mass spectrometry for screening of crude glycerins for toxic phorbol ester contaminants. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci*. 1046:226-234, 2017. DOI: https://doi.org/10.1016/j.jchromb.2017.01.005.

Hubert, J. & Kerboeuf, D. A. A microlarval development assay for the detection of anthelmintic resistance in sheep nematodes. *Vet. Rec*. 16 (30):442-446, 1992. DOI: https://doi.org10.1136/vr.130.20.442.

Khattab, M. S. A. Glycerol as feedstuff for ruminant. *Science International*. 3 (3):90-94, 2015. DOI: https://doi.org/10.17311/sciintl.2015.90.94.

Mach, N.; Bach, A. & Devant, M. Effects of crude glycerin supplementation on performance and meat quality of Holstein bulls fed high-concentrate diets. *J. Anim. Sci*. 87 (2):632-638, 2009. DOI: https://doi.org/10.2527/jas.2008-0987.

Marie-Magdeleine, Carine.; Mahieu, M.; Philibert, L.; Despois, P. & Archimède, H. Effect of cassava (*Manihot esculenta*) foliage on nutrition, parasite infection and growth of lambs. *Small Rumin. Res*. 93 (1):10-18, 2010. DOI: https://doi.org/10.1016/j.smallrumres.2010.04.024.

Monteiro, M. V.; Bevilaqua, C. M.; Morais, S. M.; Machado, L. K.; Camurça-Vasconcelos, A. L. & Campello, C. C. *et al.* Anthelmintic activity of *Jatropha curcas* L. seeds on *Haemonchus contortus*. *Vet. Parasitol*. 182 (2-4):259-263, 2011. DOI: https://doi.org/10.1016/j.vetpar.2011.04.010.

Neu, P. M.; Schober, S. & Mittelbach, M. Quantification of phorbol esters in *Jatropha curcas* by HPLC-UV and HPLC-ToF-MS with standard addition method. *Eur. J. Lipid Sci. Technol*. 120 (8):1800146, 2018. DOI: https://doi.org/10.1002/ejlt.201800146.

Neupane, D.; Bhattarai, D.; Ahmed, Z.; Das, B.; Pandey, S.; Solomon, J. K. Q. *et al.* Growing Jatropha (*Jatropha curcas* L.) as a potential second-generation biodiesel feedstock. *Inventions*. 6:60, 2021. DOI: https://doi.org/10.3390/inventions6040060.

Phulia, V.; Sardar, P.; Sahu, N. P.; Sanap, B. N.; Shamna, N.; Fawole, F. J. *et al.* Effect of detoxification methods on anti-nutritional factors and proximate composition of defatted. *Anim. Nutr. Feed Technol*. 18:67-77, 2018. DOI: https://doi.org/10.5958/0974-181X.2018.00006.9.

Pradhan, S.; Naik, S. N.; Ashhar, M.; Khan, I. & Sahoo, P. K. Experimental assessment of toxic phytochemicals in *Jatropha curcas*: oil, cake, bio-diesel and glycerol. *J. Sci. Food Agric*. 92 (3):511-519, 2012. DOI: https://doi.org/0.1002/jsfa.4599.

Rahu, M. I.; Naqvi, S. H. A.; Memon, N. H.; Idrees, M.; Kandhro, F.; Pathan, N. L. *et al.* Determination of antimicrobial and phytochemical compounds of *Jatropha curcas* plant. *Saudi J. Biol. Sci*. 28 (5):2867-2876, 2021. DOI: https://doi.org/10.1016/j.sjbs.2021.02.019.

Rizo, A. *Efectos repelentes y acaricidas del aceite de las semillas de Jatropha curcas L. en larvas de Rhipicephalus (Boophilus) microplus (Canestrini, 1887).* 2. Matanzas, Cuba: EEPF Indio Hatuey, Universidad de Matanzas, 2019.

Romero-Benavides, J. C.; Ruano, Ana L.; Silva-Rivas, R.; Castillo-Veintimilla, Paola; Vivanco-Jaramillo, Sara & Bailon-Moscoso, Natalia. Medicinal plants used as anthelmintics: Ethnomedical, pharmacological, and phytochemical studies. *Eur. J. Med. Chem*. 129:209-217, 2017. DOI: https://doi.org/10.1016/j.ejmech.2017.02.005.

Salaheldeen, M.; Mariod, A. A.; Aroua, M. K.; Rahman, S. M. A.; Soudagar, M. E. M. & Fattah, I. M. R. Current state and perspectives on transesterification of triglycerides for biodiesel production. *Catalysts*. 11:112, 2021. DOI: https://doi.org/10.3390/catal11091121.

Salles, Hévila O.; Braga, Ana C. L.; Nascimento, Maria T. dos S. C. do; Sousa, Ana M. P.; Lima, A. R.; Vieira, L. da S. *et al.* Lectin, hemolysin and protease inhibitors in seed fractions with ovicidal activity against *Haemonchus contortus*. *Braz. J. Vet. Parasitol*. 23 (2):136-143, 2014. DOI: http://dx.doi.org/10.1590/S1984-29612014050.

Yaqoob, H.; Teoh, Y. H.; Sher, F.; Ashraf, M. U.; Amjad, S.; Jamil, M. A. *et al. Jatropha curcas* biodiesel: A lucrative recipe for Pakistan's energy sector. *Processes* 9(7):1129, 2021. DOI: https://doi.org/10.3390/pr9071129.