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Original Research Article

Phytochemical and Invitro Antihelmintic Activity of Aqueous Polyherbal Seed Extract against Pheretima Postuma

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Abstract: The present study was to evaluate the phytochemical screening and anthelmintic activity of aqueous polyherbal seeds extract (APSE) against Pheretima posthuma. The seeds of Coriandrum sativum, Sesamum indicum, Glycine max, Momordica charantiana trachyspermum ammi, was identified and purchased from local market of Nuzvid. APSE was prepared from the dried seeds of five different plants using the solvent water. Initially, APSE was screened for phytochemical constituents by standard methods. Further, antihelmintic 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. The data were found statistically significant by using one way ANOVA ($P< 0.0001$). 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 has impact on global economic factor. The worms which causes helminthiasis are called as helminths and the drugs which are used for treating helminthiasis are nothing but anthelmintic. 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. 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 [1].

Coriandrum sativum which is commonly known as "dhaniya" belongs to the family Apiaceae (Umbelliferae). It is mainly cultivated through its seeds [2], sativum seeds showed the presence of 33 compounds, including monoterpenoid, monoterpenoid glycosides and glucosides, and aromatic compound glycosides such as norcarotenoid glucoside [3].

Sesame belongs to the genus Sesamum, which comprises about 20 species a few other species in the genus are occasionally cultivated for their edible seeds [4]. Sesame (Sesamum indicum L.) (Family: Pedaliaceae), one of the oldest oilseed crops, has been widely cultivated in Africa and Asia and is one of the most important oilseed crops in Vietnam [5].

Lignans refer to compounds originating from C6C3 units and possess two β, β0 linkages (8–80 bonds). Phytosterols, including sterols and stanols, are triterpenoids found in plants [6].

Carom seeds commonly called as with Genus *Trachyspermum* and species *ammi* family Apiaceae is commonly famous as Ajwain [7]. Ajwain seed analysis has revealed it to contain fiber (11.9%), carbohydrates (38.6%), tannins, glycosides, moisture (8.9%), protein (15.4%), fat (18.1%), saponins, flavone and mineral matter (7.1%) containing calcium, phosphorous, iron and nicotinic acid. Ajwain fruits yield 2% to 4% brownish essential oil, with thymol as the major constituent (35% to 60%) [8].

Soyabean with genus name *Glycine* species *max* and family Fabaceae [9]. The most common phytochemicals in food include polyphenols, flavonoids, isoflavones, phenolic acids, stilbenoids, isothiocyanates, saponins, procyanidins, phenylpropanoids, anthraquinones, ginsenosides, and others [10].

Momordica charantia Linn. (Karela) is a flowering climber of family Cucurbitaceae [11]. The fruit has a tuberculate surface presenting numerous swellings; it is emerald-green in colour when immature, turning orange-yellow during ripening [12]. The morphological characteristics, such as colour, size, form, odour, and taste, were observed [13].

MATERIALS AND METHODS

Collection

The polyherbal seeds (*coriandrm sativum, Sesamum indicum, trachyspermum ammi, Glycine max, Momordica charantia)* was identified and purchased from local market of Nuzvid.

Preparation of Extract [14]

The dried polyherbal seeds were collected, and then dried seeds were powdered to get a coarse powder. The dried powder seeds were taken in beaker and add 1000 ml of distilled water. Then it was kept for maceration 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 [14]

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

Test Organism [15]

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 where shaken vigorously. Death was included when the worms lost their motility followed by white secretions and fading away of their body colour.

Evaluation of Antihelmintic Activity:

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

- **Group I:** Control Group
- **Group II:** Standard Group – Albendazole [16] 50, 100, 200,300, 400mg/ml
- **Group III:** Test-I -Aqueous extract of polyherbal seeds 50,100, 200,300,400mg/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 colour 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 Dennett's multiple comparison test. Comparisons were made between haloperidol 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: 7.0.

RESULTS AND DISCUSSION

In this study, we found that aqueous polyherbal seeds 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	+
Steroids	+
Tannins	+
Amino acids	_
Glycosides	+
Fixed oils and fatty acids	+

+ indicate the compulsory present and – indicate the absent.

Antihelmintic Activity

The aqueous polyherbal seed extract produced a significant antihelmintic 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	261±0.97	283 ±1.30
	100mg/ml	217±0.81	239±0.51
	200mg/ml	133±0.73	147±0.91
	300mg/ml	86±0.83	111 ±0.63
	400mg/ml	72±1.07	82 ±01.12
Test-I[APSE]	50 mg/ml	190 ±0.51	215 ±0.20
	100mg/ml	155 ±0.24	173 ±1.03
	200mg/ml	115 ±0.24	127±0.83
	300mg/ml	44.0±0.63	94 ±1.50
	400mg/ml	36 ±0.58	71 ±0.97

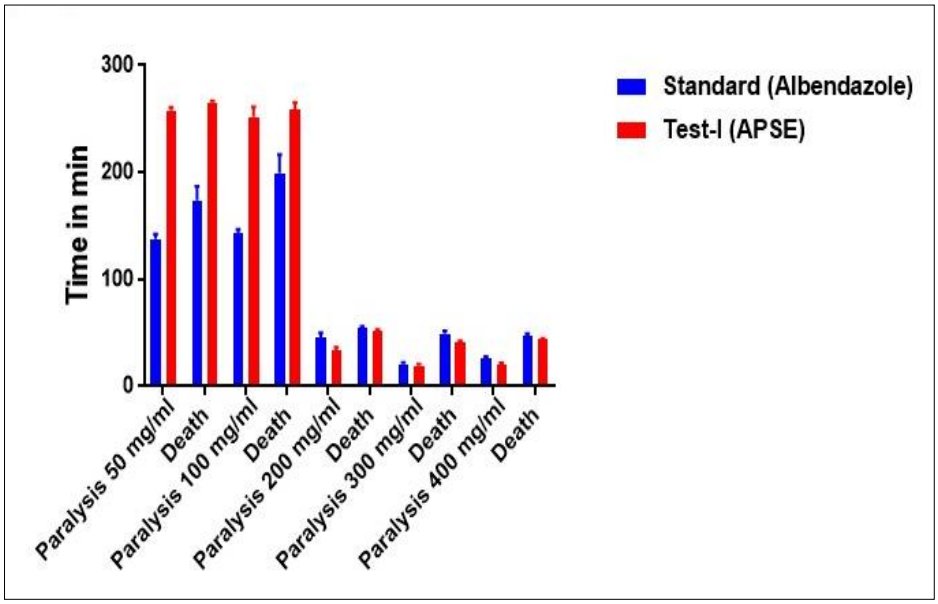

Figure 1: Anthelmintic activity of standard, APSE. Values are expressed as Mean ± SEM, P < 0.0001.

Figure 2: Standard (Albendazole) -50,100,200,300,400mg/ml- Paralysis and death

Figure 3: Test (APSE) -50,100,200,300,400mg/ml- Paralysis and death

CONCLUSION

In the present investigation, aqueous polyherbal seed extract posses the presence of alkaloids, carbohydrates, saponins, tannins, flavonoids, phenols, terpenoids, steroids, 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 400 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 400 mg/m. The study finally concluded aqueous polyherbal seed extract showed marked and potent anthelmintic activity than the standard drug Albendazole.

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