First Report on the *in vitro* Antihelminthic Activity of *Centella Asiatica* ("Gotu Kola"/ "Vallaarai")

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Abstract— *Anthelmintic resistancehas been reported in animals and also suggested in humans. Thus, there is an urgent need for the identification and development of alternative anthelmintics for human use. With this background, a study was designed to examine two medicinal plants, Vernonia anthelminthica and Punica granatum, having known antihelmintic activity with another herbal plant, Centella asiatica. The aqueous, ethanol and methanol extracts of fresh roots, seeds and leaves of the above plants were obtained separately. Anthelmintic activity was tested in vitro by using the earth worm (Eisenia fetida)as described earlier. The worms were placed in separate Petri dishes containing 30ml suspensions of methanol, ethanol and aqueous extracts (25mg/ml and 50 mg/ml) of plant extracts. Albendazole at 20 mg/ml and 1% acacia in normal saline were used as the reference standard and the control group respectively.Time taken for complete paralysis and death of individual worms was recorded. All three crude extracts of plants showed greater anthelmintic activity than that of albendazole. The solution containing 50mg/ml aqueous extract of Vernonia anthelminthica seeds paralyzed the earth worm within the shortest time (5.10±0.14 min) followed by the solution containing 50mg/ml ethanolic extract of Centella asiatica leaves (7.09±0.12 min). Though antihelmintic activity of crude extract was increased proportionately with increased dose, all three plants showed optimum antihelmintic activity at 50mg/ml, promoting paralysis and subsequent death of the worms. It can thus be concluded that in addition to other nutritional values such as iron and folic acid, this is the first report of the antihelmitic activity of Centella asiatica.*

Keywords— Antihelminthic activity, Medicinal plants, *Centella asiatica*

I. INTRODUCTION

World Health Organization guidelines for the introduction and maintenance of deworming programmes recommend the adoption of mass prophylactic chemotherapy (treatment without prior diagnosis), with one of two anthelmintics, in communities where the prevalence of soil-transmitted helminthiasis is over 20% (WHO, 2006). Deworming on such a large scale may contribute to the development of anthelmintic resistance, as has been documented in animals (Kaplan & Vidyashankar, 2012) and also suggested in humans (Albonico *et al.*, 2004). Thus there is an urgent need for the identification and development of alternative anthelmintics for human use.

A. *Objective of the study*

This study was designed to evaluate two medicinal plants, *Vernonia anthelminthica* and *Punica granatum*, having known antihelmintic activity (Mali & Mehta, 2008) with another herbal plant, *Centella asiatica* (known as "Gotu kola" in Sinhala, "Vallaarai" in Tamil), which was also expected to have anthelmintic activity (personal communication, Ayurvedic Practitioners, Jaffna).

II. METHODOLOGY AND EXPERIMENTAL DESIGN

The fresh roots of *Punica granatum*, seeds of *Vernonia anthelminthica* and leaves of *Centella asiatica* were collected and air dried in shade at room temperature. Dried plant materials were ground to coarse powder individually by electric grinder.

The crude aqueous extracts were prepared according to the standard methods. Briefly, fifty grams (50g) of the powdered plant material was mixed with 350ml of distilled water and boiled for 1.5 h and filtered after cooling to 40°C. The filtrate was concentrated in a rotary evaporator and the dried sample was stored at 4°C until used.

The solvents extraction was done by dissolving 50 g of dried plant powder in soxhlet apparatus with ethanol and methanol (350ml) separately for 10 hrs at 65°C. The extracts were concentrated to dryness in a rotary pressure evaporator and stored at 4°C until use.

The yield of the aqueous, ethanolic and methanolic crude extract (% w/w) obtained from the plant material was recorded.

Adult earthworms (*Eisenia fetida*), which resemble the intestinal roundworm parasites of humans in some of their anatomical and physiological characters, were used for this study (Dahiya *et al.*, 2012). The worms were freshly collected from the Department of Biology, Faculty of Agriculture, University of Jaffna, and washed initially with tap water, followed by normal saline to remove soil particles and debris.

Test samples for *in vitro* study were prepared by dissolving and suspending 2.5g of aqueous, ethanol and methanol extracts of plant materials in 1% acacia solution individually. Stock solutions at concentrations of 25mg/ml and 50mg/ml were prepared separately from all three extracts using normal saline.

Anthelmintic activity was tested *in vitro* on *Eisenia fetida* as described earlier. Briefly, the worms were divided into eight groups containing six earthworms of approximately equal sizes and weight in each group. The worms were placed in separate Petri dishes containing different concentrations of the plant extracts. 30ml suspensions of methanol, ethanol and aqueous extracts (25mg/ml and 50 mg/ml) of each plant material were used as test samples. Albendazole at 20 mg/ml (which a highly effective, broad spectrum anthelmintic used for the treatment of soil-transmitted helminth infections in humans) and 1% acacia in normal saline were used as the reference standard and the control group respectively.

The time taken to complete paralysis and death of individual worms was observed. The mean time for paralysis was noted when no movement was observed even with vigorous shaking of worms and death was concluded when the worms were lost their motility followed by fading away of their body colour. The results were recorded and compared.

III. RESULTS & DISCUSSION

All three crude extracts of the plants showed greater anthelmintic activity (Table 1) than that of albendazole. When the dose of the crude extract was increased, greater anthelmintic activity was observed. Crude extracts of all three plants were highly effective at 50mg/ml, promoting paralysis of the worms and their subsequent death.

The solution containing 50mg/ml aqueous extract of *Vernonia anthelminthica* seeds paralyzed the earth worm within the shortest time (5.10±0.14 min) followed by the solution containing 50mg/ml ethanolic extract of *Centella asiatica* leaves (7.09±0.12 min).

This is the first report, to our knowledge, of anthelmintic activity of *Centella asiatica*, which has already reported to have the anti parasitic activity against the protozon parasites such as *Leishmania*, *Plasmodium* and *Trypanosoma* previously (Roy *et al.*, 2013).

		Vernonia anthelminthica		**Centella asiatica**		**Punica Granatum**	
	Dose (mg/ml)	Time to paralysis (min) Mean±SEM	Time to death (min) Mean±SEM	Paralysed Time (min) Mean±SEM	Death Time (min) Mean±SEM	Paralysed Time (min) Mean±SEM	Death Time (min) Mean±SEM
Aqueous	25	8.05±0.13	25.17±0.40	17.10±0.24	61.17±0.35	25.96±0.29	29.87±0.42
	50	5.10±0.14	21.37±0.30	12.10±0.14	22.35±0.39	13.80±0.23	22.97±0.36
Ethanol	25	24.03±0.58	34.15±0.54	11.07±0.16	15.47±0.35	34.73±0.30	42.87±0.40
	50	20.63±0.42	25.32±0.53	7.09±0.12	10.35±0.21	29.83±0.40	35.80±0.59
Methanol	25	30.18±0.64	33.40±0.50	22.13±0.19	53.07±0.35	27.73±0.32	37.27±0.52
	50	18.55±0.73	22.27±0.28	10.07±0.21	25.37±0.47	18.98±0.43	31.27±0.41
Albendazole (Std)	20	47.87±0.38	68.17±0.30	49.22±0.25	71.33±0.35	46.73±0.32	74.27±0.29
Control (1% acacia with normal saline)	Neither paralysis nor death was observed until the end of experiment.						

In addition to anti-parasitic effects, *Centella asiatica* is also reported to have various other health benefits such as antibacterial, antiviral, anti-inflammatory, antioxidant, anti-asthmatic and wound healing properties (Roy *et al.*, 2013).

Although *Eisenia fetida* resembles the anatomical and physiological characteristics with the roundworms which parasitize humans, they are of two different phyla (Annelida and Nematoda, respectively) which may account for the observed lack of efficacy of albendazole against *Eisenia fetida*.

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