



Nematicidal activity of methanolic flower extract of *Tagetes patula* against *Haemonchus contortus* worms

PALLAVI BHARDWAJ¹, C VARSHNEYA² and VINOD SHARMA³

CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur, Himachal Pradesh 176062 India

Received: 4 June 2014; Accepted: 26 November 2014

Key words: *Haemonchus contortus*, Nematicidal activity, Small ruminants, *Tagetes patula*

India with plant biodiversity of about 15,000–20,000 medicinal plants has enormous potential of developing plant based anthelmintics. Oil of *Tagetes patula* (marigold; local name – *Gainda*), grown abundantly as an ornamental plant in the Indian subcontinent, possesses larvicidal, fly repellent and other biological activities including nematicidal action (Reynolds *et al.* 2000). However antiparasitic action, especially against gastro intestinal nematodes, has not been systematically studied. *Haemonchus contortus*, one of the gastro intestinal nematodes having considerable economic importance in small ruminants (Bhat *et al.* 2011), is prevalent wherever sheep and goats are raised and causes the greatest economic losses in temperate and tropic regions (Chaudary *et al.* 2007). Katoch *et al.* (1999) have reported a high incidence (97.56%) of haemonchosis among sheep grazing at high altitudes of Himachal Pradesh, with sub-zero winter temperatures. Therefore, in the present paper, we studied the *in vitro* nematicidal action of methanolic extract of *Tagetes patula* flowers against *Haemonchus contortus* worms.

Fresh flowers of *Tagetes patula* were shade dried and powdered. Methanolic extract of *T. patula* was made by cold extraction procedure and the filtered extract was dried using rotary vacuum evaporator at 40°C. The color and percent recovery of the extract was recorded on dry weight basis (w/w). The residue was used for studying adulticidal activity against *Haemonchus contortus* worms and partial-purification of the bioactive constituent(s) by chromatographic techniques. The *in vitro* trials of the methanolic extract of *T. patula* (flowers) were conducted on active and motile mature *Haemonchus contortus* (female) worms of goats by petridish method (Singh *et al.* 2005). The efficacy of the extract was evaluated at different concentrations i.e. 1.0, 2.0, 4.0 and 8.0 mg/ml at 38.5°C. The inhibition of motility of worms was used as the main criteria for judging the anthelmintic activity. The motility

of worms was observed at hourly interval up to 5 h by gross examination and motility scoring was done as 0, immotile; +, dull; ++, low motility; +++, moderate motility; +++++, high motility. To ascertain the viability of motionless worms, pinch technique was used. The per cent mortality was calculated and anthelmintic activity was scored accordingly, as nil, 0%; very low, 1–20%; low, 20–40%; moderate, 40–60%; excellent, >80% mortality (upper limits are exclusive).

The crude extract was subjected to bioactivity-guided purification by silica gel column chromatography. The various fractions were collected using different solvents and were dried *in vacuo*. Following the testing of anthelmintic activity of the fractions eluted, an active elute was identified. Rechromatography of the active fraction was attempted for further bioactivity-guided purification. The residues were taken for assay of adulticidal activity and thin layer chromatography. Thin layer chromatographic analysis of crude methanolic extract and chromatographic fractions was done using solvent systems of different polarity to develop a system, which provided the best resolution of chemical constituents. TLC was done on glass plates using silica gel G and iodine as indicator (Stahl 1969). The data were analyzed statistically by Tukey-Kramer test using Graph Pad InStat.

The crude methanolic extract of flowers of *Tagetes patula* was dark brown in appearance and showed 23.20 % recovery. The adult untreated worms in control group showed high motility with no mortality till 5 h of incubation. In the test groups, worms exhibited high to moderate motility with no mortality in all the concentrations up to 3 h of incubation. The extract exhibited dose and time dependent increase in mortality with 81.0±4.50% and 83.33±9.62% anthelmintic activity at 4.0 and 8.0 mg/ml concentration respectively. An increase in concentration from 4 mg/ml to 8 mg/ml, however, did not show any significant change in adulticidal activity. The residue obtained from 7 fractions (F₁-F₇) collected during column chromatography of crude extract was subjected to *in vitro* trials at 2mg/ml concentration. The fractions F₁, F₂, F₃ and F₇ were ineffective, 5 h post incubation. Most of the bioactive constituent(s) was eluted in fraction (F₅)

Present address: ¹Assistant Professor (bpallavi@rediffmail.com), ²Dean PGS cum Professor and Head (cvarshneya@hotmail.com), Department of Veterinary Pharmacology and Toxicology; ³Assistant Professor (vinodsharma@gmail.com), Department of Animal Nutrition.

Table 1. *In vitro* anthelmintic activity of the rechromatography fractions of the methanolic extract of *T. patula* against *H. contortus* at 2mg/ml concentration

Fraction* No.	Motility score		Per cent mortality	Adulticidal activity
	Pre-incubation	5 h post-incubation		
R4	++++	0	68.33±9.27 ^a	Moderate
R5	++++	+	13.33±6.67 ^b	Very low
R6	++++	++	0.0±0.0 ^b	Nil
R7	++++	+++	0.0±0.0 ^b	Nil
R8	++++	++++	0.0±0.0 ^b	Nil

Values are mean±SEM; Means with different superscripts vary significantly ($p < 0.001$) with each other.

*Anthelmintic activity for the fractions R1, R2 and R3 could not be determined for the want of sufficient sample.

(chloroform:methanol; 50:50) as evident from 71.66±11.66 % mortality, which was reduced to 50.00±0.0 % in fraction F6. Thus fraction F₅ was selected for further purification by rechromatography. The results of *in vitro* trials conducted on the fractions collected after rechromatography of fraction F₅ are presented in Table 1. Fraction R₄ eluted with chloroform:methanol (75:25) exhibited maximum anthelmintic activity with 68.33±9.27 % mortality at 5 h of incubation. The other fractions were ineffective. Anthelmintic activity for the fractions R₁, R₂ and R₃ could not be determined for the want of sufficient sample. For TLC, the solvent system chloroform:methanol:ethyl acetate (75:25:1) provided the best resolution. TLC profile, on exposure to iodine vapours revealed appearance of a distinct band with R_f value 0.782 in the crude extract and bioactive fraction R₄ having putative anthelmintic activity. This band was absent in inactive fractions.

The development of anthelmintic resistance to nematode parasites of sheep and goats is a serious problem in many parts of the world including India (Coles 1997, Ram *et al.* 2007). Resistance of *H. contortus* to various anthelmintics was reported from Haryana and Himachal Pradesh (Yadav 1990, Uppal *et al.* 1992). The plant derived anthelmintics offer a sustainable and environmentally accepted alternative to synthetic drugs. Several herbal products and plant based medicaments have been documented for the adulticidal activity against *Haemonchus contortus* (Bhardwaj *et al.* 2004, Eguale *et al.* 2011, Zhu *et al.* 2013). The nematocidal action of *T. patula* has been widely documented against plant nematodes (Faizi *et al.* 2011), however, there are apparently very less reports on the nematocidal action against *Haemonchus contortus* worm (Bhardwaj *et al.* 2010) and other important blood sucking nematodal parasites of the ruminants. Such study is particularly of great significance in veterinary medicine where cost and the ease with which drugs can be administered to livestock are of paramount importance. The TLC analysis of crude extract as well as different fractions was done using solvent systems of different polarity. The solvent system chloroform : methanol : ethyl acetate (75 : 25 : 1) provided the best

resolution and a distinct band with R_f value 0.782 was found in the crude extract and bioactive fraction R₄. As this band was absent in inactive fractions, thus the constituent(s) corresponding to the band seemed to be responsible for the anthelmintic activity. Similar type of investigation, on the methanolic extract of *Bauhinia variegata* leaves, was reported against *Haemonchus contortus* worm by Singh *et al.* (2005). Several other chromatographic constituents of varying polarity (Hatakeda *et al.* 1985) were isolated from *T. patula* flowers, leaves and roots. Bioassay-guided isolation studies on the extracts of yellow flowers of *Tagetes patula* L. against the *Heterodera zaeae* has identified phenolic compounds and a few fatty acids, their methyl esters, and thiophenes in polar and nonpolar extract, respectively (Faizi *et al.* 2011). Our findings corroborated with earlier workers who observed maximal nematocidal action in the methanolic extract of *Tagetes* spp (Ray *et al.* 2002, Bhardwaj *et al.* 2010).

In this study we may conclude that the methanolic flower extract of *Tagetes patula* possesses promising anthelmintic activity against adult *Haemonchus contortus* worms under *in vitro* conditions. Partial purification of the crude methanolic extract using silica gel chromatography and rechromatography revealed the appearance of a bioactive constituent with R_f value of 0.782 in TLC profile and therefore there is a need of further purification of the probable bioactive phytochemical(s) which can lead to the development of an effective green anthelmintic.

SUMMARY

The present study investigates the nematocidal activity of the methanolic flower extract of *Tagetes patula* against *Haemonchus contortus* worms and partial purification of the bioactive constituent(s). The crude methanolic extract exhibited 81.0±4.50% and 83.33±9.62% anthelmintic activity at the concentration of 4.0 and 8.0 mg/ml respectively. The fraction F5 (chloroform:methanol, 50:50) and fraction R4 (chloroform: methanol 75:25) were found to possess maximal adulticidal action against the test parasite at 2 mg/ml concentration. In TLC profile a probable bioactive constituent with R_f value 0.782 in the solvent system chloroform:methanol:ethyl acetate (75:25:1) was detected.

ACKNOWLEDGEMENT

The work was supported by a Research Grant from National Agricultural Technology Project, New Delhi, India.

REFERENCES

- Bhardwaj P, Varshneya C and Mittra S. 2010. Anthelmintic efficacy of the methanolic extracts of *Bauhinia variegata* and *Tagetes patula* against the exogenous stages of *Haemonchus contortus*. *Indian Veterinary Journal* 87(12): 1204–06.
- Bhardwaj P, Varshneya C, Singh A and Telang R S. 2004. *In vitro* effects of leaf extract of *Mentha spicata* on eggs and infective third stage larvae of *Haemonchus contortus*. *The Indian Journal of small ruminants* 10(2): 163–65.

- Bhat S A, Mir M R, Allaie S Q, Khan H M, Husain I and Ali A. 2011. Comparative resistance of sheep breeds to pasture infection in Jammu and Kashmir. *Global Veterinaria* **8**: 222–28.
- Chaudary R F, Khan F M and Qayyum M. 2007. Prevalence of *Haemonchus contortus* in naturally infected small ruminants grazing in the Potohar areas of Pakistan. *Pakistan Veterinary Journal* **27**(2): 73–79.
- Coles G C. 1997. Nematode control practices and anthelmintic resistance on British sheep farms. *Veterinary Record* **141**: 91–93.
- Eguale T, Tadesee D and Giday M. 2011. *In vitro* anthelmintic activity of crude extracts of five medicinal plants against egg-hatching and larval development of *Haemonchus contortus*. *Journal of Ethnopharmacology* **137**: 108–13.
- Faizi S, Fayyaz S, Bano S, Iqbal E Y, Lubana and Siddiqi H. 2011. Isolation of nematicidal compounds from *Tagetes patula* L. yellow flowers: structure-activity relationship studies against cyst nematode *Heterodera zae* infective stage larvae. *Journal of Agricultural and Food Chemistry* **59**: 9080–93.
- Hatakeda K, Snito N, Asano T and Kusima Y. 1985. A new nematicidal compound in French Marigold. *Japanese Journal of Nematology* **15**: 11–13.
- Katoch R, Mandial R K and Nagal K B. 1999. Outbreak of *Haemonchus contortus* in sheep of Himachal Pradesh. *Indian Veterinary Journal* **76**(10): 932–38.
- Ram H, Rasool T J, Sharma A K, Meena H R and Singh S K. 2007. Comparative efficacy of different anthelmintic against fenbendazole-resistant nematode of pashmina goat. *Veterinary Research Communications* **31**: 719–23.
- Ray D, Prasad D, Singh R P and Ray D. 2002. Chemical examination and antinematic activity of marigold (*Tagetes erecta* L.) flowers. *Annals of Plant Protection Sciences* **8**(2): 212–17.
- Reynolds L B, Potter J W and Ball Coelho B R. 2000. Crop rotation with *Tagetes* sp. is an alternative to chemical fumigation for control of root lesion nematodes. *Agronomy Journal* **92**(5): 957–66.
- Singh A, Bhardwaj P, Varshneya C and Telang R S. 2005. Anthelmintic activity of leaves of *Bauhinia variegata*. *Indian Veterinary Journal* **82**(8): 855–57.
- Stahl E. 1969. *Thin Layer Chromatography - A Laboratory Hand book*. Springer-Verlag, Berlin.
- Uppal R P, Yadav C L, Godara P and Rana J S. 1992. Multiple anthelmintic resistance in field strain of *Haemonchus contortus* in goats. *Veterinary Research Communication* **16**: 195–98.
- Yadav C L. 1990. Fenbendazole resistance in *Haemonchus contortus* of sheep. *Veterinary Record* **126**: 586.
- Zhu L, Dai J L, Yang L and Qiu J. 2013. *In vitro* ovicidal and larvicidal activity of the essential oil of *Artemisia lancea* against *Haemonchus contortus*. *Veterinary Parasitology* **195**: 112–17.