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

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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 – *Gaimda*), 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 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 83.3±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 2 mg/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)
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 RF 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 nematicidal 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 RF value 0.782 in the solvent system chloroform:methanol:ethyl acetate (75:25:1) was detected.

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**REFERENCES**


