



Optimization of Indian Medicinal Plants for Development of Prominent Efficacious Herbal Anthelmintic

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Abstract

Prunus persica (L.) leaves and *Trichosanthes dioica* (R.) seeds are mentioned in various Asian traditional texts as a drug used for anthelmintic purpose ethno pharmacologically. The objective of the present study was investigation of anthelmintic activity of different extracts of *P. persica* leaves & *T. dioica* seeds along with optimization of dose. Nematodes, *Ascaridia galli* were used to carry out experiments for anthelmintic activity. Piperazine citrate was used as a standard drug. Time required for paralysis and death (lethal time) of worms were noted for each sample of extracts of both plants as well as standard. The results demonstrated that treatment with both of the plant extracts significantly ($P < 0.01$) paralyzed and killed *A. galli*. The activity was found to be increased with dose. Ethanol and ethyl acetate extracts activity at 60 mg/ml concentration were comparable to the well known anthelmintic agent Piperazine citrate (10 mg/ml). In conclusion, the use of the leaves of *P. persica* & seeds of *T. dioica* as a anthelmintic has been confirmed. Further studies were carried out by optimizing different ratio of ethanol extract of both plants in combination to get more prominent efficacy as a herbal anthelmintic by using minimum concentration of extracts of plants.

Keywords: Anthelmintic, *Ascaridia galli*, *Prunus persica*, *Trichosanthes dioica*

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Introduction

Helminthes infections are the most common infections in man which affects the large proportions of the world's population. In the treatment of parasitic diseases, the anthelmintics drugs are used indiscriminately. Recently the use of anthelmintics produces toxicity in human beings. Hence the development and discovery of new substances acting as anthelmintics are being derived through plants which are considered to be the best source of bioactive substances. *Trichosanthes*, a genus of family Cucurbitaceae is an annual or perennial herb distributed in tropical Asia. Over 20 species are recorded in India of which *T. anguina* & *T. dioica* are cultivated as vegetable. Other important species found in throught the world are *T. palmata*, *T. cordata*, *T. nervifolia*, *T. cucumerina*, *T. wallichiana*, & *T.*

cuspidata etc (Khare, 2007). Seeds paste is used to kill worms in wounds and fungal infections.² The bark is used in leprosy and jaundice. Leaves of *T. dioica* have been investigated for their antioxidant and anti-inflammatory activities in the past (Wang et al. 2010). *Trichosanthes dioica* (Parwal) screened for the treatment of Alzheimer's disease. Fruits of *T. dioica* reported for the hypoglycemic effect for Prevention of Type-2 Diabetes (Rai et al. 2008). The seeds of *T. dioica* was used in treatment of helminthes ethnopharmacologically (Charaka samhita & Ayurveda) but no scientific data is available yet. Therefore, it was thought to investigate anthelmintic potential of seeds of *T. dioica*. The seeds extract of *T. dioica* contain 7- oxidihydrokaroundol-3-benzoate as the most predominant component in the highly polar fraction of the nonsaponifiable lipid (Toshihiro et al. 1997).

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Prunus persica L. (Peach) named as *Amygdalus persica* is a perennial & deciduous tree of the subfamily Prunoideae of the family Rosaceae. The leaves are insecticidal, sedative, diuretic, demulcent, expectorant, and vermifugal. Leaf paste is used to kill worms in wounds and fungal infections. The treatment of gastritis, whooping cough and chronic bronchitis is carried out internally with leaves . The bark is used in leprosy and jaundice. Leaves of *P. persica* have been investigated for their antioxidant and anti-inflammatory activities in the past (Deb et al., 2010) . *Prunus persica* screened for the treatment of Alzheimer's disease. Fruits of *Prunus persica* reported for the hypoglycemic effect

for Prevention of Type-2 Diabetes. *Prunus persica* seeds showed good results in the treatment of the degenerative disorders, such as hypermenorrhea and dysmenorrhea (Kritikar and Basu, 1984). Hence various activities have been reported from various parts of *P. persica*. The leaves of *P. persica* were used in treatment of helminthes ethnopharmacologically (Charaka Samhita & Ayurveda), but no scientific data is available yet. Therefore, it was thought to investigate anthelmintic potential of leaves of *Prunus persica* & *T. dioica*.

Materials and methods

Plant material

The seeds of *Trichosanthes dioica* (Parwal) and leaves of *P. persica* were collected in the month of May from a place near Meerut district and authenticated by Department of botany, Meerut College Meerut, Uttar Pradesh. (India).

Experimental worms

Ascaridia galli Schrank (Nematoda) were collected from the department of veterinary science, Pantnagar India.

Preparation of Extracts

The seeds of *T. dioica* and leaves of *P. persica* were dried separately under shade and crushed in an electric blender to form powder (200 g) and subjected to Soxhlet extraction by using ethanol as solvents.

Anthelmintic activity

Experimental Design (Kaushik et al. 2001) The fresh worms of nearly equal size were selected for the study. Worms were divided into 14 groups of six worms in each. The first group was served as positive control and kept in 9 cm Petri dishes containing 20 ml of Piperazine citrate (10mg/ml) in 2 % DMSO (dimethyl sulphoxide) in phosphate buffered saline (PBS, pH 7.2, 0.15 M). Piperazine citrate (10mg/ml) was served as reference vermicide drug in the positive control group.

Twelve groups were kept in Petri dishes containing 20 ml of each three extracts at four different concentrations (10, 20, 40 & 60 mg/ml). Time for paralysis has been noted when no movement of any sort was observed except when the worms were shaken vigorously. Time for death (lethal time) of worms has been recorded after ascertaining that worms were neither moved when shaken vigorously nor when dipped in warm water (50°C). Death has been concluded when the worms were lost their body colour. (Sharma et al., 2011, Ajaiyeoba et al., 2001)

Further to optimize the dose for better efficacy, three groups of six worms were kept in Petri dishes containing 20 ml of 2% DMSO (dimethyl sulphoxide) in phosphate buffered saline solution individually mix with two most efficacious extracts of each plant in three different ratio F₁, F₂ & F₃. The experiment repeated again to obtain the least value of paralysis & lethal time.

Statistical analysis

The result were express as Mean \pm SEM. Statistical analysis was carried out using one way ANOVA followed by Dunnett's Multiple Comparison Test. P<0.05 was considered statistically significant.

Results and discussion

The results of in vitro evaluation of different test extracts from *Prunus persica* in *A. galli* are summarized in Table 1. Against *A. galli* all the test extracts demonstrated concentration dependent paralysis and lethal effects (Table 1). Ethanol extracts of *Prunus persica* significant anthelmintic activity and it was found that the ethanol extract activity is higher than other both extracts at all the concentrations against *A. galli*. Activity was found to be increased with dose. Ethanol and ethyl acetate extract activity was comparable to the well known anthelmintic agent Piperazine citrate.

Treatment	Concentration (mg/ml)	Mean paralysis time (min) \pm SEM	Mean lethal time (min) \pm SEM
Positive control (Piperazine citrate)	10	11.16 \pm 0.30	19.00 \pm 0.36
Negative control (Vehicle only)	NIL	NIL	NIL
Ethanol extract	10	57.16 \pm 0.40	72.33 \pm 0.33
	20	28.16 \pm 0.54	30.16 \pm 0.65
	40	15.83 \pm 1.19	28.00 \pm 0.93
	60	14.00 \pm 0.25	23.00 \pm 0.57
Ethyl acetate extract	10	58.16 \pm 0.30	74.16 \pm 0.30
	20	29.00 \pm 0.57	31.16 \pm 0.40
	40	17.16 \pm 0.47	29.00 \pm 0.57
	60	14.16 \pm 0.54	25.00 \pm 0.36
Petroleum ether extract	10	265.83 \pm 1.55	383.83 \pm 0.70
	20	203.00 \pm 0.93	322.16 \pm 0.30
	40	121.50 \pm 0.99	253.16 \pm 0.54
	60	41.66 \pm 0.55	86.16 \pm 0.30

Table 1: Effects of *Prunus persica* leaves against *A. galli*.

Number of worms per group (n) = 6, SEM = Standard Error of Mean Vehicle: 2 % DMSO in PBS

Trichosanthes dioica seeds in *A. galli* are summarized in Table 2. Against *A. Gallii*, all the test extracts exhibited concentration dependent paralysis and lethal effects. Ethanol extracts of *Prunus persica* have significant anthelmintic activity and it was found that the ethanol

extract activity is higher than other both extracts Ethanol and ethyl acetate extracts activity at 60 mg/ml concentration were comparable to the well known anthelmintic agent Piperazine citrate (10 mg/ml).

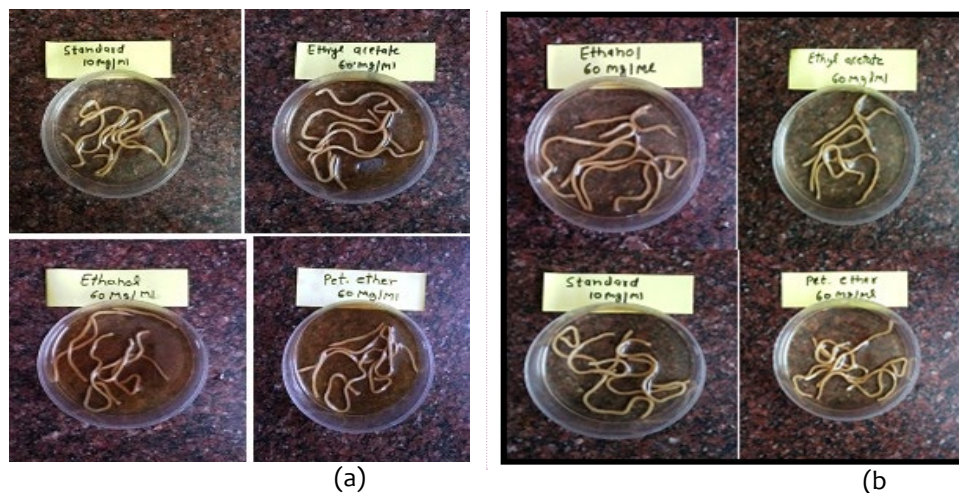


Fig.1: Photographs showing effect against A. galli: (a) T. dioica seeds , (b) P. Persica leaves

Treatment	Concentration (mg/ml)	Mean paralysis time (min) ± SEM	Mean lethal time (min) ± SEM
Positive control (Piperazine citrate)	10	11.16 ± 0.30	14.33± 0.21
Negative control (Vehicle only)	NIL	NIL	NIL
Ethanol extract	10	41.50± 0.34	53.16 ± 0.30
	20	31.83 ± 0.40	44.66 ± 0.42
	40	22.50 ± 0.56	36.33 ± 0.49
	60	15.83 ± 0.40	19.83 ± 0.47
Ethyl acetate extract	10	48.50 ± 0.76	61.50. ± 0.50
	20	35.33 ± 0.80	48.33 ± 0.55
	40	26.16 ± 0.40	41.16 ± 0.40
	60	18.50 ± 0.42	24.50 ± 0.56
Petroleum ether extract	10	175.83 ± 0.87	107.33 ± 0.80
	20	170.16 ± 3.26	101.16 ± 1.1
	40	146.83 ± 0.79	94.33 ± 0.95
	60	94.16 ± 0.94	81.00 ± 0.89

Table 2: Effects of Trichosanthes dioica seeds against A. galli. Number of worms per group (n) = 6, SEM = Standard Error of Mean Vehicle: 2 % DMSO in PBS

Ascaridia galli, all worms were found to be paralyzed and eventually killed by the all test formulations (Table 3). F1 formulation i.e. 1:1 ratio of both plants extracts exhibited maximum potency i.e. shortest pa-

ralysis and lethal times. The potency was not more than the reference drug, piperazine citrate but comparable to it.

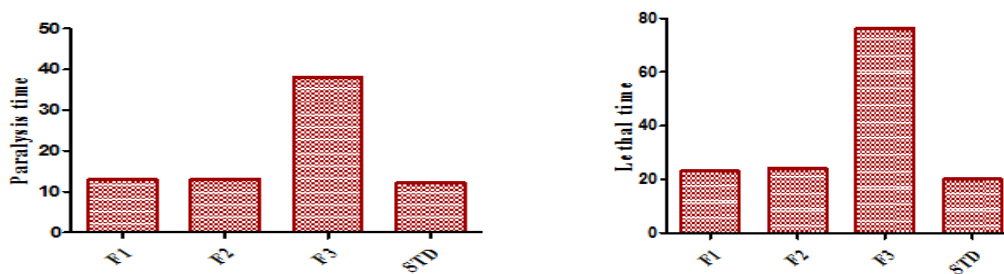


Fig 2: Effects of different formulations F1, F2 & F3 on paralysis and lethal time

Treatment	Mean paralysis time (min) ± SEM	Mean lethal time (min) ± SEM
Positive control (Piperazine citrate)	12.14 ± 0.20	20.00 ± 0.26
Negative control (Vehicle only)	NIL	NIL
F1	13.00 ± 0.25**	23.00 ± 0.57**
F2	13.16 ± 0.52**	24.00 ± 0.30 **
F3	38.16 ± 0.53	76.16 ± 0.20

Table 3: Effects of different ratio of both plant extracts against *A. Galli*

Number of worms per group (n) = 6, SEM = Standard Error of Mean, *(P<0.05), ** (P<0.01) Vehicle: 2 % DMSO in PBS

Conclusion

Ethanopharmacological use of *Prunus persica* and *T.dioica* as an anthelmintic has been confirmed. All the extracts showed the anthelmintic activity. The ethanol extract showed more potent activity as compared to ethyl acetate and petroleum ether extracts. F1 formulation (1:1 ratio of both plant extracts) exhibited maximum potency as an anthelmintic with shortest paralysis and lethal times.

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Conflict of Interest: Conflict of interest declared none.

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