In vitro anthelmintic activity of Thymus capitatus from southern Tunisia on gastrointestinal nematodes of sheep

Akkari H., Boubaker Elandalousi R., Gharbi M.

in

Baumont R. (ed.), Carrère P. (ed.), Jouven M. (ed.), Lombardi G. (ed.), López-Francos A. (ed.), Martin B. (ed.), Peeters A. (ed.), Porqueddu C. (ed.).
Forage resources and ecosystem services provided by Mountain and Mediterranean grasslands and rangelands

Zaragoza : CIHEAM / INRA / FAO / VetAgro Sup Clermont-Ferrand / Montpellier SupAgro
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 109

2014
pages 251-255

In vitro anthelmintic activity of *Thymus capitatus* from southern Tunisia on gastrointestinal nematodes of sheep

H. Akkari[1,*], R. Boubaker Elandalousi[2] and M. Gharbi[3]

[1]Laboratoire de Parasitologie, Université de la Manouba,
École Nationale de Médecine Vétérinaire, 2020 Sidi Thabet (Tunisia)
[2]Laboratoire d'épidémiologie et microbiologie vétérinaire, Institut Pasteur Tunis and Institut supérieur de biotechnologie BiotechPôle, BP-66, 2020, Sidi Thabet, Ariana, University la Manoua (Tunisia)
[3]Laboratoire de substances naturelles, Institut National de Recherche et d'Analyse physico-chimique Pôle Technologique Sidi Thabet, 2020 Sidi Thabet (Tunisia)
*e-mail: hafidh_akkari@yahoo.fr

Abstract. *Thymus capitatus* (Lamiaceae) is used traditionally by people as spices and was reported to possess some biological effects. The objective of this study was to evaluate the anthelmintic efficacy of *T. capitatus* in comparison to albendazole against the gastrointestinal (GI) nematodes of sheep. To fulfil the objectives, In vitro anthelmintic activities of crude aqueous (CAE) and ethanolic extracts (CEE) of aerial parts of *T. capitatus* were investigated on the egg and adult nematode parasite *Haemonchus contortus*. Both extract types of *T. capitatus* inhibited hatching of eggs completely at a concentration close to 2mg/ml. LC50 of ethanolic extract of *T. capitatus* was 0.368 mg/ml while that of aqueous extract was 6.344 mg/ml. There was statistically significant difference between aqueous and ethanolic extracts ($p < 0.05$). The ethanolic extract showed better in vitro activity against adult parasites than the aqueous one in terms of the paralysis and/or death of the worms at different hours post-treatment (PT). Dose dependent activity was also observed for both extract. As far as the literature could be ascertained, there is no published report on anthelmintic activity of *T. capitatus*. The results of the present study suggest that *T. capitatus* extracts are a promising alternative to the commercially available anthelmintics for the treatment of GI nematodes of small ruminants.

Keywords. *Thymus capitatus* – Anthelmintic – Haemonchus contortus – Tunisia.

Activité anthelminthique* in vitro *du* Thymus capitatus *du sud de la Tunisie sur les parasites gastro-intestinaux du mouton

Résumé. Thymus capitatus (Lamiaceae) *est traditionnellement utilisée comme épice et a été signalée posséder des effets biologiques. L'objectif de cette étude était d'évaluer l'efficacité anthelminthique de* T. capitatus *par rapport à l'albendazole contre les nématodes gastro-intestinaux (GI) des ovins. Pour atteindre ces objectifs, les activités* in vitro *des extraits bruts aqueux et éthanoliques des parties aériennes de la plante ont été testées sur les œufs et les vers adultes d'*Haemonchus contortus*. Les deux types d'extraits ont inhibé totalement l'éclosion des œufs à une concentration proche de 2 mg/ml. Les DL50 de l'extrait éthanolique et de l'extrait aqueux de* T. capitatus *étaient 0,368 mg/ml et 6,344 mg/ml respectivement. Il y avait une différence statistiquement significative entre les extraits aqueux et éthanoliques ($p < 0,05$). En outre, l'extrait éthanolique a montré une meilleure activité* in vitro *contre les parasites adultes que l'extrait aqueux en termes de paralysie et / ou de mort des vers à différentes heures post-traitement. Cet effet dose dépendant a également été observé pour les deux l'extraits. Les résultats de cette étude révèlent que les extraits de* T. capitatus *peuvent représenter une alternative prometteuse aux anthelminthiques purement chimiques pour le traitement des nématodes gastro-intestinaux des petits ruminants.*

Mots-clés. Thymus capitatus – *Anthelminthique* – Haemonchus contortus – *Tunisie*.

I – Introduction

The genus *Thymus* (Lamiaceae), which comprises about 215 species, is particularly prevalent in the Mediterranean area (Hazzit *et al*., 2009). *Thymus capitatus* is known in Tunisia as ''Zaâtr'' and was commonly used as spices and was reported to possess some biological effects such as antibacterial (Essawi and Srour, 2000), antiviral, and antioxidant activities (Miura and Nakatani, 1989; Ines *et al*., 2012).

As far as the literature could be ascertained, there is no published report on anthelmintic activity of *T. capitatus.* In the present study, an experiment was performed to test in vitro anthelmintic efficacy of crude aqueous and ethanolic extracts of aerial parts of *T. capitatus* when compared to a reference drug albendazole against *Haemonchus contortus*.

II – Material and methods

Plant material, collection and preparation. Fresh plants were collected in June 2011 from a local farm at Matmata, local government area of Gabes, Tunisia. Leaves, stems and flowers were separated and thoroughly rinsed in running tap water. The stems were cut into chunks, and all of the plant material was air dried at room temperature for a period of 14 days and pulverized. The aerial parts of the plant (leaves, stems and flowers) were air-dried and finally ground to a fine powder.

Preparation of extracts. Both crude aqueous and ethanolic extracts were used. Thus, dried and finely powdered aerial parts of *T. capitatus* (100 g) were sequentially extracted by maceration with distilled water at room temperature (20-25°C, 3 × 500 ml). The aqueous extract was collected and filtered by Whatman No.1 filter paper and then lyophilized (Alawa *et al*., 2003). For the ethanolic extract, 200 g of powdered plant were added to 500 ml of 95° ethanol. The resulting mixture was incubated for 16 hours at room temperature and frequently agitated before being filtered through Whatman No. 4 filter paper. The process is repeated 3 times. Then, all the solvent was evaporated in Rotavapor. All extracts were concentrated, dried and kept in the dark at 4°C until tested.

***In vitro* anthelmintic assays – Egg hatch assay.** Eggs used in the present assay were collected from donor sheep according to World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines (Coles *et al*., 1992). Fresh eggs were then washed repeatedly with distilled water. Aqueous and ethanolic extracts of the leaves of *T. capitatus* were used as the test treatment. Albendazole (99.8% pure standard reference) was used as the reference drug (positive control) while untreated eggs in PBS (Phosphate Buffered Saline) with DMSO (Dimethyl sulfoxide) (0.5%) were used as negative control. In the assay, approximately 200 eggs in 1.5 ml of PBS were placed in each test tube. Aqueous and ethanolic extracts of *T. capitatus* at concentrations of 2.0, 1.0, 0.5, 0.25, 0.125 and 0.0625 mg/ml in a total volume of 0.5 ml in PBS and ethanolic extracts at same concentrations in a total volume of 0.5 ml in PBS with DMSO (0.5%) were used. Albendazole was dissolved in DMSO and diluted at the concentrations of 1 µg/ml. The test tubes were then covered and kept in incubator at 27°C for 48 h. The experiment was replicated four times for each concentration. Hatched larvae (dead or alive) and un-hatched eggs were then counted under dissecting microscope with 40× magnification.

***In vitro* anthelmintic assays – Adult worms motility assay.** This test was performed according to Hounzangbe-Adote *et al*. (2005). Adult worms were collected from an experimentally infected lamb, 6 weeks after infection. Immediately after slaughtering, the abomasum was removed, opened and placed in 37° C saline. The collected parasites were then washed and kept in PBS. Five to ten actively moving worms were placed in petridishes filled with 2.0, 1.0, and 0.5 mg/ml of the aqueous and ethanolic extracts of *T. capitatus* respectively in PBS and in PBS with DMSO (0.5%) in a total volume of 4 ml. PBS with DMSO (0.5%) was used as a negative control. Albendazole dissolved in DMSO and diluted in PBS at the concentrations of 0.5 mg/ml was used as a positive control. Three replicates were performed for each treatment. Inhibition of worm mo-

tility was the rationale for anthelmintic activity. The time required for paralysis or complete inactivity and mortality was recorded at 0, 2, 4 and 8 h intervals. After 8h the extracts and albendazole were washed away and parasites resuspended in luke warm PBS for 30 min to test the revival of the worm motility. The number of motile (alive) and immotile (dead) worms were counted under dissecting microscope, and recorded for each concentration. Death of worms was ascertained by absence of motility for observation period of 5-6 s. A mortality index was calculated as the number of dead worms divided by the total number of worms per petridish.

Statistical analysis. The statistical analysis was performed using the SPSS-10.0 software package for Windows. LC_{50} for egg hatch inhibition was calculated by probit analysis. Regression was used for evaluation of dose-response relationship using Minitab® Release 14. The result of the worm motility inhibition was expressed as mean ± standard error of mean (S.E.M). Means of anthelmintic efficacy were compared by student's t test. A probability of 0.05 was used as a threshold for statistical significance.

III – Results and discussion

1. Egg hatch assay

The results of *H. contortus* egg hatching inhibition by aqueous and ethanolic extracts of *T. capitatus* are presented in Figure 1. In PBS less than 2% eggs did not hatch. With the albendazole concentration used, 92.05% eggs incubated did not hatch. Both extracts showed ovicidal activity in all tested concentrations and the histogram evolution showed dose dependency ($P<0.05$). Statistical differences ($P<0.05$) were also observed among two types of extracts.

Crude ethanolic extract (LC50 = 0.368 mg ml^{-1}) was found to have higher inhibitory effects compared with that of aqueous extract (LC50 = 6.344 mg ml^{-1}) on egg hatching.

Both extracts of *T. capitatus* inhibited egg hatching at lower concentration compared to some medicinal plants studied previously, for instance, 7.1 mg/ml of aqueous extract of *Annona senegalensis* inhibited only 11.5% eggs (Alawa *et al*., 2003) and methanol extract of *Spigelia anthelmia* induced 97.4% egg hatch inhibition at concentration of 50 mg/ml (Assis *et al*., 2003).

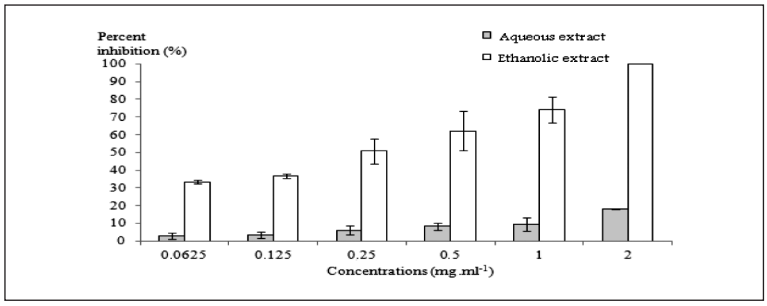

Fig. 1. Dose-dependent profile of the percent hatching egg of *Haemonchus contortus* submitted to the six increasing concentrations of plant extracts (0.0625, 0.125, 0.25, 0.5, 1 and 2 mg/ml).

2. Adult worm motility

The ethanolic extract of *T. capitatus* killed more worms than the aqueous extract in all tested concentrations. Dose dependent activity was also observed for both extract. The ethanolic extract induced 100% mortality at the highest concentration tested while the aqueous extract induced

85.71% at the same concentration (Table 1). There was 78.04% mortality of worms in albendazole (used as a reference drug) within 8 h post-exposition. There was 13.63% mortality of worms kept in PBS with DMSO 0.5% observed 8 h post-exposition.

Table 1. In vitro anthelmintic efficacy of crude aqueous and ethanolic extracts of *Thymus capitatus* on *Haemonchus contortus* of sheep

Treatment	Concentrations mg/ml	Percent of *Haemonchus contortus* worms showing mortality post-exposure to various treatments (Mean ± SEM)			
		0 h	2 h	4 h	8 h
Crude aqueous extract	0.5	0 ± 0.00	0 ± 0.00	3.57 ± 0.036	21.42 ± 0.089
	1	0 ± 0.00	9.09 ± 0.06	31.83 ± 0.099	68.21 ± 0.087
	2	0 ± 0.00	37.01 ± 0.105	45.71 ± 0.084	85.71 ± 0.128
Crude ethanolic extract	0.5	0 ± 0.00	0 ± 0.00	16.66 ± 0.089	33.33 ± 0.108
	1	0 ± 0.00	33.33 ± 0.125	50 ± 0.136	66.66 ± 0.177
	2	0 ± 0.00	76.92 ± 0.102	84.61 ± 0.25	100 ± 0.00
Albendazole	0.5	0 ± 0.00	0 ± 0.00	12.5 ± 0.051	78.04 ± 0.061
PBS with DMSO 0.5%	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	

Several studies have revealed that the effect of plant extracts on adult worms can occur only at the highest concentration, for example: methanolic extract of *Euphorbia helioscopia L* induce highest nematode mortality (98%) at 50 mg/ml (Bashir *et al.*, 2012). The greater anthelmintic activity of crude ethanolic extracts than crude aqueous extracts could be due to easier and rapid transcuticular absorption of the ethanolic extract into the body of the worms owing to its lipid soluble nature.

IV – Conclusion

Based on the results of the present study, it can be concluded that *T. capitatus* aerial parts tested in the form of crude aqueous and ethanolic extracts showed significant in vitro anthelmintic activity at concentrations and doses tested against ovine nematodes as determined by worm motility inhibition and egg hatching inhibition of *H. contortus*. These findings suggest that *T. capitatus* could form an alternative to commercially available synthetic anthelmintics. Further investigations are needed to determine the exact active components against helminths and to test it in vivo for a potential commercial development.

References

Alawa C.B.I., Adamu A.M., Gefu J.O., Ajanusi O.J., Abdu P.A., Chiezey N.P., Alawa J.N. Bowman D.D., 2003. *In vitro* screening of two Nigerian medicinal plants (Vernonia amygdalinaand Annona senegalensis) for anthelmintic activity. *Veterinary Parasitology*, 113, p. 73-81.

Assis L.M., Bevilaqua C.M.L., Morais S.M., Vieira L.S., Costa C.T.C., Souza J.A.L., 2003. Ovicidal and larvicidal activity *in vitro* of *Spigelia anthelmia* Linn. extracts on *Haemonchus contortus*. *Veterinary Parasitology*, 117, p. 43-49.

Bashir A., Lone M.Z., Chishtia F.A.B., Hida Yatullah T., Suhaib A.B., 2012. *In vitro* and *in vivo* anthelmintic activity of *Euphorbia helioscopia* L. *Veterinary Parasitology*, 189, p. 317-321.

Coles G.C., Bauer C., Borgsteede F.H.M., Greerts S., Klei T.R., Taylor M.A., Waller P.J., 1992. World association for the advancement of veter-inary parasitology (WAAVP) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. *Veterinary Parasitology*, 44, p. 35-44.

Essawi T. and Srour M., 2000. Screening of some Palestinian medicinal plants for antibacterial activity. *Journal of Ethnopharmacology*, 70, p. 343-349.

Hazzit M., Baaliouamer A., Verıssimo A.R., Faleiro M.L,. Miguel M.G., 2009. Chemical composition and biological activities of Algerian Thymus oils. *Food Chemistry*, 116, p. 714-721.

Hounzangbe-Adote M.S., Paolini V., Fouraste I., Moutairou K., Hoste H., 2005. *In vitro* effects of four tropical plants on three life-cycle stages of the parasitic nematode, *Haemonchus contortus*. *Res. Vet. Sci,* 78, p. 155-160.

Miura K and Nakatani N., 1989. Antioxidative activity of flavonoids from thyme (*Thymus vulgaris* L.). *Agricultural and biological Chemistry*, 53, p. 3043-3045.