

## **Anthelmintic Activity of Fruit Peel and Root Extracts of *Trapa natans* L. var. *bispinosa* Roxb**

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**Abstract:** The present study was aimed to evaluate anthelmintic activity of aqueous and methanolic extract of *Trapa natans* L. var. *bispinosa* Roxb fruit peels and root. The plant commonly known as “Water Chestnut” in India and is an annual aquatic floating herb occurring throughout the Indian subcontinent and used traditionally for several medicinal purposes. The effect of this plant was evaluated for anthelmintic activity on adult Indian earthworms *Pheretima posthuma* and aquarium worm *Tubifex tubifex*. Three concentrations viz., 5, 10 and 20 mg/ml of each extracts were studied to determine the time of paralysis and time of death. Results showed that the alcoholic extracts exhibited significant anthelmintic activity at highest concentration of 20 mg/ml. Piperazine citrate in 10mg/ml concentration was evaluated as standard reference and distilled water as control. Methanolic fruit peel extract showed anthelmintic activity in dose-dependent manner giving shortest time of paralysis (P) and time of death (D) with 20 mg/ml concentration, for both worms. The alcoholic extract of peel shows more potent activity (20 mg/ml) against both worms and demonstrated first time fruit peel extract of *Trapa natans* possess potential antihelminthic activity.

**Key words:** Anthelmintic Activity • *Trapa natans* • *Pheretima posthuma* • *Tubifex tubifex*

### **INTRODUCTION**

In developing nations helminths infections are most widely founds and commonest infection in man, affecting basically people who are in below poverty level and does not maintain hygienic condition. Helminths infections caused by infestation with one or more intestinal parasitic worms. Helminths infections are global problems with serious social and economic repercussions. The worms commonly found in gastrointestinal tract but may cause threat in to other body organ by burrowing into them. Major threats caused by the parasite to public health as well as cattle and contribute to the prevalence of malnutrition, anemia, eosinophilia and pneumonia. The drug which either kill or expel infesting helminthes called anthelmintic drugs, the parasitic worms expel from the body by either killing or stunning them also called vermicides or vermifuges. The gastrointestinal track is the prime house of many helminthes, but some also live in various tissues, or their larva transferred to tissues. Resistant developed from currently available drugs is a problem to treatment of this disease. However,

development of anthelmintic resistance chemotherapy is still the most widely used option for the control of helminthes in parasites of high economic significance. In addition, after treatment with albendazole, ormebendazole, several side effects have been reported in hosts such as gastrointestinal symptoms (epigastric pain, diarrhea, nausea, vomiting), nervous system symptoms (headache, dizziness) and allergic phenomena (edema, rashes, urticaria). Some anthelmintic drugs, such as praziquantel and albendazole, are contraindicated for certain groups of patients like pregnant and lactating woman [1, 2, 3, 4].

*Trapa natans* L. var. *bispinosa* Roxb is commonly known as “Water Chestnut”, in India and is an aquatic plant, which is usually rooted in the mud; it bears a rosette of floating leaves at the tip of the submerged stem and plant having both vegetative reproduction and seed production take place. However, modern molecular research places *Trapa* species in the Lythraceae in the order Myrtales. The *Trapa* species are determined by fruit morphology and plants with four stout horns on the fruit most often are called *Trapa natans*. The seeds of water

chestnut is having ample *Trapa* starch used in making liniments for the cure of rheumatism, sunburn, sore throat, bilious affections, bronchitis, fatigues, inflammation, nutrient, appetizer, astringent, diuretic, aphrodisiac, cooling, antidiarrhoeal tonic and it also having cancer preventing properties reported and used in many Ayurvedic preparation [5, 6, 7]. Plant stem have been also used in eye disorders. The dried peels of fruits are used in bleeding disorders, threatened abortion, dysuria, polyuria and oedema. [8, 9, 10].

The present work aimed to find out the anthelmintic activity of *Trapa natans* L. peels and establish scientific evidence for its ethnobotanical use.

## MATERIALS AND METHODS

**Plant Collection and Identification:** The plant *Trapa natans* L. var. *bispinosa* Roxb was collected in the month of September to October 2010 from the river and lakes of village Janjgir -Champa, Chhattisgarh, care was taken to obtain a root from healthy condition plant. The plant material was taxonomically identified and authenticated as a root of *Trapa natans* L.var. *bispinosa* Roxb at the Botanical Survey of India, Shillong. Reference number of the Authentication Letter is CNH/ I-I (10)/ 2010/ Tech. II / 65. Samples were preserved in Department of Pharmacognosy, Royal College of Pharmacy Science Raipur, (C.G) for future reference.

**Preparation of Extract:** The peels were removed from fruits by hand and shade-dried. Dried peels then converted into powdered with mechanical grinder and passed through sieve No. 40, finally stored in an airtight container for further study. The plant root were cut and dried in shade, then cut into small pieces, powdered with mechanical grinder and macerated with solvent. The dried coarse powder of root and peels were charged separately in to different soxhlet apparatus for hot extraction and extracted with different solvent according to the solvent polarity chloroform, ethyl acetate, methanol and water successively. Aqueous and methanolic extract of roots and peels were filtered and dried under reduced pressure in vacuum dryer to get a sticky mass and were then used for anthelmintic effects.

**Experiment:** The anthelmintic activity of aqueous and methanolic extracts of peel and root of plant were carried out as per the procedure of Ajaiyeoba *et al.* [11] with some minor modifications. Indian earthworm i.e., *Pheretima posthuma* and aquarium worm *Tubifex tubifex* were used in the present study. The earthworm *Pheretima*

*posthuma* used as a model for the anthelmintic activity assay because of anatomical and physiological resemblance of *Pheretima posthuma* with the intestinal round worm parasite of human being and easy availability. The aquarium worm *Tubifex tubifex* was used because it belongs to the same group of annelida [12-18].

The Indian earthworm *Pheretima posthuma* collected from slum area soil of Raipur. An average sized earthworm 5.5-6 cm were washed with raise tap water for the removal of the adhering dirt and cultured using commonly practical method. Aquarium worm *Tubifex tubifex* were collected from the local market of Raipur, average size of worms 2-2.5 cm. were taking study.

In experiment both worms were divided into 16 groups, 4 worms each approximately same size were placed in open glass beaker along with the standard and test sample 50ml each. The standard drug Piperazine citrate 10mg/mL, distilled Water as control and three different concentrations of aqueous and methanol extracts of peel and root (5, 10 and 20 mg/mL) in double distilled water were prepared freshly and used for the study of anthelmintic activity. The anthelmintic activity was determine at two different stage 'time of paralysis' and 'time of death' of the worms. Time taken for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time taken for death was recorded when the worms lost their motility completely and death was ascertained when the worm neither moved at vigorously shaken nor dipped into warm water 50°C and followed with fading away of their body colors. A maximum time period 120min was observed for the ascertain time for the paralysis as well as time for death of both worms.

**Statistical Analysis:** All experiment was repeated thrice. The mean and  $\pm$  SEM, were calculated by one-way ANOVA followed by Dunnett's test and values of  $P < 0.05$  being considered as statistically significant.

## RESULTS AND DISCUSSION

The predominant effect of piperazine citrate which is used as the reference standard drug in experiment produces hyperpolarisation of muscles by its GABA agonistic action opening  $Cl^-$  channels that causes relaxation and depresses responsiveness to contractile action of acetylcholine occurs flaccid paralysis and thereby expulsion of the worm by peristalsis. The worms recovered normal stage if they placed in a piperazine free medium [19]. The results of experiment shown in Table 1, revealed that methanolic peel extract showed

Table 1: Anthelmintic activity of fruit peel and root aqueous and methanolic extract of plant *Trapa natans* L.

Group	Conc. (mg/mL)	<i>Pheretima posthuma</i>		<i>Tubifex tubifex</i>	
		Paralysis (min.)	Death (min.)	Paralysis (min.)	Death (min.)
Control (Dist. Water )	-	-	-	-	-
Piperazine citrate	10	28.0±0.57	66.3±0.88	29.0±0.57	43.3±0.66
Fruit Peel Extract (Methanol)	5	65.0±1.15	82.3±1.20	65.3±0.88	83.3±2.90
	10	56.3±0.88	73.0±0.57	56.33±0.88	57.6±1.45
	20	38.0±0.57	69.0±0.57	43.0±0.57	49.0±0.57
Fruit Peel Extract (Aqueous)	5	102.6±1.52	117.0±0.52	86.6±0.88	101.6±0.88
	10	86.0±0.57	103.0±0.57	74.0±0.57	98.3±0.88
	20	65.6±1.20	77.6±0.14	47.3±1.20	72.0±1.15
Root Extract (Methanol)	5	□120	NA	□120	NA
	10	□120	NA	□120	NA
	20	□120	NA	□120	NA
Root Extract (Aqueous)	5	□120	NA	□120	NA
	10	□120	NA	□120	NA
	20	□120	NA	□120	NA

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Each value of result are represents as mean ± SEM (n=3) \* P<0.05, as compared to standard and NA= Not applicable

significant anthelmintic activity out of all tested extracts. Result demonstrated that time of paralysis as well as death of worms of methanolic peel extract shown as compared to piperazine citrate especially at higher concentration of 20 mg/mL.

By employing one-way ANOVA, all data were found to be statistically significant at 5% level of significance (p<0.05). The extent of activity shown by the crude peel methanolic extracts was found to be better than methanolic and aqueous root extract and aqueous peel extract of *Trapa natans* plant. However, less than the standard drugs Piperazine citrate and confirmed that the methanolic extract of fruit peels of plant *Trapa natans* is having anthelmintic activity.

The literature have been reported that the presence of flavonoids, tannins and polyphenolic compounds show anthelmintic activity, [20, 21] as they can bind to free protein in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and thereby causes death [22, 23]. Some synthetic phenol anthelmintics e.g. niclosamide, oxiclozanide and bithionol are shown effects to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation and phosphorylation [24]. Phytochemical analysis of the crude seed extracts revealed presence of carbohydrates phytosterol, saponins, fixed oil and fats and flavonoids, tannins and glycoside in pericarp extract of fruits of *Trapa natans* [25].

It is expected that tannin content of the peel methanolic extracts of *Trapa natans* produced similar effects therefore; further studies would be interesting and

required to identify the active chemical constituents actually responsible for the anthelmintic activity and to study its further pharmacological action. The wormicidal activity of the alcoholic extract against the earthworms suggested that resembling same effectiveness against the human parasitic infections.

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