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Original Research Article

Invitro Antihelmintic Activity of Aqueous Extract of *Punica granatum* Fruit Peel against Pheritima Posthuma

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Abstract: In the present work aqueous fruit peel extract of *Punica granatum* was evaluated for its Anthelmintics activity against earthworm at three different concentrations (25, 50, 100 mg/ml). The Antihelmintic activity of extract was comparable with Marketed allopathic drugs (i.e. Piperazine citrate, Albendazole, Ivermectin, Levamisole- 25, 50, 100 mg/ml). 2% v/v Tween 80 in water was taken as a normal control. Marketed allopathic drugs concentrations were prepared using 2% v/v Tween 80. Antihelmintic activity was evaluated by considering the time required for mobility, paralysis and death of worms by the prepared extracts and marketed allopathic drugs concentrations. The data were found statistically significant by using one way ANOVA (P< 0.001).

Keywords: *Punica granatum*, *Pheretima posthuma*, Anthelmintic activity, allopathic drugs.

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 [1].

Punica granatum belonging to family Lythraceae, is a fruit bearing deciduous shrub. Fruits are consumed fresh or used for the preparation of fresh juice, jelly and jam, and beverage products. In several systems of medicine *Punica granatum* fruit is used for variety of ailments. Its fruit juice have various phytoconstituents whose functional and medicinal effects such as hepatoprotective, antibacterial, antioxidant, anticancer, antidiabetic, anti-atherosclerotic effects, estrogen-like activity had been confirmed. In Ayurveda, the peels of the fruit are used for stomach ailments including diarrhoea and dysentery. The peels has wide range of therapeutic properties and can be used in treatment of diabetes, cancer, cardiovascular disease, dental conditions, erectile dysfunction and male infertility, infectious diseases, Alzheimer's disease and dermal wounds [2].

MATERIALS AND METHODS

Collection of Plant Material

The fruits of *Punica granatum* was identified and purchased from local market of Nuzvid.

Preparation of Aqueous Extract (Maceration method) [3]

The 500gm of dried fruits peel of *Punica granatum* was 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 [4-6]

The preliminary phytochemical investigation was carried out with aqueous extract of *Punica granatum* fruit peel for identification of phytochemical constituents. Phytochemical tests were carried out by standard methods.

Test Organism [8]

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: Aqueous fruit peel extract of *Punica granatum* (PGAE-25, 50,100 mg/ml)
Group II: Albendazole-*25, 50, 100* mg/ml
Group III: levamisole-25, 50, 100 mg/ml
Group IV: Piperazine Citrate -25, 50, 100 mg/ml
Group V: Ivermectin- 25, 50, 100 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 [7].

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 tests and marketed branded antihelmintic drugs. P-values <0.05 was considered statistically significant. The statistical analysis was done by using Graph pad prism version no: 7.04.

RESULTS AND DISCUSSION

In this study, we found that aqueous fruit peel extract of *Punica granatum* possess the following chemical constituents (Table 1).

Table 1: Phytochemical screening of aqueous fruit peel extract of *Punica granatum*

Phytochemical Constituents	Aqueous fruit peel Extract of *Punica granatum*
Alkaloids	+ve
Flavonoids	+ ve
Carbohydrates	+ ve
Saponins	-ve
Triterpenoids	-ve
Sterols	- ve
Tannins	+ ve
Glycosides	+ ve
Fixed oils and fats	-ve

+ve indicate the compulsory present and –ve indicate the absent.

Antihelmintic Activity

The Aqueous fruit peel extract of *Punica granatum* produced a significant antihelmintic activity in dose dependent manner as shown in below table.

Table 2: Anthelmintic activity of aqueous fruit peel extract of *Punica granatum*

Type of Sample	Dose (mg/ml)	Time (min) taken for paralysis of earthworms Mean ± S.E.M	Time (min) taken for death of earthworms Mean ± S.E.M
Aqueous Extract of leaves of *Punica granatum* [PGAE]	25	47.4±±0.812	71±0.707
	50	33.8±0.583	62±0.836
	100	33.4±0.927	39.6±0.509
Albendazole	25	79.6±0.509	86.6±0.510
	50	31.4±0.510	61.8±0.583
	100	14.2±0.374	29.6±0.509
Levamisole	25	23.4±0.509	16.4±0.510
	50	10.6±0.509	6.8±0.583

Type of Sample	Dose (mg/ml)	Time (min) taken for paralysis of earthworms Mean ± S.E.M	Time (min) taken for death of earthworms Mean ± S.E.M
	100	6.8±0.489	4.6±0.245
Piperazine citrate	25	31.4±0.509	31.2±0.583
	50	14.4±0.678	15.8±0.374
	100	10±0.316	11.2±0.374
Ivermectin	25	31.2±0.583	51±0.837
	50	15.8±0.374	26±0.447
	100	11.4±0.509	16.2±0.583

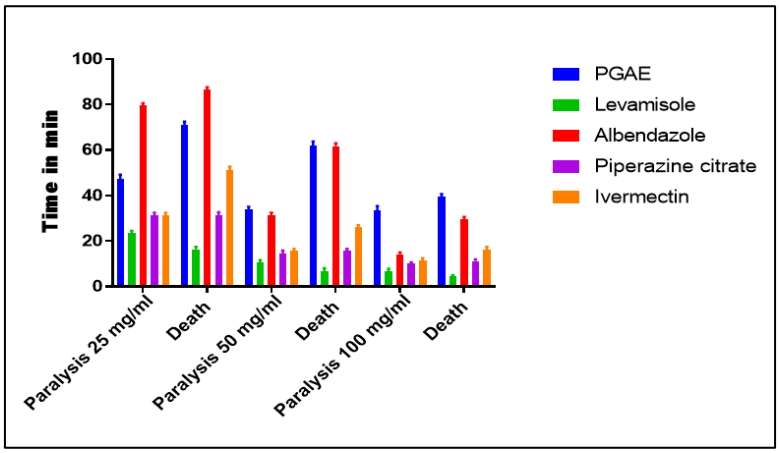

Figure 1: Graphical representation of time taken for paralysis& death of earthworm by using PGAE& Different marketed branded drugs

CONCLUSION

In the present investigation, aqueous fruit peel extract of *Punica granatum* possess the presence of alkaloids, carbohydrates, tannins, Flavonoids and glycosides. 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 fruit peel extract of Punica granatum showed the antihelmintic effect by above mentioned mechanisms. From the result shown in table-2 aqueous fruit peel extract of Punica granatum showed anthelmintic activity in dose dependent manner giving shortest time of paralysis and death. The study finally concluded Levamisole>Piperazine citrate> *Punica granatum* fruit peel extract>Ivermectin> Albendazole.

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REFERENCES

1. Baravkar, A. A., Shende, M. V., Nalawade, N. A., & Aher, N. B. (2020). In vitro anthelmintic activity of aqueous and organic extract of roots of *Punica granatum linn*. *International Journal of Advanced Research*, 8(7), 459-463.
2. Mahajan, D. C., Satyapal, U. S., Tatke, P. A., & Naharwar, V. (2014). Antimicrobial and anthelmentic activity of Punica granatum fruit peel extracts. *Int. J. Pharmacogn. Phytochem. Res*, *6*, 482-487.
3. Apte, A. K., Khot, V. S., Biradar, N. S., & Patil, S. B. (2014). Anthelmintic activity of Trachyspermum ammi (l) extract. *International Journal of Pharmacy and pharmaceutical sciences*, *6*(2), 236-238.
4. Sharada, L. D. (2018). Pharmacognosy and phytochemistry: A Companion Handbook. *Pharma. Med. Press*, *2nd edition*, *69-77*.
5. Beena, P., Rajesh, K. J., & Arul, B. (2016). Preliminary phytochemical screening of Cicer arietinum in folklore medicine for hepatoprotection. *J Innov Pharm Biol Sci*, *3*, 153-9.
6. Manohar, V. R., Chandrashekar, R., & Rao, S. N. (2012). Phytochemical Analysis of Ethanolic Extract of Fruits of Terminalia chebula (EEFTC). *Drug Invention Today*, *4*(10).
7. Durgawale, T. P., Khanwelkar, C. C., Durgawale, P. P., & Kakade, S. V. (2017). Comparative Anthelmintic Activity of Different Extracts of Portulaca Oleraceae L. Whole Plant. *Biomedical & Pharmacology Journal*, *10*(4), 2013.
8. John, J., Mehta, A., Shukla, S., & Mehta, P. (2009). A report on anthelmintic activity of Cassia tora leaves. *Songklanakarin Journal of Science & Technology*, *31*(3).