ANTHELMINTIC ACTIVITY OF THE LEAVES OF MURRAYA KOENIGII LINN AND MUSSAENDA FRONDOSA LINN.

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ABSTRACT
The aim of the present study was to evaluate the anthelmintic potential of crude extracts of leaves of Murraya koenigii and Mussaenda frondosa in different fractions namely petroleum ether, chloroform and methanol using Pheretima posthuma (Annelida) as test worms. Various concentrations (2.5-20.0 mg /ml) of the different fractions were tested in the bioassay which involved determinations of the time of paralysis (P) and the time of death (D) of the worms. Albendazole (10.0mg / ml) solution was included as standard reference and Tween 80 (0.1%) as control. The result of the present study indicated that the petroleum ether, chloroform and methanolic fractions of Murraya koenigii and Mussaenda frondosa significantly demonstrated the slowing down of the movement and caused death of the worms at a concentration of 20.0mg /ml as compared to the standard reference, Albendazole. MFC fractions showed the minimum time required for death. In conclusion, the traditional use of the Murraya koenigii and Mussaenda frondosa as an anthelmintic has been confirmed and further studies are suggested to isolate the active principles responsible for the activity.

KEYWORDS: Albendazole, Anthelmintic, Murraya koenigii, Mussaenda frondosa, Pheretima posthuma.
INTRODUCTION

Helminthic infections are now being recognized as cause of much chronic ill health and sluggishness among the tropical people. More than half of the population in the world suffers from worm infection of one or the other kind, which is a matter of great concern.

Traditional system of medicine reports the efficiency of several natural products eradicating helminthes. Keeping this in view, the present study deals with the evaluation of anthelmintic activity of the leaves of Murraya koenigii (Rutaceae) and Mussaenda frondosa (Rubiaceae).

Murraya koenigii (Rutaceae) is an unarmed, small aromatic tree with dark grey bark and closely crowded spreading dark green foliage. The roots, barks and leaves have been used since ayurvedic times for different therapeutic purposes.\textsuperscript{[1]} The roots, barks and leaves are bitter, acrid, astringent, aromatic, demulcent, depurative, anthelmintic, febrifuge, stomachic, appetizing, carminative, anodyne, constipating, anti-inflammatory, antisepctic and tonic.\textsuperscript{[2]} Ancient literature shows that it has been used in helminthes.\textsuperscript{[2]}

Another garden plant Mussaenda frondosa (Rubiaceae) is a handsome erect or scandent shrub with grey barks, leaves simple and the calyx lobes becomes enlarged into white, pink or orange foliaceous structure. The plant is astringent, sweet, expectorant, febrifuge, anti-inflammatory, vulnerary, alterant, demulcent, ophthalmic and cardiotonic.\textsuperscript{[3]} The plant has shown several potential activities like the anti-microbial activity\textsuperscript{[4]}, antioxidant\textsuperscript{[5]}, wound healing\textsuperscript{[6,7]}, hypolipidemic\textsuperscript{[8]} and radical scavenging.\textsuperscript{[9]} Siju et al (2010) has reported the anthelmintic activity in the ethyl alcohol and aqueous extract of the plant.\textsuperscript{[10]}

MATERIALS AND METHODS

Plant collection and Authentication

The stems and leaves of Murraya koenigii and Mussaenda frondosa were collected from mature trees and its botanical identification was confirmed by Dr. M.K. Panigrahi, Professor and Dean, KMIPS and a voucher specimen was deposited in herbarium of KMIPS, Chhend colony, Rourkela, Odisha.

Plant Extraction: The plant material of Murraya koenigii and Mussaenda frondosa were shade dried for several days and powdered material was extracted successively by petroleum ether (60\textdegree-80\textdegree C) and with chloroform using soxhlet and by methanol by cold percolation method. It was evaporated and dried in vacuo to yield a dark coloured material soluble in
DMSO or Dimethyl Sulfoxide. All the fractions were preserved in the refrigerator till further use.

**Worms Collection and Authentication:** Indian earthworm Pheretima Posthuma (Annelida) was obtained as gift samples from Centre of P.G. Studies, Department of Microbiology, Orissa University of Agriculture and Technology, Bhubaneswar, Odisha.

**Preparation of Test sample:** Samples for in–vitro studies were prepared by dissolving 200 mg of each extract in 100 ml of distilled water using 0.1 % Tween 80 as suspending agent to obtain a stock solution. From this stock solution, different working dilutions were prepared to get concentration range of 2.5 , 5.0, 10.0 and 20.0 mg/ml.

**Anthelmintic Assay:** The anthelmintic assay was carried as per the method of Ajaiyoeba E.O.et al [11] with minor modifications. The assay was performed on adult Indian earthworm, Pheretima posthuma of length 8-10cms due to its anatomical and physiological resemblance with intestinal roundworm parasite of human being.[12-15] Due to easy availability, earthworms have been widely used for in vitro evaluation of anthelmintic compounds.[16-19]

50ml formulation containing concentrations, each of crude extracts of Murraya koenigii and Mussaenda frondosa in petroleum ether, chloroform and methanol were prepared in distilled water using 0.1% Tween 80 as suspending agent and six worms (same type) were placed in it. Time for paralysis was noted when no movement of any sort could be observed except where the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water (50°C)[20-21] Albendazole (10mg/ml) was used as reference standard while 0.1% Tween 80 in distilled water as the control.

**RESULT AND DISCUSSION**

Preliminary phytochemical screening of crude extracts and its different fractions revealed the presence of flavanoids, alkaloids, saponins and tannins. As shown in Table 1 the methanolic extract of the two plants exhibited activity in dose-dependent manner giving shortest time of death within 20mg/ml. In most of the cases the initial movement was fast but slowed down with time, but paralysis was not seen and ultimately death was observed after some time. The reference drug, Albendazole showed the same after 48 minutes and immobilization in 29 minutes. This shows that the test drug had minimum effect on the muscles of the helminthes.
The benzimidazole exemplified by mebendazole and albendazole are versatile anthelmintic agents, particularly against gastrointestinal nematodes and hematodes where their action is not dictated by the systematic drug concentration. Appropriate doses of mebendazole and albendazole are highly effective against both larval adult stages of hematides. Immobilization and death of suspendible gastrointestinal parasites occur slowly and the clearance from the gastrointestinal tract may not be complete until a few days after treatment.

Benzimidazole produce many biochemical change in susceptible nematodes e.g. inhibition of mitochondrial fumarate reductase, reduced glucose transport and uncoupling of oxidative phosphorylation. There is strong evidence that the primary action of these drugs is to inhibit mitochondrial polymerization by binding β–tubulin.

The selective toxicity of these agents derives from the fact that specific high affinity binding to parasite β- tubulin occur at a much lower concentration than dose binding to the mammalian parasite. Thus the two identified mechanism of drug resistance to nematodes involve both a progressive loss of ‘susceptible’ β- tubulin gene isotypes together with emergence of a ‘resistance’ isotypes with a conserved point mutation that encodes a tyrosine instead of a phenylalanine at position 200 of β tubulin.

The two extracts fractions demonstrated slowly down of the movement and caused death at a concentration of 20mg/ml in a time almost near to the reference drug, albendazole. Phytochemical analysis of the crude extract revealed the presence of tannins among the other chemical constituents contained within them. Tannins were shown to produce anthelmintic action chemically tannins are polyphonolic compounds. Some synthetic phenolic anthelmintic are reported to interfere with energy generation in helminthic parasites by uncoupling oxidative phosphorylation. It is possible that tannins present in the extracts produced similar effects. Another possible anthelmintic effect of tannins is that they can bind to the proteins in the gastrointestinal tract of the host animals or glycoprotein on the cuticle of the parasite and may cause death.

The traditional medicines hold a great promise as a source of easily available effective anthelmintic agents in people, particularly in developing countries including India. It is in the context that the people consume several plants or plant derived preparations to cure helminthic infections e.g. decoctions of Murraya koenigii leaves are given to children to cure worms, without causing resistance to the worms. The origin of many effective drugs
have been found in the traditional medicine practices and in view of this it is important to undertake studies pertaining to screening of the folk medicinal plants for their claimed anthelmintic efficiency.

**Table 1: Anthelmintic activity of different extracts of Murraya Koenigii and Mussaenda frondosa**

<table>
<thead>
<tr>
<th>Sl.no</th>
<th>Test Substance</th>
<th>Concentration Mg/ml</th>
<th>Time Taken</th>
<th>Time Taken for death (D)in minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Murraya Koenigii</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Standard (Albendazole)</td>
<td>10.0</td>
<td>29±0.09</td>
<td>48±1.09</td>
</tr>
<tr>
<td>3</td>
<td>Petroleum Ether Extract</td>
<td>2.5, 5.0, 10.0, 20.0</td>
<td>-</td>
<td>109.50±0.43, 98.33±0.42, 90.00±0.37, 85.00±0.26</td>
</tr>
<tr>
<td>4</td>
<td>Chloroform extract</td>
<td>2.5, 5.0, 10.0, 20.0</td>
<td>-</td>
<td>105.17±0.31, 101.33±0.49, 94.83±0.31, 89.67±0.42</td>
</tr>
<tr>
<td>5</td>
<td>Methanolic Extract</td>
<td>2.5, 5.0, 10.0, 20.0</td>
<td>-</td>
<td>89.67±0.42, 85.83±0.48, 77.67±0.42, 69.83±0.30</td>
</tr>
</tbody>
</table>

All Values represent Mean ± SEM . n = 6 in each group, p < 0.01 and p < 0.05

**Graph 1: Anthelmintic activity of different extracts of Murraya Koenigii and Mussaenda frondosa**
CONCLUSION
The traditional use of leaves of Murraya koenigii has been confirmed as the leaves, stem extract displayed profound anthelmintic activity in the study. Mussaenda frondosa leaf extracts has shown good activity and can be promising newer medicine for use for the above action. Further it would be interesting to isolate the possible phytocontituents which may be responsible for the anthelmintic activity and to the possible the mechanism of action.

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