

ANTIHELMINTIC EFFECT OF EMBELIA TSJERIAM-COTTAM**Manjusha Suresh Nikam¹, Md. Rageeb Md. Usman^{*}, Gautam P. Vadnere¹**

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ABSTRACT

The present study was undertaken to investigate the anthelmintic activity of extract of *Embeliatsjeriam-cottam* using earthworm. Different concentrations of standard drug (Albendazole) and extract of *Embeliatsjeriam-cottam fruits* were employed and the average time required for paralysis and death was noted. It was found that the Paralysis time & Death time was lowest for 5% concentration of Ethanolic extract and Death time was slightly better than Albendazole Standard solution. Though Ethanolic Extract can be compared to the Standard hence establishing the pharmacological anthelminthic activity of *Embelia tsjeriam-cottam*.

Keywords: *Embelia tsjeriam-cottam* Anthelmintic Activity, Albendazole, Ethanolic Extract.

INTRODUCTION

Helminthes infections are among the most widespread infections in humans, distressing a huge population of the world. The human roundworm *A. lumbricoides* is one of the most common parasites in the world, infecting 1.2 billion people globally. Infections are most commonly documented in Asia, sub-Saharan Africa, the Americas and China. The spectrum of disease associated with *A. lumbricoides* infection is known as ascariasis, and morbidity assessed as disability adjusted life years (DALYs) is approximately 10.5 million. Furthermore, morbidity with serious health consequences is observed in 122 million cases per year [1,2] The World Health Organization reports that 35% diseases are because of roundworm, which is a typical parasitic worm. More than 1.5 billion individuals or 24% of the total population are tainted with soil-transmitted (STH) helminth contaminations around the world. [3] However, ascariasis is still considered a neglected tropical disease (NTD).

The community-based control of STHs is based on mass drug administration by two synthetic anthelmintics, albendazole and mebendazole. [4] A wide spread resistance to the commercially available anthelmintic treatments has been observed in multiple nematode species. [5] Therefore, alternative anthelmintic strategies are urgently needed. In addition anthelmintic strategies such as grazing management, biological control with nematophagous fungi or food supplementation with leguminous plants accumulating high amounts of condensed tannins, phytotherapy could be a part of an integrated control system. The family

Myrsinaceae consists of nearly 1000 species of trees and shrubs spread over 33 genera including four genera namely *Myrsine*, *Maesa*, *Rapanea* and *Embelia*, which are widely used in herbal medicines. *Embelia tsjeriam-cottam* is a large scandent shrub, distributed throughout India and belongs to the family *Myrsinaceae*. It is commonly known as *Barberang* in Hindi and *Vidangain* Sanskrit. The dried fruits are being used for the preparation of medicine. [6] *Embelia* species identified by *Susruta* (Father of surgery) as anthelmintic, alternative and tonic. [7] Further Dr Harris found in ancient Arabian writing as *birang-I-kabauli* for remedy of tapeworm. Embelin has been isolated and quantified in *Embelia tsjeriam-cottam* Burm. f. and other species of *Myrsinaceae* family. Embelin such evaluated against *Heligmosomoide spolygyrus* in mice significantly reduced the total worm counts. In the present study, the anthelmintic potential of traditionally used medicinal plant *Embelia tsjeriam-cottam* was scientifically explored against Earth worm model to substantiate the folklore claims.

MATERIALS AND METHODOLOGY

Plant Collection and Authentication

E. tsjeriam fruits and seeds were collected with the help of manual like clippers, diggers, scrapers, etc by the lab technicians from Department of Pharmacognosy, College of Pharmacy, Chopda (Jalgaon, Maharashtra) and Herbarium sheets were prepared. The student collected the requisite from the department. Further, Botanical Survey of India, Pune, authenticated the plant and its constituents. *E. tsjeriam-cottam* were taxonomically identified and authenticated by (Dr. C R. Jadhav, Botanist) at Botanical Survey of India, Pune, Maharashtra.

Experimental Animal

For the experiment, earthworms were collected from moist soil from local Earthworm Project and washed with normal saline to remove soil and fecal matter during the experiment. The earthworms of 5-7cm length and 0.2-0.3cm width were used for the experimental protocol.

METHODOLOGY FOR THE STUDY

Extraction of Active Constituents from *Embilia Tsjeriam-Cottam* Fruits [8-12]

1. Collected plant parts were air dried under shade and then ground to a coarse powder using a grinder. Finely powdered *Embelia tsjeriam-cottam* fruit-samples were extracted using Soxhlet method.

2. Powdered sample (100g) was extracted through Soxhlet apparatus for 16-18 hrs with acetone, ethanol and methanol solvent systems separately. The total extract was condensed in dry bath and kept as embelin sample stock solution.

Standardization of Plant Extract and Its Bioactive Fractions Embelin by Using Phytochemical and Analytical Methods

1. FTIR [13,14]

Fixed amount of plant specimen was mixed with KBr salt, using a mortar and pestle, and compress into a thin pellet. Infra red spectra were recorded as KBr pellets on a Thermo scientific Nicot iS5 iD1 transmission, between 4000-400 cm^{-1} . The powdered sample of the plant extract was treated for FT-IR spectroscopy. The result was analyzed based on peak obtained.

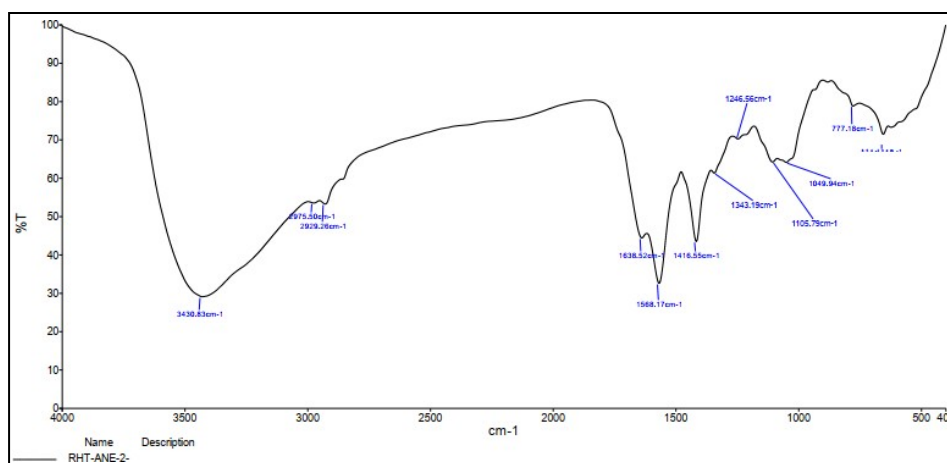


Figure 1: FTIR of *E. tsjeriam*

Table 1: FTIR of *E. tsjeriam* peaks interpretation

Frequency (cm-1)	Type of Vibration	Functional Group	Intensity
3480.83	O-H stretch, H-bonded	Alcohol	Strong, broad
2975.50	C-H stretch	Alkane	Strong
2929.26	C-H stretch	Alkane	Strong
1638.52	C=C stretch	Aromatic	Medium-weak
1568.17	N-H bending	Amide	Strong
1416.55	C-C stretch	Aromatic	Medium
1343.19	N-O symmetric stretch	Nitro compounds	Medium
1246.56	C-O stretch	Alcohol,	Strong
1105.75	C-O stretch	Alcohol,	Strong

The leaf powder of *E. tsjeriam-cottam* exhibited 12 characteristic bands. The highest band occurred at 3480.83 cm⁻¹ indicating the presence of functional groups like alcohols and phenols having O–H stretch and H–bonded groups, 2975.50 cm⁻¹ indicating the presence of alkenes (C–H stretch) group, 1638.52 cm⁻¹ indicating the presence of aromatic (C=C stretch), 1416.55 cm⁻¹ indicating the presence of amide (N–H bending), 1343.19 cm⁻¹ indicating the presence of nitro compounds (N–O symmetric stretch), 1049.94 cm⁻¹ indicating the presence of aliphatic amine (C–N Stretch).

2. TLC [15]

Crude embelin extract (10 µl each) solution was used for the chromatographic method to identify and isolate the pure embelin. The mobile phase selected for use was n–Propanol: n–Butanol: Ammonia in a ratio of 7:1:2. Spots were detected by using 1% solution of vanillin in methanolic sulfuric acid as chromatogenic reagents and viewed under ultraviolet light at 365 nm wavelength for identification of the separated compounds.

The R_f value of sample was determined to ensure presence of embelin in the extracted sample against the standard. The R_f values were found to be Standard which established the efficacy of the Extraction process.

In Vitro Pharmacological Evaluation Of *EmbiliaTsjeriam-Cottam* Extract For Its Antihelminthic Activity For Treatment Of Ascaris (Intestinal Ring Worm) By Using Earthworms As Culture [16,17,18]

Pheretima posthuma were used because of its anatomical and physiological similarity with intestinal roundworm parasites of human beings and them for the fact that they belong to same group of Annelids. All the test solutions and standard drug solutions were prepared freshly before starting the experiment. Albendazole (10 mg/ml) was used as reference standard while saline water served as a control. 20 worms were used to carry out the experiment. 100 ml formulations containing concentrations of acetone, methanol & ethanol extracts of were used. The time for paralysis (in min) was noted when no movement of any sort could be observed except when the worms were shaken vigorously. The time of death of the worms (in min) was recorded after ascertaining that worms neither moved when shaken vigorously or when dipped in warm water (50°C).

RESULTS

The results of anthelmintic activity of different concentrations of Standard drug and extract of *Embiliatsjeriam- cottam* fruits are depicted in Table 2.

Table 2: In-Vitro Pharmacological Activity Results

Extracts Used	Concentration of Extracts used (%)	Time (sec)	Death
		Paralysis	
Acetone	1	200	280
	2.5	180	240
	5	120	190
Methanol	1	350	420
	2.5	290	350
	5	210	190
Ethanol	1	240	300
	2.5	180	270
	5	100	140
Albendazole Standard	1	340	450
	2.5	200	300
	5	90	150

The results revealed concentration dependent activity. The average paralysis time (in sec) in different concentrations of Standard drug was found to be 340, 200 and 90 while the average death time (in sec) was found to be 450, 300 and 150. Ethanol extract of *Embelia tsjeriam-cottam*, in 1%, 2.5% and 5% was found to cause paralysis of worms in 240, 180 and 100 seconds respectively.

It was found that the Paralysis time & Death time was lowest for 5% concentration of Ethanolic extract and Death time was slightly better than Albendazole Standard solution. Though Ethanolic Extract can be compared to the Standard hence establishing the pharmacological antihelminthic activity of *Embeliatsjeriam-cottam*.

CONCLUSION

Albendazole (10mg/ml) was used as reference standard while saline water served as a control. 20 worms were used to carry out the experiment. 100 ml formulations containing concentrations of acetone, methanol & ethanol extracts of *Embiliatsjeriam- cottam* fruits

were used. The time for paralysis (in min) was noted when no movement of any sort could be observed except when the worms were shaken vigorously. The time of death of the worms (in min) was recorded after ascertaining that worms neither moved when shaken vigorously or when dipped in warm water (50°C). It was found that the Paralysis time & Death time was lowest for 5% concentration of Ethanolic extract and Death time was slightly better than Albendazole Standard solution. Though Ethanolic Extract can be compared to the Standard hence establishing the pharmacological antihelminthic activity of *Embeliatsjeriam-cottam*.

CONFLICT OF INTEREST

Authors have no conflicts of interest to declare.

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