

## ANTHELMINTIC ACTIVITY OF THE ALCOHOLIC EXTRACT OF THE DRIED FRUITS OF *Piper nigrum* L.

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### ABSTRACT

*Piper nigrum* L., commonly known as Black pepper, is a flowering climber native to South India. The dried fruits of this plant is usually used as spice and seasoning and is famed as 'world's most traded spice'. In addition to its use as a spice, black pepper is known to possess ethno-medical properties too. A study was carried out to analyze the anthelmintic properties of the alcoholic extract from the sun-dried fruits of *P. nigrum*, so that, the plant can be used as a safe and easily available medicine against helminthiasis in human beings. Adult Indian Earth worm, *Phertima Prosthuma*, being similar to intestinal round worm parasites of human beings in anatomy and physiology, was used as the experimental animal. The helminthocidal activity of the fruit extracts was compared with the reference standard, Albendazole. The study revealed that, the alcoholic extracts from the sun-dried fruits of *P. nigrum* possess dose-dependent anthelmintic activity and is more effective than Albendazole against *P. prosthuma*.

**KEYWORDS:** Anthelmintic, Intestinal Parasites, *Phertima prosthuma*, *Piper nigrum*

Helminthiasis or intestinal worm infection is regarded as the most common public health problem in developing countries. Worms, commonly known as 'helminths', include pinworm, roundworm and tapeworm which live parasitically inside the gastrointestinal tract of animals and human beings. These worms alter their hosts' nutrient absorption while receiving nourishment from the host and cause diseases like anaemia, malnutrition, eosinophilia and pneumonia. Most of the diseases caused by helminths are of a chronic, debilitating nature; they probably cause more morbidity and greater economic and social deprivation among humans and animals than any single group of parasites (Bundy, 1994). Helminths also harm the hosts by secreting some toxins that lead to serious diseases. Control of helminths infection is widely based on synthetic drugs. But the prolonged use of a limited number of chemical compounds against helminths has led to the additional problem of drug resistance in helminths (Sondhi *et al.*, 1994; Tagbota & Townson, 2001). Henceforth, it is important to evaluate the efficacy of folklore medicinal plants on their proclaimed anthelmintic efficacy.

A large number of medicinal plants are claimed to possess anthelmintic properties and are utilized by ethnic people worldwide. As plants and plant products are free from side-effects, they are preferable than synthetic drugs in curing diseases. Moreover, plant-based drugs are easily available and are least expensive. There are reports on the efficacy of a number of medicinal plants in curing intestinal parasitism in human beings (Sarkar *et al.*, 2000; Vidya *et al.*, 2012; Ullah *et al.*, 2013; Sheeja *et al.*, 2014). Among the large number of plants that are claimed to

possess anthelmintic properties, *Piper nigrum* is the most commonly used one in our locality to check helminthiasis.

*Piper nigrum*, commonly known as black pepper, is a flowering vine that belongs to the family Piperaceae. The plant is native to South India and is cultivated there and elsewhere in tropical regions for its fruit, which is usually dried and used as a spice. Black pepper is the world's most traded spice. It is one of the most common spices added to cuisines around the world. The spiciness of black pepper is due to the chemical piperine (Johri & Zutshi, 1992; Meghwal & Gowsami, 2013). Piperine is an alkaloid found naturally in the fruit of black pepper and long pepper (*Piper longum* L) that possess some antimyco-bacterial, anticonvulsant, anti carcinogenic and anthelmintic properties (Sudhakar *et al.*, 2013). Present study was conducted to analyze the anthelmintic property of *P. nigrum* against intestinal round worm parasites of human beings *in vitro* so as to reveal its potentialities to be used as a safe and easily available medicine against helminthiasis in human beings.

### MATERIALS AND METHODS

#### Preparation of Plant Extracts

Fruits of *P. nigrum* were collected fresh from the plant from Manrothuruthu village of Kollam District, Kerala, South India and were identified and authenticated in the Department of Botany, SN College for Women, Kollam, Kerala, South India. Fruits were separated out from the pepper corm, spread in a clean plate and sun-dried for 1 week. The dried fruits were ground into fine powder using a mixer grinder (Philips Mixer Grinder, India) and passed through sieve no. 40. The fine powder

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was then subjected to Soxhlet extraction using ethanol as solvent. The obtained liquid extracts were subjected to Rotary evaporator and subsequently concentrated under reduced pressure (in vacuum at 40°C). The dried powder was stored at 4°C in air tight bottle for further use.

### Experimental Animals

Adult Indian Earth worm, *Pheritima prosthuma* (Plate.1), being anatomically and physiologically similar with the round worm parasites of human beings (Vigar 1984; Dash et al., 2002; Mali & Mehta, 2007; Satish & Ravindra, 2009) was used as the experimental animal to evaluate the anthelmintic activity of *P. nigrum* fruits *in vitro*. Earthworms with an average size of 6-8 cm were collected from swampy area in our campus and were identified in the Department of Zoology, SN College for Women, Kollam. The worms were kept in normal saline solution.

### Drugs and Chemicals

The reference drug, albendazole with the trade name 'Zentel' (Glaxo SmithKline Pharmaceuticals Ltd, Mumbai, India) was used during the experimental protocol. In all the experiments 50 mg ml<sup>-1</sup> albendazole was used as the reference standard and distilled water was used as the control. To prepare a concentration of 50 mg ml<sup>-1</sup> albendazole, the tablets were crushed using mortar and pestle and 5 mg of it was accurately weighed out and dissolved in 100 ml distilled water.

### Anthelmintic Activity

The anthelmintic assay was carried out as per the method of Ajaiyeoba *et al* (2001). To test the anthelmintic activity of *P. nigrum* fruit extracts, test solutions of three different concentrations (15, 30 and 50 mg/ml) were prepared by dissolving required quantity of the fruit extract in distilled water. The equi-sized worms were divided into five groups; each group is with two worms. Group 1 to group 3 worms were placed in each 9 cm Petri dish containing 25 ml of the three test solutions, group 4 was placed in the reference standard and group 5 was placed in control solution. All the test solutions and standard drug solution were prepared freshly before starting the experiments. Observations were made for time taken for paralysis when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50°C). The experiments were repeated thrice and the observations were expressed as mean ± SD.

## RESULTS AND DISCUSSION

The results of the experiments conducted to analyze the anthelmintic activity of the alcoholic extracts of the sun-dried fruits of *P. nigrum* are given in Table 1. From the observations made it is clear that, the time taken for paralysis and death of the worms decrease with increase in the concentration of the extract (Plate 2). The results reveal that, the alcoholic extract shows anthelmintic activity in dose-dependent manner giving shortest time of paralysis (7 minutes) and death (12 minutes) with 50 mg/ml concentration of the test solution. It was also observed that, the fruit extract exhibits more potent activity against the test animal even at its lowest concentration of 15 mg/ml (paralysis in 15 minutes and death in 20 minutes) when compared to the reference standard (50mg/ml Albendazole) in which paralysis and death were observed in 40 minutes and 50 minutes respectively. From the above results, it is concluded that, the fruits of *P. nigrum* possess dose-dependent anthelmintic activity and the activity of *P. nigrum* fruits against *P. prosthuma* is much greater than that of the commonly used commercial drug Albendazole. The experimental evidence obtained in the laboratory model could provide a rationale for the use of *P. nigrum* fruits as an easily available anthelmintic.

**Table 1: Anthelmintic activity of the fruit extracts of *P. nigrum***

Sl No.	Treatment	Paralysis time (minutes)	Death time (minutes)
1	Normal Saline	--	--
2	Albendazole (50 mg ml <sup>-1</sup> )	40 ± 2.64	50 ± 2.83
3	Fruit extract (15 mg ml <sup>-1</sup> )	15 ± 2.43	20 ± 3.42
4	Fruit extract (30 mg ml <sup>-1</sup> )	10 ± 2.52	16 ± 3.21
5	Fruit extract (50 mg ml <sup>-1</sup> )	7 ± 2.44	12 ± 2.63



Plate 1: Experimental animal, *Pheritima prosthuma*



Plate 2: *P. prosthuma* on different concentrations of the test solution

## ACKNOWLEDGEMENTS

The author is thankful to Dr. Sherly P. Anand, Principal, SN College for Women, Kollam, Kerala for providing necessary facilities to carry out present research work. The author is grateful to Dr. S. Sekaran, Head, Department of Botany, SN College for Women, Kollam, Kerala for authentication of plant specimens and Dr. S. Sreeja, Head, Department of Zoology, SN College for Women, Kollam, Kerala for identification of worms. The author is grateful to the authorities of 27<sup>th</sup> Swadeshi Science Congress for giving an opportunity to present this research work.

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