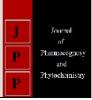


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# *In vitro* anthelmintic evaluation of ethanolic extract of *Punicag ranatum* Linn.

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#### Abstract

*Punica granatum* Linn. is also known as Pomegranate is a fruit-bearing deciduous shrub, having potential anthelmintic activity as, *In-vitro* anthelmintic activity of fruit ethanolic extract of *Punica granatum linn*. was evaluated using Paramphistomum *cervi*, *Moniezia expansa*, *Avitellina Centripunctata* and *Trichuris ovis*, sustained on time and concentration of extract, standard anthelmintic used was fenbendazole. Complete cessation of motility and mortality were recorded contemplating parasitic activity. Study records complete cessation of motility of worms after increase in concentrations of the *Punica granatum linn*. fruit extract. After 15, 14 & 13 hours of exposure in concentrations of 25, 50 & 75 mg/ml respectively, there was complete mortality of study group of parasites. Before which, complete cessation of motility of worms after 9.5, 8.5 & 7.5 hours of exposure detected.

Keywords: Punica granatum Linn., In-vitro, ethanolic extract, anthelmintic, complete cessation, motility, mortality

#### Introduction

Infestation with helminths is an important factor in the health and productivity of livestock throughout the world, intensive systems of management often lead to high levels of infestation particularly in grazing animals. Direct losses result either from mortalities and/ or from reduced productivity caused by both clinical and subclinical parasitism. The search for new drug is still continued, in view of increasing cost of management and side effects produced by synthetic drugs. Efforts to improve the existing drug by reducing their toxic side effects and increasing spectrum of activity through the production of new analogue.

Several modern researches have elaborated the use of plant drugs being safer than synthetic chemicals. Their synergistic effects due to the presence of active ingredients and presence of minerals and salts beneficial in the treatment (Atal & kapoor,1982)<sup>[1]</sup>

The plant *Punica granatum* Linn. is claimed to possess anthelmintic action singly or in combination as reviewed from various sources. Accordingly the present study was planned and the ethanolic extract of dried fruit rind of plant *Punica granatum* Linn. was screened for its *invitro* anthelmintic action against *Paramphistomum cervi*, *Moniezia expansa*, *Avitellina centripunctata And Trichuris ovis* in goat as main objective.

# **Material and Methods**

The ripe fruit of *Punica granatum* Linn. were procured from local market and the fruit was removed and dried at room temperature to remove the moisture. It was then powdered and sieved to obtain a powder of uniform grade. Then this powder of fruit rind of *Punica granatum* linn. was subjected to ethanolic extraction.

# **Preparation of ethanolic extract**

Ethanolic extract of powder of dried fruit rind of *Punica granatum* Linn. was prepared by the method described by Rosenthaler (1930)<sup>[2]</sup>. One-hundred-gram powder of dried fruit rind of *Punica granatum* Linn. was subjected to extraction in the Soxhlet's apparatus using ethanol. This was heated on a heating mantle till the colorless solvent started running back to the reservoir. Then the contents were poured in a dry, clean petridish and ethanol was then evaporated off from the extract by keeping at room temperature. The petridish with the extract was preserved in a cool and dry place.

# *In vitro* anthelmintic screening of ethanolic extract of dried fruit rind of *Punica granatum* Linn

The rumen, reticulum, small intestine and colon of goats were collected from the slaughter house governed by Nagpur Municipal Corporation.

The rumen, reticulum, small intestine and caecum of goats were incised longitudinally *Paramphistomum cervi*, *Moniezia expansa*, *Avitellina Centripunctata* and *Trichuris ovis* were collected by blunt forceps and these were placed in a beaker containing tyrode solution. These worms were identified from university Dept. of Vet. Parasitology, NVC, MAFSU, Nagpur. These worms were used for screening the *in-vitro* anthelmintic activity of ethanolic extract of powder of dried fruit rind of *Punica granatum Linn*. by the petridish method (Agarwal *et al.*, 1977)<sup>[3]</sup>.

The activity of ethanolic extract of powder of dried fruit rind of *Punica granatum* Linn. was tested at three different dilutions. The ethanolic extract of powder of dried fruit rind of *Punica granatum* Linn. was used in concentrations of 25, 50 and 75 mg/ml of tyrode solution (Jawahar *et al.*,1976)<sup>[4]</sup>. The ethanolic extract of powder of dried fruit rind of Punica *granatum* Linn. being water insoluble was transformed into homogenous emulsion by using twenty (20 polysorbate). This homogenous emulsion was then further diluted to 100 ml with tyrode solution. Tyrode solution alone & Fenbendazole dissolved in tyrode solution were used as negative and positive control (Standard) respectively. The Fenbendazole was used in concentration of 5mg/ml of tyrode solution.

Two controls and the three concentrations of the ethanolic extract of powder of dried fruit rind of *Punica Granatum* Linn. were poured in the clean and sterile petridishes with 100ml of tyrode solution each. Then, ten worms irrespective of sex were transferred in each petridish and the time of transfer was noted.

Observations were made with the test drug at an interval of 30 minutes till the mortality of worms occurred. During observations the time required for complete

cessation of motility (Paralysis) was noted. The worms were pressed at either end with a blunt glass rod to confirm complete cessation of motility. At the end of trial, the worms were transferred to the petridish containing tyrode solution at  $40 \leftarrow C$  to check the motility if any and those worms showing no motility were considered as dead worms (Sharma & Sisodia, 1976)<sup>[5]</sup>. The findings were confirmed by taking no. of observations. All the experiments were conducted at room temp. varying between 97 and 104  $\leftarrow$  F (Garg And Mehta, 1958)<sup>[6]</sup>.

## Results

Table 1: Shows activity of ethanolic extract of *Punica granatum* Linn. at various concentration

Sr. No.	Drug and concentration	Number of worms exposed	<b>Complete cessation of motility (hours)</b>	Mortality of worms (hours)
1.	Tyrode solution (Plain)	10	36	48
2.	Fenbendazole 5mg/ml	10	6.5	13
Ethanolic extract of Punica granatum Linn				
	a) 25mg/ml	10	9.5	15
3.	b) 50mg/ml	10	8.5	14
	c) 75mg/ml	10	7.5	13

## Discussion

The Table1 is indicative of the ethanolic extract of powder of *Punica granatum* Linn. caused complete cessation of motility of worms after 9.5, 8.5 & 7.5 hours of exposure while mortality of worms occurred after 15, 14 & 13 hours of exposure in concentrations of 25, 50 & 75 mg/ml respectively. The results are supported by the studies of Prakash *et al.*, 1980<sup>[7]</sup> where he reported that, the activity of alcoholic extract of *Punica granatum* Linn inhibited the development of eggs of *Haemonchus contortus* to filariform larvae.

# Conclusion

Considering the various medicinal properties of *Punica* granatum Linn. The ethanolic extract of fruit rind of *Punica* granatum Linn. Was subjected to *in-vitro* anthelmintic screening. The ethanolic extract of powder of *Punica* granatum Linn. Caused complete cessation of motility of worms after 9.5, 8.5 and 7.5 hours of exposure while mortality of worms occurred after 15, 14, 13 hours of exposure in concentrations of 25, 50 & 75mg/ml respectively. The results are supported by the studies of *in-vitro* anthelmintics activity of alcoholic extract of *Punica* granatum Linn. (Prakash *et al*, 1980)<sup>[7]</sup>.

From the present observation it is concluded that the ethanolic extract of powder of dried fruit rind of *Punica granatum* Linn. was found effective in complete cessation of motility of worms and thereafter death of worms *viz. Paramphistomum cervi, Moniezia expansa, Avitellina Centripunctata* and *Trichuris ovis.* Hence, powder of fruit of *Punica granatum* 

Linn. Can be used as a wormicidal agent and need further clinical study.

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