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Haemonchus contortus protease inhibition by *n*-alkyl ferulates from *Maprounea guianensis*

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ABSTRACT

This report describes the inhibition of the proteolytic activity of *Haemonchus contortus* lysate by a mixture of *n*-alkyl ferulates isolated from *Maprounea guianensis*. These compounds were evaluated *in vitro* for the ability to inhibit the *H. contortus* proteases using SDS–PAGE copolymerized with gelatine (zymography) and by assaying the hemoglobinolytic activity of these enzymes in solution. The ferulates abrogated the proteolysis compared to E-64, suggesting the activity is the result of a cysteine protease.

Parasite proteases have been implicated both in vital parasite functions and in host–parasite relationships, with a key role in the parasite pathology (Knox, 2007). Several authors have proposed proteases as targets for parasitism control (Knox, 2007). Plant compounds are an attractive solution (Hounzangbe-Adote et al., 2005; Githiori et al., 2006) because the use of anthelmintics leads to the emergence of resistance (Kaplan, 2004; Coles, 2005). One strategy is to use low-molecular-weight compounds as protease inhibitors for parasite control.

In this study, we use *in vitro* assays to evaluate the protease inhibition activity of an *n*-alkyl ferulate mixture (Fig. 1) isolated from *Maprounea guianensis aublet* (Euphorbiaceae) against *Haemonchus contortus*, a gastrointestinal nematode of small ruminants that causes substantial production losses (Rinaldi et al., 2007).

Dried stems of *M. guianensis* (600 g) were powdered and extracted with 2 l of MeOH at room temperature. The MeOH extract was partitioned using hexane:MeOH/H_2O (9:1), and the hexanic extract obtained (8.68 g) was applied to a silica gel (40–60 mm) chromatography column and eluted with hexane:EtOAc. The fractions eluted with 60% of EtOAc contained dodecosyl, tetracosyl, hexacosyl, octacosyl, and triacontyl ferulates. The chemical structures were determined using spectroscopic data, as described by David et al. (2004).

The inhibition of the *H. contortus* proteolytic activity was determined using adult worm lysates as protease the source. Water-soluble extracts from adult worms were obtained by homogenization in a manual Potter tissue grinder followed by sonication at 4 °C with 30-s periods of ultrasonic treatment. The extract was centrifuged (6000 g min^{-1}, 15 min, 4 °C), and the recovered supernatant was either used immediately or stored at −20 °C. The amount of recovered protein was determined by the Bradford assay (Bio-Rad, CA, USA).

The protease inhibition was evaluated by zymography (Heussen and Dowdle, 1980) using an SDS–PAGE gel (7.5%) copolymerized with 0.1% gelatine to fractionate the protein (20 μg) under non-reducing conditions. After electrophoresis, washed gels (2.5% Triton X-100 buffered solution) were incubated with or without ferulates (18 h/37 °C in 0.1 M citrate-phosphate, pH 5, 2 mM β-mercaptoethanol). The gels were stained with 0.1% Coomassie Blue R-250 to visualize the proteolytic band intensities for the samples with and without ferulates. To evaluate the hemoglobinase activity, *H. contortus* lysate (72 μg) and caprine hemoglobin (Hb) (2 mg) were mixed with 10, 20, or 200 μM of ferulates in the same buffer. After incubation (18 h/37 °C), samples were fractionated on 15% SDS–PAGE gels, and the gels were stained to visualize the inhibition. E-64 (20 μM) was used as an inhibitor control.

The present work describes a preliminary assessment of the inhibition of *H. contortus* proteases with a mixture of ferulates using gelatinolytic and hemoglobinolytic assays. The analysis of the protease activity by gelatin-SDS–PAGE revealed complete inhibition of gelatinolysis (Fig. 2A), and a similar result was found for the inhibition of the hemoglobinolytic activity by the mixture of *n*-alkyl ferulates (Fig. 2B). The results show that the ferulates abrogated the proteolytic activity compared to an untreated control and to E-64.

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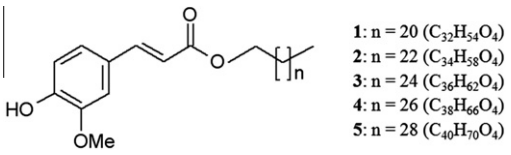

Fig. 1. *n*-Alkyl ferulate mixture from *M. guianensis* (David et al., 2004) made up of tetracosyl (1), octacosyl (2), hexacosyl (3), triacontyl (4), and dodecosyl (5) ferulates.

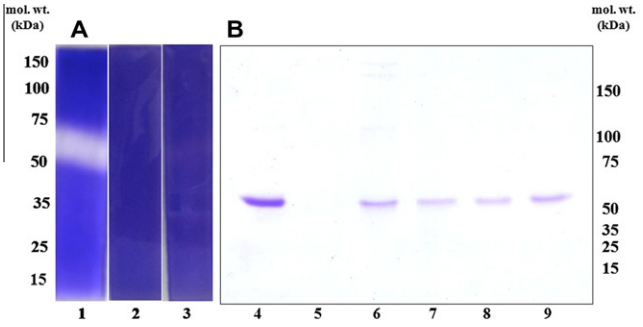

Fig. 2. (A) Zymography and (B) inhibition of hemoglobinolytic activity of *H. contortus* lysates by the *n*-alkyl ferulate mixture. (1) Proteinase activity; (2) inhibition by E-64; (3) inhibition by ferulates; (4) Hb control; (5) Hb incubated with parasite lysate (without inhibitor); (6) Hb incubated with parasite lysate and E-64; (7–9) Hb incubated with parasite lysate and 50, 100, and 200 μM ferulates, respectively.

The proteolytic activity was abolished by E-64, suggesting that the activity is due to a cysteine protease. Thus, the ferulate interference with the protease activity is relevant because it is important in the host–parasite interaction, as parasites require proteases to penetrate and degrade host proteins (Knox, 2007). In this context, it has been reported that phenolic compounds inhibited proteases (Jedinák et al., 2006). Furthermore, as cysteine proteases play an important role in the vital functions of parasites, inhibitors of these enzymes may be a novel treatment for use against parasites, particularly helminths (Izuhara et al., 2008). Thus, ferulates may be a potential drug for hemonchosis treatment. However, further studies are required to evaluate the potential therapeutic uses of ferulates.

Conflict of interest

All the authors agree that they do not have any financial, personal or organizational conflict with respect to the contents of this manuscript.

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