In Vitro Anthelmintic Activity of Lagenaria siceraria Leaves in Indian Adult Earthworm

Amit Kumar*, Sangh Partap

1. Department of Pharmaceutical chemistry, Teerthanker Mahaveer College of Pharmacy, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India. [E-mail: prm.amit.kumar@gmail.com]

Different extracts of Lagenaria siceraria 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 methanolic extract exhibited more activity as compared to others. The anthelmintic activity of Lagenaria siceraria leaves extract has therefore been demonstrated for the first time.

Keyword: Anthelmintic, Lagenaria siceraria, Pheretima posthuma, Methanolic extract.

1. Introduction

Medicinal plants and derived medicine are widely used in traditional cultures all over the world and they are becoming increasingly popular in modern society as natural alternatives to synthetic chemicals[1]. In the last few decades there has been an exponential growth in the field of herbal medicine. It is getting popularized in developing and developed countries owing to its natural origin and lesser side effects[2].

At the present juncture, the modern conventional healthcare is burdened with great problems of unsafe medicines, chronic diseases, resistant infections, auto immune disorders and degenerative disorders of ageing, despite great scientific advances. More than 70% of India’s 1.1 billion populations still use these non-allopathic systems of medicine[3]. India possesses almost 8% of the estimated biodiversity of the world with around 0.126% million species[4].

The World Health Organization (WHO) estimated that approximately 80% of world population relies mainly on traditional medicines, mostly plant drugs in their health care. Today, Ayurveda coexists with modern system of medicine, and is still widely used and practiced. About 30% of the currently used therapeutics is of natural origin[5].

Lagenaria siceraria commonly known as Bottle gourd is also called as Doodhi in Gujarati, Lauki (Hindi), Kadoo (Marathi) and is official in Ayurvedic Pharmacopoeia. It is one of the excellent fruit for human being made and gifted by the nature having composition of all the essential constituents that are required for normal and good human health[6]. Leaves of L. siceraria are taken as emetic in the form of leaf juice or decoction. This by adding sugar is also used in Jaundice. Crushed leaves are used for baldness and applied on the head for the headache. Leaves are also used as alternative purgative[7-8]. The
edible portion of fruits is fair source of ascorbic acid, β-carotene and good source of vitamin B complex, pectin dietary soluble fibers and contains highest source of choline level-anisotropic factor, a healer of mental disorders, along with required metabolic and metabolite precursors for brain function. Amongst any other vegetable known to man till date, it is also good source of minerals and amino acids[9-10]. Two sterols were identified and isolated from petroleum ether fractions of ethanol extract of dried fruit pulp of L. siceraria namely fucosterol and campesterol[11]. HPLC analysis of extract of flowering plant of Lagenaria siceraria shows presence of flavone-C glycosides[12]. The effect of semi purified dietary fibers isolated from the fruit of L.S on fecal steroid excretion was reported[13]. In the present work we evaluate hepatoprotective activity of methanolic extract of L. Siceraria leaves in experimental rodents.

2. Material and Method
2.1 Plant material
The leaves of Lagenaria siceraria were collected in locally from local area of Bareilly, Uttar Pradesh, India in the month of August 2010 and were authentified by Dr. Beena Kumari, Taxonomist, Hindu College, Moradabad (India) as Lagenaria siceraria (Cucurbitaceae) leaves. A voucher specimen has been kept in the herbarium (HC.MBD/HAP/BK/2010/7/167) of the Department of Botany, Hindu college, Moradabad (India).

2.2 Experimental worms:
All the experiments were carried out in Indian adult earthworms (Pheretima posthuma) due to its anatomical resemblance with the intestinal roundworm parasites of human beings. They were collected from moist soil and washed with water to remove all fecal matters.

2.3 Preparation of Extracts:
The leaves of Lagenaria siceraria were dried under shade and crushed in an electric blender to form coarse powder and subjected to Soxhlet extraction (Continuous hot extraction) by using methanol and water as solvent. The extracts were concentrated by rotary evaporator and used for testing anthelmintic activity. Preliminary phytochemical screening was carried out to assess the presence of phytoconstituents in the extract.

2.4 Administration of Albendazole:
Albendazole (20 mg/ml) was prepared by using 0.5% w/v of CMC as a suspending agent as administered as per method of extract.

2.5 Administration of extract:
The suspension of Methanolic and aqueous extract of leaves of Lagenaria siceraria of different concentration (50,100 mg/ml) were prepared by using 0.5% w/v of CMC as a suspending agent and final volume was made up to 10 ml for respective concentration. Albendazole was used as standard. Groups of approximately equal size worms consisting of two earthworms individually in each group were released into in each 10 ml of desired concentration of drug and extracts in the petri dish.

2.6 Experimental Design:
The anthelmintic activity was performed according to the method[14]. On adult Indian earthworm Pheretima posthuma as it has anatomical and physiological resemblance with the intestinal round worm parasites of human beings. Pheretima posthuma was placed in petridish containing two different concentrations (50 &100mg/ml) of methanolic & aqueous extract of leaves of Lagenaria siceraria. Each petridish was placed with 2 worms and observed for paralysis or death. Mean time for paralysis was noted when no movement of any sort could be observed, except when the worm was shaken vigorously; the time death of worm (min) was recorded after ascertaining that worms neither moved when shaken nor when given external stimuli. The test results were compared with Reference compound Albendazole (20 mg/ml) treated samples.
Table 1: Anthelmintic potency of methanolic and aqueous extract of *Lagenaria siceraria*

<table>
<thead>
<tr>
<th>Extract</th>
<th>Concentration (mg/ml)</th>
<th><em>Pheretima posthuma</em></th>
<th>Paralysis (P)</th>
<th>Death (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (0.5% CMC)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard (Albendazole)</td>
<td>20 mg/ml</td>
<td>7.71 ± 0.927</td>
<td>15.80 ± 1.158</td>
<td></td>
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<tr>
<td>Methanolic extract</td>
<td>50 mg/ml</td>
<td>55.8 ± 2.518</td>
<td>80.4 ± 2.909</td>
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</tr>
<tr>
<td></td>
<td>100 mg/ml</td>
<td>30.6 ± 1.364</td>
<td>56.8 ± 1.497</td>
<td></td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>50 mg/ml</td>
<td>124.6 ± 3.059</td>
<td>177.4 ± 3.501</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100 mg/ml</td>
<td>111.8 ± 2.557</td>
<td>156.66 ± 5.636</td>
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</tr>
</tbody>
</table>

All Values represent Mean ± SD; n=6 in each group. Comparisons made between standard versus treated groups, P<0.05 was considered significant.

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.

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 paralyse / death of the earth worms.

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 has 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 *Lagenaria siceraria* extracts in intestinal nematode control. The anthelmintic activity of methanol extracts could be due to the constituents present.

3. Results and 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.
4. Conclusion
The results of the present study clearly indicated that the crude methanol extract of *Lagenaria siceraria* 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 paralyse / death of the earth worms. The current investigation leads to conclusion that the leaves of *Lagenaria siceraria* 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 *Lagenaria siceraria* as an anthelmintic drug.

5. Reference