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Anthelmintic studies on the leaves of *Urena lobata* Linn.

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Abstract

In the present study, alcoholic and aqueous extracts of the leaves of Urena lobata were investigated for their anthelmintic activity against Tubifex tubifex, Ecinia foeitida and Pheretima posthuma. The parameters analysed include time of paralysis and time of death of parasites. The alcoholic extract of the leaves of the plant showed significant activity when compared with the aqueous extract of the same plant.

Key Words: Urena lobata, Tubifex tubifex, Ecinia foeitida, Pheretima posthuma, Piperazine citrate.

Introduction

Helminths of one species or another are harboured by a large proportion of mankind. In some cases these infections result mainly in discomfort and do not cause ill health. In many countries, particularly those in tropical and subtropical regions, almost all the indigenous population is infected with hookworms or other helminths. Therefore, the treatment of helminthiasis is of great significance [1].

Helminths or worm infection is generally limited to the intestinal lumen but worms can migrate to other tissues in certain situations. The life cycle of each helminth varies. Helminths are divided into 3 groups [1],

1. Cestodes (Flatworms or Tapeworm)
2. Nematodes
3. Trematodes (Flukes)

Anthelmintics are classified according to the above said three groups. Anthelmintic drugs are used to eradicate or reduce the number of helminthic parasites in the intestinal tract or tissues of the body. These parasites have many biochemical or physiologic processes in common with their mammalian hosts, yet there are some differences that help to find pharmacologically active substances which have little or no action on the host [1].

Cestodes are commonly referred to as tape worms. The common tapeworms that infest man are *Taenia saginata*, *Taenia solium* and *Diphyllobothrium latum*. Cestodes have complex lifecycles. Majority of the patients do not show any symptoms but some experience abdominal discomfort, indigestion, anorexia, and vitamin B deficiency [1].

Nematodes are commonly referred to as round worms, *Ascaris lumbricoides* in the intestinal nematode whereas filarial and guinea worms live in blood, lymphatics and other tissues. The other nematode are hookworms (*Ancylostoma duodinale*) and pin worms (*Enterobius vermicularis*) [1]**.** Infection of *Ascaris lumbricoides* in man is called Ascariasis. Hookworm infection caused by the parasitation of the small intestine with *Ancyclostoma duodinale* or *Necator americanus* (Ancyclostomiasis). Filariasis caused by 8 species of filarial parasite. Disease can be lymphatic filariasis or non- lymphatic filariasis. Lymphatic filariasis caused by *W.bancrofti*, *B.malayi*, *B.timori*.[2]**.** Trematodes are non-segmented flattened helminths. The common infection is schistosomiasis by schistosome (blood fluke), group of trematodes such as *Schistosoma haematobium*, *Schistosoma mansoni*, and *Schistosoma japonicum* [1].

Urena lobata is a plant from malvaceae family and the plant is used traditionally in treating many diseases. The scientific reports on the plant also suggest that *Urena lobata* may be a good source of promising phytotherapeutic chemical moieties [3,4].

Fig 1: Urena lobata Linn

Material and Methods

Plant Material

The fresh leaf were collected from the medical college campus of Pariyaram in the month of March-April and was authenticated by Dr.V.Abdul Laleel, Asst. Professor, Department of P.G.Studies and Research in Botany, Sir Syed College, Thaliparamba, Kannur, Kerala. Leaves were then shade dried and a specimen of bearing voucher no.APSC/COG/02/2018-2019 has been deposited in the department of Pharmacognosy, Academy of Pharmaceutical Sciences, Pariyaram Medical College, Kannur District, Kerala State, and South India.

Preparation of Extracts

The shade dried leaves were powdered mechanically and there after subjected to the extraction process. For preparing the aqueous extract, the leaf powder was extracted with water at room temperature till the exhaustion. The mixture was kept for 12 hours with constant agitation at 30 minutes intervals. The extract was filtered and the filtrate thus collected was concentrated under reduced pressure. The semisolid extract obtained was stored in a refrigerator for further use. The alcoholic extract was prepared by hot extraction method by using soxhlet apparatus.

Preliminary Phytochemical Screening [5,6]

1) Successive solvent extraction

Petroleum ether extract: The powdered plant material (50g) was extracted with petroleum ether by hot extraction process (soxhlet) for 6 hr. After completion of extraction, the solvent was removed by distillation and subjected to concentration.

Benzene extract: The marc left after petroleum ether extraction was dried and extracted with benzene by hot extraction process (soxhlet) for 6hr. After completion of extraction, the solvent was removed by distillation and subjected to concentration.

Chloroform extract: The marc left after benzene extraction was dried and extracted with chloroform by hot extraction process (soxhlet) for 6hr. After completion of extraction, the solvent was removed by distillation and subjected to concentration.

Acetone extract: The marc left after chloroform extraction was dried and extracted with acetone by hot extraction process (soxhlet) for 6hr. After completion of extraction, the solvent was removed by distillation and subjected to concentration.

Ethanolic extract: The marc left after acetone extraction was dried and extracted with (95%) ethanol by hot extraction process (soxhlet) for 6hr. After completion of extraction, the solvent was removed by distillation and subjected to concentration.

Aqueous extract: The marc left after ethanol extraction was dried and extracted with distilled water by maceration process for 7 days. After completion of extraction, the solvent was removed by evaporation and subjected to concentration.

The above extracts were used for phytochemical studies and the extractive value for each extract was calculated and recorded.

Organisms Selected for the evaluation

The organisms selected for the investigation include *Pheretima posthuma*, *Eicinia fetida*, and *Tubifex tubifex*. *Pheretima posthuma* comes under Indian earthworm category [12]. An adult earth worm is having an average size of 15-25 cm in length with long elongated cylindrical narrow body with brown colour due to the presence of porphyrin skin pigment. *Eisenia fetida* is also comes under the category of earthworms which lives on the surface of the soil that are adapted to decaying organic material. These are also known as red worm, tiger worm etc. The worms are having 3-4 inches length with light and dark bands of red colour. *Tubifex tubifex* is also known as sewage worm. They generally live in the sediments of river, lake and aquariums. An adult worm will be having 20cm length. Because of the haemoglobin pigment the worms appears in red colour.

Collection of the organisms

Indian earthworm like *Pheretima posthuma* and *Eisenia fetida* were collected from the water logged soil from the fields of Cheruthazham village of Kannur district, Kerala and *Tubifex tubifex* were collected locally from the aquarium markets. The authenticities of the collected parasites were confirmed before the evaluation. Parasites were washed with water for removing the adhering sand and other elements. They were also washed with saline for final cleaning before the evaluation.

Experimental

The evaluation of the leaves of *Urena lobata* for its anthelmintic activity has been carried out with slight modifications as suggested by Ajayieoba E. O. et al. All the worms selected for the evaluation belongs to annelida group. The worms bearing almost equal size were released in to petridishes containing 20 ml of solutions of aqueous and alcoholic extracts of three different concentrations like 100,150,250 mg/ml. The worms were observed for the time taken for paralysis as well as death. The paralyses of the worms were confirmed by the absence of any sort of movement even after vigorous shaking. No movement of the parasites even after dipping in warm water confirms the death of the worms. The worms were also observed for their colour and any sort of changes within their body. Saline was the control and piperazine citrate was the reference standard used during the evaluation [7].

Statistical Analysis

The statistical analyses were done by using Dunnett's test.

Results and Discussion

Table 1: Extractive values of various extracts of Urena lobata

Solvents used	Percentage (%) of extracts (w/w)
Petroleum ether (60-80)	0.68
Benzene	0.62
Chloroform	0.71
Acetone	0.65
Ethanol (95%)	2.50
Chloroform water	2.35

The extract of the plant displays a significant anthelmintic activity in a dose dependent manner. The activities of alcoholic and aqueous extracts were comparable with that of piperazine citrate. Piperazine citrate is a broad spectrum anthelmintic used in treating round, hook and pin worm infection which acts by inhibiting the glucose uptake system results in the lethal depletion of energy reserves in the helminthes. An anthelmintic drug can act mainly by two mechanisms. Either by causing paralysis of the parasite or by causing damage to the cuticle of the parasite, which results in the partial digestion or rejection by immune mechanism [8].We have to be more careful in selecting the dose as well as the route of administration of an anthelmintic drug because the parasitic worms may not be consume sufficient amount of drug to be effective.

The phytochemical screening of the leaf extracts shows the presence of carbohydrates, phytosterols, glycosides, phenolic compounds, tannins and flavonoids in both alcoholic and aqueous extracts. Phytochemicals such as alkaloids, tannins, phenols etc. are reported to have significant anthelmintic activity [9].Tannins and Phenolics are known to interfere with the energy generation in helminth parasites by uncoupling oxidative phosphorylation [10].Tannins were reported to interfere with energy generation of worms by uncoupling oxidative phosphorylation or they bind to the free protein of the gastrointestinal tract and leads to the death of the worms [11]

Table 2: Qualitative preliminary phytochemical screening of various extracts of Urena lobata

Test	Petroleum Ether	Benzene	Chloroform	Acetone	Ethanol	Water
Alkaloids	-	-	-	-	-	-
Carbohydrates	-	-	-	-	+	+
Phytosterols	+	-	+	-	-	-
Glycosides	-	-	-	-	+	+
Fixed oils & fats	-	-	-	-	-	-
Saponins	-	-	-	-	-	-
Phenolic compounds & Tannins	-	-	-	-	+	+
Proteins & amino acids	-	-	-	-	+	+
Flavonoids	+	-	-	-	+	+

Gums & mucilages	-	-	-	-	+	-
Resins	-	-	-	-	-	-

(+ Presence , - Absence)

Table 3: Anthelmintic activity of the leaves of Urena lobata Extracts.

Extracts	Conc. (mg/ml)	*Tubifex tubifex*		*Ecinia foeitida*		*Pheretima posthuma*	
		Paralysis Time in minutes	**Death Time in minutes**	**Paralysis Time in minutes**	**Death Time in minutes**	**Paralysis Time in minutes**	**Death Time in minutes**
Control (Saline)	…… ……..	…… …..	…… …..	…… …..	…… …..	…… …..	…… …..
Aqueous Extract	100	93±0.258	179±0.058	77±0.259	119±0.029	89±0.146	118±0.025
	150	87±0.059	91±0.025	59±0.021	90±0.0258	71±0.035	96±0.017
	250	69±0.0147	66±0.049	39±0.043	60±0.025	67±0.099	74±0.035
Alcoholic extract (Ethanol)	100	82±0.0236	159±0.085	69±0.025	149±0.026	97±0.024	156±0.024
	150	69±0.039	59±0.039	51±0.032	72±0.022	75±0.013	84±0.049
	250	40±0.078	47±0.013	29±0.021	59±0.036	59±0.057	69±0.037
Piparazine Citate (STANDARD)	100	168±0.032	201±0.285	162±0.021	210±0.027	162±0.025	249±0.029
	150	141±0.784	139±0.057	112±0.024	199±0.039	144±0.064	221±0.064
	250	129±0.025	121±0.049	99±0.018	187±0.073	129±0.037	189±0.042

**Values are mean ± S.E.M. from six observations.*

Anthelmintic drugs act by paralysing the parasite by damaging the worm such that the host immune system can eliminate it, or by altering parasite metabolism. Because the metabolic requirements of these parasites vary greatly from one species to another, drugs that are highly effective against one type of worm may be ineffective against others. To bring about its action, the drug must penetrate the tough exterior cuticle of the worm or gain access to its alimentary tract. This may present difficulties, because some worms are exclusively haemophagous, while others are best described as tissuegrazers. A further complication is that many helminths possess active drug efflux pumps that reduce the concentration of the drug in the parasite. Most commonly used anthelmintic drugs include mebendazole, albendazole, piperazine, levamisole, ivermectin etc [13].

Unwanted effects are few with albendazole or mebendazole, although gastrointestinal disturbances can occasionally occur. For tiabendazole, the commonest being gastrointestinal disturbances, although headache, dizziness, and drowsiness have been reported and allergic reactions may also occur. Unwanted effects of piperazine include gastrointestinal disturbances, urticaria and bronchospasm. Some patients experience dizziness, paraesthesias, vertigo and incoordination. Diethylcarbamazine is a piperazine derivative. Unwanted effects are common but transient. Side effects from the drug itself include gastrointestinal disturbances, joint pain, headache, and a general feeling of weakness. Levamisole can cause gastrointestinal disturbances but also more serious effects, notably agranulocytosis. Ivermectin associated with skin rashes and itching [13].

From the findings we can state that the leaves of the plant have varying degree of anthelmintic action and the actions of both the extracts were dose dependent too. Hence the probable mechanism of action of *Urena lobata* could be due to the presence of tannin contents in the leaves. Since almost all synthetic anthelmintic drugs possess one or other side effects, usage of herbal drugs to treat the worm infection has to be entertained and encouraged. The study strongly supports the traditional use of the plant as an anthelmintic.

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