

Assessment of *in vitro* anthelmintic activity of *Heracleum afghanicum* Kitamura leaves

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Abstract

Aim and Objective: The present study was carried to determine the anthelmintic activity of *Heracleum afghanicum* Kitamura (Umbelliferae) collected from the mountainous region of Karmi, Afghanistan. **Material and Methods:** Different extracts of *H. afghanicum* Kitamura were screened for anthelmintic activity against Indian earthworm *Eisenia foetida*. Different concentrations (2–10 mg/ml) of various extracts were tested, and results were expressed in terms of time for paralysis and time for the death of worms. Albendazole (20 mg/ml) was used as the reference standard and normal saline (0.9%) as a control group. **Results and Discussion:** Dose-dependent activity was observed in the plant extracts. Ethyl acetate extract was found to exhibit more activity as compared to others. The anthelmintic activity of *H. afghanicum* Kitamura leaves extract has therefore been demonstrated for the first time. The efficacy of the extracts may be due to the presence of various phytoconstituents. Further studies on isolation of phytoconstituents and their biological activities *in vitro* and *in vivo* are to be carried out.

Key words: Antimicrobial, anthelmintic, *Heracleum afghanicum* Kitamura, Umbelliferae

INTRODUCTION

Helminths contaminations, over and over entitled helminthiasis, are among the most inescapable contamination and a preeminent degenerative malady upsetting a vast extent of world's populace. In creating nations, they represent a substantial risk to general well-being and add to the pervasiveness of lack of healthy sustenance, frailty, eosinophilia what's more, pneumonia.^[1] The helminths parasites predominantly subsist in the human body in the intestinal tract, but they are likewise found in tissue, as their hatchlings relocate toward them.^[2] Most infections caused by helminths are of an incessant, incapacitating nature; they presumably cause greater grimness and more prominent financial and social hardship among people and creatures than any single gathering of parasites. Chemical control of helminths combined with the enhanced administration has been the imperative worm control system all over the world.^[3,4] In any case, advancement of resistance in helminths against regular anthelmintics is a preeminent issue in the treatment of helminths infections.^[5,6]

From this time forward, it is imperative to search for alternative strategies against gastrointestinal nematodes, which have prompted the proposition of screening therapeutic plants for their anthelmintic action. *Heracleum afghanicum* Kitamura belonging to the genus *Heracleum*, family Umbelliferae, is an endemic plant of Afghanistan and grows wildly in different cold and mountainous regions of the country.^[8,9] It is a fast-growing perennial robust herbaceous plant, with an erect hollow stem up to 5 cm in diameter at the bottom and up to 1.5 m tall. Its leaves are alternate, petiolate, large, broad, and having 5–7 lobes. Its flowers are white and aggregated in large flat and compound umbels up to 30 cm in diameter. The marginal flowers of the umbel are larger than those

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located in the center. The plant starts blooming from April to August according to climatic condition of its natural habitat. The fruits are obovate achenes up to 8 mm long and often mature in July and August. Fruit ripening also depends on climatic conditions. Local rural people call *H. afghanicum* by several vernacular names such as Balderghan,^[8] Balder ghoo, Sofi, and SafidSarak (personal communication with common villagers).

The plant grows abundantly in some regions of different provinces of Afghanistan, which have aforesaid criteria or geoclimatic specification such as Schibar Pass of Badakhshan and north slope of Hindu Kush^[8,9].

The volatile oil present in the fruits of the plant has been reported to have a sedative effect on mice. The young stems are also used as a digestive aid. *H. afghanicum* leaves are used as a pain killer and antipyretic.^[8]

MATERIALS AND METHODS

Plant Material

The leaves of *H. afghanicum* leaves were collected in July from Holang of Parwan, Afghanistan, and were authenticated by Dr. Noor Ahmed Mirazai, Head of Biology, Kabul University, Afghanistan. A voucher specimen has been kept in the herbarium (4500/KU-005255 and 31939-II/KU-5267) of the Department of Botany, KUFs Herbarium (Afghanistan).

Experimental Worm

All the experiments were carried out on Indian adult earthworms (*Eisenia foetida*) due to its anatomical resemblance with the intestinal roundworm parasites of human beings. They were procured and authenticated from Mahavir organic manure, Jalandhar, and washed with water to remove all fecal matters.

Extracts Used

Five extracts were used, namely, petroleum ether, dichloromethane, ethyl acetate (EA), methanol, and aqueous extracts which were prepared by adopting the successive solvent extraction method using the Soxhlet apparatus.

Administration of Standard

Albendazole (ALBZ) oral suspension IP 200 mg/5 ml was procured from local pharmacy and was diluted with normal saline to prepare a suspension of 20 mg/ml as stock suspension. The suspension was then used in the preparation of working suspensions of different concentrations (2–10 mg/ml) as standards in anthelmintic assay.

Administration of Extract

Stock solution of the different extracts was prepared at a concentration of 200 mg/ml in dimethyl sulfoxide. The dilution of aqueous extract (200 mg/ml) was prepared in normal saline (0.9% NaCl solution). The prepared stock solutions were stored at 4°C in refrigerator, until being used in the preparation of diluted working formulations used in anthelmintic activity assay.

Experimental Design

Test samples of all the four extracts were prepared in the concentrations 2, 4, 6, 8, and 10 mg/ml. Standard ALBZ dilutions at the same concentration range (2, 4, 6, 8, and 10 mg/ml) in normal saline were prepared and poured into 5 Petri plates (15 ml per plate), separately. Normal saline (15 ml/plate) and mixture of dimethyl sulfoxide (0.5 ml) with normal saline (14.5 ml) were placed into two separate Petri plates as negative controls (higher doses of dimethyl sulfoxide also exert anthelmintic effect on earthworms). The adult earthworms (*E. foetida*) having 6–10 cm length and 0.2–0.3 mm diameter were first rinsed with tap water to remove the clay and then were sorted into different groups of 3 worms of almost equal size in each group.

Then, in each Petri plate containing 15 ml of either standard/sample solutions or negative controls, one group of the worms (3 worms) was released. The earthworms were observed individually at room ambience for recording the time (in minute) taken for paralysis and the time taken for death of each individual worm. Paralysis of the worms was confirmed when they did not show sensitivity in terms of motion, contraction, or bending, except when pressed vigorously by a finger at one of their ends. The death of the worms was confirmed by complete loss of their motility even by keeping them in warm water at 50°C and subsequently fading of their body color.^[7,10] Mean time for paralysis was noted when no movement could be observed, except when the worm was shaken vigorously. The time death of worm (min) was recorded after ascertaining that worms do not show any movement when shaken or external stimuli were given. The test results were compared with the reference compound ALBZ (20 mg/ml)-treated samples.

The inhibition of motility (paralysis, Figure 1) and/or mortality (Figure 2) of the earthworms subjected to the treatment were considered as the criteria for the anthelmintic activity of tested extract.^[7,10] For all tested samples, the experiments were carried out in triplicates and the results (paralysis time [PT] and death time) were recorded as mean±standard deviation of the three experiments ($n = 3$).

RESULTS AND CONCLUSION

The results of the present study clearly indicated that the crude EA extract of *H. afghanicum* did produce anthelmintic

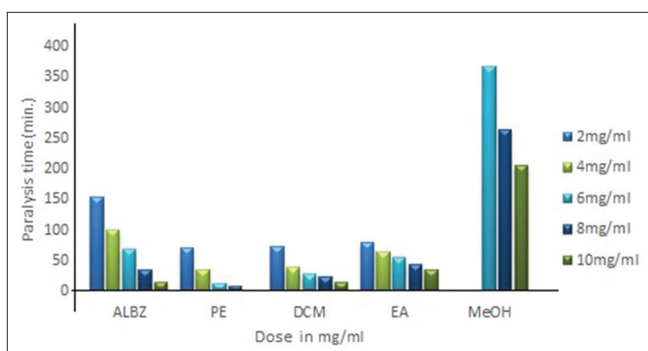


Figure 1: Bar diagram indicating the time taken for paralysis of *Eisenia foetida* during anthelmintic assay, ALBZ: Albendazole, PE: Petroleum ether, DCM: Dichloromethane, EA: Ethyl acetate, MeOH: Methanol

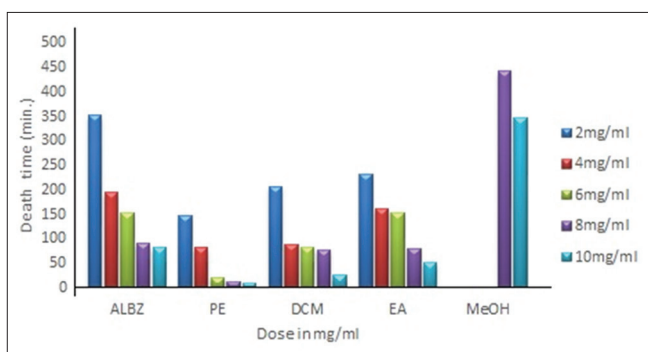


Figure 2: Bar diagram indicating the time taken for the death of *Eisenia foetida* during the anthelmintic assay. ALBZ: Albendazole, PE: Petroleum ether, DCM: Dichloromethane, EA: Ethyl acetate, MeOH: Methanol

activity against Indian earthworm *E. foetida*. All of the tested extracts (except the aqueous extract) cause paralysis and subsequent death of the worms (Table 1). The EA extract possesses significant anthelmintic activity at 10 mg/ml concentration measured by the time taken for paralysis/death of the earthworms. At dose 10 mg/ml, EA extract was almost 3.5-fold potent than ALBZ suspension used at the same dose against the earthworms. PT for the EA extract was recorded 4.83 ± 0.17 min, respectively, at dose 10 mg/ml while for standard ALBZ, PT was 14.9 ± 1.0 min. DCM extract produces comparable (13.7 ± 0.75 min) slightly stronger activity, but both petroleum ether and methanol extracts were found to have very weak anthelmintic activity against the tested worms. These data also indicated that anthelmintic action of *H. afghanicum* is totally due to potent lipophilic components of the crude drug extracted by EA extract, dichloromethane extract, and petroleum extract. The current investigation leads to the conclusion that the leaves of *H. afghanicum* have potent anthelmintic activity when compared with the standard drug. The results did not, however, exclude the possibility that doses of the extract with lower anthelmintic activity in this study might be efficacious against other species of helminths. Further studies using *in vivo* models and to isolate active constituents from extract are required to carry out and established the effectiveness and

Table 1: Anthelmintic activity

Concentration mg/ml	Standard					Extracts																			
	ALBZ					PE					DCM					EA					MeOH				
	PT (min)	DT (min)	PT (min)	DT (min)	DT (min)	PT (min)	DT (min)	PT (min)	DT (min)	DT (min)	PT (min)	DT (min)	PT (min)	DT (min)	DT (min)	PT (min)	DT (min)	PT (min)	DT (min)	DT (min)	PT (min)	DT (min)			
2	153.6±1.86	352.2±5.42	71.1±9.67	146.6±9.9	207.3±4.4	72.5±4.37	207.3±4.4	79.2±9.8	233.3±15.1	--	--	--	--	--	--	--	--	--	--	--	--	--	--		
4	100.7±3.8	194.2±1.26	34.7±2.62	82.1±2.34	89.5±4.67	38.8±2.59	89.5±4.67	64.7±1.8	162.7±5.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
6	68.6±5.29	152.8±3.4	12.6±0.60	21.6±0.67	83.2±2.22	27.2±1.26	83.2±2.22	54.6±2.6	153.2±5.06	368.5±2.22	368.5±2.22	368.5±2.22	368.5±2.22	368.5±2.22	368.5±2.22	368.5±2.22	368.5±2.22	368.5±2.22	368.5±2.22	368.5±2.22	368.5±2.22	368.5±2.22	368.5±2.22		
8	34.4±3.01	91.1±2.87	7.89±0.25	13.2±0.75	78.4±4.35	23.1±2.50	78.4±4.35	42.6±0.8	80.6±1.33	264.6±4.06	264.6±4.06	264.6±4.06	264.6±4.06	264.6±4.06	264.6±4.06	264.6±4.06	264.6±4.06	264.6±4.06	264.6±4.06	264.6±4.06	264.6±4.06	264.6±4.06	264.6±4.06		
10	14.9±1.06	82.1±8.23	584.83±0.17	10.3±0.29	25.7±0.96	13.7±0.75	25.7±0.96	33.8±1.8	53.1±1.35	206.6±8.35	206.6±8.35	206.6±8.35	206.6±8.35	206.6±8.35	206.6±8.35	206.6±8.35	206.6±8.35	206.6±8.35	206.6±8.35	206.6±8.35	206.6±8.35	206.6±8.35	206.6±8.35		

Values are expressed as mean±SD. PT: Paralysis time, DT: Death time *extracts were used in mg/ml, -: Not determined, SD: Standard deviation, EA: Ethyl acetate, ALBZ: Albendazole, PE: Petroleum ether, DCM: Dichloromethane, MeOH: Methanol

pharmacological rationale for the use of *H. afghanicum* as an anthelmintic drug.

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