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Original Research Paper

INVESTIGATION OF ANTHELMINTIC ACTIVITY OF *PERGULARIA DAEMIA* LEAVES

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ABSTRACT

The present study was carried out to assess the anthelmintic activity of *Pergularia daemia* leaves. The different extracts of *Pergularia daemia* leaves were evaluated separately on earthworm (Eudrillus eugeniae), roundworm (Ascaris lumbricoides) and tapeworm (Taenia solium) using albendazole as reference standard. The extracts caused paralysis followed by the death of warms at tested dose levels. The both extracts at the highest tested concentration significantly exhibits ($P<0.01$) paralysis and also caused death of worms as comparable with that of standard drug albendazole. The study displayed that ethanol extract of *Pergularia daemia* leaves shown most significant anthelmintic activity than the aqueous extract. Further studies are suggested to isolate the active principles from the plant extracts responsible for the activity.

Keywords: Albendazole, Anthelmintic, Death, Paralysis, *Pergularia daemia*.

INTRODUCTION

The World Health Organization reveals that over two billion people are suffering from parasitic worm infections (Mulla *et al*., 2010). It is estimated that by the year 2025, about 57% of the population in developing countries will be influenced (Clewes and Shaw, 2000). Most of the existing anthelmintics produce side effects such as abdominal pain, loss of appetite, nausea, vomiting, headache and diarrhea (Devi *et al*., 2009). Anthelmintics from the natural sources may play a key role in the treatment of these parasite infections without side effects, when compare to synthetic drugs (Aswar *et al*., 2008). Increasing problems of development of resistance in helminths against anthelmintics have led to the proposal of screening medicinal plants for their anthelmintic activity (Iqbal *et al*., 2004). The plant *Pergularia daemia* (Asclepiadaceae) in Tamil Veliparuthi; It's distributed throughout India. Traditionally the plant *Pergularia daemia* is used as anthelmintic, astringent, emetic, laxative, antipyretic and expectorant, also used to treat infantile diarrhoea and malarial intermittent fevers. The plant leaves decoction is given as an anthelmintic, the juice of the leaves is used as expectorant in the treatment of catarrhal affection (Kirtikar and Basu, 2005; Nadkarani, 1976). Aerial parts of this plant reported the various pharmacological activities like hepatoprotective (Sureshkumar and Mishra, 2006), antifertility (Golam Sadik *et al*., 2001a), anti-diabetic (Wahi *et al*., 2002, analgesic, antipyretic and anti-inflammatory (Sathish *et al*., 1998). The anthelmintic studies have not been reported for the leaves of this plant. Based on this, an attempt has been made to evaluate the anthelmintic

potential of *Pergularia daemia* leaves against earthworm, roundworm and tapeworm by in-vitro model.

MATERIALS AND METHODS

Plant Materials

The plant material (leaves) was collected from the town of B. Komarapalayam. The plant was identified and authenticated by joint director, botanical survey of India, southern circle, Coimbatore. The collected leaves were dried in the shade, leaves are crushed to coarse powder. The powdered mass was passed through sieve no 60 and used for extraction.

Ethanolic Extract Preparation

The crude ethanolic extract of the *Pergularia daemia* leaves was prepared according to the standard method. One hundred grams of the powder leaves material of *Pergularia daemia* was placed in a thimble and extracted with 70% ethanol in a Soxhlet apparatus for 8-12 h. Solvents were removed at temperature below 50ºC in an oven. The residue (extract) of respective plant material was stored at 4°C until used.

Aqueous Extract Preparation

The crude aqueous extract of the *Pergularia daemia* leaves was prepared according to the standard method. The powdered plant material was mixed with 500 mL of distilled water in a round bottom flask apparatus for 8-12 h. The filtrate was then concentrated in a rotary evaporator and the extract stored at 4ºC until required.

Phytochemical Screening

The extracts of *Pergularia daemia* leaves were subjected to preliminary phytochemical screening (Khandelwal 2008, Harborne, 1998).

ANTHELMINTIC ACTIVITY

Selection of Worms

Adult earthworms (*Eudrillus eugeniae*), Roundworm (*Ascaris lumbricoids*) and Tapeworms (*taenia solium*) were used to evaluate anthelmintic activity because it's having anatomical and physiological resemblance with intestinal earthworm, roundworm and tapeworm parasite of the human being. Earthworms were collected from the moist surface soil and under stones in Agriculture University, Coimbatore, India. Roundworms and tapeworms were obtained from intestine of freshly slaughtered fowls. Infested intestines of fowls were collected from the local slaughter house and washed with normal saline solution to remove all the faecal matter. These intestines were then dissected and worms were collected and kept in normal saline solution. The average size of earthworm was 5-8 cm, average size of round worm was 7-25 cm and average size of tapeworm was 10-20 cm (Seema Nakhare and Garg 1991, Venkatachalam *et al*., 2010).

Anthelmintic Activity

The anthelmintic assay was carried out as per the standard method with minor modifications (Gaind and Budhiraja 1967, Satish *et al*., 2009). Test samples of the extracts was prepared at the concentrations (25, 50, 100 mg/ml) in distilled water and six worms i.e. *eudrillus eugeniae, ascaris lumbricoids and taenia solium* of approximately equal size (same type) were placed in each nine cm Petri dish containing 25 ml of above test solution of extracts. Albendazole was used as reference standard and distilled water as control. This procedure was adopted for all three different types of worms. All the test solution and standard drug solution were prepared freshly before starting the experiments. Observations were made for the time taken for paralysis was noted 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 (50ºC).

Statistical Analysis

Data were analyzed using one way factorial ANOVA tests followed by Dennett's t - tests on each group. P values under 0.01 were considered highly significant (shown as **).

RESULTS

Phytochemical Analysis

Preliminary phytochemical screening of the extracts of *Pergularia daemia* leaves revealed the presence of carbohydrate, flavonoids, glycosides, tannins & phenolic compounds and saponins (Table 1).

Anthelmintic Activity

The anthelmintic activity of ethanolic and aqueous extract of leaves of *Pergularia daemia* exhibited anthelmintic activity using *Eudrillus eugeniae* (Earthworm), *Ascaris lumbricoids* (Roundworm), *Taenia solium* (Tapeworm) in dose-dependent manner giving shortest time of paralysis (P) and death (D) with 100 mg/ml concentration. The ethanolic extract caused paralysis and time of death was (16.86 ±0.74 min), (27.12±0.52 min) for earthworm and (20.86±0.54 min), (61.84±0.54 min) for roundworm and also (54.12±0.49 min), (110.17±0.59 min) for tapeworm while aqueous extract revealed paralysis and time of death was (20.91±0.31 min), (30.89 ±0.45 min) for earthworm and (32.33±0.67 min), (76.19±0.56 min) for roundworm and also (64.44±0.54 min), (172.14±0.81 min) for tapeworm respectively was compare with standard drug albendazole showed the paralysis and time of death was (12.21±0.31), (21.89±0.49) for earthworm and (18.21±0.54), (52.98±0.75) for roundworm and also (47.51±0.39), (158.94±0.51) for tapeworm respectively. From the above result tape worm will take more time to paralysis and death at all test doses as compared to earth worm and round worm. From the above results extracts of *Pergularia daemia* leaves produced a significant ($P<0.01$) anthelmintic activity in dose dependent manner as shown in Table 2.

DISCUSSION

Preliminary phytochemical screening of the extracts of *Pergularia daemia* leaves revealed the presence of carbohydrate, flavonoids, glycosides, tannins & phenolic compounds and saponins. Albendazole by increasing chloride ion conductance of worm muscle membrane produced hyper polarization and reduced excitability that led to muscle relaxation and flaccid paralysis (Mali *et al*., 2005). The extracts of *Pergularia daemia* leaves not only demonstrated paralysis, but also caused death of worms especially at higher concentration of 100 mg/ml, in shorter time as compared to standard drug Albendazole. Phytochemical analysis of the crude extract revealed the presence of tannins and phenolic compounds is one of the chemical constituents contained within them. Tannins were shown to produce anthelmintic activities. Chemically tannins are polyphenolic compounds (Martin, 1985). Tannins and Phenolics are known to interfere with the energy generation in helminth parasites by uncoupling oxidative phosphorylation (Athnasiadou *et al*., 2001) and also bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite, leading to death. It is possible that tannins and phenolic compounds also having in the extracts of *Pergularia daemia* leaves may be responsible for the anthelmintic activity.

CONCLUSION

From the above results, it is concluded that *Pergularia daemia* leaves extracts have significantly determined anthelmintic activity. The ethanolic extracts of *Pergularia daemia* leaves shown most significant anthelmintic activity as compare to the aqueous extracts. The plant may be further explored for its phytochemical profile to recognize the active constituent responsible for anthelmintic activity.

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Table 1: Preliminary phytochemical screening of different extracts of *Pergularia daemia* leaves

S. No	Test of extract	Ethanol extract	Aqueous extract
1	Carbohydrates	+	+
2	Proteins & amino acids	-	-
3	Glycosides	+	+
4	Alkaloids	-	-
5	Phytosteroids	-	-
6	Favonoids	+	+
7	Saponin	+	+
8	Tannins & phenolic Compounds	+	+
9	Fixed oils & fats	-	-
10	Gums & mucilages	-	-

(+) Present, (-) Absent

Table 2: Anthelmintic activity of different extracts of *Pergularia daemia* leaves

S. No	Groups	Concentration (mg/ml)	*Eudrillus eugeniae* (Earthworm)		*Ascaris lumbricoids* (Roundworm)		*Taenia solium* (Tapeworm)	
			Time taken for paralysis (P) in min. (Mean & SEM)	Time taken for death (D) in min. (Mean & SEM)	Time taken for paralysis (P) in min. (Mean & SEM)	Time taken for death (D) in min. (Mean & SEM)	Time taken for paralysis (P) in min. (Mean & SEM)	Time taken for death (D) in min. (Mean & SEM)
01	Control (Water Only)	-----	-----	-----	-----	-----	-----	-----
02	Albendazole	25 mg/ml	20.61±0.48	30.94±0.39	37.42±0.42	90.86±0.81	69.42±0.29	201.14±0.35
		50 mg/ml	16.43±0.51	29.52±0.58	21.22±0.42	63.61±0.54	54.86±0.29	172.02±0.43
		100 mg/ml	12.21±0.31	21.89±0.49	18.21±0.54	52.98± 0.75	47.51±0.39	158.94±0.51
03	Ethanolic extract	25 mg/ml	22.84±0.52**	37.14±0.72**	41.86±0.31**	98.42±0.57**	73.12±0.52**	210.00±0.52**
		50 mg/ml	20.12±0.39**	33.14±0.31**	31.84±0.34**	71.40±0.30**	61.89±0.24**	194.98±0.49**
		100 mg/ml	16.86±0.74**	27.12±0.52**	20.86±0.54**	61.86±0.54**	54.12±0.49**	110.17±0.59**
03	Aqueous extract	25 mg/ml	29.94±0.31**	42.98±0.49**	52.17±0.67**	109.91±0.49**	84.58±0.56**	221.12±0.61**
		50 mg/ml	26.48±0.54**	37.52±0.45**	37.67±0.67**	84.10±0.56**	72.52±0.69**	203.94±0.59**
		100 mg/ml	20.91±0.31**	30.89±0.45**	32.33±0.67**	76.19±0.56**	64.44±0.54**	172.14±0.81**

Values are expressed as mean ± SEM. Values were find out by using ONE way ANOVA followed by Dunnett's *t* -test. ** Values are significantly different from control at (P<0.01).

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