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Anthelmintic Activity and Phytochemical Analysis of *Chenopodium album* against *Haemonchus contortus*

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Research Article

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ABSTRACT

Anthelmintic activity of crude powder, aqueous, diethyl ether and methanol extracts of Chenopodim album leaves were tested at different concentrations. All extracts showed 100% percent mortality of the worms at 0.5%, 1% and 2% concentrations. Maximum corrected mortality of 100% was observed in crude powder and aqueous extract at 2% concentration. Overall crude powder and aqueous extracts showed better anthelmintic activity than diethyl ether and methanol extracts. Phytochemical analysis of aqueous extract of C. album reveals the presence of alkaloids, saponins, tannins, flavonoids, sterols resins and triterpenes. However, methanol and diethyl ether extracts were found positive for saponins, tannins and proteins only.

INTRODUCTION

Gastrointestinal (GI) nematodosis is a common problem of small ruminants throughout the world including India. It is caused by mixed infection of GI nematodes that causes severe anaemia, damage to the gastric mucosa and villous atrophy resulting into death of the animals. Among various GI nematodes, *Haemonchus contortus* is a predominant and highly pathogenic G.I. nematode responsible for impaired productivity in small ruminants throughout the world [1,2]. In general, GI nematodosis is controlled by the use of chemical drugs that belong to benzimidazole, imidothiazole and avermentin groups. Farmers or veterinarians often treat infected animals, with chemical drugs, which showed non-specific clinical signs like diarrhoea or are found positive on fecal examination without estimation of intensity of infection. This has led to indiscriminate and frequent use of these drugs resulting in the emergence of drug resistance [3]. The rapid emergence of resistance to these drugs associated with high cost, food residue and environmental pollution have compelled researchers to switch over to discover new agent from natural origin as an alternative source of chemical drug. Plant material is the only option to meet out this goal. Therefore, exploitation of plant having anthelmintic activity is prerequisite in this direction.

Chenopodium album belonging to family Chenopodiaceae commonly known as Bathua is widely distributed in tropical and sub-tropical parts of India. It is commonly found in Gujarat, Haryana, Uttar Pradesh, Himachal Pradesh, Madhya Pradesh, Karnataka, Maharashtra, Rajasthan, West Bengal, Sikkim and Jammu & Kashmir. A number of uses have been reported for *C. album*. The leaves may be taken in the form of an infusion or decoction as a laxative and anthelminthic. It has also been recommended for the treatment of hepatic disorders and splenic enlargement [4]. The finely powdered leaf is used as a dusting powder to allay irritation about the external genitalia of children [5]. It is also used in abdominal pains, eye disease, throat troubles, piles, diseases of the blood, heart and spleen and biliousness [6]. The aim of present study was to evaluate the *in-vitro* effect of various extracts of *C. album* leaves on adult *H. contortus*.

METHODS & MATERIALS

Collection and processing of plant material

Leaves of *Chenopodium album* were collected from local herbs situated in and around Pantnagar. The leaves of selected plant were cleaned manually by removing the coarse impurity by hand and blowing the air to remove the dust and fine impurities, these were then shade dried in laboratory and further dried in incubator at 390ºC for 6 hours to remove moisture; if any. The leaves were grinded in electric grinder machine at room temperature to obtain coarse powder, which were used for extraction. Three extracts viz. cold aqueous, methanol and diethyl ether were prepared for evaluation of anthelmintic activity.

Preparation of organic solvent extract

50 gm powder was taken for each solvent viz. methanol and diethyl ether extracts and soaked in 400 ml of the respective solvent and stirred properly at every one hour interval in clean glass beaker covered with aluminium foil at room temperature. These were later filtered through several layers of muslin cloth and using separating funnels. The filtrate was concentrated by evaporation at lower temp. (40-500°C) and reduced pressure by using rotatory vacuum evaporator at 50-550°C [7].

Preparation of aqueous extract

50 gm of powdered sample was soaked in 400 ml. of distilled water and stirred every one hour interval initially for 2-3 times and left undisturbed for 8 hrs at room temperature and then filtered through muslin cloth and separating funnel. Then after, filtrate was concentrated by using rotatory vacuum evaporator at 50-550°C.

Evaluation of anthelmintic efficacy

Adult *Haemonchus contortus* worms were procured from the abomasii of freshly slaughtered goat. Then after they were kept in wide mouth container having lukewarm normal saline and brought to laboratory. The motile worms were cleaned with lukewarm normal saline solution. The cleaned worms were transferred in beaker containing Lock's solution at 370°C [8]. Different concentrations viz. 0.25%, 0.5%, 1% and 2% of various extracts were prepared in Lock's solution for evaluation of their anthelmintic activity. Ten adult *H. contortus* were taken in each small petridishes having different dilutions of test extract in Lock's solution viz. 0.25%, 0.5%, 1% and 2%.

Total volume of each petridishes was kept at 15 ml. Exclusive 15 ml Locks solution was taken as control. It was then incubated at 370°C ± 10°C for hours and number of live and dead adult worms was counted at 2, 4, 6, 12, 18 and 24 hours interval. The minimum lethal time for all the ten worms in each extract was recorded. The viability of the worms was determined by pinch technique as described by Neogi et al., [9] and Eguale and Giday, [10]. The corrected mortality for each extract was calculated by taking into account the mortality of worms, if any, in the Locks solution. Corrected mortality was calculated as per the formula [11].

$$\%\,\text{corrected morality} = \frac{\text{Total morality} - \text{Control morality}}{\text{Total morality}}\ \text{X}100$$

Phytochemical analysis of extract residue

The various extracts residue of *C. album* were analysed to detect twelve major phytochemical groups viz. alkaloids, anthraquinones, flavonoids, saponins, tanins, sterols, reducing sugars, glycosides, resins, triterpenes, proteins and coumarins by standard method [12-14].

Data analysis

Statistical analysis of the data was performed using two way anova [15].

RESULTS & DISCUSSION

The anthelmintic activity of different extracts of *Chenopodium album* at various concentrations is presented in **Tables 1-4.** Crude powder showed 80, 100,100 and 100% anthelmintic activity at 0.25%, 0.5%, 1% and 2% concentrations with percent corrected mortality of 25, 40, 50 and 100, respectively whereas aqueous extract showed 100 percent mortality in all concentrations with percent corrected mortality of 40, 50, 50 and 100, respectively.

Methanol extract showed 60,100, 100 and 100 percent mortality at 0.25%, 0.5%, 1% and 2% concentrations with corrected mortality of 0, 50, 50 and 50%. However, diethyl ether extract showed 80%, 100%, 100% and 100% mortality at 0.25%, 0.5%, 1% and 2% concentrations with percent corrected mortality of 25, 50, 50 and 50, respectively. The two way ANOVA analysis revealed non-significant differences between concentrations and different extracts of the plant. The dose dependent anthelmintic activity of aqueous and hydroalcoholic extracts of *Chenopodium ambrosiodes* against *H. contortus* has also been reported by Eguale and Giday [10]. Anthelmintic activity of Chenopodium has also been reported by Ketzis [16] and Jabbar A et al. [17].

Table 1. *In-vitro* effect of *Chenopodium album* extracts at 0.25% (2.5 mg/ml) on *Haemonchus contortus.*

Treatment	No. of parasites exposed	Time of exposure (number of parasite found dead)								
		2 hrs	4 hrs	6 hrs	12 hrs	18 hrs	24 hrs	30 hrs	% corrected mortality	% corrected mortality
Crude powder	10	0	0	0	0	3	8	8	80	25
Cold aqueous	10	0	0	0	0	5	8	10	100	40
Methanol	10	0	0	0	0	5	6	6	60	0
Diethyl ether	10	0	0	0	0	7	8	8	80	25
Negative control	10	0	0	0	0	2	5	6	60	-

Table 2. *In-vitro* effect of *Chenopodium album* extracts at 0.5% (5 mg/ml) on *Haemonchus contortus.*

Treatment	No. of parasites exposed	Time of exposure (number of parasite found dead)								
		2 hrs	4 hrs	6 hrs	12 hrs	18 hrs	24 hrs	30 hrs	% corrected mortality	% corrected mortality
Crude powder	10	0	0	0	0	2	4	10	100	40
Cold aqueous	10	0	0	0	2	4	10	-	100	50
Methanol	10	0	0	0	8	8	10	-	100	50
Diethyl ether	10	0	0	0	0	0	10	-	100	50
Negative control	10	0	0	0	0	2	5	6	60	-

Table 3. *In-vitro* effect of *Chenopodium album* extracts at 1% (10 mg/ml) on *Haemonchus contortus.*

Treatment	No. of parasites exposed	Time of exposure (number of parasite found dead)								
		2 hrs	4 hrs	6 hrs	12 hrs	18 hrs	24 hrs	30 hrs	% corrected mortality	% corrected mortality
Crude powder	10	0	0	0	8	8	10	-	100	50
Cold aqueous	10	0	0	8	8	9	10	-	100	50
Methanol	10	0	0	4	6	9	10	-	100	50
Diethyl ether	10	0	0	0	4	7	10	-	100	50
Negative control	10	0	0	0	0	2	5	-	50	-

Table 4. *In-vitro* effect of *Chenopodium album* extracts at 2% (20 mg/ml) on *Haemonchus contortus.*

Treatment	No. of parasites exposed	Time of exposure (number of parasite found dead)								
		2 hrs	4 hrs	6 hrs	12 hrs	18 hrs	24 hrs	30 hrs	% corrected mortality	% corrected mortality
Crude powder	10	0	0	4	10	-	-	-	100	100
Cold aqueous	10	0	4	10	-	-	-	-	100	100
Methanol	10	0	0	4	6	7	10	-	100	50
Diethyl ether	10	0	6	6	6	8	10	-	100	50
Negative control	10	0	0	0	0	2	5	-	50	-

Phytochemical analysis of various extracts residue is presented in **Table 5.** Phytochemical analysis of aqueous extract of *C. album* reveals the presence of alkaloids, saponins, tannins, flavonoids, sterols, resins and triterpenes. However, methanol and diethyl ether extracts were found positive for saponins, tannins and proteins only. These findings are partially in agreement with the finding of Nedialkova et al. [18] where they reported phenolics, flavonoids, saponins, ecdysteroids and triterpenoids were the major phytoconstituents of the genus. Silveira et al. [19] found that about 60% of plant material is composed of liquid, containing mostly sapogenins, the nonglycosidic portion of saponins, having significant anthelmintic activity. Tannins are responsible for interfering with energy generation in helminthic parasites by uncoupling oxidative phosphorylation and binding to glycoprotein on the cuticle of the parasite, thereby may cause death [20]. Alkaloids isolated from higher plants, have been used as anthelmintic agents [21]. A wide variety of alkaloids have shown a potent activity on parasitic protozoa and on several helminthes species *in vitro* and some specifically inhibit essential enzymatic systems in parasites. Alkaloids also play an important role in inhibition of mobility (larval paralysis) and low cytotoxicity.

Table 5. Phytochemical groups present in extracts residue of *C. album.*

Phytochemical groups	Cold aqueous	Diethyl ether	Methanol
Alkaloids	+	-	-
Anthraquinone	-	-	-
Flavonoids	+	-	-
Saponins	+	+	+
Tannins	+	+	+
Sterols	+	-	-
Reducing sugars	-	-	-
Glycosides	-	-	-
Resins	+	-	-
Triterpenes	+	-	-
Proteins	-	+	+
Coumarins	-	-	-

In the present study, crude powder and aqueous extract showed 100 percent corrected mortality at 2% concentration. It could be due to synergistic effects of alkaloids, saponins, tannins, flavonoids, sterols resins and triterpenes. *Chenopodium album* may be used as an alternative treatment of gastrointestinal nematodosis in small ruminants under field conditions.

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