

Parasitocidal and brine shrimp cytotoxicity potential of crude methanolic extract of rind of *Punica granatum* Linn against round worms and tape worms

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Abstract: Rind of *Punica granatum* is traditionally used for anthelmintic purposes. The current work describes the possible anthelmintic activity of crude methanolic extract of *Punica granatum* (Pg. Cr) against round worms (*Ascaridia galli*) and the tape worms (*Raillietina spiralis*). Brine shrimp cytotoxicity is also performed. Brine shrimp cytotoxic activity was tested using different concentrations (1000µg/ml, 100µg/ml and 10µg/ml) of Pg.Cr. *In vitro* anthelmintic activity of Pg. Cr was determined against the parasites using albendazole and piperazine citrate as standard anthelmintic drugs in concentration 10 mg/ml. LC₅₀ value for Brine shrimp cytotoxicity was 189.44±28µg/ml. In test concentration of 40mg/ml of the Pg. Cr, *Raillietina spiralis* was paralyzed in 23 minutes. However, for parasitocidal activity (death of the parasite), it took less time (40 minutes) as compared to standard Albendazole. Time taken for death of the parasite *Raillietina spiralis*, in concentration 40 mg /ml, is 40 min. While standard drugs took more time to kill the *Raillietina spiralis*. Pg. Cr took 19 minutes to paralyze the *Ascaridia galli* at concentration 40 mg/ml whereas; it took 48 minutes for to kill the parasite *Ascaridia galli*. The current work confirms the traditional use of rind of *Punica granatum* as anthelmintic against *Raillietina spiralis* and *Ascaridia galli*. Results of brine shrimp cytotoxicity assay warrant for the isolation of cytotoxic compounds. List of abbreviation: Pg. Cr = Crude methanolic extract of *Punica granatum*.

Keywords: *Punica granatum*; Parasitocidal; *Raillietina spiralis*; *Ascaridia galli*, albendazole, brine shrimp cytotoxicity; piperazine citrate.

INTRODUCTION

Punica granatum belongs to the order of Myrtales and family of Lythraceace that has more than 500 cultivators throughout the world (Das and Barman, 2012).

In Pakistan, it grows as wild plant at an altitude of 1000-2000 m, over the whole of western and northern built (South Waziristan, Khyber PukhtunKhwa, Dir, Kurram, Chitral, Baluchistan). It also grows in Hazara regions; and has been found in Kashmir and in Himalayan regions. In addition to treat obesity, arthritis and ischemia of brain in infants, *Punica granatum* has been reported to have anti-oxidant, anticancer and anti-inflammatory activities (Kotamballi *et al.*, 2003; Jurenka, 2008). Its peels have antimicrobial activity (Al-Zoreky, 2009). Relatively resistant infections caused by *Staphylococcus aureus*, *Streptococcus epidermidis*, *Lactobacillus acidophilus* and *E. coli* have been successfully treated with *Punica granatum* (Abdollahzadeh *et al.*, 2011). Besides its role in treatment of diabetes mellitus, *Punica granatum* has also been used to treat erectile dysfunctions. Its preparations are used as antiseptic as well as to provide protection against ultraviolet rays induced skin infections and injuries (Das and Barman, 2012; Kotamballi *et al.*, 2003;

Jurenka, 2008; Park *et al.*, 2010). Phytochemical analysis reveals that *Punica granatum* has anthocyanins, ascorbic acid, caffeic acid, catechin, Epigallocate-chingallate (EGCG), quercetin, sterols, punicic acid, ellagic acid, phenolic punicalagins and flavonoids (Jurenka, 2008). The juice of the pulp is rich in anthocyanins, ascorbic acid, EGCG and iron (Jurenka, 2008). The seed oil has 95% punicic acid, ellagic acid and ilagic acid, and sterols (Jurenka, 2008). The leaves are rich in tannins (punicalin and punicafolin) and flavones glycosides, which include apigenin and luteolin (Jurenka, 2008). The extract of leaves is used as eye wash, as astringent for diarrhea and dysentery (Qnais *et al.*, 2007). The rind (peel) is rich in phenolic punicalagins, EGCG, quercetin, rutin, flavones, flavonols, flavonones, anthocyanidins gallic acid and other fatty acids (Mohammed and Yahya, 2005). Methanolic extract of *Punica granatum* in concentration (50, 100 and 150 mg/ml) has been evaluated for possible anthelmintic activity against *Pheretima posthuma* (Indian earthworm) (Swarnakar *et al.*, 2013). The extract of dried rind (peel) is used traditionally in stomach ache and colitis. In Indian traditional system of medicine, *Punica granatum* is used as an astringent, anthelmintic, diuretic and cardio tonic (Abdollahzadeh *et al.*, 2011). The current work describes rationale for use of Pg.Cr as anthelmintic against the common round worms and the tape worms.

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MATERIALS AND METHODS

Collection, identification and preparation of plant materials

Mature fruits of *Punica granatum* were collected from the board Bazar of Peshawar, and Batkhela Bazar, Malakand agency, Khyber Pakhtunkhwa. The fruits were identified by plant taxonomist professor Dr. Jehandar Shah. The fruits were washed with distilled water to remove dirt. The fleshy juicy materials were removed. The rind was retained and subjected to shade drying. After shade drying, the rind materials (1.5 kg) were grinded with grinder. The pulverized materials were soaked in four liters methanol (80%) for 5 days. The materials were filtered using a porcelain filter. The filtrates were concentrated using a rotary evaporator on 45 °C till a semisolid brownish extract (20 g) was obtained.

Preliminary phytochemical screening

Preliminary phytochemical screening for the presence of tannins, glycosides, saponins, alkaloids, flavonoids, proteins, carbohydrates and steroids was performed (Evans, 2002; Kokate *et al.*, 1994).

Drugs and chemicals

Analytical grade (E. Merck) chemicals were used in the experiments. Piperazine citrate and albendazole (GSK) were used as standard reference drugs in the experiments at concentration 10 mg/ml as per our previous reports (Ali *et al.*, 2011b). Ethical committee of the Khyber Medical University approved the study protocols.

Statistical analysis and calculations

Microsoft XL sheet was used to calculate mean and SEM. Graphical curves were drawn for EC₅₀ and LC₅₀ using graph pad prism.

Brine shrimps cytotoxicity

For Brine Shrimp lethality bioassay of Pg.Cr, shrimp (*Artemia salina*) eggs were hatched in sterile sea water (salt content =38.0g/L; pH adjusted to 8.5). After hatching, active nauplii were collected from the brighter side of the hatching chamber. Different concentrations of tests samples (1, 10, 100 and 1000 µg/ml) of Pg.Cr along with Dimethyl Sulfoxide (DMSO 1% in water) were prepared for bioassay. After the vehicle solvent evaporation, six nauplii (Brine shrimp larvae) were added to each of the test jar. These jars were maintained on normal room temperature for a period of 24 hours. During the 24 hours, the death toll of nauplii at second stage of development was noted. Their percent mortality was determined to calculate LC₅₀ as per our reported procedures (Ali *et al.*, 2011a; Ali *et al.*, 2011b). The experiments were performed three times.

Collection of parasites and anthelmintic activity

Tape worms (*Raillietina spiralis*) and adult round worms (*Ascaridia galli*) were collected from the infested

intestines of the fowls (chickens) obtained from the nearby slaughter house of Chakdara, Dir lower. The worms were placed and maintained in normal saline throughout the experimental procedures. Average length of the tape worms was 6-7.8 cm, round worms 4.8-7cm. Veterinary practitioner identified the worms.

Ajaiyeoba *et al.* (2001) method was used to determine anthelmintic activity of Pg. Cr. Different concentrations of Pg. Cr (10 mg/ml, 20 mg/ml and 40 mg/ml) were prepared in normal saline. Six worms each of approximately equal length of both the stated species were placed in the Petri dish containing 25ml of freshly prepared test solution of Pg. Cr. Similarly, six round worms and six tape worms were placed in the other Petri dishes containing 25 ml of albendazole (10 mg/ml) and 25 ml of Piperazine citrate (10 mg/ml) as reported by Ali *et al.* (2011 a & b). The worms were declared to be paralyzed only when they could not move at all unless shaken vigorously (Ajaiyeoba *et al.*, 2011; Ali *et al.*, 2011a, 2011b). Time of death of the worms was considered when the worms did not exhibit any movement upon vigorous shaking or upon shifting the worms into warm water (50°C). The petri dishes were checked at 5 minutes intervals. The experiments were performed in quadruplicate.

RESULTS

Phytochemical analysis of *Punica granatum*

The plant tested positive for tannins, carbohydrates, proteins, flavonoids and steroids. The plant tested strongly positive for the presence of saponins However, it tested negative for triterpenoids and alkaloids (table 1).

However, it took more time (48 minutes) for parasitocidal activity against *Ascaridia galli*. Self-explanatory, % parasitocidal activity against the test parasite is expressed in fig. 2.

DISCUSSION

The anthelmintic activity may be attributed to these phytochemical constituents particularly the saponins that have been reported, in general, for anthelmintic activity (Sparg *et al.*, 2004). This suggests that the plant extract could be a possible source of cytotoxic compound(s) that may justifies its use as anticancer drug because of the positive correlation of Brine shrimps cytotoxicity assay and effects on human KB cell lines of naso-pharynx carcinoma (Tawaha, 2006) that requires further work. Hence the cytotoxic effect may be attributed to the phytochemicals present in the plant.

Thus the efficacy of Pg. Cr is almost comparable with efficacy of standard drug, Albendazole. However, Pg.Cr took 40 minutes for parasitocidal activity (death of the parasite) as compared to Albendazole which took 55

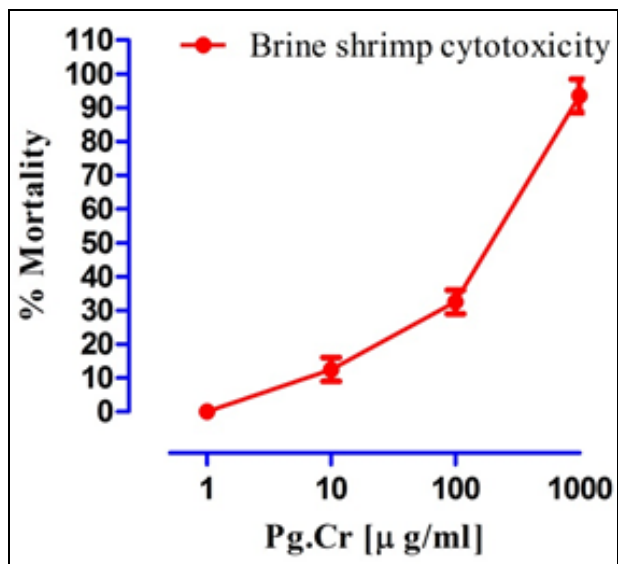


Fig. 1: Brine shrimp cytotoxicity report of *Punica granatum*.

Similarly, on concentration 40 mg/ml, Pg. Cr took 19 minutes for paralysis of the said parasite *as* compared to albendazole which took 17 minutes.

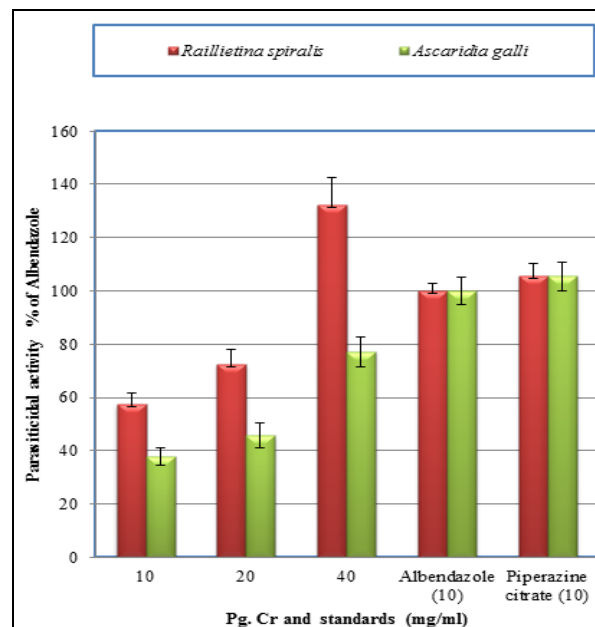


Fig. 2: Parasitocidal activity of *Punica granatum* against *Raillietina spiralis* and *Ascaridia galli* (calculated on based on time taken for paralysis, n=4).

Table 2: Anthelmintic activity of crude methanol extract of *Punica granatum* against *Raillietina spiralis* and *Ascaridia galli*

Time taken for paralysis (P) and death (D) in minutes (n= 4).					
Test samples and standards	Conc. (mg/ml)	<i>Raillietina spiralis</i>		<i>Ascaridia galli</i>	
		P	D	P	D
<i>Punica granatum</i>	10	38±4	92±3.2	31±4	98±6
	20	27± 5	73±4.9	27±5.3	81±5
	40	23±4	40±4	19±3	48±4
Albendazole	10	22±4.3	55±5	16±2	39±4
Piperazine citrate	10	18±4	52±5	14±2	37±3.6
Negative control	-	-	-	-	-

Results of Brine shrimps cytotoxicity assay are expressed in fig. 1. The extract produced, respectively, 16%, 33.33 % and 100% cytotoxic effect in 10, 100 and 1000 μ g/ml.

LC₅₀ value of Pg. Cr is 189.44±28 μ g/ml.

On concentration 20 mg/ml, Pg.Cr took 27 minutes to paralyze the *Ascaridia galli* (Table 2).

Similarly, Pg.Cr took 27 minutes to paralyze *Raillietina spiralis* in concentration 20 mg/ml.

On 40 mg/ml, Pg. Cr took 23 minutes for paralysis of *Raillietina spiralis*.

minutes to kill the *Raillietina spiralis*. This suggests that the extract has significant anthelmintic activity against *Raillietina spiralis*. Pg. Cr showed 77.08±10% parasitocidal activity against *Ascaridia galli* on concentration 40 mg/ml (Ali et al., 2011a, 2011b, 2011c).

The results suggest that the extract of rind of *Punica granatum* has good anthelmintic activity against *Raillietina spiralis* and *Ascaridia galli* that has not been reported earlier. This activity may be attributed to the presence of phytochemicals present in the plant particularly the saponins, which are cytotoxic and anthelmintic in general (Ali et al., 2011c).

The results strongly confirm the traditional use of rind of *Punica granatum* as anthelmintic. Hence the plant species is a potential target for isolation of pharmacologically active anthelmintic constituents.

CONCLUSIONS

The anthelmintic activity of *Punica granatum* at concentration 40 mg/ml against *Raillietina spiralis* and *Ascaridia galli* is comparable to standard albendazole that confirms its traditional use as anthelmintic. Based on the LC₅₀, extract of rind of *Punica granatum* shall not be used in large amount as it is cytotoxic.

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