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## Pharmacognostic evaluation and Anthelmintic activity of different extracts of *Euphorbia humifusa*

Gomase Pravin V, Shaikh Jishan, Shaikh Maaz, Shaikh Mujeeb, Mansuri Ramjan

Department of Pharmacognosy, Ali-Allana College of Pharmacy, Akkalkuwa, Nandurbar, India

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### ABSTRACT

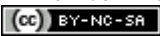
The plant of *Euphorbia humifusa* was evaluated for anthelmintic activity using adult earthworm. The plant was dried for few days in sunlight and made into powder using grinder. The successive extraction of the drug powder was done by using solvent in the sequence of increasing polarity using Soxhlet apparatus. Anthelmintic activity was performed by pin-prick method and inhibition of spontaneous paralysis was observed. With the higher doses of 40 mg/ml of extracts of (Pet. Ether, Chloroform and Methanol). The effects were comparable with Albendazole. However, there was no final recovery in case of worms treated with extracts as well as Albendazole.

**Keywords:** Phytochemical Screening, Medicinal plants, *Euphorbia humifusa*.

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**Address for Correspondence:** Mr. Gomase Pravin V, \*Department of Pharmacognosy, Ali-Allana College of Pharmacy, Akkalkuwa, Nandurbar, India; Email: [jishanshaikh77785@gmail.com](mailto:jishanshaikh77785@gmail.com)

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## INTRODUCTION

Helminth infections are the most common infections in man, and exaggerated worldwide population. It may cause anemia, eosinophilia, pneumonia and prevalence of malnutrition. *Helminthiasis* is the most common infection caused by worms that is contaminant to human body parts. Normally, the worms not only live in the gastrointestinal tract but may also reside into liver and other organs. When infected people excrete faeces with helminth eggs, the soil in the areas with poor sanitation will be contaminated. There are two clinically important types of worm infections, one is the worms live in the host's alimentary canal and the other is worms live in other tissues of the host's body. Tapeworms or cestodes (*Taenia saginata*, *Taenia solium*, *Hymenolepis nana*, *Diphyllobothrium latum*) and intestinal roundworms or nematodes (*Ascaris lumbricoides*, *Enterobius vermicularis*, *Trichuris trichiura*, *Strongyloides stercoralis*, *Necator americanus*, *Ankylostoma duodenale*) are live in the host's alimentary canal while trematodes or flukes (*Schistosoma haematobium*, *Schistosoma mansoni*, *Schistosoma japonicum*), tissue roundworms (*Trichinella spiralis*, *Dracunculus medinensis*) and hydatid tapeworm (Echinococcus species) are live in the host's tissues. Several nematodes that usually live in the gastrointestinal tract of animals may communicate a disease to humans and penetrate tissues. A skin infestation, termed creeping eruption, is caused by the larvae of dog and cat hookworms. Toxocariasis is caused by larvae of cat and dog roundworms of the *Toxocara* genus<sup>1</sup>.

### Epidemiology<sup>1</sup>

*Helminthiasis* is the most common infection mainly caused by the helminths. It is observed in various tropical and subtropical areas, and it is also classified as neglected tropical diseases. They spread the majority of common parasitic infection of human in developing countries. *Ascaris lumbricoides*, *Trichuris tritura*, *Necator americanus*, *Ancylostoma duodenale*, *schistosomes* and filarial worms cooperatively infect more than one billion people, rivaling AIDS and malaria. As the recent evaluations, over a billion people have been infected due to at least one helminth species in Asia, Africa, America and Su-saharan, which leads to severe morbidity, accompanied by persistent shortage, decreased efficiency, and poor socioeconomic development. *Helminthiasis* has immunomodulatory effects on the host cells, with implications for many affecting pathogens. In fact, in endemic areas, AIDS, malaria and tuberculosis are recognized to be caused by *helminthiasis*. In most cases, they can induce severe hypersensitivity reaction that leads to chronic allergic reactions called anaphylaxis.

### Etiology of helminthiasis<sup>1</sup>

Helminths have a complex life cycle that often links several species. Helminth infections are mainly caused due to improper sanitation. They enter by mouth in unpurified drinking water or in poorly cooked meat from infected animals. It is also enter through the skin by a skin cut, an insect bite or even after swimming or walking on polluted soil. Humans are the primary hosts for the helminth infections and most of the worms reproduce sexually in the human host, producing eggs or larvae that pass out of the body and infect the secondary host. In some cases, the eggs or larvae may persevere in the human host and become encysted, enclosed with granulation tissue, giving rise to cysticercosis. This is characterized by encysted larvae in the muscles, viscera<sup>1</sup>

**Collection and identification of plant:** Plant *Euphorbia humifusa* was collected from Akkalkuwa District Nandurbar, Maharashtra. The plant was authenticated by Dr. V. R. Jogdand, Dept. of Botany, RFNS, Senior Science College, Akkalkuwa, Dist.- Nandurbar and submit the plant specimen for further reference.

### Pharmacognostic Investigation of *Euphorbia humifusa*

#### Organoleptic / Macroscopic evaluation<sup>2,3</sup>

In the present study the bark of *Euphorbia humifusa* was investigated for its macroscopic characteristics. Macroscopic characters, which were observed, are given follows.

**Color:** Greenish red

**Odor:** Aromatic, Pleasant

**Taste:** Astringent, mucilaginous

**Texture:** Smooth

#### EXTRACTION METHOD<sup>4,5,6</sup>

##### Extraction Procedure by Using Soxhlet Apparatus

##### Technique: Soxhlet extraction

##### Solvent:

Using three different solvents in the following sequence of increasing polarity extraction was done.

1. Petroleum ether (60-80°C)
2. Chloroform
3. Methanol

The extraction was carried out in several batches. Total quality of dried plant processed = 400 gm.

**Petroleum ether extraction:** The extraction with non-polar solvent like petroleum ether was called as defatting. Extraction carried out by continuous hot extraction method using Soxhlet extractor till all constituents are removed. The end of completion of extraction was indicated by no color with iodine in iodine chamber. After completion of

extraction, solvent was distilled out and dried extract was obtained. This extract was kept in desiccators till further use. Petroleum ether extract mainly contain coloring matter like chlorophyll, carotenoids, lipids, free sterols and triterpenes.

**Chloroform:** The marc after exhaustive petroleum ether extraction was air dried and subjected to exhaustive Soxhlet extraction with chloroform.

**Methanolic extraction (successive):** The marc after exhaustive chloroform extraction was air-dried and subjected to exhaustive Soxhlet extraction with methanol (high polarity solvent). The Methanolic extract usually contains glycosides, tannins, resinous substances, acids-like cinnamic acid, benzoic acid, alkaloids and free

alkaloids. The extract was subjected to solvent recovery by vacuum distillation.

**Extraction of whole plant of *Euphorbia humifusa*:** The plant of *Euphorbia humifusa* was reduced to fine powder (#40 size mesh) and around 400 gm of powder was subjected to successive continuous hot extraction (Soxhlet) with petroleum ether, chloroform and methanol. Each time before extracting with next solvent the powdered material was dried in hot air oven at 50° C for 1 hour. After effective extraction, the solvents were distilled off, the extract then concentrated on water bath and extract obtained with each solvent weighed. Its percentage is calculated in terms of air-dried weight of plant material.

## EVALUATION OF EXTRACTS FOR ANTHELMINTIC ACTIVITY<sup>7</sup>

### METHODOLOGY

#### Selection of worms



**Fig: 1** Adult African earthworm *Eudrilus eugeniae*



The assay was performed on adult earthworm, *Eudrilus eugeniae* due to its anatomical and physiological resemblance with the intestinal round worm parasites of human beings. Because of easy availability, earthworms have been widely used for the initial evaluation of Anthelmintic compounds *invitro*. Adult earthworm *Eudrilus eugeniae* were collected from a local supplier and authenticated by Prof. Dr. Md. Zuber Shaikh, Head Department of Zoology, RFNS, Senior Science College Akkalkuwa. Dist.- Nandurbar. Maharashtra, India. They were washed with normal saline to remove all the traces of fecal matter and waste surrounding their body. The African earthworm (*Eudrilus eugeniae*) 3 – 7 cm in length and 0.1 – 0.3 cm in width weighing 0.6 – 5.01 g were used for all experiment protocols<sup>7</sup>.

#### Preparation of samples<sup>8</sup>

Petroleum ether, Chloroform and Methanol extracts from the plant *Euphorbia humifusa* were investigated for their Anthelmintic activity against *Eudrilus eugeniae*. Various concentrations (10, 20, 30, 40 mg/ml) of each extract were tested in the bioassay, which involved determination of time of

paralysis and time of death of the worms. Albendazole was included as standard reference. Required quantity of different extracts of plant and standard drug (Albendazole) were dissolved in DMSO and then diluted with distilled water to get different concentration. (10, 20, 30, 40 mg/ml)<sup>8</sup>.

#### In-vitro evaluation of Anthelmintic activity<sup>8,9</sup>

The anthelmintic activity was carried out on adult earthworm *Eudrilus eugeniae* were collected from a local supplier. Sixteen groups (four concentrations 10, 20, 30, 40 mg/ml of all extract and standard) each containing six worms of approximately equal size were subjected to 10 ml of desired concentration. Observations were made for the time taken for paralysis and death of individual worms. Time of paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms was recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50 °C). 1% gum acacia was used as control. Results are expressed as mean ± SEM. The statistical analysis was performed by one-way

analyses of variance (ANOVA) followed by Bonferroni multiple comparisons test with GraphPad Instant.<sup>8,9</sup>

**Table 1: Distribution of extracts in groups with worms**

Groups	Extracts	Concentration	No of worms
Groups- 1	Petroleum Ether	10 mg/ ml	6
Groups- 2	Petroleum Ether	20 mg/ ml	6
Groups- 3	Petroleum Ether	30 mg/ ml	6
Groups- 4	Petroleum Ether	40 mg/ ml	6
Groups- 5	Chloroform Extract	10 mg/ ml	6
Groups- 6	Chloroform Extract	20 mg/ ml	6
Groups- 7	Chloroform Extract	30 mg/ ml	6
Groups- 8	Chloroform Extract	40 mg/ ml	6
Groups- 9	Methanol Extract	10 mg/ ml	6
Groups- 10	Methanol Extract	20 mg/ ml	6
Groups- 11	Methanol Extract	30 mg/ ml	6
Groups- 12	Methanol Extract	40 mg/ ml	6
Groups- 13	Standard (Albendazole)	10 mg/ ml	6
Groups- 14	Standard (Albendazole)	20 mg/ ml	6
Groups- 15	Standard (Albendazole)	30 mg/ ml	6
Groups- 16	Standard (Albendazole)	40 mg/ ml	6

**RESULT**

**Extraction of whole plant of *Euphorbia humifusa*:** The percentage yield (w/v) of the dried plant *Euphorbia humifusa* was found with petroleum ether, chloroform and methanol respectively. The percentage yield of the dried plant of *Euphorbia humifusa* of methanol was found to be greater than other extracts (Table 2).

**In-vitro evaluation of Anthelmintic activity**

The anthelmintic activity was carried out by taking Sixteen groups (four concentrations 10, 20, 30, 40 mg/ml of all extract and standard) each containing six worms of approximately equal size were subjected to 10 ml of desired concentration. Observations were made for the time taken for paralysis and death of individual worms it found to be that methanolic extract shows the best Paralysis Time  $32.84 \pm 0.75$  and Death Time  $39.8 \pm 0.77$  in 40 mg/ml solution when it compare with the standard Albendazole (Table 3, Fig. 2).

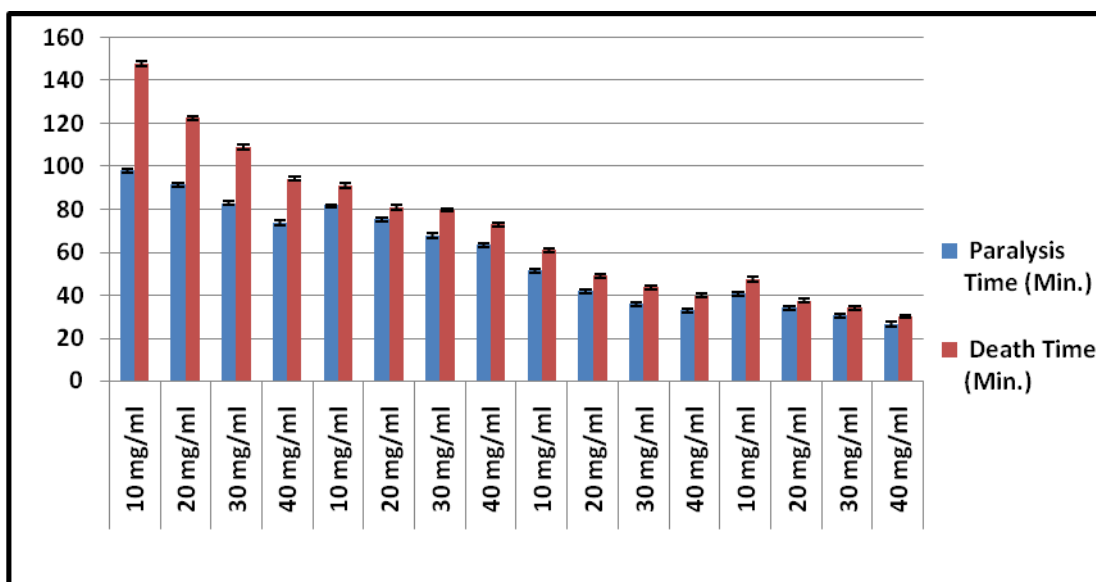
**Table 2: Yield of various extracts obtained from Plant of *Euphorbia humifusa***

Sr. No.	Part Use	Method of Extraction	Extract	Color	Yield (%) w/w
1	Whole Plant	Successive continuous hot extraction (Soxhlet)	Petroleum ether	Yellowish brown	4.7
2			Chloroform	Dark Green	6.92
3			Methanol	Dark brown	9.13

**Table 3: Paralysis and death time of Earthworm (*Eudrilus eugeniae*) for Different extracts of *Euphorbia humifusa***

Test/ Substance/ Extract	Concentration	Earthworm ( <i>Eudrilus eugeniae</i> )	
		Paralysis Time (Min.)	Death Time (Min.)
Pet. Ether	10 mg/ml	$98.35 \pm 0.33$	$147.79 \pm 1.61$
	20 mg/ml	$91.75 \pm 0.55$	$122.62 \pm 0.59$
	30 mg/ml	$82.98 \pm 1.12$	$109.11 \pm 0.95$

	40 mg/ml	73.7 ±1.08	94.36 ±1.0
<b>Chloroform</b>	10 mg/ml	81.96 ±0.42	90.96 ±1.22
	20 mg/ml	75.53 ±0.52	80.91 ±0.98
	30 mg/ml	67.87 ±0.99	79.97 ±0.64
	40 mg/ml	63.6 ±0.77	73.03 ±0.94
<b>Methanol</b>	10 mg/ml	51.73 ±0.62	60.96 ± 0.95
	20 mg/ml	41.79 ±1.03	48.91 ±0.9
	30 mg/ml	36.06 ±0.89	43.61 ±0.94
	40 mg/ml	32.84 ±0.75	39.8 ±0.77
<b>Albendazole</b>	10 mg/ml	40.89 ±0.87	47.41 ±0.96
	20 mg/ml	34.38 ±0.78	37.44 ±1.07
	30 mg/ml	30.45 ±1.04	34.21 ±0.91
	40 mg/ml	26.1 ±1.31	30.26 ±0.62



**Fig 2: Paralysis and death time of Earthworm (*Eudrilids eugenia*) for Different extracts of *Euphorbia humifusa***

## DISCUSSION AND CONCLUSION

On the complete Anthelmintic study of different extract, it reveals that all extracts including petroleum ether, chloroform and methanolic of plant *Euphorbia humifusa* have shown anthelmintic activity against adult earthworm but methanolic extract have significant anthelmintic activity as compare to other extracts.

Under the phytochemical investigation of extracts of plant *Euphorbia humifusa* reveals that Methanolic extract contain high concentration of flavonoids, tannins and phenolic compounds and it contain alkaloids as compare to other. The plants have anthelmintic activity mainly due to their phytoconstituents such as phenolic compounds,

flavonoids, tannins and alkaloids. Hence, it may conclude that anthelmintic activity of different extracts of plant will be due to presence of phenolic compounds, flavonoids, tannins and alkaloids compounds which may act separately or jointly.

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## CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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