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Research Article

PRELIMINARY ANTHELMINTIC STUDIES ON THE TWO VARIETIES OF *PUNICA GRANATUM* FRUIT EXTRACTS

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ABSTRACT

Resistance to anthelmintic drugs has become a big challenge in developing nations as well as developed countries. According to WHO only few drugs are available till yet for the treatment of helminth infection. Helminths can affects any age group of person whether it may be a child or adult. *Punica granatum* is a fruit bearing plant of family Punicaceae which aims for the treatment of Helminthesiasis, Its English name is Pomegranate. In ancient time its various parts were used to treat many kinds of ailments. In the present study the *Punica granatum* fruit extracts of two varieties were taken to explore comparative anthelmintic potential. The two were *Punica granatum* Nashik (India) and *Punica granatum* Kandhari (Afgan). Isolation and extraction of peel, seed and juice of both varieties were done in laboratory. Only ethanolic extract of Epicarp and seed were taken. It was found that the Epicarp of Nasik variety has great potential for anthelmintic activity and juice possesses no anthelmintic activity. Albendazole, Piperazine citrate and Pyrantel pamoate were taken as standard drugs. Dose of all extracts and standard drugs were taken as 20 mg/ml for *in vitro* study.

Keywords: Anthelmintic resistance, *Punica granatum,* Piperazine citrate, Pyrantel pamoate.

INTRODUCTION

Helminthesiasis is a severe and challenging gastrointestinal infection at present time. It affects more than half of the population of developing nation's worldwide.[1] The main cause of helminth infection is poor personal and environmental sanitation habits, eating and drinking of contaminated food and water.[2] Under such circumstances the incident rates may reach 90 %. There are several kinds of helminthiases named after the helminth causing them. Ascariasis is the most common one and is endemic in Africa, Latin America and the Far East, although the morbidity rate varies according to the region. Almost 73 % of *A. lumbricoides* infections occur in Asia, while about 12 % occur in Africa and only 8 % in Latin American. Even though the mortality rate is low, most of the people infected are children under 15 years with problems of faltering growth and/or decreased physical fitness. Children infected with Ascaris have proven to be lower in weight and height and have lower haemoglobin concentration and I.Q. than the control group. Helminthiases are transmitted through: (i) the ingestion of polluted crops, (ii) contamination with polluted sludge, faeces or wastewater, and (iii) the ingestion of polluted meat. Sanitary programmes based only on medical drugs are not enough to control Helminthiasis; wastewater and sludge treatment also need to be practiced. Additionally, long-term chemotherapy may have negative effects if sanitary conditions are not addressed. Most common helminths that cause infection to GIT are Ascaris, Hookworm, Roundworm, Threadworm, Tapeworm, Enterobius, Trichuris, Necator, Ancylostoma and Strongyloids etc. Some of the above worms can also infect to the organ -tissues and their larvae may enter in it. They may cause intestinal injury, enterobiasis and lymphatic obstruction by secreting different toxins.[3] In the treatment of parasitic diseases, the anthelmintic drugs are used indiscriminately. Recently the use of anthelmintic produces toxicity in human beings. Hence the development and discovery of new substances acting as anthelmintic; are being derived through plants which are considered to be the best source of bioactive substances. Anthelmintics are those drugs that are used in expelling out the worms that are parasitic in nature by either stunning them or by killing them. They are also known as vermifuges or vermicides. Natural anthelmintic includes the following list of components: Tobacco, walnut, clove, kalonji seeds etc. The selection of drug for the treatment is not only based on its efficacy, but also on its side effect/toxicity. The suitability in dose regime and the cost related intervention are the issues in the people of developing countries. Since, they rely on traditional and folkloric medicines for the treatment of their various ailments. The present study explores the same in a scientific way for the fruits epicarp and seed of two different varieties of *Punica granatum.*

MATERIALS AND METHODS

Collection and Authentication of Plant Material

Experiment was performed on the two varieties of Pomegranate, namely Kandhari (Afgan variety) and Nasik (Indian variety). Nasik variety was authentified by Beerbal Sahni Institute of Paleobotany, Lucknow, India (Regi. no. 13363) and Kandhari from NISCAIR, Delhi, India (Regi.no. NISCAIR/RHMD/Consult/-2011-12/1876/176). Separation of seeds and epicarp was done manually on the same day of collection. Further, the seeds with juicy testa were treated with a light juicer machine in order to separate the juicy material from the seeds. The seed residue and epicarp of both fruits were collected and dried under shade. The dried mass were processed into coarse powder and sieved by mesh size 10. The granules were stored separately in a closed vessel for further use.

Drug and Chemicals

The Standard drugs Piperazine citrate, Pyrantel pamoate and Albendazole were procured from commercial sources and other chemical used such as NaCl and DMSO were taken from department's laboratory of analytical grade.

Animals

The Indian earthworms *Pheretima posthuma* were collected from moist soil and their skin surface thoroughly washed with fresh water followed by normal saline to remove all extraneous matter. The earthworms of 6-8 cm in length and 0.1-0.2 cm in width were selected for the study due to their anatomical and physiological similarity with the intestinal parasites of human being.[4-5]

Preparation of Extracts

The shade dried material was subjected to Soxhlet apparatus for successive extraction by increasing polarity of solvents like Petroleum ether, chloroform, ethanol and water in successive manner.[6] Temperature of the soxhlet assembly was maintained throughout the extraction process. Each time the marc was air dried to make free from solvent and refilled in the apparatus for further extraction. After each extraction, the extracts were cooled at room temperature, filtered and concentrate under reduced pressure. The dried extract was kept in desiccators for further use. On the basis of preliminary evaluation, the ethanolic (epicarp and seed) and aqueous (juice) extracts of the both varieties were taken for the main study.

Anthelmintic Activity Study

The Anthelmintic activity was evaluated on adult Indian earthworm, which was collected locally from moist soil. The experiment was carried out at normal room temperature.

Experimental Protocol

Group 1- Control (Vehicle)
Group 2- Standard I (Albendazole- 20 mg/mL)
Group 3- Standard II (Piperazine citrate- 20 mg/mL)
Group 4- Standard III (Pyrantel pamoate- 20 mg/mL)
Group 5- Ethanolic extract of *Punica granatum* Nasik variety epicarp (20 mg/mL)
Group 6- Ethanolic extract of *Punica granatum* Kandhari variety epicarp (20 mg/mL)
Group 7- Ethanolic extract of *Punica granatum* Nasik variety seed (20 mg/mL)
Group 8- Ethanolic extract of *Punica granatum* Kandhari variety seed (20 mg/mL)
Group 9- Aqueous extract of *Punica granatum* Nasik variety juice (20 mg/mL)
Group 10- Aqueous extract of *Punica granatum* Kandhari variety juice (20 mg/mL)

Observation Table

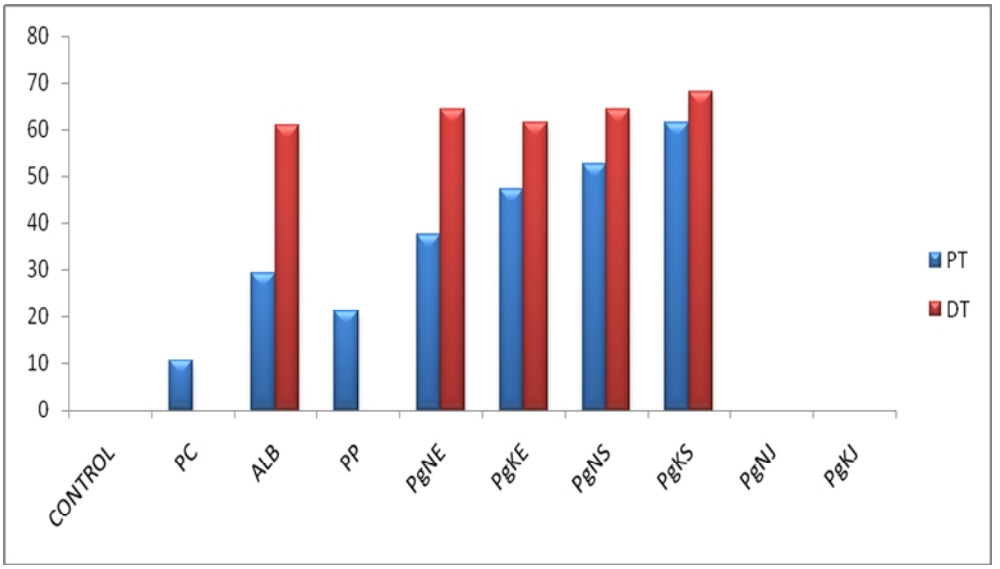
Treatment /Groups	Conc.(mg/mL)	Paralysis time (min.)	Death time (min.)
Control (Vehicle)	20 mg/mL	Abs.	Abs.
Piperazine citrate	20 mg/mL	10.6 ± 0.33	Abs.
Albendazole	20 mg/mL	29.3 ± 0.25	61.02 ± 0.22
Pyrantel pamoate	20 mg/mL	21.2 ± 0.19	Abs.
Ethanolic extract (PgN) Epicarp	20 mg/mL	37.5 ± 0.25	64.1 ± 0.06
Ethanolic extract (PgK) Epicarp	20 mg/mL	47.3 ± 0.01	61.4 ± 0.17
Ethanolic extract (PgN) Seed	20 mg/mL	52.5 ± 0.59	64.2 ± 0.21
Ethanolic extract (PgK) Seed	20 mg/mL	61.5 ± 0.48	68.2 ± 0.40
Juice (PgN)	20 mg/mL	Abs.	Abs.
Juice (PgK)	20 mg/mL	Abs.	Abs.

Values are expressed as mean ± SEM (n = 6). *Punica granatum* Nasik (PgN), *Punica granatum* Kandhari (PgK)

Table 1: Phytochemical Test for Fruits of *Punica granatum*

S. No.	Chemical test	*Punica granatum* (Nasik)					*Punica granatum* (Kandhari)				
		Epicarp		Seed		Juice	Epicarp		Seed		Juice
		Aq.	EtOH	Aq.	EtOH	Aq.	Aq.	EtOH	Aq.	EtOH	Aq.
1.	**Carbohydrate** Molisch's test	+	+	+	+	+	+	+	+	+	+
2.	**Reducing Sugar**										
	Fehling's test	+	+	+	+	+	+	+	-	+	+
	Benedict' test	+	+	+	+	+	+	+	+	+	+
3.	**Amino acids**										
	Ninhydrin test	+	+	+	+	+	+	+	+	+	+
	Tyrosin test	-	-	+	+	+	-	-	+	+	+
4	**Flavonoid's**										
	Shinoda	+	+	+	+	+	+	+	+	+	+
	Alkaline reagent test	+	+	+	+	+	+	+	+	+	+
5.	**Mucilage**										
	Ruthenium red	+	+	+	+	+	+	+	+	+	+
6.	**Tannins and Phenolic compound** 5 % $FeCl_3$	+	+	+	+	+	+	+	+	+	+
7.	**Alkaloids**										
	Dragendroff's	+	+	+	+	+	+	+	+	+	+
	Mayer's	+	+	-	-	-	-	-	-	-	+
	Hager's	+	+	-	-	+	-	-	-	-	+
	Wagner's	+	+	+	+	+	-	-	-	-	+
8.	**Glycoside**										
	Froath formation test	+	+	+	+	+	+	+	+	+	+
9.	**Steroids**	-	-	-	-	-	-	-	-	-	-

Note: Salkowaski and Liebermann burchard reaction shows presence of steroids in Petroleum ether extract of seed of both Varieties



[Piperazine citrate (PC), Albendazole (ALB.), Pyrantel pamoate (PP), *Punica granatum* Nasik Epicarp (PgNE), *Punica granatum* Kandhari Epicarp (PgKE), *Punica granatum* Nasik Seed (PgNS), *Punica granatum* Kandhari Seed (PgKS), *Punica granatum* Nasik Juice (PgNJ), *Punica granatum* Kandhari Juice (PgKJ), Paralysis time (PT), Death time (DT)]
*In the case of Control, PgNJ and PgKJ there was No Paralysis, No Death

Extracts and drug solutions were prepared at the same dilution (20 mg/mL). Experiment was performed in petridish at room temperature and uniform volume of 20 ml was taken in the petridish and the observation was made with the test subject (i.e. *Pheretima posthuma*). Mean time of paralysis and death was recorded (Observation Table). Paralysis time was noted down when there was no self movement in the body of the worm, except when the worm was shaken vigorously, and death time was measured when there was no movement of body even after it was shaken vigorously and a sharp pin touched to the mouth region of worm followed by fading away of their body colour.[7-8]

RESULTS AND DISCUSSION

Phytochemical studies on Pomegranate fruits showed the presence of alkaloids, tannins, flavonoids, glycosides and other phenolic compounds (Table 1). Seeds also showed the presence of steroids. It may be possible that some of the phytoconstituents mentioned above have Anthelmintic activity. From the experiment it was observed that Ethanolic extract of the epicarp of both varieties showed potent Anthelmintic activity but lesser than the standard drugs. *Punica granatum* Nasik epicarp paralyze the worm in shorter duration of time than *Punica granatum* Kandhari epicarp. Seed of both varieties also showed moderate Anthelmintic activity and juice neither produced paralysis nor death. Piperazine citrate cause flaccid paralysis to the worm may be by increasing the chloride ion conductance[9] and make the worm to expel out from intestine through fecal matter, therefore Piperazine citrate is considered as vermifuge not vermicidal. Pyrantel pamoate also caused spastic paralysis to worm hence it is also vermifuge. Albendazole is vermicidal drug because it produced death to the worm. From the observation table, it was revealed that epicarp and seed have vermicidal effect whereas epicarp was shown potent vermicidal effect because death time for epicarp was measured approx. 64 and 61 min for Nasik and Kandhari pomegranate respectively and it was 61 min. for Albendazole. Phytochemical analysis of epicarp showed the presence of tannins as chief constituents and tannins were shown to possess Anthelmintic activity.[10] Chemically tannins are polyphenolic compounds.[11] Potent Anthelmintic effect of ethanolic extract of epicarp may be due to presence of high amount of tannins because they can bind with the free protein available in the GIT of host[12] or to the glycoprotein present on the cuticle of parasite[13] and cause death to the worm.

CONCLUSION

Though Pharmaceutical market is full of anthelmintic drugs like Albendazole, Piperazine, Ivermectin etc; but these drugs are expensive and full of various side effects. Natural drug may play an important role here to minimize the side effects and cost effectiveness. Hence from the above result it is concluded that the both varieties Pomegranate epicarp and seed have anthelmintic activity and overall epicarp showed potent anthelmintic activity than seeds and among epicarp *Punica granatum* Nasik epicarp showed significant activity. Juice of the both varieties did not show any anthelmintic effect. It will be fruitful for society to isolate that particular compound of epicarp which showed Anthelmintic potential.

Order of Anthelmintic activity:

Albendazole > Piperazine citrate > Pyrantel pamoate > PgNE > PgKE > PgNS > PgKS

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