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SHORT COMMUNICATION

IN VITRO ANTHELMINTIC EFFICACY OF NATIVE PLANTS AGAINST *HAEMONCHUS CONTORTUS*

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Abstract: The current study aimed to investigate *in vitro* anthelmintic efficacy of two medicinally important plants against *Haemonchus contortus* in small ruminants. Fruit peel of *Punica granatum* Linn. (vern. Anar), leaves and roots of *Berberis lycium* Royle (vern. Sumbal) were tested for their anthelmintic efficacy. Methanolic extracts of the test plants from various plant parts were tested for anthelmintic efficacy against the *Haemonchus contortous* using albendazole as a reference standard. The results revealed that both the plant extracts exhibited potent anthelmintic activity at concentrations higher than 50 mg/mL when tested against their respective standard drug. In case of *Berberis lycium* Royle when the results were compared, methanolic roots extracts showed more potent activity as compared to leaves extracts at the same concentration. It was observed that the *in vitro* anthelmintic potential of *Punica granatum* Linn. fruit peel and *Berberis lyceium* Royale root can be used to treat helminth infections after *in vivo* trails.

Keywords: anthelmintic activity, small ruminants, *Punica granatum, Berberis lycium,* phytomedicine

Small ruminant farming has a prominent role in the sustainability of rural communities around the world (1), as well as being socially, economically and politically highly significant at national and international levels, as with all livestock species (2). The factors that negatively affect the livestock production, infections with parasites and in particular with gastrointestinal nematodes continue to represent a serious challenge to the health, welfare, productivity and reproduction of grazing ruminants throughout the world (2). Helminthiasis is a term stating to various types of parasitic worms that inhibit inside the body of humans and small ruminants and adversely effects the immune system of the host (1). Helminthiasis has an antagonistic effect on production of small ruminants and hence, causes heavy economic losses especially in developing countries including Pakistan, where mismanagement and poor control practices are prevalent (3). Controlling the helminthiasis can result in greater economic productivity caused by better ruminant growth and thus directly improve the well being of animal farmers.

Anthelmintics are used to overcome the problem of gastrointestinal parasites. Due to the poor use of conventional anthelmintics, resistance has been developed in many parasitic strains (4). Plants are used as medicines by humans since the ancient times. In the early stages of human civilization medicinal plants have been used to cure various diseases (5). To contest parasitism in many parts of the world, medicinal plants have been used for centuries and their usage is reported throughout the world till present day as in Asia (6) and Africa (7). *Haemonchus contortus* is one of the major gastrointestinal pathogens of small ruminants (8). This species was used by several authors to evaluate the anthelmintic effects of various medicinal plant species (9-12).

Natural flora is quite rich in biogenic compounds which serve as useful bioresources for the extraction of herbal medicines. Kumar et al. (13)

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extracted anthelmintic extracts from three plant species viz. *Amaranthus spinosus, Amaranthus caudatus* and *Amaranthus viridis* L. belonging to the Amaranthaceae family. Piperazine was used as a reference standard at a concentration of 10 mg/mL. *Berberis lycium* (*B. lycium*) Royle (family: Berberidaceae), a native to Pakistan, India and whole region to Himalayas is widely used like food and in folk medicine (14). *B. lycium* contains berberine, plamitine, berbamine, vitamin C, saponins, β-carotene, and various minerals including sodium and postassium (15). Previously, *B. lycium* showed antimicrobial activities (16). *Punica granatum* (*P. granatum*) belongs to taxonomic family Lythraceae, and has a number of medicinal uses. Its common name is pomegranate. Pomegranate juice, seed oil and aerial part extracts contain vitamin C, ellagic acid, quercetin, and rutin (17). Pomegranate has been used for thousands of years to cure a wide range of diseases across different cultures and civilizations. It has great nutritional values and numerous health benefits. Pomegranates are used as treatment for cancer, osteoarthritis and other diseases. The pomegranate has been used in natural and holistic medicine to treat sore throats, coughs, urinary infections, digestive disorders, skin disorders, arthritis, and to expel tapeworms.

Keeping in view the role of medicinal plants having anthelmintic efficacy; the present study was conducted to screen the anthelmintic efficacy of native plants of Sub Himalayan regions of Pakistan.

MATERIALS AND METHODS

The study area

Plant samples were collected from Sub Himalayan regions of Pakistan, situated between North Latitude 33° 65' 714" and East latitudes of 73° 03' 008" in Rawalpindi District at an elevation of 1523 m above sea level (18).

Collection of plant material and identification

The plant samples were collected in the month of October. The taxonomic position of the collected plants was identified and authenticated by the Department of Botany, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi.

Preparation of methanolic plant extracts

The collected plant samples were brought to the Applied Biotechnology and Genetic Engineering Lab, International Islamic University, Islamabad, Pakistan for further studies. The required plant material was roots and leaves in case of *B. lycium* and fruit peel in case of pomegranate. Required parts were separated by using fine cutter. They were washed thoroughly with running tap water followed by autoclaved distilled water. The samples were well dried under shade. Each part was coarsely powdered to get one kilogram chopped plant material, which was soaked in 5 L of methanol for 2 weeks in a glass container. The suspension was shaken three times a day. After 15 days, the suspension was filtered in muslin cloth and this practice was repeated three times. After getting clear filtrate, 500 mL of the filtrate was subjected to rotary evaporation maintaining the bath temperature at 40°C followed by transfer of the obtained extract (about 245 mL) to beaker (500 mL). On subjecting to further evaporation on water bath at 100°C, a gelly mass weighing about 43 g was achieved. The crude extracts of each part were labeled and stored in refrigerator for further study (18).

Collection of adult parasites

Adult parasites *(Haemonchus contortous*) from the abomasum of freshly slaughtered sheep and goat were collected from a local attributor and brought them to laboratory. These parasites were thoroughly washed with tap water followed by distilled water. The clean parasites were placed in PBS in incubator at 27°C as long as *in vitro* trails were started.

Anthelmintic efficacy

Anthelminthic activity of two medicinal plants such as methanolic extract of fruit peel of *P. granatum* Linn. and methanolic extract of roots and leaves of *Berberis lyceium* Royle was evaluated by using the assay described by Ajaiyeoba et al. (19) with certain modifications. The worms were distributed into 6 groups. The worms were distributed into 6 groups for the three categories of methanolic extract tested (fruit peel of *P. granatum* Linn., leaves and root of *Berberis lycium* Royle). First group was treated with normal saline and was used as a control. Second group was treated with anthelminthic drug suspension and albendazole was used as a reference standard. Remaining four groups were used as tests and were treated with four different concentrations of methanolic extract. All the test suspensions were prepared freshly before starting the experiment. The parameters studied were paralysis time (PT) and death time (DT). Time of paralysis was noted when the parasites were shaken vigorously and no movement of any type could be observed. Death time was recorded when motility of the parasites was completely lost and their body colors were faded away. All the results were expressed as the mean ± standard deviation (SD) of six animals in each group.

Statistical analysis

For statistical analysis ANOVA statistical significance test LSD was employed using SPSS on data to draw conclusion. Difference between means was considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Results showing the anthelmintic efficacy of methanolic extract of fruit peel of *P. granatum* Linn, are summarized in Table 1. At 15 mg/mL, mean time of paralysis was 54.67 and 69.33 min death time was recorded, whereas reference drug albendazole showed 21.67 min paralysis and 38.67 min death time at the same concentration. For 25 mg/mL, 46.67 mean time of paralysis and 61.67 min death time was observed. At the concentration of 50 mg/mL, the mean time of paralysis was reduced to 39 min and and to 57 min of death time which was comparable with the standard. The results also showed that activity was dose dependent giving the shortest mean time 32.33 min of paralysis and 42 min fot mean death time with concentration 75 mg/mL. Methanolic extracts at concentration of 75

Table 1. Anthelmintic efficacy of methanolic extract of fruit peel of *Punica granatum* Linn.

Treatment (mg/mL)	Mean time of paralysis (min) ± SD	Mean time of death (min) ± SD
Albenzadole 15	$21.67^{d} \pm 0.88$	$38.67^{c} \pm 0.89$
CME 15	$54.67^{b} \pm 1.45$	$69.33^{a} \pm 0.89$
25	$46.67^{c} \pm 1.42$	$61.67^{a} \pm 0.78$
50	$39.00^{d} \pm 0.77$	$57.00^{b} \pm 0.89$
75	$32.33^{c} \pm 1.15$	$42.00^{b} \pm 1.17$
Control (normal saline)	0.00 ± 0.00	0.00 ± 0.00

Note: Values having the same superscripts means statistically non-significant difference.

Table 2. Anthelmintic efficacy of crude methanolic leaves extract of *Berberis lycium* Royle

Treatment (mg/mL)	Mean time of paralysis (min) ± SD	Mean time of death (min) ± SD
Albenzadole 15	$21.67^{d} \pm 0.88$	$38.67^{b} \pm 0.89$
CME 15	$47.33^{b} \pm 0.81$	$65.00^{a} \pm 1.19$
25	$36.33^{d} \pm 0.57$	$56.33^{a} \pm 0.88$
50	$31.00^{d} \pm 0.57$	$51.00^{a} \pm 0.57$
75	$23.33^{d} \pm 0.20$	$41.67^{b} \pm 0.88$
Control (normal saline)	0.00 ± 0.00	0.00 ± 0.00

Note: Values having the same superscripts means statistically non-significant difference.

Table 3. Anthelmintic efficacy of crude methanolic root extract of *Berberis lycium* Royle.

Treatment (mg/mL)	Mean time of paralysis (min) ± SD	Mean time of death (min) ± SD
Albenzadole 15	$21.67^{d} \pm 0.88$	$38.67^{b} \pm 0.88$
CME 15	$39.00^{b} \pm 1.15$	$59.00^{a} \pm 0.57$
25	$33.33^{d} \pm 0.88$	$52.00^{a} \pm 0.44$
50	$27.67^{d} \pm 1.20$	$45.33^{a} \pm 0.71$
75	$22.00^{c} \pm 0.57$	$38.33^{d} \pm 0.67$
Control (normal saline)	0.00 ± 0.00	0.00 ± 0.00

Note: Values having the same superscripts means statistically non-significant difference.

mg/mL were most effective when compared with the reference drug against *Haemonchus contortus*. Our results are in agreement with the number of earlier studies from different regions (20-22).

Anthelmintic efficacy of crude methanolic leaves and roots extract of *B. lycium* Royle (Sumbal)

In the present study, the second plant to evaluate for anthelmintic efficacy was *B. lycium* Royle. Its leaves and roots were separately tested against the parasite. Leaves exhibit dose dependent activity. At concentration 15 mg/mL the mean paralysis time was recorded as 47.33 min whereas mean time taken by parasites for death was 65 min (Table 2). The standard drug albendazole caused paralysis at 21.67 min and death at 38.61 min for the same concentration of 15 mg/mL. At concentration 25 mg/mL, the mean time for paralysis was 36.33 min and death at 56.33 min. whereas at concentration 50 mg/mL the mean time for paralysis was recorded as 31 min and death at 51 min, which was comparable with the standard drug albendazole at the same concentration. The leaves extract showed the highest activity at 75 mg/mL with the mean paralysis time of 23.33 min and death time 41.67 min. The results suggested that leaf extract of Sumbal is more effective than the synthetic drug to kill the parasites at concentrations greater than 50 mg/mL.

Table 3 showed that the anthelmintic efficacy of root extract is dose dependent. The shortest mean time for paralysis (22 min) and death (38.33 min) was observed at concentration 75 mg/mL. The concentration at 15, 25 and 50 mg/mL showed the mean time of paralysis at 39, 33.3 and 27.67 min, respectively. The mean death rate at the same concentrations was recorded as 59, 52 and 45.33 min, respectively. Data revealed that at 25 mg/mL the activity was comparable with that of standard drug whereas it increases with an increase in concentration.

In the present study, it was observed that crude methanolic extracts of fruit peel of *P. granatum* Linn. exhibit positive response to certain degree of anthelmintic efficacy against *H. contortus*. At concentration higher than 50 mg/mL, extracts exhibited more effective activity. Our results are in agreement with the number of earlier studies from different regions (20-22) but there is no report on the native plant of Sub Himalayan regions of Pakistan. Different times of paralysis were observed for various trials as presented in Tables 1-3. This can be explained on the basis of strain differences employed in the experiment.

Our results and earlier reports revealed that the peel extract of *P. granatum* Linn. possesses potent anthelmintic efficacy irrespective of their origin and can be used as a very good replacement of synthetic drug. It is also observed that methanolic root extracts of *B. lycium* are more effective than the leaf extracts. *B. lycium* Royle contains an active alkaloid berberine. Berberine has already been reported to have promising anti-inflammatory (23), antineoplastic (24), hypoglycemic and immunomodulating (25) activities.

However, there are very few reports on the presence of anthelminthic efficacy in this plant, especially from Pakistan. Our results suggested that due to the presence of anthelminthic efficacy in this plant it can be used to develop broad spectrum drugs. Berberine has a capacity to form complexes with DNA and topoisomerase (26). It has also been reported that compounds that are cytotoxic or have the capability to interact with DNA typically show antiparasite activity. The anthelminthic efficacy observed in the methanolic extracts of this plant may be due to the ability of berberine to interact with DNA of parasites.

CONCLUSION

It was concluded that methanolic extracts of both the plants possess potent anthelmintic efficacy. However, *in vitro* anthelmintic potentials of *P. granatum* Linn. fruit peel and *B. lycium* Royle root were vivid and could be used for treating helminth infections after *in vivo* trails. The infection in small ruminants can be controlled by the cultivation of these plants in the sheep-goat's management areas.

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