

## PHARMACOLOGICAL STUDYS FOR INVESTIGATION OF ANTIHELMINTIC ACTIVITY OF *PUNICA GRANATUM* PULP AGAINST ADULT INDIAN EARTHWORM *PHERITIMA POSTHUMA*

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### ABSTRACT

The present research programme was carried out to investigate the anthelmintic activity of the aqueous and methanolic extract of *punica granatum* pulp against adult Indian earthworm *Pheritima posthuma*. Various concentrations (50 and 100 mg/ml) of aqueous and methanolic extract evaluated for anthelmintic activity by recording the time required for paralysis and death of worms. Albendazole (20mg/ml) was used as standard. Result indicates aqueous and methanolic extracts of *Punica Granatum* pulp significantly ( $p < 0.01$ ) exhibited anthelmintic activity in dose dependent manner when compared with standard. The shortest time required for paralysis and death was observed with concentration of 100mg/ml. aqueous extract showed maximum effect when compared with methanolic extract. Further studies are under progress to confirm the possible chemical constituents responsible for activity.

### Keywords:

### INTRODUCTION

*Punica granatum* L. is a member of the family Punicaceae. The root and bark contain tannin (20-22%) and alkaloids (0.5-1%). The seeds contain steroidal oestrogen. The fruit pulp contains protein, carbohydrate, fat, fibre, minerals, oxalic acid and vitamins A, B and C<sup>1</sup>. In early times Greeks and Romans used *Punica* for tanning leathers, which is obtained from root, bark, stem, leaves and fruit rind<sup>2</sup>. Almost all parts of this plant are used in traditional medicine for the treatment of various ailments. Bark and rind of the fruit are used in dysentery, diarrhea, piles, bronchitis, to reduce the risk of cardiovascular disease, and as an anthelmintic<sup>3</sup>. The literature indicates the plant extract of different parts of *Punica granatum* and its isolated compounds were studied for various biological activities like Antibacterial and antifungal activity<sup>4</sup>, Antioxidant activity<sup>5</sup>, In vitro antimalarial activity<sup>6</sup>, Wound healing activity<sup>7</sup>, Cardioprotective<sup>8</sup> and Anticancer activity<sup>9,10</sup>.

### MATERIALS AND METHODS

#### Plant material

The plant of *punica granatum* was collected from local area of Ahmednagar dist., Maharashtra. The herbarium of this plant was identified & authenticated at M.J.S.M.college shrigonda by Mr. Pawar, department of Botany.

#### Preparation of extract

Fresh pulp of *punica granatum* was collected and air dried in shade at room temperature. The powder pulp material was extracted with water and methanol by maceration for 72 hrs. The extract was dried at low temperature under reduce pressure.

#### Worm collection and authentication

Indian adult earthworm (*Pheritima posthuma*) was collected from moist soil of the vermiculture plant, Rahuri krishi mahavidhyapith, Dist.Ahmednagar, and authenticated at M.J.S.M.college shrigonda by Mr. Pawar, department of Botany.

#### Anthelmintic activity

The anthelmintic activity will be perform according to method of Ghosh *et al.*<sup>11</sup>

The anthelmintic activity was performed according to the method of Ghosh *et al.* on adult Indian earthworm *Pheritima posthuma* as it has anatomical and physiological resemblance with the intestinal roundworm parasites of human beings<sup>12, 13</sup>. *Pheritima posthuma* worms are easily available and used as a suitable model for screening anthelmintic drugs<sup>14</sup>. In the 50ml of formulation containing different concentration of Aqueous and methanol extract (50,100mg/ml in saline water) and standard (20mg/ml) were

prepared and approximately equal size five earthworms were released in each group. Observations were made for the time taken to paralysis or death of individual worms. Paralysis was said to occur when the worms do not revive even in normal saline. Death was concluded when the worms lose their motility followed with fading away of their body color. Albendazole (20mg/ml) was used as standard while normal saline as control.

### RESULT

Experimental data showed that, the Aqueous and Methanolic extracts of *Punica Granatum* pulp has anthelmintic activity in dose dependent manner as shown in table no 2. The shortest time required for paralysis and death was observed with concentration of 100mg/ml. higher concentration of aqueous extract showed maximum effect when compared with methanolic extract.

The statistical analysis of result was carried using One way ANOVA followed by Dunnett's test with  $P < 0.01$  indicating the results were significant.

**Table 1: shows anthelmintic activity of aqueous and methanolic extract of punica granatum pulp.**

Test substance	Concentration (mg/ml)	Time taken for paralysis(min.)	Time taken for Death(min.)
Vehicle	-	-	-
Albendazole	20	13.44±0.14	18.48±0.13
Aqueous extract	50	43.50±0.16**	75.33±0.122**
	100	20.22±0.007**	24.47±0.13**
Methanolic extract	50	45.30±0.13**	76.25±0.06**
	100	24.33±0.05**	37.31±0.07**

Values are expressed as MEAN±SEM, One way ANOVA followed by Dunnett's test n=5 in each group \*\* $P < 0.01$



**fig.1: it shows death of worms after addition of methanolic extract of punica granatum pulp (100mg/ml)**



fig.2: it shows death of worms after addition of methanolic extract of punica granatum pulp (50 mg/ml)



fig.3: it shows death of worms after addition of aqueous extract of punica granatum pulp (100mg/ml)



fig.4: it shows death of worms after addition of aqueous extract of punica granatum pulp (50 mg/ml)



fig.5: it shows death of worms after addition of standard drug albendazole. (20 mg/ml)

## DISCUSSION

Adaptation of locally available herb or herbal product in the treatment of human ailment of great advantages as far as cost availability of treatment concern. Hence the study was planned to investigate utility of locally and abundantly available plant that is *Punica Granatum* in treatment of helmenthic infection.

The Aqueous and Methanolic extracts of *Punica Granatum* pulp not only showed paralysis but also caused death of worms at different concentration, and when concentration increases anthelmintic

activity increases, and shortest time required for paralysis and death was observed with aqueous extract when compared with methanolic extract of *Punica Granatum* pulp.

Albendazol by increasing chloride ion conductance of worms muscle membrane produced hyperpolarisation and reduced excitability that which led to muscle relaxation and flaccid paralysis<sup>15</sup>.

## CONCLUSION

The present study has shown that, the Aqueous and Methanolic extract of *Punica granatum* pulp have been confirmed to display anthelmintic activity. Further studies are in progress to identify the possible chemical constituent responsible for anthelmintic potential.

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