

Original Research Article

Phytochemical and Invitro Antihelmintic Activity of Aqueous Polyherbal Seed Extract Against Indian Earthworms

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Abstract: The present study was to evaluate the phytochemical screening and anthelmintic activity of aqueous polyherbal seed extract (APSE) against *Pheretima posthuma*. The seeds of *Helianthus annuus*, *Cucurbita pepo*, *Linum usitatissimum*, *Citrullus lanatus* and *Cucumis melo* was identified and purchased from local market of Nuzvid. APSE was prepared from the dried seeds of five different seeds using the solvent water. Initially, APSE was screened for phytochemical constituents by standard methods. Further, anthelmintic study was conducted against *Pheretima posthuma*, collected from local Vermicomposting Farm, Nuzvid. In the phytochemical screening, APSE showed presence of glycosides, alkaloids, flavonoids, phenols, phytosterols and tannins. In the anthelmintic study, mortality was produced in earth worm populations by APSE. The use of APSE as an anthelmintic was confirmed by using standard method against *Pheretima posthuma*. The results indicated that the test drug has significant anthelmintic properties. Hence, it can be concluded that the APSE can be used as a novel drug for the treatment of worm infestations.

Keywords: Aqueous polyherbal seed extract (APSE), phytochemical screening, *Pheretima posthuma*, Anthelmintic activity, Albendazole.

INTRODUCTION

Helminthiasis is a worm infestation of humans and other animals even life stock and crops affecting health and food production respectively and have impact on global economic factor (Kumar, 2014). The worms which cause helminthiasis are called as helminths and the drugs which are used for treating helminthiasis are nothing but anthelmintics (Abongwa, 2017). There are various types of worms such as hook worms, fluke worms, round worms, tape worms which causes helminthiasis. The names are given according to their shapes. The major organs which get affected in helminthiasis are stomach and intestine and major symptoms of sever helminthiasis include diarrhea, abdominal pain, general malaise and impaired cognitive development. Chronic helminthiasis by hook worm lead to intestinal bleeding and anemia (Hedley, 2015). *Pheretima* is a genus of earthworms. *Pheretima posthuma* are long cylindrical shaped worms having length of 15-30cm. they are mostly found in moist soil and responsible for vegetables and humus. Their life span is 3 to 10 years (Kutschera, 2010) [1].

Sunflower (*Helianthus annuus* L.) belongs to the family Asteraceae. *Helianthus* genus contains 65 different species (Andrew *et al.*, 2013). The name *Helianthus*, being derived from *helios* (the sun) and *anthos* (a flower), has the same meaning as the English name Sunflower, which has been given these flowers from a supposition that they follow the sun by day, always turning towards its direct rays. The sunflower that most people refer to is *H. annuus*, an annual sunflower. In general, it is an annual plant which possesses a large inflorescence (flowering head), and its name is derived from the flower's shape and image, which is often used to depict the sun. The plant has a rough, hairy stem,

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broad, coarsely toothed, rough leaves and circular heads of flowers (Khaleghizadeh, 2011). The heads consist of many individual flowers which mature into seeds on a receptacle base (Seghatoleslami *et al.*, 2012) [2].

Pumpkin (*Cucurbita*) belongs to the family Cucurbitaceae generally grown in the regions of the globe as a vegetable. These are grown-up in the tropical and sub-tropical regions and including the cucumbers and squash. Worldwide there are three types of the pumpkins are present name as "*Cucurbita pepo*", "*Cucurbita maxima*" and "*Cucurbita moschata*" Lee *et al.*, For the purpose of vegetable and medicinal pumpkins are grown throughout world. In many countries the pumpkin has been conventionally used as remedy like China, Pakistan, India, Yugoslavia, Argentina, Mexican regions, America and Brazil Jia *et al.*, The pumpkin seeds are utilized for the cure of different diseases the herbal remedies separately or combine with medicines are used for the medical treatment. The pumpkin is the one of the famous edible plant that is utilized as the cure of many disorders due to the occurrence of many edible components and phytochemicals Yadav *et al.*, [3].

Flaxseed, or Linseed (*Linum Usitatissimum*), popularly known as Alsi, Jawas, Aksebija in Indian languages, (Anonymous, 2000). Flax (*Linum usitatissimum*) belonging to family Lineaceae, is a blue flowering annual herb that produces small flat seeds varying from golden yellow to reddish brown color. Flaxseed possesses crispy texture and nutty taste (Morris 2007; Rubilar *et al.*, 2010). The whole flaxseed is flat and oval with pointed tips, varies in color from dark to yellow, and measures approximately $2.5 \times 5.0 \times 1.5$ mm (Freeman, 1995). Flaxseed contains a seed coat or true hull (also called testa), a thin endosperm, two embryos, and an embryo axis. Embryos form 55% of the total weight of hand-dissected flaxseed, the seed coat and the endosperm account for 36% of the total weight, and the embryo axis is 4% (Bhatty, 1995). Flaxseed continues to surge forward in its recognition as a functional food, being rich in the essential omega-3 fatty acid, alpha linolenic acid and many phytochemicals. Flaxseed also provides dietary fiber and protein (flax primer) was a singled out as one of six nutraceuticals (Oomah 1995). Almost all parts of linseed plant are utilized for various purposes. Seed contains oil which after refining is used for edible purpose (Singh *et al.*, 2011a, b). The stem yields fiber of good quality possessing high strength and durability. Humans have been consuming flaxseed since ancient times. It has been cultivated for fiber as well as for medicinal purposes and as nutritional product (Tolkachev and Zhuchenko, 2000) [4].

Citrullus lanatus (water melon) produces a fruit that is about 93% water, hence the name "water" melon. The "melon" part came from the fact that the fruit is large and round and has a sweet, pulpy flesh. The scientific name of the watermelon is derived from both Greek and Latin roots. The *Citrullus* part comes from a Greek word "citrus" which is a reference to the fruit. The *lanatus* part is Latin, and has the meaning of being wooly, referring to the small hairs on the stems and leaves of the plant [5]. *Citrullus lanatus* (watermelon) family Cucurbitaceae is one of the largest fruit crop in the world, in 2013 watermelon global production approximated 109 million tons. Watermelon fruit is composed of flesh (68%), seeds (2%) and rind approximately 30% of the total mass of the fruit [5].

Musk melon is a beautiful, juicy, tasty and delicious fruit popular for its nutritive and medicinal properties. The Cucurbitaceae family includes squash, pumpkins, cucumbers, Musk melons, watermelons, and gourds. *Cucumis melo* (Cantaloupe or Musk melon) is one of the most important cultivated cucurbits, which is native to India and Africa. It is a spreading, annual, more or less hairy vine. It grows well in all the tropical and subtropical areas of the world, but prefers hot climate. Musk melon is recommended for the treatment of cardiovascular disorders, as a diuretic, stomachic, antitussive and as a vermifuge [6].

MATERIALS AND METHODS

Collection of Plant Material

The seeds of *Helianthus annuus*, *Cucurbita pepo*, *Linum usitatissimum*, *Citrullus lanatus* and *Cucumis melo* was identified and purchased from local market of Nuzvid.

Preparation of Aqueous Extract (Maceration Method) [7]

The 500gm (100gm/each) of dried poly herbal seeds were collected powdered to get a coarse powder and was kept for maceration with 1000 ml of distilled water for 7 days. The extract was double filtered by using muslin cloth and Whatman no.1 filter paper and concentrated by evaporation on water bath. The extract was dried and used.

Preliminary Phytochemical Screening [8-10]

The preliminary phytochemical investigation was carried out with aqueous polyherbal seed extract for identification of phytochemical constituents. Phytochemical tests were carried out by standard methods.

Test Organism [11]

Indian adult earthworms (*Pheretima posthuma*) were used during the experiment. The earthworms were collected from a local supplier. Worms were washed with normal saline to remove all fecal matter. The earthworms of 8-

10 centimeter (cm) in length and 0.2 -0.5 cm width were used for all the experiment protocol. Ready availability, anatomical and physiological resemblance of (*Pheretima posthuma*) made it to be used initially for *in-vitro* evaluation of anthelmintic activity. Time for paralysis was noted either when any movement could not be observed except when the worms were shaken vigorously. Death was included when the worms lost their motility followed by white secretions and fading away of their body colour.

Evaluation of Anthelmintic Activity

The anthelmintic activity was evaluated on adult Indian earthworm. The earthworms were randomly chosen and divided into 3 groups having five earthworms in each as follows:

Group I: Control Group.

Group II: Standard Group – Albendazole [12] -50,100, 200 mg/ml.

Group III: Test-I -Aqueous polyherbal seed extract [APSE- 50,100, 200 mg/ml].

Observations were made for the time taken by worms to paralyze and death was observed. Time for paralysis was noted when no movement could be observed with a slight pin prick method. Death was ascertained by applying external stimuli which stimulate and induce movements in worms as well as fade of the body color was noted.

Statistical Analysis

The values are expressed as mean± SEM. The statistical analysis was performed using one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. Comparisons were made between control group and test/standard groups. P-values <0.05 was considered statistically significant. The statistical analysis was done by using Graph pad prism version no: 6.0.

RESULTS AND DISCUSSION

In this study, we found that aqueous polyherbal seed extract possess the following chemical constituents (Table 1).

Table 1: Phytochemical screening of APSE

Phytochemical constituents	Aqueous polyherbal seed extract [APSE]
Alkaloids	+
Carbohydrates	+
Flavonoids	+
Phenols	+
Saponins	+
Terpenoids	+
Steroides	+
Tannins	+
Amino acids	-
Glycosides	+
Fixed oils and fatty acids	+

+ indicate the compulsory present and – indicate the absent.

APSE-Aqueous polyherbal seed extract

Anthelmintic Activity

The aqueous polyherbal seed extract produced a significant anthelmintic activity in dose dependent manner as shown in below table.

Table 2: Anthelmintic activity of aqueous polyherbal seed extract (APSE) & Standard drug on earth worm

Groups	Concentration	Time Taken in minutes	
		Paralysis (P)	Death (D)
Control		-	-
Standard (Albendazole)	50mg/ml	270.0±0.32	5290.8±1.07
	100mg/ml	207.4±0.87	281.6±0.93
	200 mg/ml	133.2±0.73	232.2±1.35
	300 mg/ml	86.0±0.84	111.0±0.63
	400 mg/ml	72.4±1.03	91.6±0.93
Test-I [APSE]	50 mg/ml	190.4±0.51	214.8±0.20
	100 mg/ml	155.4±0.24	305.4±0.25
	200 mg/ml	115.4±0.24	205.0±0.31
	300 mg/ml	44.0±0.63	100.4±0.67
	400 mg/ml	36.4±0.87	81.2±0.46

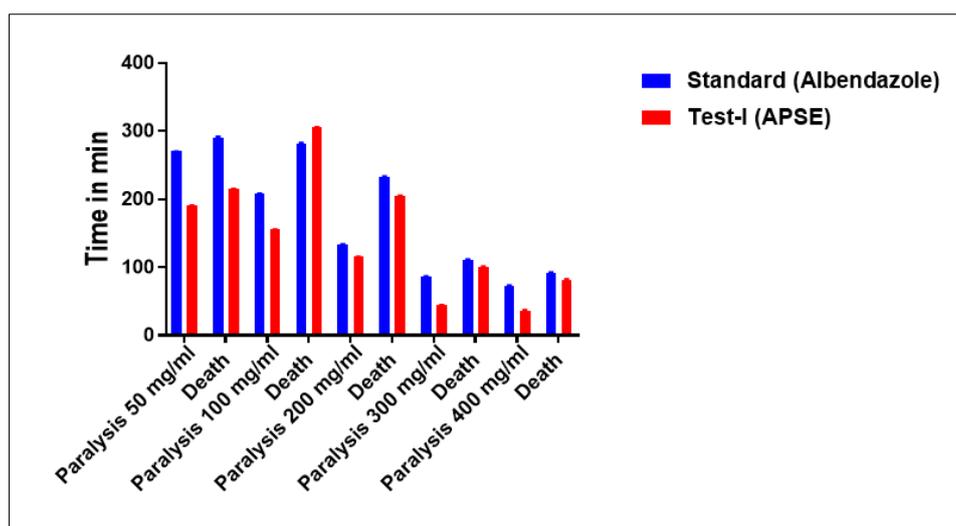


Figure 2: Anthelmintic activity of standard, APSE, Values are expressed as Mean \pm SEM, $P < 0.0001$

CONCLUSION

In the present investigation, aqueous polyherbal seed extract possess the presence of alkaloids, carbohydrates, saponins, tannins, flavonoids, phenols, terpenoides, steroides, glycosides and fixed oils & fats. Tannins are chemically polyphenolic compound and where shown to produce anthelmintic activities and reported the effect of tannin can bind to free proteins in gastro intestinal tract of host animal or glycoproteins on the cuticle of parasite and may cause death. These facts suggest that tannins present in the aqueous polyherbal seed extract showed the antihelmintic effect by above mentioned mechanisms. From the result shown in table-2 aqueous polyherbal seed extract showed anthelmintic activity in dose dependent manner giving shortest time of paralysis and death. From the anthelmintic activity study, the aqueous polyherbal seed extract at a dose of 100 mg/ml has significant anthelmintic activity where as 50mg/ml has showed moderate activity. The aqueous polyherbal seed extract at normal concentration i.e. 50 mg/ml to higher concentration i.e. 400mg/ml showed good anthelmintic activity and this is compared with effect produced by reference standard drug albendazole. The aqueous polyherbal seed extract demonstrated paralysis as well as death of worms in a less time as compared to albendazole especially at higher concentration of 200 mg/ml. The study finally concluded aqueous polyherbal seed extract showed marked and potent anthelmintic activity than the standard drug albendazole.

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