

## Anthelmintic efficacy of aqueous extract of *Zanthoxylum armatum* DC. seeds against *Haemonchus contortus* of small ruminants

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**Abstract** *Haemonchus contortus* infection is the major hurdle in growth and productivity in small ruminants especially in tropical countries. Indiscriminate and long term use of currently available chemotherapeutic agents lead to development of resistance in microorganisms and residual problems in population. Therefore, present study was undertaken to evaluate the anthelmintic efficacy of aqueous extract of *Zanthoxylum armatum* DC. seeds against adult *H. contortus* worms of small ruminants. Proximate analysis of seed powder shows high crude protein, Cu, Zn and Ca concentration. Phytochemical ingredients of extract revealed high total phenolic, flavonoids and tannin contents. In vitro experimental trial revealed complete mortality of *H. contortus* worms at the concentration of 100 mg/ml at the time exposure of 8 h. At 50 mg/ml concentration the mortality at 6 and 8 h were  $45.45 \pm 4.55$  and  $63.64 \pm 4.54$  %. Median lethal concentration (LC<sub>50</sub>) at 6 and 8 h were 59.90 and 28.92 mg/ml, respectively. Levamisole at concentration of 0.5 mg/ml caused 50 % mortality at 2 h post exposure and 100 % mortality at 4 h post exposure. The results confirm the cidal effect of the aqueous extract of *Z. armatum* DC. seeds on adult *H. contortus* worms.

**Keywords** Anthelmintic · *Haemonchus contortus* · Phytochemistry · *Zanthoxylum armatum*

### Introduction

Haemonchosis has been reported as the most prevalent amongst gastrointestinal parasitic diseases affecting sheep and goats across all seasons, agro-ecological zones and production systems in India (Khajuria 2010). The drugs like fenbendazole, levamisole, morantel and ivermectin are routinely used for therapeutic and prophylactic purposes in sheep and goats. But, due to their free, indiscriminate and inappropriate use, the parasite is developing resistance against currently used anthelmintics (Alvarez-Sanchez et al. 2006, Easwaran et al. 2009, Domke et al. 2012). A new synthetic drug, monopantel of amino-acetonitrile derivative group has been developed with promising claim in experimental trials, but, marketing and practice as anthelmintic is in infancy stage (Sager et al. 2009). Therefore, it is important to establish a successful programme and to look for the search of alternative, newer, inexpensive, safe and environmental friendly antinematodal agent that may act for longer period, before resistance set in. Anthelmintic derived from plants used for treatment of parasitic infections in human and animals can use a major accessible and alternative source to minimize some of these problems has led to the resurgence in the use of medicinal plants to treat cases of parasitism in ruminants and much of the success has been achieved in this direction against a variety of parasites.

The screening of the relevant ethno-botanical literature, revealed that, seeds of the *Zanthoxylum armatum* DC. have promising anthelmintic property especially for roundworms (Mehta et al. 1981, Siddiqui and Garg 1990,

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Hounzangbe-Adote et al. 2005). The *Z. armatum* DC., a shrub to small tree of family Rutaceae is widely distributed in India from Jammu & Kashmir to North East states. In Jammu and Kashmir it is commonly known as Timbru. The plant is also found in Pakistan, Nepal, Bhutan and China up to an altitude of 1,500 m (The Wealth of India 1976, Sarver et al. 2009). The different parts of the plant are extensively used in indigenous system of medicine as a carminative, stomachic and anthelmintic. The fruits and seeds are employed as an aromatic tonic in fever and dyspepsia. An extract of the fruits is reported to be effective in expelling roundworms. The fruits have deodorant, disinfectant and antiseptic properties, and are used in dental troubles, and their lotion for scabies. The essential oil has antiseptic, disinfectant and the larvicidal potential against three medically important species of mosquito vectors, *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*. The ethanolic extract of *Z. armatum* DC. leaves possess hepatoprotective effect (Tiwary et al. 2007, Verma and Khosa 2010, Singh and Singh 2011). The plant contains various phytochemical constituents including alkaloids, sterol, phenolics, lignins, coumarins, terpenoids, flavonoids and their glycosides and benzenoids responsible for various activities (Ahmad et al. 1993). Based on the fact that the origin of many effective drugs is found in traditional treatment practices and *Z. armatum* DC. seeds have anthelmintic activity against round worms. Thus, the present study was drafted with the objective to evaluate phytochemical ingredients and anthelmintic efficacy of the aqueous extract of *Z. armatum* DC. seeds against the adult *Haemonchus contortus* worms of sheep and goats.

## Materials and methods

### Proximate analysis of seeds powder and preparation of extract

The seeds of *Z. armatum* DC. were purchased from the local shop after proper identification, cleaned of adulterants, dried and milled to a fine powder using an electric mixer grinder (Fig. 1). The seed powder was analyzed for the crude protein, minerals, calcium and phosphorus as per the standard protocol described in AOAC (1995). The trace minerals, zinc and copper were analysed in 1.0 g plant sample in polarized Zeeman atomic absorption spectrophotometer (Z-2300, HITACHI) as per standard method described Kolmer et al. (1951). The aqueous extract was prepared using standard protocol (Singh et al. 2012). The fruits powder was soaked in distilled water in 1:5 ratio in the glass flask and kept for 48 h at room temperature with intermittent shaking. After 48 h, the content was sieved through filter paper (2 mm). Filtrate was concentrated at



**Fig. 1** Seeds of *Zanthoxylum armatum* DC.

60 °C into semisolid viscous mass using vacuum rotary evaporator. The semisolid extract was stored in airtight container at refrigerated condition –20 °C till further uses.

### Phytochemical analysis of extract

The total phenolic and flavonoids contents in aqueous extract of seeds of *Z. armatum* DC. were determined (Savitree et al. 2004; Zhishen et al. 1999). Total phenolic content in extract was determined with the Folin–Ciocalteu reagent using gallic acid as a standard and the values were expressed as mg/g gallic acid equivalents (GAE). Similarly, quercetin was used as a standard for the estimation of the flavonoids and results were expressed as quercetin equivalents (mg quercetin/g dried extract). Non-tannin content was determined using insoluble polyvinyl-pyrrolidone (Makkar et al. 1993) and tannin content was calculated by subtracting non-tannin content from the total phenolic content in the extract (Velioglu et al. 1998).

### In vitro anthelmintic activity

The in vitro anthelmintic evaluation was conducted on mature, live *H. contortus* worms from sheep and goats, to determine the effect of aqueous extract of *Z. armatum* DC. seeds (Tariq et al. 2009). The worms were collected from the abomasa of freshly slaughtered sheep and goats, washed and finally suspended in Hank's balanced salt solution (HBSS). The 20–25 adult, actively moving *H. contortus* worms constituting approximately 100 mg of weight were exposed in triplicate in each petri dish containing 10, 25, 50 and 100 mg/ml concentration of the aqueous extract prepared in 5 ml of HBSS and HBSS alone for the negative control group. The levamisole at the rate of 0.5 mg/ml was used as reference drug of positive control. The petri dishes were kept in an incubator at 37 °C. The inhibition of motility, activeness and mortality of the

worms was observed at an interval of 0.5, 1, 2, 4, 6, 8 h. The number of motile (alive) and non motile (dead) worms were counted and recorded for each concentration. Death of worms was ascertained by absence of motility for an observation period of 30 s in the lukewarm fresh HBSS.

### Statistical analysis

The phytochemical determinations were conducted in triplicate and results were expressed as mean  $\pm$  SE. Statistical analyses were done by one-way ANOVA followed by Dunnett's test with  $P < 0.05$  as a limit of significance. Median lethal concentration ( $LC_{50}$ ) was calculated by the method of Probits using the programme SPSS 8.0 for windows at different hour intervals of each extract.

## Results and discussion

The proximate composition of seeds powder revealed high crude protein content and trace minerals like calcium, phosphorus, copper and zinc (Table 1). The phytochemical analysis of the aqueous extract revealed total phenolic content of  $3.67 \pm 0.07$  mg of gallic acid equivalent (GAE)/g of extract. The flavonoids content was  $37.52 \pm 4.04$  mg of Quercetin/g extract. The per cent mortality along with the observations on activeness, motility of adult *H. contortus* was recorded in each concentration of 10, 25, 50 and 100 mg/ml of aqueous extract at the intervals of 0.5, 1, 2, 4, 6 and 8 h, respectively and results have been presented in Table 2. The results revealed that parasites were very sluggish and movement was little at 6 h post exposure of 100 mg/ml concentration. The aqueous extract of *Z. armatum* DC. showed complete mortality of the adult *H. contortus* worms at the concentration of 100 mg/ml at the time exposure of 8 h. At 50 mg/ml concentration the mortality at 6 and 8 h were  $45.45 \pm 4.55$  and  $63.64 \pm 4.54$  %, whereas the respective values at 25 mg/ml concentration were  $27.27 \pm 4.55$  and  $36.36 \pm 2.68$ . The  $LC_{50}$  at 6 and 8 h were 59.90 mg/ml and 28.92 mg/ml, respectively. Levamisole at concentration of 0.5 mg/ml caused 50 % mortality at 2 h post exposure and 100 % mortality at 4 h post exposure. The results confirm the cidal effect of aqueous extract of *Z. armatum* DC. seeds on adult *H. contortus* worms.

A World Health Organization survey indicated that about 70–80 % of the world's population are still rely on non-conventional medicine, mainly of herbal source, for their primary healthcare (Chan 2003). The requirement of quality feed with ingredients having diversified activities against pathogenic microbes and parasites of gastrointestinal tract has now necessitated the holistic exploration of alternate unconventional plants/plant parts having

**Table 1** The Phytochemical ingredients in the aqueous extract and mineral composition in the seed powder of *Zanthoxylum armatum* DC.

Active ingredients	Quantity (unit)
Total phenolic content	$3.67 \pm 0.07$ mg of GAE/g of extract
Total flavonoids content	$37.05 \pm 4.04$ mg Quercetin/g of extract
Tannin content	$2.69 \pm 0.07$ mg of GAE/g of extract
Non-Tannin content	$0.98 \pm 0.14$ mg of GAE/g of extract
Crude protein <sup>a</sup>	$19.95 \pm 0.41$
Calcium (Ca) <sup>a</sup>	$0.88 \pm 0.10$
Phosphorus (P) <sup>a</sup>	$0.57 \pm 0.05$
Zinc (Zn)	$22.37 \pm 6.13$ ppm
Copper (Cu)	$7.83 \pm 1.21$ ppm

Values are expressed as mean  $\pm$  SE of three replicates

Values of copper and zinc are expressed as ppm

<sup>a</sup> Crude protein, calcium, phosphorus are expressed on dry matter basis

medicinal value for their incorporation in livestock ration. This needs the detailed analysis of plant for bioactive molecules, proximate contents and minerals having different role in restoring normal physiology and boosting defense mechanism through their nutritional potential. Therefore, the local herb was analyzed for proximate composition and also for mineral estimation. The findings of proximate analysis revealed that the *Z. armatum* DC. has considerable amount of crude protein and has potential of replacing protein ingredients of ration. However, in view of paucity of reports on this aspect of local herbs detailed comparison cannot be made with the previous works. The mineral therapy has a direct role through wormicidal activity as well as indirect role through strengthening of host defense, improving blood parameters, etc. Thus minimizes economic losses due to parasitism. The seed powder of *Z. armatum* DC. has high content of copper and zinc. Both the minerals have role in blood formation as well as in body defense through activation of various enzymes of synthetic and defense activity. The recent studies have shown that copper administered orally to sheep and goats has an anthelmintic effect against *H. contortus* with extended protection up to 8 weeks (Chartier et al. 2001, Waruiru et al. 2004, Burke et al. 2005). A similar study shows that copper treatment or supplementation reduced female worm length, number of eggs in utero and prolificacy of *H. contortus* resulting into reduced pasture infectivity (Martinez Ortis-de Montellano et al. 2007). The copper also have effect on other gastrointestinal nematodes of sheep and goats and can provide additional benefit of improvement in losses of growth rate and blood parameters in parasitic enzootic areas.

In vitro tests are used as preliminary studies for the search of new anthelmintics (Akhtar et al. 2000). In

**Table 2** Mean per cent of dead parasites  $\pm$ SD and LC<sub>50</sub> of different concentrations of *Zanthoxylum armatum* DC. seeds aqueous extract in an in vitro trial on adult *Haemonchus contortus* worms mortality at different time intervals

Conc. (mg/ml)	Time						
	1 h	2 h	3 h	4 h	6 h	8 h	
LC <sub>50</sub>	–	–	–	–	59.90	28.92	
100	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	22.73 $\pm$ 4.55	63.64 $\pm$ 4.54	100.00 $\pm$ 0.00	
50	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	13.64 $\pm$ 2.63	45.45 $\pm$ 4.55	63.64 $\pm$ 4.54	
25	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	22.73 $\pm$ 4.55	27.27 $\pm$ 4.55	36.36 $\pm$ 2.68	
10	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	
HBSS –ve	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	00.00 $\pm$ 0.00	
Levamisol (0.5 mg/ml)	00.00 $\pm$ 0.00	20.00 $\pm$ 4.00	50.00 $\pm$ 4.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	

Observations: Parasites were very sluggish and movement was very little at 6 h post exposure in 100 mg/ml concentration

these tests plant extracts are directly exposed in contact with the parasite to evaluate the effect of extract. In the present test of efficacy of the aqueous extract *Z. armatum* DC. seeds in different dose dependent concentrations revealed that the aqueous extract has good efficacy at the concentrations of 100 and 50 mg/ml at the exposure time of 6 and 8 h against the adult *H. contortus* worms of sheep and goat origin. Though, the efficacy was not comparable with the levamisole, the reference drug used for the control of the haemonchosis of sheep and goat in field conditions but research out is of considerable importance. The present finding is in agreement with the previous published research work (Hounzangbe-Adote et al. 2005). Seed extract also contains high concentration of total phenolic flavonoid and tannin contents. Increased dietary supplementation of these phytochemical ingredients also contributes in improving the immune defense of host to destroy or expel the parasites. Further, these ingredients protect the cellular damage to cell membrane lipid, protein and nucleic acid caused by parasites due to their strong scavenging property of free radicals or reactive oxygen species (Atoui et al. 2005).

The high nutritional value as indicated in proximate and phytochemical analysis, the presence of rational concentrations of copper and zinc, which have direct wormicidal and indirect immune boosting potential. Therefore, the plant extract may be used for the in vivo trials for the control of haemonchosis in sheep and goats. The presence of activity in aqueous extract with high phenolic and flavonoid contents additionally allows widespread application of research outcome of the study.

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