In Vitro Anthelmintic Activity of Aqueous and Alcoholic Extracts of *Citrus Medica* Leaves

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**ABSTRACT**

*Citrus medica* (Rutaceae) commonly known Bada nimbu and it is available in allover India. Literature survey revealed that this plant reported for the antioxidant activity, antimicrobial activity and anti fungal activity. In this present study we planned to evaluate the anthelmintic activity by using both alcoholic and aqueous leaf extracts. Preliminary phytochemical screening of the both extracts revealed the presence of volatile oils, carbohydrates, glycosides, flavanones, citric oxide and glycosides. From this present study the aqueous and alcoholic extracts of *Citrus medica* were found to be active as anthelmintic with reference to both the paralysis and death times as compare to the piperazine citrate. The aqueous extract shows more activity than alcoholic extract.

**Key words:** *Citrus medica*, anthelmintic, Piperazine citrate.

**INTRODUCTION**

Helminthosis play a crucial role in small ruminant production leading to enormous economic losses particularly in areas where extensive grazing is practiced. It causes loss of production through mortality, weight loss, reduced milk and wool production. Parasitic helminths affect animals and man, causing considerable hardship and stunted growth. 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. Development of resistance to most of commercially available anthelmintic became a severe problem worldwide. The gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases. Hence there is an increasing demand towards natural anthelmintics.
Citrus medica (Rutaceae) commonly known Bada nimbu and it is available in allover India. Literature survey revealed that this plant reported for the antioxidant activity, antimicrobial activity and anti fungal activity. Phytochemical screening states that it contains fixed oils, volatile oils, citric oxide and flavanone glycosides are abundant constituents of citrus leaves and fruits. In this present study we are planned to evaluate the anthelmintic activity of aqueous and alcoholic extracts of C. medica leaves5.

MATERIALS AND METHODS

Plant Collection and Authentication
Citrus medica leaves were collected from the local farms of Kanpur, Uttar Pradesh state, India, and authenticated by the authority of the botany department, Shri Gandhi Inter College, Nonari, Kanpur.

Preparation of aqueous and alcoholic extracts6
The collected leaves were air-dried under the shade in laboratory for 7-12 days. After complete drying, leaves were powdered. The aqueous extracts were prepared by dissolving 100 g of powdered plant material in 500 ml of distilled water in a glass percolator. It was allowed to macerate for 24 h at room temperature and the brew was filtered using whatman number one filter paper. The process of percolation was repeated three times (500 ml). The combined filtrate was then concentrated in a water bath to ensure the complete evaporation of the solvent. The final crude aqueous extract was transferred to a vial and kept air tight. The leaf powder packed in soxhlet apparatus was extracted with 95% alcohol for 18 h and appearance of colourless solvent in the siphon tube was taken as the termination of extraction. The extracts were preserved properly before subjecting to anthelmintic activity.

Phytochemical screening
Preliminary phytochemical screening of the aqueous extract revealed the presence of volatile oils, carbohydrates, glycosides, flavanones. Alcoholic extract shoes the presence of flavonones, citric oxide and glycosides.

Preparation of test sample
Samples for in-vitro study were prepared by dissolving the extracts in distilled water to obtain different working solutions as 5, 10, 20 and 25 mg/ml.

Worm’s collection
Indian earthworm Pheritima posthuma (Annelid) were collected from the V.L. College of Pharmacy, Raichur. Earthworm were washed with normal saline solution to remove all the fecal Matter and kept in normal saline solution. The average size of earthworm was 6-8 cm.

Anthelmintic Activity
The anthelmintic activity was performed according to the method of Meenal SK et al7 on adult Indian earthworm Pheritima posthuma as it has anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. Ten groups of approximately equal sized Indian earthworms consisting of six earthworms in each group were released into 50 ml of desired formulation. The groups were prepared as control i.e distilled water, reference i.e
Preliminary Phytochemical screening of the crude extracts revealed the presence of carbohydrates, glycosides, flavonoids, flavanones. Flavanone glycosides are abundant constituents of citrus leaves and fruits. As shown in Table 1, the different extracts exhibited anthelmintic activity in dose dependent manner giving shortest time of paralysis and death with 25 mg/ml concentration. The alcoholic extract of *C. medica* caused paralysis of 10.67 min and time of death of 60.56 min while aqueous extract revealed paralysis of 10.33 min and death of 29.82 min against the earthworm *Pheritima posthuma*. The reference drug piperazine citrate showed the same at 9 and 22 minutes respectively. The predominant effect of piperazine citrate on worm is to cause a flaccid paralysis which results in expulsion of the worm by peristalsis. Piperazine citrate by increasing chloride ion conductance of worm muscle membrane produces hyper polarization and reduced excitability that leads to muscle relaxation and flaccid paralysis. The crude extracts of *C. medica* not only demonstrated paralysis but also caused death of worms especially at higher concentration of 25 mg/ml.

<table>
<thead>
<tr>
<th>Test substances</th>
<th>Concentration (mg/ml)</th>
<th>Time taken for paralysis(min.) (X±SEM)</th>
<th>Time taken for death(min.) (X±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>00</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>Alcoholic Extract</td>
<td>5</td>
<td>80.35±0.68</td>
<td>110.03±0.61</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>45.54±0.97</td>
<td>98.34±0.79</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>23.89±1.02</td>
<td>76.81±0.62</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>11.67±0.64</td>
<td>56.56±0.84</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>5</td>
<td>44.72±0.11</td>
<td>65.62±0.58</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>31.43±0.53</td>
<td>57.67±0.25</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>19.34±0.16</td>
<td>39.33±0.15</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>10.33±0.98</td>
<td>25.82±0.53</td>
</tr>
<tr>
<td>Piperazine citrate</td>
<td>10</td>
<td>9.22±0.7</td>
<td>22.61±0.39</td>
</tr>
</tbody>
</table>

*All values represent Mean ± SEM*

**CONCLUSION**

From this present study the aqueous and alcoholic extracts of *Citrus medica* were found to be active as anthelmintic with reference to both the paralysis and death times as compare to the Piperazine citrate. The aqueous extract shows more activity than alcoholic extract.
REFERENCES