In-Vitro Anthelmintic Activity of Different Extracts of Luffa Cylindrica Leaves

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Abstract

Different extracts of L. cylindrica were taken for anthelmintic activity against Indian earthworm Pheretima posthuma. Two concentrations (50 and 100 mg/ml) of various extracts were tested and results were expressed in terms of time for paralysis and time for death of worms. Albendazole (20 mg/ml) was used as reference standard and carboxy methyl cellulose (0.5%) as a control group. Dose dependent activity was observed in the plant extracts but hydroethanolic extract exhibited more activity as compared to others. The anthelmintic activity of Luffa cylindrica leaves extract has therefore been demonstrated for the first time.

Keywords

Luffa cylindrica, Pheretima posthuma, anthelmintic activity.

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1.1 INTRODUCTION

Helminthes infections, repeatedly entitled helminthiasis are among the most pervasive infection and a foremost degenerative disease distressing a large proportion of world’s population. In developing countries, they pose a large threat to public health and contribute to the prevalence of malnutrition, anemia, eosinophilia and pneumonia [1]. The helminths parasites mainly subsist in human body in intestinal tract, but they are also found in tissue, as their larvae migrate towards them [2]. Most diseases caused by helminths are of a chronic, debilitating nature; they probably cause more morbidity and greater economic and social deprivation among humans and animals than any single group of parasites. Chemical control of helminthes coupled with improved management has been the important worm control strategy throughout the world. However, development of resistance in helminthes [3, 4] against conventional anthelmintics is a foremost problem in treatment of helminthes diseases [5, 6]. Henceforth it is important to look for alternative strategies against gastrointestinal nematodes, which have led to the proposal of screening medicinal plants for their anthelmintic activity. Luffa L. cylindrica commonly called sponge gourd, loofa, vegetable sponge, bath sponge or dish cloth gourd, is a member of cucurbitaceous family. The plant is widely distributed throughout India. Its fruit is used in the traditional
medicine as an anthelmintic, carminative, laxative, depurative, emollient, expectorant and diuretic and lactagogue and are useful in fever, syphilis, tumours, bronchitis, splenopathy and leprosy [7]. It is used as a vegetable either prepared like squash or eaten raw like cucumbers [8, 9]. Its seeds have been used in the treatment of asthma, sinusitis and fever [10]. The seed oil is reported to be used for skin infections in the form of tincture. The fruit used in the treatment of ascites, jaundice and biliary and intestinal colitis and also in enlarged spleen and liver. The plant is reputed to have anti-tubercular and antiseptic properties. The extract of leaves has been used in snakebites. Keeping in mind such astounding properties exhibited by the plant, the present study was intended to investigate anthelmintic activity of methanolic and aqueous extract of *L. cylindrica* leaves in Indian earthworm.

**1.2 MATERIAL AND METHOD**

**1.2.1: Preparation of Extracts**

The dried powdered leaves were extracted by maceration for seven days with 70% ethanol and the solvent portion was evaporated under reduced pressure to yield 70% hydroethanolic fraction. This dried fraction was further extracted by maceration with *n*-butanol. The organic layer was evaporated under reduced pressure to yield *n*-butanol fraction. The residue was again macerated with chloroform and the organic layer was evaporated under reduced pressure to yield chloroform fraction. The prepared extracts were kept under refrigeration for screening of anthelmintic activity. [11]

**1.2.2: in-vitro Anthelmintic activity**

The anthelmintic activity was evaluated on adult Indian earthworm *Phaeritima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. [12-15] The earthworms are collected and washed with normal saline with removal of fecal matter. The earthworms are 5 to 6 cm length and 0.2-0.3 cm widths were used for experiment protocol. [16, 17] Different extracts that were prepared from *Luffa cylindrica* leaves were examined systematically for them in-vitro anthelmintic activity against *Phaeritima posthuma*. The in-vitro anthelmintic assay procedures were carried out as per method of Mathew et al. [18] and Dash et al. [19] with slight modifications. [20-23] Five groups of equal size Indian earthworm consisting of six earthworms in each groups were released into 50ml of desired formulation. Each group was treated with one of the following: Vehicle (0.9%w/v NaCl), piperazine citrate (40mg/ml), and different extracts of (100 mg/ml, 50mg/ml, and 25mg/ml) in normal saline. Observations were made for the paralysis time and subsequently for death time of the worms. The mean paralysis and/or death time for each group was recorded (each reading taken for 6 times). The time taken by the worms to become motionless, consider as paralysis was recorded and the lethal time was recorded by observing the time taken to become motionless on application of external stimuli by pricking with pin. Piperazine citrate (40mg/ml) was taken as reference drug. (Table-1.1, 1.2 & 1.3)

**1.3 RESULTS**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Piperazine-citrate</th>
<th>Chloroform extract</th>
<th>Chloroform extract</th>
<th>Chloroform extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc (mg/ml)</td>
<td>40.0</td>
<td>100.0</td>
<td>50.0</td>
<td>25.0</td>
</tr>
<tr>
<td>Paralysis Time (min.)</td>
<td>28.32±0.360</td>
<td>11.62±0.143</td>
<td>24.53±0.704</td>
<td>37.18±0.64</td>
</tr>
<tr>
<td>Death Time (min.)</td>
<td>60.10±0.230</td>
<td>26.15±0.232</td>
<td>53.10±0.207</td>
<td>60.05±0.628</td>
</tr>
</tbody>
</table>

*Fig.1: Anthelmintic activity of 70% hydroethanolic extract of Luffa cylindrica leaves*
Table 1.2: Anthelmintic activity of n-butanol extract of *Luffa cylindrica* leaves

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Piperazine-citrate</th>
<th>Chloroform extract 1</th>
<th>Chloroform extract 2</th>
<th>Chloroform extract 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc (mg/ml)</td>
<td>40.0</td>
<td>100.0</td>
<td>50.0</td>
<td>25.0</td>
</tr>
<tr>
<td>Paralysis Time (min.)</td>
<td>29.32±0.360</td>
<td>13.62±0.26</td>
<td>17.03±0.20</td>
<td>21.03±0.22</td>
</tr>
<tr>
<td>Death Time (min.)</td>
<td>60.10±0.230</td>
<td>29.25±0.12</td>
<td>61.52±0.61</td>
<td>100.26±0.16</td>
</tr>
</tbody>
</table>

Fig. 2: Anthelmintic activity of n-butanol extract of *Luffa cylindrica* leaves

Table 1.3: Anthelmintic activity of chloroform extract of *Luffa cylindrica* leaves

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Piperazine-citrate</th>
<th>Chloroform extract 1</th>
<th>Chloroform extract 2</th>
<th>Chloroform extract 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc (mg/ml)</td>
<td>40.0</td>
<td>100.0</td>
<td>50.0</td>
<td>25.0</td>
</tr>
<tr>
<td>Paralysis Time (min.)</td>
<td>27.32±0.360</td>
<td>14.11±0.497</td>
<td>31.00±0.290</td>
<td>53.53±0.350</td>
</tr>
<tr>
<td>Death Time (min.)</td>
<td>61.10±0.230</td>
<td>25.0±0.369</td>
<td>59.20±1.90</td>
<td>108.25±0.25</td>
</tr>
</tbody>
</table>
1.4 DISCUSSION
Preliminary phytochemical analysis of methanolic extracts showed the presence of Flavonoids, Saponins, Tannins, Steroids, Terpenoids & Alkaloid whereas aqueous revealed the Tannins, Steroids & Alkaloid active phytoconstituents. The data revealed that the methanol extract showed anthelmintic activity at a concentration of 100 mg/ml, whereas the aqueous extract also showed paralysis and death at similar concentrations. The other test concentrations of both the extracts showed marked degree of anthelmintic activity. The anthelmintic effect of extracts is comparable with that of the effect produced by the standard drug Albendazole. Parasitic helminths affect animals and man, causing considerable hardship and stunted growth. Hundreds of millions if not billions of human infections by helminthes exist worldwide and increased world travel and immigration from the developing countries. However tremendous advances have been made during the previous decade and substantial number of synthetic precursors have been derived to cope up the damage caused by parasite, but unfortunately no effective medicine has been developed so far. Moreover, the problems associated with the use of such drugs like some serious side effects and development of resistance drives the severity of infection to the next level. These factors paved the way for herbal remedies as alternative anthelmintics. Evaluation of activities of medicinal plants claimed for possessing the anthelmintic property is getting the attention these days. Screening and proper evaluation of the claimed medicinal plants could offer possible alternatives that may be both sustainable and environmentally acceptable. The results of this study have shown promising anthelmintic activity suggesting the possible use of L. cylindrica extracts in intestinal nematode control. The anthelmintic activity of methanol extracts could be due to the constituents present. The present study suggested that the methanol extract was more effective than the other extracts, even though all the extract were endowed with anthelmintic property. The activity was concentration dependent of the different extracts. The activity of the extracts was found to be inversely proportional to the time taken for paralysis / death of the earth worms.

1.5 CONCLUSION
The results of the present study were clearly indicated that the crude methanol extract of Luffa cylindrica did produce anthelmintic activity against Indian earthworm Pheretima posthuma. The plant possesses significant anthelmintic activity at 100 mg/ml concentration measured by time taken for paralysis / death of the earth worms. The current investigation leads to conclusion that the leaves of L. cylindrica have potent anthelmintic activity when compared with the conventionally used drug. The results did not, however, exclude the possibility that doses of the extract with lower anthelmintic activity in this study might be efficacious against other species of helminths. Further studies using in vivo models and to isolate active constituents from extract are required to carry out and established the effectiveness and pharmacological rational for the use of L. cylindrica as an anthelmintic drug.

1.6 ACKNOWLEDGEMENT
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1.7 REFERENCE