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Comparative *In vitro* Anthelmintic Activity of Aqueous and Organic Extract of Root and Bark of *Punica Granatum* Linn

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ABSTRACT

Introduction: *Punica granatum* (Punicaceae) has a wide variety of medicinal application owing to its different parts such as leaves, the rind of the fruits, seeds, roots, stems, bark. Up till now very less or no research has been done on the comparative evaluation of the anthelmintic activity of the bark and roots of *Punica granatum*.

Aim: This research study aimed to evaluate aqueous and organic extracts of bark and root of *Punica granatum* for their anthelmintic activity using Indian adult earthworm called *Pheretima Posthuma* and to perform comparative evaluation between aqueous and organic extract.

Methods: Different concentrations of aqueous and organic extract ranging from 10 to 100 mg/ml were made and tested on a said earthworm. These extracts were tested for bioassays which includes time for paralysis and time for the death of the worms.

Results: Aqueous as well as organic extract of bark exhibited strong anthelmintic activity at a concentration of 100 mg/ml as compared to the root. Lower concentrations did not produce significant anthelmintic activity. The standard reference drug which is used for comparing the anthelmintic activity of these extracts was piperazine citrate at a concentration of 10 mg/ml.

Conclusion: Both aqueous and organic extracts of bark and root of *Punica granatum* are evaluated for and showed potential anthelmintic activity.

Key Words: *Punica granatum*, Extraction, Worms, Anthelmintic activity, Piperazine citrate, Helminthiasis, *Pheretima Posthuma*

INTRODUCTION

Helminthiasis is a worm infestation of humans and other animals even life stock and crops affecting health and food production respectively and has an impact on global economic factor.¹ The worms which cause helminthiasis are called as helminths and the drugs which are used for treating helminthiasis are nothing but anthelmintics.² There are various types of worms such as hookworms, fluke worms, roundworms, tapeworms 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 severing helminthiasis include diarrhoea, abdominal pain, general malaise and impaired cognitive development. Chronic helminthiasis by hookworm lead to intestinal bleeding and anaemia.³ *Pheretima* is a genus of earthworms. *Pheretima Posthuma* is long cylindrical shaped worms having a length of 15-30 cm. they are mostly found in

moist soil and responsible for vegetables and hummus. Their life span is 3 to 10 years.⁴

Punica granatum Linn (family Punicaceae) commonly known as the pomegranate is easily available and widely spread plant in the world. It is widely cultivated throughout the Mediterranean region and all tropical regions including temperate, tropical and subtropical regions due to its higher commercial scale.⁵ Due to its health effects, it is widely used in the universe. According to Ayurveda, as all parts of *Punica granatum* has active chemical constituent, it's all parts has medicinal value. Juice of the pomegranate is used as diuretic activity and cooling effect. It is also used to improve vitamin A level, as antipyretic and mineral supplement.⁶ Its bark decoction acts against piles bleeding, leaves can be used for threatened abortion, flower buds can be used for nasal bleeding, fruit rind is recommended for gum and teeth bleeding, seed powder is effective in dissolving renal calculi.⁷ Other

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beneficial medicinal activities of various parts of *Punica granatum* are immunomodulatory, anti-inflammatory, anti-diabetic, anticancer, to treat conjunctivitis etc.⁸

Literature review revealed that in Ayurveda it is mentioned that its roots also have various medicinal activities but till now no research work was done on extraction and evaluation of extract of roots of *Punica granatum*. Secondly, no research paper has been published for such comparative activity of root and bark of *Punica granatum*. Hence the said research work is undertaken for determining comparative anthelmintic activity against the human adult worm.

MATERIALS AND METHODS

Collection, authentication, drying and extraction of plant material

The roots of pomegranate were collected in June 2020 from Krushi Vigyan Kendra (KVK), the Baramati area. The roots were authenticated from Department of Botany, Shardabai Pawar Mahila Mahavidyalaya, Baramati. For cleaning purpose, dirt and dust from the roots are removed washed with flowing water and dried in shade for one month. After one month bark of the roots is also removed and roots are finely powdered in the domestic grinder. The powder is subjected to Soxhlet extraction (continuous hot percolation). For getting aqueous extract double distilled water is used and for getting organic extract petroleum ether was used. After 3 cycles of each, the mixture is subjected to rotary evaporation and the solvent is recollected. The dried extract is removed by scratching method and collected in a clean glass bottle. The dried extract is subjected to various preliminary chemical tests to detecting the presence of active phytochemical/constituents present in it.

Collection of worms and identification

Pheretima Posthuma of 6-9 cm length and weighing about 0.5 to 4 gms were collected at night from burrows of moist soil from farms of Baramati area and were identified from microbiology department, Shardabai Pawar Mahila Mahavidyalaya, Baramati. After authentication, they were used for anthelmintic activity. The reason behind selecting these worms is that they resemble by anatomy and physiology with intestinal worm parasite of the human being. The worms were cleaned using a saline solution before their use in the activity.

Drugs and chemicals

All chemicals used in the study are of analytical pure grade chemicals. 500 mg Avizine tablet from Taj Pharmaceutical Ltd, Mumbai was used for preparing standard piperazine citrate solution. Tablet was dissolved in 50 ml double distilled

water to get the concentration of 10 mg/ml.

Anthelmintic Activity

The activity was carried out by using the method reported by Ajaiyeoba.⁹ Necessary changes were done in the method as per requirement. 20 ml formulation containing three different concentrations of 10, 50 and 100 mg/ml of aqueous and organic extract were prepared and transferred in 9 different Petri dishes. Six *Pheretima Posthuma* worms of the approximately same size and same length were placed in these Petri dishes. Time for paralysis i.e. duration in which movement of worms stop is noted when Petri dishes are shaken vigorously and also the time of death i.e. period required for death worms is noted which is confirmed by shaking Petri dishes vigorously as well as by dipping Petri dishes in hot water for few minutes. Piperazine citrate was used in the concentration of 10 mg/ml as the reference standard and distilled water was used as control. Results are expressed in mean \pm SEM of six worms in each group and are tabulated and graphically represented.

RESULTS

Aqueous and petroleum ether extracts of stem bark and root of *Punica granatum* showed the effective anthelmintic activity. These extracts took the least time for paralyzing and death of the earthworms. As per figure 1, aqueous and petroleum ether extract of root showed prominent anthelmintic activity at a dose of 100 mg/ml taking the time of 11 ± 0.3 min and 9 ± 0.9 min respectively for paralysis of earthworm while aqueous and petroleum ether extract of stem bark also showed anthelmintic activity at a dose of 100 mg/ml taking the time of 17 ± 0.5 min and 11 ± 0.5 min respectively for paralysis of earthworm. From the results, it is evident that petroleum ether extract of root, as well as bark, is more effective as compared to aqueous extract for paralyzing the earthworm. Secondly, it is also observed that the root extract is more potent than stem bark extract as an anthelmintic.

DISCUSSION

Aqueous and petroleum ether extract of the root of *Punica granatum* showed prominent anthelmintic activity at a dose of 100 mg/ml taking the time of 27 ± 0.5 min. and 29 ± 0.1 min respectively for the death of earthworm. Aqueous and petroleum ether extract of stem bark of *Punica granatum* at a dose of 100 mg/ml took the time of 31 ± 0.7 min and 31 ± 0.4 min respectively for the death of earthworm (Figure 1). Comparatively, the petroleum ether extract of the root of *Punica granatum* is more potent than the petroleum ether extract of stem bark of *Punica granatum*. The function of the anthelmintic drugs like piperazine citrate is to paralyze

the worms so that they are expelled in the faeces of animals and human. The extracts not only confirmed this activity but also results in the death of the worms, especially at a dose of 100 mg/ml when compared with standard drug Piperazine citrate. Both aqueous and petroleum ether extract of stem bark and root of *Punica granatum* showed significant anthelmintic activity at 100 mg/ml dose. From this study, it can be concluded that the root extract of *Punica granatum* has been confirmed to exhibit potent anthelmintic activity than the stem bark extract.

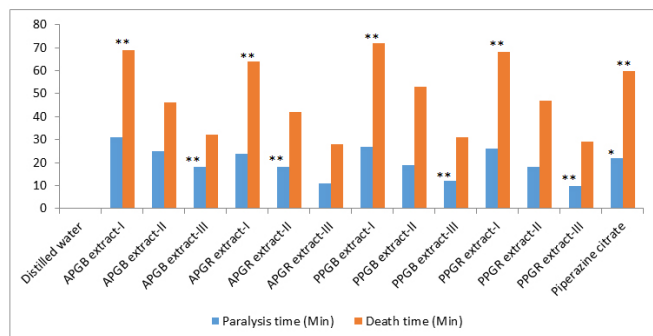


Figure 1: Paralysis time and death time in minutes. Values represent mean \pm SEM, One way ANOVA, $P < 0.0001$, followed by Dunnett's t-test, $**P < 0.01$. extract-I: Aqueous *Punica granatum* stem bark (APGB) extract, Aqueous *Punica granatum* root (APGR) extract, Petroleum ether *Punica granatum* stem bark (PPGB) extract and Petroleum ether *Punica granatum* root (PPGR) extracts were administered at the dose of 10 (I), 50 (II) and 100 (III) mg/ml.

CONCLUSION

Anthelmintic activity of aqueous and organic extract of root and bark of *Punica granatum* (*Punicaceae*) has been evaluated for anthelmintic activity against *Pheretima Posthuma* and showed the potential effect. Owing to the results obtained, development of a herbal formulation of roots of pomegranate and its characterization by pharmaceutical parameters can be a future scope. Secondly detecting active phytochemical constituents responsible for the activity using chemical analysis and determining their pharmacological mechanism of action is a future challenge.

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Conflict of Interest

No conflict of interest. Authors have performed research work at their expenditure and there is no financial support from any funding agency.

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