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INVESTIGATION OF IN VITRO ANTHELMINTIC ACTIVITY OF BARK OF EMBLICA OFFISNALIS

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ABSTRACT

The aim of the present study was investigation of *in vitro* antihelminthic activity of crude methanolic and aqueous extract of bark of *Emblica officinalis* using *Pheretima posthuma* as test worm. Various concentrations of (25, 50, 100 mg/ml) of methanolic and aqueous extracts were tested in the bioassay, which involved the determination of time of paralysis (P) and time of death (D) of the worms. Albendazole was included as standard reference and distilled water as control. The both extracts showed greater P and D time when compared with the standard reference Albendazole. The results of present study indicated that the crude aqueous extract significantly paralysis and also caused death of worm in short time, as compared to methanolic extract. In conclusion, the traditional use of stem bark of the plant of *Emblica officinalis* as an antihelminthic activity have been confirmed and further studies are suggested to isolate the active principles responsible for activity.

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INTRODUCTION

The world population use herbal medicine for health care because of safety, efficacy, easily and abundantly available without side effects. The conditions appear to be worsening with respect to the number of persons affected. Helminthiasis infections are particularly in patients residing in tribal areas lack of knowledge of sanitation. Plant *Emblica officinalis* belongs to the family Leguminosae and it is popularly known as Fenugreek used for the treatment of and helminthiasis.^[1] The present study was aimed to evaluate the scientific basic for the traditional use of antihelmentic activity of crude methanolic and aqueous stem bark extract of *Emblica officinalis* against *Pheretima posthuma*. Albendazole was included as standard reference and distilled water as control. Scientific literature survey revealed that the *in vitro* antihelmentic activity of *Emblica officinalis* has not been reported.

Causative Agents:

Antihelminthic drugs are used in the treatment of helminthiasis (vomiting of worms).

The different types of worms include

i) **Round worms:** They are of two types, Adult Intestinal Nematodes and Larval Tissue Nematodes.

ii) **Tape worms:** They are found in the intestine or larva in the tissues.

iii) **Flukes:** These are hermaphrodites with an exception of blood flukes. They are found in blood vessels, the intestine, billiary tract, lungs.

Symptoms:

Symptoms of Helminthiasis are as follows

- 1) Abdominal pain
- 2) Diarrhea
- 3) Fever
- 4) Fatigue
- 5) Enlarged liver
- 6) Gastrointestinal inflammation
- 7) Eosinophilia
- 8) Dehydration.

Mode of Transmission:

The disease is transmitted through

- 1) Fecal - oral route for ascaris, trichuris and hookworm
- 2) Skin penetration for hookworms

Prevention and Control:

Following measures can be taken for prevention and control of the disease

- Personal hygiene
- Environmental sanitation
- Clean food and drinking water.
- Use of slippers or shoes
- The drugs used are Albendazole, Mebendazole and Levamisole Helminthiasis.^[2]

Description of plant:

Phyllanthus emblica, also known as emblic, emblic myrobalan, myrobalan, Indian gooseberry, Malacca tree, or amla from Sanskrit amalaki is a deciduous tree of the family Phyllanthaceae.^[3]

Characteristics of *Emblica officinalis*



Figure.1: Diagram of *Emblica officinalis* plant.

Traditional and other medicinal uses

Ayurveda, Siddha, Unani systems of India, Tibetan, Sri Lankan and Chinese systems of medicine utilize Amla for a variety of ailments. It is considered as rasayana (rejuvenator) and used in delaying the degenerative and senescence related processes. In folk medicine, the fruits, which are sour, astringent, bitter, acrid, sweet and anodyne. Exert several beneficial effects include cooling, ophthalmic, carminative, digestive, stomachic, laxative, dyspepsia, aphrodisiac, rejuvenative, diuretic, antipyretic and tonic. They are useful in vitiated conditions of tridosha, diabetes, cough, asthma, bronchitis, cephalalgia, ophthalmopathy, dyspepsia, colic, flatulence, hyperacidity, peptic ulcer, erysipelas, skin diseases, leprosy, haematogenesis, inflammations, anaemia, emaciation, hepatopathy, jaundice, diarrhoea, dysentery, haemorrhages, leucorrhoea, menorrhagia, cardiac disorders, intermittent fevers and premature greying of hair (Hair tonic). Amla is also stated to have hepato, cardio, nephro and neuroprotective effects; antioxidant, anti-inflammatory, analgesic, antipyretic and restorative properties.^[4]

Phytochemistry:

Although these fruits are reputed to contain high amounts of ascorbic acid (vitamin C), up to 445 mg per 100 g, the specific contents are disputed, and the overall bitterness of amla may derive instead from its high density of ellagitannins, such as emblicanin A (37%), emblicanin B (33%), punigluconin (12%) and pedunculagin (14%).^[14] It also contains punicafolin and phyllanemblinin A, phyllanemblin other polyphenols, such as flavonoids, kaempferol, ellagic acid, and gallic acid.^[5]

MATERIALS AND METHODS

Collection of plant:

Emblica officinalis (Bark) were collected from Vangapally, Yadagirigutta and were identified by Prof. Dr. S. Srinivas Rao.

Preparation of plant extract:

Powdered dried bark (100 g) was extracted with 250 mL methanol and water using a Soxhlet apparatus. Then plant material was powdered finely and the net weight of the powdered plant material was (1.2 kg).^[6] The methanol extract was carried out by soxhlation for 72 hrs & aqueous extraction was carried out by maceration method for 7 days.^[7] Then the ethanol soluble materials were filtered off using Whatman filter paper No.1. Filtrate were intermingled and decoctured under vacuum at 40° C using rotary evaporator. The yield was 3 g per 100 g of *Emblica officinalis* bark. The *Emblica officinalis* extract was then subjected to characterization and phytochemical screening.^[8]

Collection of worm and authentication:

Indian earthworm *Pheretima posthuma* (annelida) were collected from the water logged areas of soil and authenticated Prof. Dr. T. Ramesh.

Helmintholytic activity:

- The activity was performed on Indian earth worms due to its anatomical and physiological resemblance with the intestinal round worm parasite of human intestine.
- Indian adult earth worm (*Pheretima posthuma*) of 5-8 cm in length and 0.2-0.3 cm in width were used.^[9]
- Eight groups of approximately equal sized earth worms each containing six earth worms were selected.^[10]
- All the earth worms were washed in normal saline solution before they were used.
- The control group was treated with distilled water.
- The earth worms were placed in a standard and extracts and time of paralysis (P) and time of death (D) were calculated.^[11]
- The time of paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously.^[12]
- The time of death were recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water (50° C).^[13]

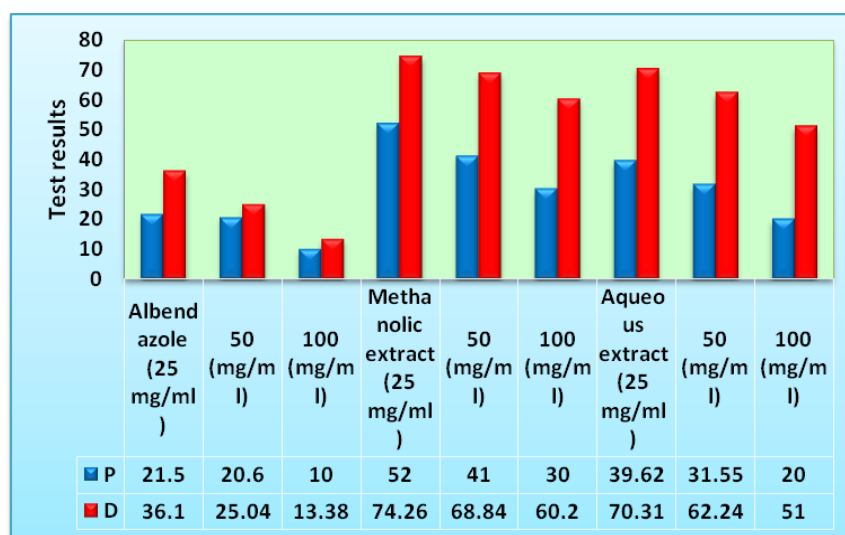
RESULTS AND DISCUSSION

Results of the preliminary phytochemical screenings of crude alcoholic extract detect tannins, alkaloids, Phenol compounds, amino acids and carbohydrates. Some of the traditionally used herbs have scientifically proved a mark and potent antihelminthic activity by using suitable experimental models. Both methanolic and aqueous extract of stem bark of the plant *Emblica officinalis* exhibited antihelminthic activity in dose-dependent manner giving shortest time of paralysis (P) and death (D) with 100 mg/ml concentration. Similar effects were observed for the tested standard drug (Albendazole) by using adult Indian earth worm, *Pheretima posthuma*. Aqueous extract had shown promising result (paralysis time is 20.00 ± 0.98 min and death time is 51.00 ± 0.24 min) as antihelminthic activity and methanolic extracts has also shown activity (paralysis time is 30.00 ± 0.16 min and death time is 60.20 ± 0.17 min) up to lesser extent.

Table: 1 Anthelmintic activity of methanolic and aqueous extract of stem bark of *Embolica officinalis*.

Test substance	Concentration (mg/ml)	Time taken for paralysis (P) Death (D) of earth worms (min)	
		P	D
Albendazole	25	21.5 ± 0.78	36.10 ± 0.57
	50	20.6 ± 0.65	25.04 ± 0.42
	100	10.0 ± 0.32	13.38 ± 0.29
Methanolic extract	25	52.00 ± 0.26	74.26 ± 0.23
	50	41.00 ± 0.50	68.84 ± 0.94
	100	30.00 ± 0.16	60.20 ± 0.17
Aqueous extract	25	39.62 ± 0.68	70.31 ± 0.83
	50	31.55 ± 0.33	62.24 ± 0.75
	100	20.00 ± 0.98	51.00 ± 0.24
Control	-	-	-

Values are mean ± S.E.M. from six observations. P: Time for Paralysis (min), D: Time for Death of worms (min) Control worms were alive for up to 24 hrs of the experimentation.

**Figure.2:** Diagrammatic presentation of Anthelmintic activity of methanolic and aqueous extract of bark of *Embolica officinalis* in different concentrations.

CONCLUSION

From the above result it is concluded that aqueous extract of bark of *Embolica officinalis* have a potent anthelmintic activity when compared with conventionally used drug. It is comparable with standard drug. Further studies using *in vivo* model are required to find out and to establish effectiveness and pharmacological rationale for the use of stem bark as anthelmintic drug. Further studies to isolate active constituent from extracts and to establish (s) mechanism of action is required.

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