ANALYSIS OF ANTHELMINTIC ACTIVITY OF CRATEVA UNILOCULARIS BUCH.-HAM. LEAF

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ABSTRACT

Crateva unilocularis (Capparaceae), commonly known as siplegan in Nepal and widely distributed throughout at an altitude of 100 - 1800 meters, is popularly known for its anthelmintic property in local area and in Ayurveda system of medicine. Present study was done to rationalize its traditional use. Anthelmintic activity of methanolic extract of C. unilocularis leaves was performed on adult earthworms viz Pheretima posthuma and Eisenea foetida. Test solutions of the main methanolic extract of C. unilocularis leaves were prepared at the concentrations of 10, 20 and 50 mg/ml in normal saline. Albendazole (20 mg/ml) was used as reference standard and normal saline was used as negative control. Each test worms P. posthuma and E. foetida were divided into five groups, each consisting of six adult worms of same size and type. Each group of six worms were placed in 25 ml of test and standard solutions. Physical activity of the worms was observed and the time taken for complete paralysis and death were recorded. All the test extracts exhibited significant paralytic and lethal actions in a concentration-dependent manner. Extract of concentration 50 mg/ml showed mean paralysis time and mean death time of 21.67 minutes and 25.67 minutes respectively to P. posthuma and 17.5 minutes and 22.16 minutes respectively to E. foetida. The result supports the traditional use of this plant as an anthelmintic and thus has a high potential for the isolation of anthelmintic compounds.

Keywords: Crateva unilocularis, anthelmintic, Nepal, Ayurveda.
INTRODUCTION

The medicinal value of plants lies in the specific chemical substances that produce a definite physiologic action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds.\(^1\) It is necessary to determine the nature of chemical constituents of the plant to determine its biological activity. The phytochemical research based on ethno pharmacological information is an effective approach in the discovery of new anti-infective agents from higher plants.\(^1\)

*Crateva unilocularis* (Capparaceae) is commonly known as siplegan in Nepal. It is a widely used medicinal plant distributed throughout Nepal at an altitude of 100 - 1800 meters. Various traditional uses of *C. unilocularis* are reported. The bark has laxative, stomachic and antiperiodic properties and the juice of young leaves is used traditionally for the treatment of helminth infestations.\(^2\)

![Figure 1: C. unilocularis Leaves with flowering parts](image)

Helminthiasis or infection with parasitic worms is a pathogenic condition in which the immature forms of the parasites invade human beings via the skin or gastrointestinal tract (GIT) and evolve into well differentiated adult worms that have characteristic tissue distribution. Anthelmintics are drugs that may act locally to expel worms from the GIT or systemically to eradicate adult helminthes or development forms that invade organs and tissues. Synthetic anthelminthics produces side effects such as abdominal pain, loss of appetite, nausea, vomiting, headache and diarrhea.\(^3\) Moreover, indiscriminate use of synthetic anthelmintics can lead to resistance of the parasitic worms. Herbal remedies are popular since ancient times for the treatment of helminth infestations in humans and could be of value in preventing the development of resistance.\(^4\)
The juice of young leaves of *C. unilocularis* is traditionally known for its anthelmintic properties. Therefore scientific characterization of anthelmintic activity of *C. unilocularis* is necessary to rationalize its traditional use.

**MATERIALS AND METHODS**

**Plant Materials**

Leaves of *C. unilocularis* were collected from Lekhnath Municipality-12, Kaski, Nepal during July, 2011. Only mature and healthy leaves were collected and any dirt, dust or insects on leaf surfaces were removed. The herbarium specimen of the plant was identified by National Herbarium and Plant Laboratory, Godawari, Lalitpur Nepal as *Crateva unilocularis*. 1.25 Kilograms of crude and dried leaves of *C. unilocularis* was macerated with 5.5 liters of methanol and was filtered. 57.55 grams of methanolic extract was obtained in dried form after drying the filtrate in vacuum rotary evaporator.

**Anthelmintic Assay**

To determine the anthelmintic activity of methanolic extract of *C. unilocularis* leaves, *in vitro* anthelmintic assay was performed on adult earthworms *Pheretima posthuma* and *Eisenea foetida* due to their anatomical and physiological resemblance with the intestinal roundworm parasite of human beings. These common earthworms are suitable for preliminary *in vitro* evaluation of anthelmintic activity because of their easy availability.

Test solutions of the main methanolic extract of *C. unilocularis* leaves were prepared at the concentrations of 10, 20 and 50 mg/ml in normal saline. Albendazole (20 mg/ml) was used as reference standard and normal saline was used as negative control. Each test worms *P. posthuma* and *E. foetida* were divided into five groups, each consisting of six adult worms of same size and type. Each group of six worms were placed in 25 ml of test or standard solutions (either test solution 10 mg/ml, test solution 20 mg/ml, test solution 50 mg/ml, albendazole 20 mg/ml or normal saline).

All the test solutions and standard drug solution were freshly prepared before starting the experiments. Physical activity of the worms was observed, and the time taken for complete paralysis and death were recorded. The mean paralysis time and mean lethal time for each group were determined. Time for paralysis was noted when no movement of any sort could be observed except when the worms were stimulated gently by a blunt pin or by vigorous shaking to activate them. Death of the worms was confirmed when complete immobility was
noted upon vigorous shaking and dipping the worms in warm water (50°C). All the results are expressed as a mean ± SEM of six worms in each group.

**Statistical analysis**
The data are presented as mean ± standard error of the mean (SEM).

**RESULTS AND DISCUSSION**
The results for *in vitro* effects of methanolic extract of *C. unilocularis* leaves in *P. posthuma* and *E. foetida* are summarized in Table 1. Against both *P. posthuma* and *E. foetida*, all the test extracts exhibited significant paralytic and lethal actions in a concentration-dependent manner. The anthelmintic activity was similar in both the test worms. The results are shown in Table 1 and Figure 2.

**Table 1**: Evaluation of Anthelmintic activity

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Concentration (mg/ml)</th>
<th><em>P. posthuma</em></th>
<th><em>E. foetida</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>P1 (min)</td>
<td>D1 (min)</td>
</tr>
<tr>
<td>1</td>
<td>Normal Saline</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Methanolic Extract</td>
<td>10</td>
<td>60.5 ± 1.83</td>
<td>82.5 ± 1.72</td>
</tr>
<tr>
<td>3</td>
<td>Methanolic Extract</td>
<td>20</td>
<td>48.83 ± 1.35</td>
<td>54.67 ± 0.98</td>
</tr>
<tr>
<td>4</td>
<td>Methanolic Extract</td>
<td>50</td>
<td>21.67 ± 0.49</td>
<td>25.67 ± 0.66</td>
</tr>
<tr>
<td>5</td>
<td>Albendazole</td>
<td>20</td>
<td>8 ± 0.73</td>
<td>16.67 ± 0.49</td>
</tr>
</tbody>
</table>

Where, P1 and D1 are the time for paralysis and time for death of *P. posthuma* and P2 and D2 are the time for paralysis and time for death of *E. foetida* respectively.
Figure 2: The time for paralysis and death of test worms against extracts and the standard

_P. posthuma_ and _E. foetida_ were found to be paralyzed and eventually killed by the test extracts in a concentration-dependent manner, showing differential toxic activities that diminished with lowering concentration. The anthelmintic activity of the methanolic extract of _C. unilocularis_ leaves against _P. posthuma_ and _E. foetida_ can be attributed to the presence of phenols and polyphenols in the extracts.[6]

The outer mucilaginous layer of earthworms is composed of complex polysaccharides which enable them to move freely. Any damage to this layer will restrict the movement of the earthworm and can cause paralysis. Thus one of the mechanisms of the anthelmintic activity of the extract may be by damaging the outer polysaccharide layer of worms.[6]

Since worms have no means of storing energy, they must eat almost continuously to meet their metabolic needs. Therefore, interfering with feeding or paralyzing the worms eventually leads to their death.[7]

The phytochemical evaluation has shown the presence of alkaloids and saponins in the extracts of _C. unilocularis_ both of which may have a role in anthelmintic activity. Alkaloids may act on central nervous system and cause paralysis of the earthworm to exhibit anthelmintic activity.[8] Saponins act by changing the membrane permeability of the worms and pore formation, which is similar with the conventional anthelmintic drug such as praziquantel. This would affect the permeability of the cell membrane of the worms.[6]
CONCLUSION

*C. unilocularis* is an important medicinal plant in the traditional Ayurvedic system of medicine and is widely used traditionally for the remedy of various ailments and diseases. The methanolic extract of *C. unilocularis* leaves showed promising *in vitro* anthelmintic activity against *P. posthuma* and *E. foetida* in a concentration dependent manner although the activity was weaker than standard anthelmintic. This supports the traditional use of this plant as an anthelmintic. *C. unilocularis* thus has a high potential for the isolation of anthelmintic compounds. Therefore further research on this plant is necessary to flourish its medicinal properties and therapeutic uses.

REFERENCES