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**In Vitro Evaluation of Anthelmintic activity of the roots and leaves of *Pavetta indica* Linn.**

Satkar Prasad<sup>\*a</sup> and Anand Chaurasiya<sup>b</sup>

<sup>a</sup> PhD Research Scholar, Suresh Gyan Vihar University, Jaipur, (Rajasthan) - India

<sup>b</sup> Asso. Prof., Swami Vivekanand College of Pharmacy Indore, (MP) - India

**Abstract**

The present study was to evaluate the anthelmintic activity of petroleum ether, chloroform & methanol extracts of roots and leaves of *pavetta indica* linn. (Rubiaceae) against Indian adult earthworms (*Pheretima posthuma*) and roundworm (*Ascaridia gali*). The parameters like the time of paralysis and the time of death were determined by using the different extract at different concentration (25, 50, and 100 mg/ml). Albendazole (in 5% aqueous DMF) was used as reference standard and 5% aqueous in DMF as a control group. Higher activities were observed at the higher concentration. Dose dependent activity was observed in all extracts. The shortest time required for paralysis and death was observed with concentration of 100 mg/ml of methanol extract of roots of plant. The studies indicate that the root extract of plant exhibited more potent activity as compared to leaves extracts.

Key-Words: *Pavetta indica*, *Pheritima posthuma*, *Ascardia galli*, Albendazole

**Introduction**

Helmentic infections are among the most widespread infections in humans, distressing a huge population of the world. Although the majority of infections due to helminthes are generally restricted to tropical regions and cause enormous hazard to health and contribute to the prevalence of under nourishment, anaemia, eosinophilia and pneumonia. Parasitic diseases cause ruthless morbidity affecting principally population in endemic areas. The gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases. Hence there is an increasing demand towards natural anthelmintic.

*Pavetta Indica* linn. belonging to family Rubiaceae is called in hindi Kankara. It is a bushy shrub 0.6-12 m in height. Distributed the greater part of India ascending to an altitude of 1500m in the Himalayas, it has also recorded from Andamans (2). The literature survey reveals that the plant *Pavetta indica* linn. has been used as diuretic, purgative, anticephalagic & prescribed in jaundice, headache, haemorrhoidal pain in dropsy (3). *Pavetta Indica* having anti-inflammatory activity (4), analgesic activity (5), diuretic and purgative activity (6), antimicrobial activity of leaves, antidiabetic activity of methanolic extract (7). More than 400 species of *pavetta* genus is available.

Some other species showed different activity like *P. crassipes*, *P. pyroids* & *P. gradenifolia* is reported to antimalarial activity (8, 9). The present paper reports the anthelmintic activity of roots & leaves extracts of *pavetta indica* against *Pheritima posthuma* and *Ascardia galli*.

**Material and Methods**

**Plant material**

The leaves & Roots of the plant *Pavetta Indica* linn were collected from Bhopal (Vindya herbal medicinal garden) in the month of September and authenticated by the department of Botany, Safia College Bhopal and voucher specimen 087/bot.1/saf/13 deposit in the college. The leaves were dried under shade for 15 days & roots were dried under shade for 30 days. Then the dried material was homogenized to coarse powder and was stored in airtight container.

**Preparation of extract**

Extraction of plant material was done by successive solvent extraction method using various organic solvent as according to non-polar to polar. The plant material is placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the Soxhelt extractor. This extractor is placed onto a distillation flask containing the solvent. The Soxhelt is then equipped with a condenser, and the solvent is heated to reflux. The warm solvent vapor travels up a distillation arm and floods into the chamber housing the thimble. When the chamber is almost full, it gets

**\* Corresponding Author**

E.mail: satkarprasad@yahoo.co.in

automatically emptied by a siphon side arm back down to the distillation flask. This cycle may be allowed to repeat many times so that the desired compound gets concentrated in the distillation flask. 150 grams of this powdered material were extracted using soxhlet apparatus using 250 ml of various solvents (Pet ether, Chloroform and ethanol) successively for 24 hours (40 °C). The extracts were filtered and used for phytochemical screening and excess filtrate was filtered through a single layer of muslin cloth, and then final filtrate was collected by passing it through a Whatman grade 1 filter paper in a Buchner funnel under vacuum. The filtrate was evaporated to dryness. The extracted materials were stored in air tight container until the time of use. (10, 11)

#### Collection of worms

Indian adult earthworms (*Pheretima posthuma*) were collected from moist soil and washed with water to remove all fecal matters. Roundworm (*Ascaridia gali*) was obtained from intestine of freshly slaughtered fowls. Infested intestine of fowls were collected from the local slaughter house and washed with normal saline solution to remove all the fecal matters. These intestines were then dissected and worm were collected and kept in normal saline solution. The average sizes of earthworm are 4-6 cm length and roundworms are 5-7 cm.

#### Drug used

Albendazole was used as standard reference drug for anthelmintic study.

#### Anthelmintic activity.

S.no	Group	Concentration (mg/ml)	<i>Pheretima posthuma</i> (Earthworm)		<i>Ascaridia gali</i> (Roundworm)	
			Paralysis (In Min.)	Death (In Min.)	Paralysis (In Min.)	Death (In Min.)
1	Control	-	-	-	-	-
2	Standard (Albendazole)	25	19.52±1.08	30.06±1.76	21.60±1.16	37.26±1.32
		50	11.57±1.78	25.16±2.47	15.86±1.93	33.42±1.22
		100	2.55±0.76	4.37±1.06	2.50±0.74	4.28±0.66
3	Petroleum ether extract of leaves	25	32.12±1.26	50.25±1.58	39.48±2.08	62.60±1.04
		50	23.01±1.07	45.65±1.65	33.45±1.50	59.86±1.34
		100	15.56±0.56	17.62±0.14	12.53±0.16	14.16±0.05
4	Chloroform	25	26.22±1.82	54.26±0.88	34.18±1.24	62.24±2.02

The anthelmintic activity of the leaves and root extracts were performed as per the method of Ajaiyeoba et al.(12).. with slight modification on indian earthworm (*Pheretima posthuma*), due to its anatomical & physiological resemblance with the intestinal roundworm parasite of human being(13) and roundworm (*Ascaridia gali*) are easily available from intestine of freshly slaughtered fowls.

The petroleum ether, chloroform and methanol extracts of leaves and roots of *pavetta indica* were suspended in 5% aqueous DMF. The worms are divided into 40 groups (20 for *Pheretima posthuma* & 20 for *Ascaridia gali*) with each containing six worms were placed in petridish. Two group serves as control & two group serves as standard for both type of worms and 18 sets of two different type of group was treated with extracts with desired concentration. Observation were made for the time taken for paralysis and death of individual for worm and mean time was taken for all the groups(12).

#### Statistical Analysis

The results were analyzed for statistical significance using one way ANOVA followed by student t- test. The P value (<0.001) was considered significant.

#### Results and Discussion

Table- 1 Anthelmintic activity of leaves and roots extracts of *Pavetta indica* on *Pheretima posthuma* & *Ascaridia gali*.

	extract of leaves	50	21.66±2.18	46.33±1.98	26.22±1.89	57.64±2.12
		100	11.43±0.64	13.11±0.26	10.43±0.11	13.19±0.25
5	Methanol extract of leaves	25	18.06±1.26	26.06±1.64	21.08±1.24	38.18±1.90
		50	14.07±1.66	22.07±1.66	17.03±2.42	35.02±2.79
		100	4.27±0.02	6.43±0.07	4.55±0.23	6.53±0.22
6	Petroleum Ether extract of Roots	25	30.16±0.60	48.28±1.14	38.28±1.22	60.24±2.02
		50	22.76±1.67	40.62±2.24	30.67±2.03	653.51±1.42
		100	12.43±0.88	16.12±0.45	7.41±0.01	9.23±0.41
7	Chloroform extract of roots	25	26.24±1.11	50.28±2.28	33.08±1.24	61.28±1.42
		50	20.66±2.18	44.33±1.98	28.87±2.64	52.33±2.54
		100	9.16±0.66	12.17±0.16	4.12±0.54	6.11±0.03
8	Methanol extract of roots	25	17.32±1.95	25.36±1.24	19.08±1.04	35.90±1.62
		50	12.36±2.02	20.53±2.54	16.89±1.43	25.97±1.26
		100	3.19±0.11	5.11±0.09	3.12±0.54	4.76±0.03

Results are expressed as Mean ± SEM, n= (6), student‘t’ test vs control

Anthelmintic activity of *Pavetta indica* linn is confirmed by examining the time taken for paralysis and death for *Pheretima posthuma* & *Ascaridia gali* worms were reported in table 1. The extracts were showing dose dependent anthelmintic activity. From the above result it is observed that at higher concentration methanolic extract showing better activity as compare to other two extracts (pet ether & chloroform). In case of *Pheretima Posthuma*, methanol extract of leaves at concentration of 100 mg/ml showed paralysis time is 4.27 Min & death time is 6.43 min, while methanol extract of roots at concentration of 100 mg/ml showed paralysis time is 3.19 min & death time is 5.11 min. In case of *Ascaridia gali*, methanol extract of leaves at concentration of 100 mg/ml showed paralysis time is 4.55 Min & death time is 6.53 min, while methanol extract of roots at concentration of 100 mg/ml showed paralysis time is 3.12 min & death time is 4.76 min, which showed that root extract having more potent anthelmintic activity against *Pheretima posthuma* and *Ascardia gali* as compare to leaves extracts

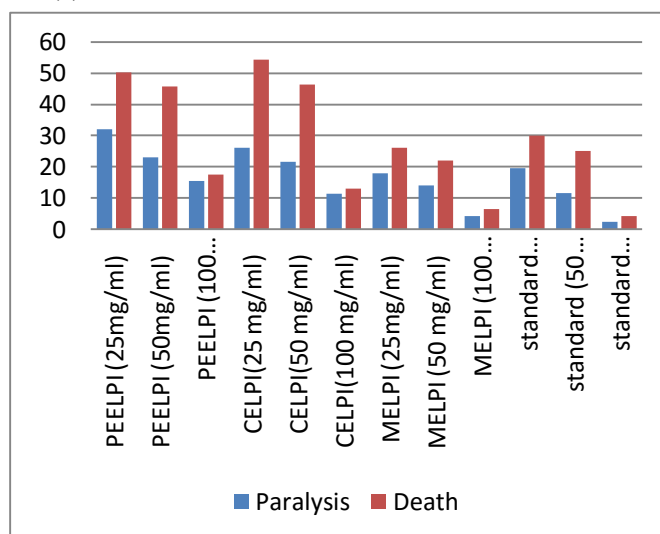


Fig. 1: Paralysis and death time for different extracts in different concentration of leaves of *Pavetta indica* Linn. against *Phertima posthuma*

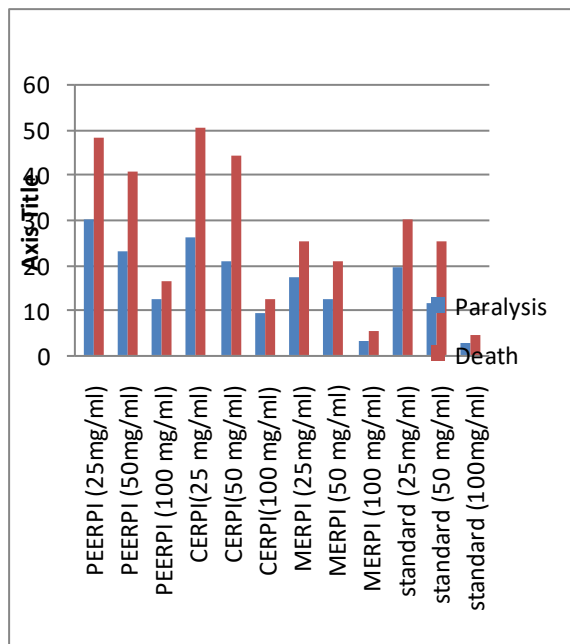


Fig. 2: Paralysis and death time for different extracts in different concentration of roots of *Pavetta indica* Linn. against *Phertima posthuma*

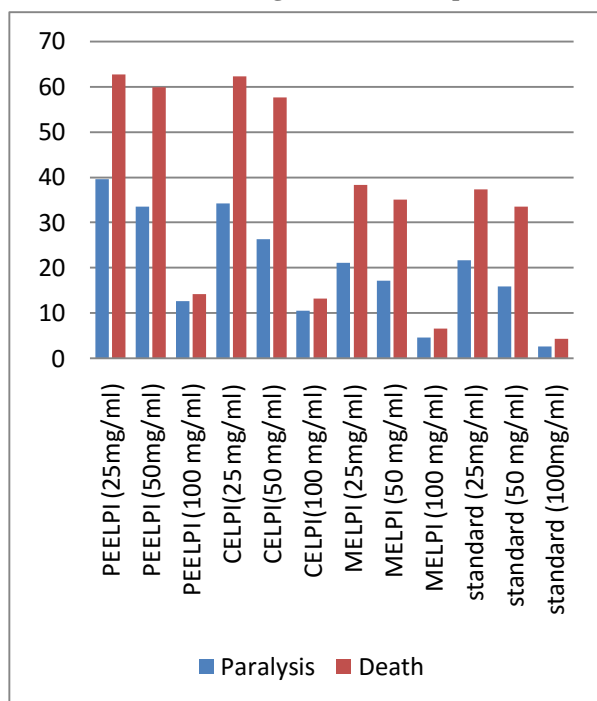


Fig. 3: Paralysis and death time for different extracts in different concentration of leaves of *Pavetta indica* Linn. against *Ascardia gali*.

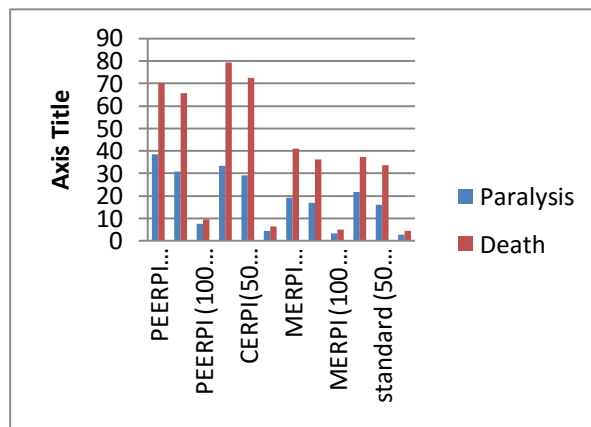


Fig. 4: Paralysis and death time for different extracts in different concentration of roots of *Pavetta indica* Linn. against *Ascardia gali*.

### Conclusion

The result of present study clearly indicate that leaves & roots extracts of *pavetta indica linn.* having anthelmintic activity against *Pheretima posthuma* and *Ascardia gali*. The plant possesses significant anthelmintic activity of methanolic extracts of roots. The current investigation leads to conclusion that the root of *pavetta indica linn* has potent anthelmintic activity, when compared with leaves of *pavetta indica*. Further, in future it is necessary to identify & isolate the possible active phytoconstitute responsible for the anthelmintic potential.

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