In vitro anthelmintic activity of some Mediterranean plants against Haemonchus contortus infective stage

Aïssa A., Manolaraki F., Pardo E., Znaïdi I.A., Ben Salem H., Kraiem K., Hoste H.

in

Ben Salem H. (ed.), López-Francos A. (ed.).
Feeding and management strategies to improve livestock productivity, welfare and product quality under climate change

Zaragoza : CIHEAM / INRAT / OEP / IRESA / FAO
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 107

2013
pages 183-187

In vitro anthelmintic activity of some Mediterranean plants against *Haemonchus contortus* infective stage

A. Aissa*, F. Manolaraki, E. Pardo**, I.A. Znaïdi*, H. Ben Salem***, K. Kraiem* and H. Hoste****

*Laboratoire des Productions Animales, Institut Supérieur Agronomique de Chott-Mariem 4042 Chott-Mariem (Tunisie)
**UMR 1225 INRA/ENVT. Ecole Nationale Vétérinaire de Toulouse, 23 Chemin des Capelles 31076 Toulouse Cedex (France)
***Laboratoires des Productions Animales et Fourragères, Institut National de la Recherche Agronomique de Tunisie (INRAT), Rue Hédi Karray, 2049 Ariana (Tunisie)
e-mail: asaissa@laposte.net

Abstract. The use of bioactive tanniniferous plants seems a promising alternative to control infections parasitic nematodes of the gastro intestinal tract in small ruminants. Both *in vitro* and *in vivo* studies, have confirmed the anthelmintic properties of several forage legumes, or tropical and browse plants. The aim of the present study was to evaluate the *in vitro* anthelmintic (AH) effect of 3 Tunisian plants (*Trigonella foenum graecum, Periploca angustifolia* Labill*., Ceratonia siliqua*) on *Haemonchus contortus* infective larvae. The larval exsheathment inhibition assay (LEI) was used to determine the potential inhibitory effects of 5 plant extracts at different concentrations (1200, 600, 300, 150µg/ml). The inhibition effect at the highest concentration ranged between 82.97-100% for *C. siliqua* (leaves), *P. angustifolia* (pods and leaves)*, T. foenum graecum* (whole plant) and at 53.5% for *C. siliqua* (fruits). The possible implication of polyphenols and/or tannins in the anthelmintic activity was showed after measuring the total phenols, total tannins and the biological activity of the extracts tested.

Keywords. Tannins – Larval exsheathment assay – Third stage larvae (L3) – Tunisia.

***Activité anthelminthique* in vitro *de quelques plantes de Tunisie contre les larves infestantes d'*Haemonchus contortus**

Résumé. *L'utilisation de plantes bioactives riches en métabolites secondaires, comme les tannins, semble une alternative pour la lutte contre les nématodes parasites gastro-intestinaux chez les petits ruminants. Des études,* in vitro *et* in vivo*, ont confirmé les propriétés anthelminthiques de plusieurs plantes légumineuses fourragères, tropicales ou de plantes de parcours. L'objectif de notre étude était l'évaluation* in vitro *des effets anthelminthiques (AH) de 3 plantes tunisiennes (*Trigonella foenum graecum*,* Periploca angustifolia *Labill.,* Ceratonia siliqua*) sur les larves infestantes d'*Haemonchus contortus*. Le test d'inhibition de dégainement larvaire a été utilisé pour déterminer les effets anthelminthiques de 5 extraits de plantes aux concentrations suivantes (1200, 600, 300, 150 µg/ml). L'effet d'inhibition de dégainement, à la plus forte concentration utilisée, a varié de 82,97-100% pour* C. siliqua *(feuilles),* P. angustifolia *(gousses et feuilles), et* T. foenum graecum *à 53,5% pour* C. siliqua *(fruits). L'implication possible de polyphénols ou/et de tannins dans l'activité anthelminthique a été soupçonnée après avoir mesuré les phénols totaux, tannins totaux et l'activité biologique des extraits testés.*

Mots-clés. *Tannins – Dégainement des larves L3 – Larve infestante (L3) – Tunisie.*

I – Introduction

The parasitic nematodes of the gastrointestinal tract remain a major worldwide concern for the health and welfare of grazing ruminants because of the pathological problems and the

production looses that they cause. For more than 50 years, the control of these parasitic diseases has relied on the repeated use of chemical anthelmintics (AH). However this extensive use of synthetic molecules, is nowadays facing some limits. The increasing concern of consumers on the use of chemical drugs in farm animals, the possible impact of chemical residues in the environment (Mc Kellar, 1997) but mainly the development of resistance to AH in worm populations (Jackson and Coop, 2000; Kaplan, 2004) explain the need to seek alternative solutions. Among those solutions, the possible exploitation of bioactive plants, rich in secondary metabolites such as tannins, seem one of the option for the sustainable control of these parasites. Many *in vitro* and *in vivo* studies, have shown that the use of bioactive plants seems to modulate the biology of nematode parasites, either by reducing the parasitic infection in the host or the pasture contamination (Hoste *et al.,* 2006). Most of these studies have focused on the anthelmintic properties of legume forages such as sulla (*Hedysarium coronarium*), (Niezen *et al.* 1995, 1998, 2002), or sainfoin (*Onobrychis viciifolia)* (Paolini *et al*., 2005; Heckendorn *et al*., 2007; Manolaraki *et al.,* 2010). Moreover, some other studies have explored the potential AH properties of several browses exploited in Mediteranean area such as *Ceratonia siliqua*, *Pistacia lentiscus*, *Castanea sativa* (Manolaraki *et al*., 2010) or heather (*Calluna vulgaris*) (Osoro *et al*., 2007).

The main objective of the current study was to verify the possible AH activity of three Tunisian browse plants by evaluating in vitro 5 plant acetonic extracts on *Haemonchus contortus* third stage larvae.

II – Materials and methods

1. Plant samples and preparation of extracts

The plant samples were collected from the field in spring-summer 2011. In total 5 samples from 3 different plants were collected: *Trigonella foenum graecum* (whole plant), *Periploca angustifolia* (leaves and pods), *Ceratonia siliqua* (leaves and fruits). After freeze drying, 10g of each plant samples were extracted by shaking (1 hour, T≤35°C) in 100 ml of acetone:water (70:30). The filtrate was concentrated under low pressure (T≤35°C) and washed 3 times with dichloromethane in order to eliminate lipids and chlorophylls. The final extract tested was obtained after freezing and lyophilisation.

2. Bioassay: Larval exsheathment inhibition

The larval exsheathment inhibition assay (LEI) (Bahuaud *et al*., 2006) was used to evaluate the AH activity of the samples on ensheathed third-stage larvae (L3) of *H. contortus.* The L3s were obtained from donor goats monospecifically infected with *H. contortus* (MAFF,1986). The larvae were then stored at 4°C before use. One thousand 3-month-old L3 were incubated for 3 h at 20°C either with four different concentrations (1200, 600, 300, 150 of extract µg/ml) of each plant extract diluted in phosphate buffer saline solution (PBS: 0.1M phosphate, 0.05M NaCl, pH 7.2) or with PBS (used as a negative control). After washing with PBS, the larvae were submitted to the artificial process of exsheathment by contact with a solution of sodium hypochloride (2% w/v) and sodium chloride (16.5% w/v) diluted in 1 to 400 in PBS. The kinetics of L3 exsheathment, according to the different treatments, was identified under microscopic observation at a magnification of x 200 at 0, 20, 40 and 60 min after contact with the solution to induce the artificial exsheathment. These measurements of the exsheathment rates at regular time (20 minutes) interval were performed because it is important to assess the linearity of the response for the control (PBS) and to verify that nearly 100% of the infective larvae were exsheathed after 60 minutes. This is a prerequest to make possible the calculation of IC50 and their comparison/interpretation.

3. Evaluation of tannin content

A. Biological activity

The biological activity of the plant samples, related to the tannin content was measured using the radial diffusion method (Hagerman, 1978) which is based on the property of tannins to form complexes with proteins. We used Bovine Serum Albumin (BSA) (Sigma Aldrich Ltd) as protein source and tannic acid (Sigma Ltd) as a standard. The results were expressed in g-equivalents of tannic acid/100 g of dry plant (DP)

B. Folin-Ciocalteu assay

The Folin Ciocalteu method (Makkar, 2003) was used to determine the total polyphenols (TP) and total tannins (TT) in the extracts. After the initial measurements of TP, an inhibitor of tannins, the polyvinylpolypyrrolidone (PVPP) (Sigma Aldrich Ltd) was added to the extract and the measurement was repeated. Then TT was calculated as the difference between TP measured with or without addition of PVPP in the same extract. The TP and TT were determined by recording the absorbance at 725 nm using of a spectrophotometer (UV-Visible Spectronic Unican, Genesys 8). A tannic acid standard curve was performed and the results were expressed as tannic acid equivalents/100 g of dry plant (DP).

III – Results and discussion

Under Mediterranean conditions, the breeding of animals relies on the exploitation of rangelands covered by several plants from various families. In Tunisia, and especially in arid zones, which cover more than 70% of the total area (Floret and Pontanier,1982), shrub species such as *P. angustifolia, C. siliqua* and *T. foenum graecum* constitute feeding resource for livestock especially during the summer, when the alternative herbaceous species have wilted (Papanastasis *et al*., 1998). The AH properties of *C. siliqua* have previously been shown *in vitro* and *in vivo* (Manolaraki et al., 2010). In contrast, according to our knowledge this is the first time that the AH activity of *P. angustifolia and T. foenum graecum* was examined. Most of the browse plants found in rangelands are rich in plant secondary metabolites (PSMs) including tannins. This observation was confirmed in our study, since 3 out of 5 plant extracts [*C. siliqua* (leaves), *P. angustifolia* (pods and leaves)] have shown high TP and TT values reaching 11.63 and 6.11 g equivalent Tannic acid/100 g of dry plant for TP and TT, respectively (Table1).As far as the two other plant, *C. siliqua* (fruits) and *T. foenum graecum,* is concerned the lower TP, TT values that have shown corresponds to lower AH (Table 2).

Table 1. Polyphenolic compounds and biological activity of the tannin content for the five plant samples

	TP	TT	BA
C. siliqua (leaves)	11.63 (±0.14)	6.11 (±0.28)	8.97 (±0.99)
P. angustifolia (pods)	9.01 (±0.17))	2.19 (±0.19)	2.00 (±0.17)
P. angustifolia (leaves)	6.09 (±0.25)	0.72 (±0.06)	2.42 (± 0.23)
C. siliqua (fruits)	3.58 (±0.09)	2.07 (±0.08)	0.70 (± 0.05)
T. foenum graecum	1.37 (±0.39)	0.59 (±0.15)	0.16 (±0.04)

TP, TT, BA values are expressed as g of tannic acid equivalent/100g of dry plant.

During the last decade, repeated evidence tend to confirm the hypothesis that AH properties are associated with some PSMs and especially tannins (Hoste *et al.*, 2006; Rochfort *et al.*, 2008). Our results tend to confirm this hypothesis since the plant sample with the highest BA and TP

and TT content [*C.siliqua* (leaves)] has shown the highest AH activity. Manolaraki *et al.* (2010) have shown the implication of tannins in the AH activity of *C.siliqua* (fruits) by using PVPP, since the results were restored to control values after addition. The overall results in the current study show the AH activity of the 5 plant samples, as measured by the LEI assay, which ranged from 100 to 53.5 % at the highest concentration (Table 2). These results complete previous data obtained from studies that show the AH properties of various plants browsed by small ruminants in the Mediterranean basin (Manolaraki *et al.*, 2010; Hoste *et al.,* 2009; Bahuaud *et al.*, 2006).

Table 2. Mean values (±SD) of proportion exhseathed larvae and inhibition values (%) compared to the PBS control at different concentration and at 60 minutes after the start of the LEI assay

	Kinetics of L3 exsheathment			
	150µg/ml	**300µg/ml**	**600µg/ml**	**1200µg/ml**
C. siliqua (leaves)	24.2 (±24.4)	8.3 (±7.2)	6.0 (±13.0)	0.0 (±0.0)
Inhibition (%)	74.14	91.12	93.63	100.0
P. angustifolia (pods)	15.6 (±12.5)	5.9 (±4.9)	3.9 (±4.4)	2.6 (±2.4)
Inhibition (%)	84.16	94.02	96.09	97.82
P. angustifolia (leaves)	24.4 (±32.4)	6.7 (±4.4)	4.0 (±3.2)	2.6 (±3.7)
Inhibition (%)	72.05	93.2	95.92	97.35
C. siliqua (fruits)	91.1 (±6.5)	73.7 (±13.2)	77.2 (±32.6)	40.3 (±25.7)
Inhibition (%)	0	14.99	10.94	53.5
T. foenum graecum	89.0 (±7.4)	76.5 (±21.9)	59.1 (±42.1)	15.5 (±8.8)
Inhibition (%)	1.47	15.88	35	82.97

IV – Conclusions

Over all the present study confirmed the potential AH properties of the 3 browse Tunisian plants tested, although further studies are needed to better understand the nature of active compounds and the possible mode of action on parasites. This knowledge is essential to acquire for the correct use of plants rich in PSM and/or tannins in small ruminant livestock.

References

Bahuaud D., Martinez-Ortiz De Montellano C., Chauveau S., Prevot F., Torres-Acosta F., Fouraste L. and Hoste H., 2006. Effects of four tanniferous plant extracts on the in vitro exheathment of the third – stage larve of parasitic nematodes. In: *Parasitology*, 123, p. 545-554.

Floret C. and Pontanier R., 1982. L'aridité en Tunisie pré-saharienne, climat, sol, végétation et aménagement. Travaux et documents de l'ORSTOM.

Hagerman E.A. and Bulter G.L., 1978. Protein precipitation method for the quantitative determination of tannins. In: *Journal of Agricultural and Food Chemistry*, 26, p. 809-812.

Heckendorn F., Haring D.A., Maurer V., Senn M. and Hertzberg H., 2007. Individual administration of three tanniferous forage plants to lambs artificially infected with *Haemonchus contortus* and *Cooperia curticei*. In: *Veterinary Parasitology*, 146, p. 123-134.

Hoste H., Jackson F., Athanasiadou S., Thamsborg S.M. and Hoskin S.O., 2006. The effects of tannin-rich plants on parasitic nematodes in ruminants. In: *Trends in Parasitology*, 22, p. 253-261.

Hoste H., Brunet S., Paolini V., Bahuaud D., Chauveau S., Fouraste I. and Le Frileux Y., 2009. Compared *in vitro* anthelmintic effects of eight tannin-rich plants browsed by goats in the southern part of France. In: *Options Méditerranéennes*, 85, p. 431-436.

Jackson F. and Coop R.L., 2000. The development of anthelmintic resistance in sheep nematodes. In: *Parasitology*, 120, p. 95-107.

Kaplan R.M., 2004. Drug resistance in nematodes of veterinary importance : astatus report. Trends in In: *Parasitology*, 20, p. 477-481.

MAFF (Ministry of Agriculture, Fisheries and Food), 1986. *Manual of Veterinary Parasitological Laboratory Techniques.* Her Majesty's Stationery Office, London.

Manolaraki F., Sotiraki S., Stefanakis A., Skampardonis V., Volanis M. and Hoste H., 2010. Anthelmintic activity of some Mediterranean brouse plants against parasitic nematodes. In: *Parasitology,*137 (4), p. 685-696.

Makkar H.P., 2003. *Quantification of tannins in tree and shrub foliage*. *A laboratory manual.* Food and Agriculture Organization of the United Nations/International Atomic Energy Agency (FAO/IAEA), Vienna, Austria, p. 49-53.

Mckellar Q.A.,1997. Ecotoxicology and residues of anthelmintic compounds. In: *Veterinary Parasitology,* 72, p. 413-435.

Niezen J.H., Waghorn T.S., Charleston W.A. and Waghorn G.C., 1995. Growth and gastointestinal nematode parasitism in lambs grazing either lucerne (*Medicago sativa*) or sulla (*Hedysarum coronarium*) which contains condensed tannins. In: *Journal of Agricultural Science,* 125, p. 281-289.

Niezen J.H., Robertson H.A., Waghorn G. and Charleston W.A., 1998. Production, faecal egg counts and worm burdens of ewe lambs which grazed six contrasting forages. In: *Veterinary Parasitology,* 80, p. 15-27.

Niezen J.H., Charleston W.A.G., Robertson H.A., Shelton I.D., Waghorn G. and Green R., 2002. The effect of feeding sulla (*Hedysarum coronarium*) or lucerne (*Medicago sativa*) on lamb parasite burdens and development of immunity to gastrointestinal nematodes. In: *Veterinary Parasitology,* 105, p. 229-245.

Osoro K., Mateos-Sanz A., Frutos P., García U., Ortega-Mora L.M., Ferreira L.M., Celaya R. and Ferre I., 2007. Anthelmintic and nutritional effects of heather supplementation on Cashmere goats grazing perennial ryegrass-white clover pastures. In: *Journal of Animal Science,* 85, p. 861-870.

Paolini V., de La Farge F., Prevot F., Dorchies P. and Hoste H., 2005. Effects of the repeated distribution of sainfoin hay on the resistance and the resilience of goats naturally infected with gastrointestinal nematodes. In: *Veterinary Parasitology,* 127, p. 277-283.

Papanastasis V.P., Platis P.D. and Dini-Papanastasi O., 1998. Effects of age and frequency of cutting on productivity of Mediterranean deciduous fodder tree and shrub plantations. In: *Forest Ecology and Management*, 110, p. 283-292.

Rochfort S., Parker A.J. and Dunshea F.R., 2008. Plant bioactives for ruminant health and productivity. In: *Phytochemistry,* 69, p. 299-322.