EVALUATION OF *IN-VITRO* ANTHELMINTHIC ACTIVITY OF ETHANOLIC AND AQUEOUS EXTRACT OF BARK AND LEAVES OF *Crataeva religiosa*

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Abstract

Crataeva religiosa belongs to the family capparidacea. It exhibits a varity of pharmacological properties and therefore has been used in indigenous medicine for treatment of different aliments. Aqueous and ethanolic extracts of bark and leaves of this plant were evaluated for *in-vitro* anthelmintic activity using an adult earth worm, *Eisenia fetida*. Different concentrations (10, 20, 50, 100 mg/mL) of aqueous and ethanolic extract of *Creataeva religiosa* were used in this study. Mebendazole at the concentration of 20 mg/mL was used as a standard, and saline was used as a control. *In-vitro* anthelmintic activity was evaluated by determining the time taken to paralyze and death of *Eisenia fetida* against different concentrations of extracts, mebendazole and saline. All tests were performed in triplicate. One way ANOVA followed by Tukey's post hoc was used to compare the activities between extracts and mebendazole. According to the results, concentration-dependent activities were observed for all extracts on paralyzing or killing of worms. The ethanol extract of leaves and aqueous extract of bark have shown higher efficiency in anthelmintic activity than their counterparts. Both leaves and bark extracts exhibited a significant difference in anthelminthic activity compared to mebendazole. We also observed that the death time of worms was lower in aqueous and ethanol extracts of bark at 100 mg/mL than in mebendazole ($p<0.05$). In conclusion, the anthelminthic activity of the leaf and bark of *Crataeva religiosa* is observed in this study. Further studies are needed to screen the phytoconstituents of the plant that are responsible for the anthelmintic activity.

Keywords: *Crataeva religiosa*, Anithelminthic activity, Evaluation, Bark, Leave

I. INTRODUCTION

Crataeva religiosa is a branched deciduous tree, belongs to the family capparidacea. It is distributed in the countries in tropical zone such as India and Sri Lanka. This plant is called as 'lunuwarana' in sinhala and 'mavilanku' in tamil. Root bark, stem bark and leaves of this plant are used for various medicinal treatemnts in indigenous medicine The different parts of this tree exhibit various pharmacological properties like diuretic, anti-inflammatory, laxative, antioxidant, antioxaluric, hepatoprotectant, lithotriptic, antirheumatic, antiperiodic, antimycotic, contraceptive, antipyretic, antilithitic, rubefacient and vasicant properties. The bark of the *Crataeva religiosa* is used in treatment of urinary disorders and kidney stone [1].

Parasitic infections affect human health causing various complications such as gastrointestinal disorders, malnutrition, anaemia, allergies and sometimes life-threatening if untreated. Although several synthetic drugs are available in treating helminth infections, discovery of new drugs needed against them due to resistance emerging against them and side effects of the synthetic drugs. Plant-based molecules have been shown promising results in the discovery of new drugs against various ailments. Even modern pharmacopoeia still consists at least of 25% of drugs derived from plants. [2] Several *in-vivo* trials have been conducted for the evaluation of the anthelminthic activity of compounds from plant origin. They showed activity by the expulsion of worms from their hosts or reducing no eggs per faeces compared to standard drugs. [3] Therefore, the present study was undertaken to evaluate *in-vitro* anthelmintic activity of leaf and bark extracts of *Crataeva religiosa*.

II. MATERIAL AND METHODS

A laboratory-based experimental study was conducted. The aerial parts (leaf and bark) of *C. religiosa* were collected from the Jaffna district.

Collection and preparation of sample

The leaf and bark of *C. religiosa* were collected and air-dried in the shade at room temperature. Dried plant material was grounded to powder using an electric grinder. The extract was prepared with distilled water and ethanol using soxhlet apparatus.

Preparation of extracts

One hundred grams of the powdered plant material was mixed with 250 mL of solvents and extraction carried out using soxhlet apparatus for 1.5 hours. Then it was allowed to cool to room temperature. The resultant extract was concentrated using a rotary evaporator, and the extract was kept at 4 ^{o}C until further usage. The extract yield (%W/W) from plant material was calculaed [4].

Experimental worm

The adult earthworms (*Eisenia fetida*) wereas used due to its anatomical and physiological resemblance with the intestinal roundworm parasites of humans [5]. The worms were collected from the Department of Biology, Faculty of Agriculture, University of Jaffna.

They were washed initially with tap water followed by normal saline to remove the soil particles, debris and faecal matters.

In-vitro anthelmintic activity

Figure 1. Adult earthworm (*Eisenia fetida)*

The anthelmintic activity was performed on adult earthworm *E. fetida* according to the method described in previous studies [6]. This test was performed in triplicate. The worms were placed in a Petri dish containing different concentrations (10, 20, 50, 100 mg/ml) of aqueous and ethanolic extracts of *C. religiosa*.

Two worm were placed in each petri dish and observed for paralysis or death of the worms (Figure 2). The time taken for paralysis was noted when no movement was observed in the worms. It was further confirmed by shaking or giving external stimuli to the worms. Death was confirmed by loss of their motility followed by fading of their colour. Mebendazole (20 mg/ml) was used as standard drug, and saline was used as control.

Figure 2. *In-vitro* study of anthelmintic activity using *Eisenia fetida*

Statistical analysis

All resulted were presented as mean with standard deviaton. One-way ANOVA followed by Tukey's post hoc was used to compare the activities between extracts and mebendazole.

III. RESULTS AND DISCUSSION

Percentage extraction yield of *C. religiosa* leaves using ethanol and water were 4.92% and 5.82% respectively. The percentage yield of *C. religiosa* bark extracted using ethanol and water were 5.27% and 4.56% respectively.

Time durations to exhibit Paralysis and death - for water and ethanol extracts of leaf and bark of *C. religiosa* at different concentrations were given in Tables 1 and 2.

According to the results, concentration-dependent activities were observed for all extracts on paralyzing or killing of worms. As shown in the Figure 3, among the leave extract of *C. Religiosa*, aqueous extract at 100 mg/ml and ethanolic extract at 50 and 100mg/ml showed activity comparable to mebendazole. As shown in Figure 4, among the bark extracts, aqueous and ethanolic extracts at 50 and 100 mg/ml showed comparable activity to mebendazole. The ethanol extract of leaves and aqueous extract of bark have shown more efficiency.

Leaves and bark extracts exhibited a significantly higher in anthelminthic activity compared to mebendazole. We also observed that the death time of worms was lower in aqueous and ethanol extracts of bark at 100 mg/mL than in mebendazole ($p \leq 0.05$).

Table 1. Anthelmintic activity of *Crataeva religiosa* leaf extract

Extracts	Concentration (mg/mL)	Paralyzed Time (min) Mean ± SD	Death Time (min) Mean ± SD
Aqueous extract	10	_	SWAM
	20	_	SWAM
	50	_	SWAM
	100	19.13 ±0.15	23.5±0.4
Ethanol extract	10	_	SWAM
	20	_	SWAM
	50	17.5± 0.3[a]	29.93±0.83 [a]
	100	4.33± 0.15[b]	7.2±0.3[b]
Mebendazole 20mg/mL	20	4.53± 0.5	8.47±0.5
Saline	_	_	SWAM

SWAM-survived with active movement even after one hour. Mebendazole: standard; Saline: control. Means within the column without a common superscript letter differ at $p \leq 0.05$ compare to mebendazole.

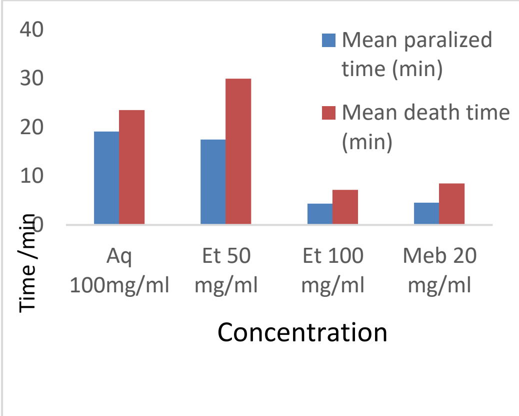

Figure 3. Mean time duriation for paralizing and death in earthworms in the presence of leaves extracts of *C. religiosa*

Table 2. Anthelmintic activity of *Crataeva religiosa* bark extract

Extracts	**Concentration (mg/mL)**	**Paralyzed Time (min) Mean ± SD**	**Death Time (min) Mean ± SD**
Aqueous extract	10	_	SWAM
	20	_	SWAM
	50	5.83± 0.15[a]	12.60±0.65[a]
	100	1.97± 0.15[b]	3.37± 0.32[b]
Ethanol extract	10	_	SWAM
	20	80.23± 1.32[a]	172.13±2.97[a]
	50	25.77± 0.25[b]	50±0.3[b]
	100	4.70±0.2[c]	5.40± 0.17[c]
Mebendazole 20 mg/mL	20	4.53±0.5	8.47± 0.5
Saline	_	_	SWAM

SWAM-survived with active movement. Mebendazole: standard; Saline: control. Means within the column without a common superscript letter differ at $p \leq 0.05$ compare to mebendazole.

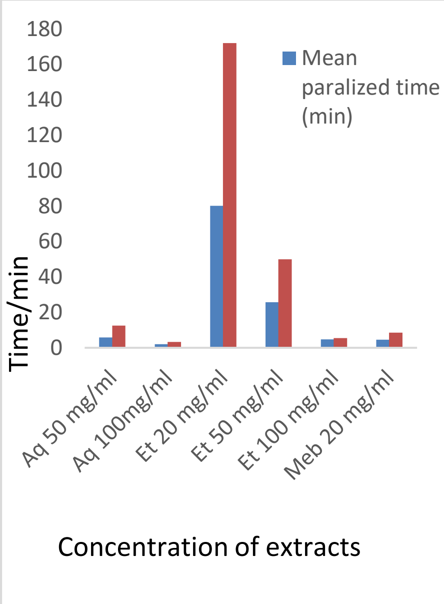

Figure 4. Mean paralized and death times of worms by bark extracts of *C. religiosa*

Anthelmintic activity of C. religiosa leaf extract

The mean time take to paralysis the worm in aqueous extract of *C. religiosa* leaf at 100 mg/mL (19.13±0.15 minutes) was significantly higher than the time take to paralysis in ethanol extract at 100 mg/mL (4.33±0.15 minutes), $p<0.05$. However, in ethanol extract, the mean time taken to paralysis the worm at 50 mg/mL was approximately four-fold higher (17.5±0.3 minutes) than the time take to paralysis at 100 mg/mL (4.33±0.15 minutes) ($p<0.05$). It was observed that the time taken to paralysis the worm in the aqueous extract at 100 mg/mL was slightly higher (19.13±0.15 minutes) than the time taken to paralysis in ethanol extract at 50 mg/mL (17.5±0.3), indicating the efficiency of ethanol extract on a paralysis of worm.

We also observed that the paralyzing time in mebendazole at 20 mg/mL (positive control) was 4.53±0.5 minutes, which was not significantly different from the time taken to paralysis in ethanol extract at 100 mg/mL (4.33±0.15) ($p>0.05$), whereas killing time was significantly reduced compared to positive control. However, the time is taken to paralysis in ethanol at 50 mg/ml, and in the aqueous extract at 100 mg/mL was significantly higher than the positive control $p<0.05$).
Conversely, in aqueous extract at 10, 20, 50 mg/mL and in ethanol extract at 10, 20 mg/mL, the worms survived with active movements, similar to saline (0.9%) solution (negative control).

In the aqueous extract of *C. religiosa* leaf, the time take to kill the worms at 100 mg/mL was increased by 22.84% compared to paralyzing time at the same concentration, whereas in ethanol extract, the time take to kill the worms at 50, and 100 mg/mL was increased by 71.0% and 66.28% compared to paralyzing time in respective concentrations ($p<0.05$). In positive control, the time to kill the worms at 20 mg/mL of mebendazole was increased by 87.0% compared to paralyzing time ($p<0.05$).

The killing time in mebendazole at 20 mg/mL (positive control) was 8.47±0.5 minutes, which was significantly different from the time taken to kill in ethanol extract at 100 mg/mL (7.2±0.3), $p<0.05$. The paralyzing time of the worm was not significantly different from positive control ($P>0.05$)

However, the time is taken to paralysis in ethanol at 50 mg/ml and in aqueous extract of a leaf at 100 mg/mL was significantly higher than the positive control $p<0.05$).

Anthelmintic activity of Crataeva religiosa bark extract
The mean time taken to paralysis the worm in aqueous extract of *Crataeva religiosa* bark at 100 mg/mL (1.97±0.15 minutes) was significantly lower than that at 50 mg/mL (5.83±0.15) minutes ($p<0.05$). A similar trend was observed in ethanol extract of bark that the paralyzing time and killing time was gradually decreased with increasing concentration *of Crataeva religiosa* bark extract (Spearman's rho correlation for paralysis and killing=-0.949 and 0.953; $p<0.000$).

The mean time taken to paralysis the worm in aqueous extract of *Crataeva religiosa* bark at 50 and 100 mg/mL was significantly lower than the time take to paralysis in ethanol extract at 50 and 100 mg/mL, respectively (At 50 mg/mL: 5.83±0.15 vs 25.77±0.25 minutes; at

100 mg/mL: 1.97±0.15 vs 4.7±0.2 minutes), $p<0.05$. Similarly, the killing time was lower in aqueous extract than in ethanol extract at 50 and 100 mg/mL (at 50 mg/mL: 12.6±0.65 vs 50±0.3 minutes; at 100 mg/mL: 3.37±0.32 vs 5.4±0.17 minutes), indicating aqueous extract is better than ethanol extract from the bark. The paralyzing time taken in ethanol extract of bark at 100 mg/mL (4.7±0.2 minutes) was slightly higher than in positive control ($p>0.05$), whereas killing time was significantly lower in ethanol extract at 100 mg/mL than positive control ($p<0.05$).

Comparison between leaf and Bark on the effectiveness

The killing time of worms at 100 mg/mL of *C. religiosa* leaf in the aqueous extract was significantly higher than that in bark extraction ($p<0.05$). Conversely, the killing time of *Crataeva religiosa* bark in ethanol extract was significantly lower than that in bark extraction at 50 mg/mL ($p<0.05$), whereas the killing time of *C. religiosa* bark in ethanol extract was significantly higher than that in bark extraction at 100 mg/mL ($p<0.05$).

Anthelminthic activity and phyto constituents of C. religiosa

Several studies reported that the phytocompounds that show the anthelminthic activity include saponins, alkaloids, polyphenols, tannins, etc. Different phytoconstituents act as anthelminthic agents in different mechanisms. *Crataeva religiosa* consists of various phytoconstituents such as alkaloids, flavonoids, glycosides, terpenoids and saponins [8]. Isolation of individual phytoconstitutents and screening for activity is needed in further studies to find new chemical entities with anthelminthic activities.

Anthelminthic activity of other genus of Crataeva

Anthlminthic activity of *C. religiosa* was first time reported in this study. However other genus of crataeva such as *Crataeva unilocularis* (9) and *Crataeva nurvala* (10) also showed anthelminthic activity in *in-vitro* studies.

IV. CONCLUSION AND RECOMMENDATIONS

Higher concentration of extracts of Leaf and bark of *Crataeva religiosa* showed higher anthelminthic activity compare to standard. Further studies are needed to isolate the phytoconstituents of the plant that are responsible for the anthelminthic activity and their efficacy in *in-vivo* studies.

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