In-vitro Anthelmintic Activity of Aqueous Extract of Leaves of Cleome Rutidosperma DC. (Capparidaceae) Against Haemonchus Contortus

Md. Shahed-Al-Mahmud 1* Hasib Al Hasan 2

1Department of Microbiology and Immunology, School of Medicine, Tzu Chi University, No. 701, Sec. 3, Zhongyang Rd. Hualien 97004, Taiwan
2Department of Pharmacy, State University of Bangladesh, Dhaka-1205, Bangladesh

ABSTRACT

Background: Cleome rutidosperma is an annual herb found in different places in Bangladesh. Its leaves used as the treatment for Helminthiasis associated with Haemonchus contortus. This parasite is one of the most pathogenic nematodes and responsible for anemia, edema, and death of cattle’s, mainly during summer in warm, humid climates as like Bangladesh. The aim of the study to investigate the in-vitro anthelmintic activity of aqueous extract C. rutidosperma (AECR) leaves against H. contortus. Methods: We performed phytochemical analysis and in-vitro anthelmintic assay to determine the possible phytochemicals, caused by anthelmintic activity against H. contortus. Results: In-vitro anthelmintic assay, the AECR at the doses of 25; 50 and 100mg/mL significantly (p<0.01) and (p<0.001) paralyzed and caused the death of H. contortus in a dose-dependent manner. Reference standard drug Albendazole (15 mg/mL) exerts almost same effect as AECR. No paralyzed and death observed at the control group when treated with 0.9% normal saline. Conclusion: This data confirmed that the aqueous extract C. rutidosperma has the in-vitro anthelmintic activity of against H. contortus. C. rutidosperma may offer an alternative source for the control of gastrointestinal nematodes of cattle’s.

Keywords: Cleome rutidosperma; Haemonchus contortus; Anthelmintic; Albendazole; Paralyzed; Nematodes

INTRODUCTION

The rapid development of resistance in helminths against anthelmintic has led to the proposal of screening medicinal plants for their anthelmintic activity. The success rate of Helminthiasis curing by gastrointestinal nematodes become small ruminants due to its resistance. [1] Haemonchus contortus is one of the highly pathogenic parasite and is capable of causing acute disease and high mortality in all classes of livestock compared with other parasitic nematodes. [2] A lot of money are spent annually worldwide for controlling parasitic nematodes using anthelmintic drugs. [3] However, anthelmintic resistance has influenced on the success of conventional anthelmintic drugs for the control of GI nematode. [4] Therefore, new alternatives of treatment, including the use of medicinal plants as they are less toxic, biodegradable and environmentally friendly. [5]

Cleome rutidosperma DC. generally known as Fringed Spider Flower or Purple Cleome is a species of flowering plant in the genus Cleome of the family Capparidaceae, native to Tropical Africa. [6] It has become naturalized in various parts of tropical America as well as Southeast Asia. In Bangladesh, it is locally known as Beguni Hururia Ful. It is a ubiquitous weed of lawns. C. rutidosperma has been well-studied by different researchers.

Traditionally, the roots, leaves, and seeds of the plants of Cleome genus used as antiscorbutic, anthelmintic, rubefacient, vesicant and carminative. [7] In Malaysia, the reason of planting of C. rutidosperma around field edges considered as part of an insect control planned. The antiplasmodial activity of the chloroform-methanol (1:1) extracts from the leaves reported by. [8] Similarly, diuretic, laxative, analgesic, anti-inflammatory, antipyretic, antimicrobial, antioxidant and free radical scavenging activities of the aerial parts of C. rutidosperma reported earlier. [6] Also, the root of C. rutidosperma shows the significant association between the hypoglycemic effect. [9] C. rutidosperma is traditionally popular for use in the treatment of paralysis, epilepsy, convulsions, spasm, earache, pain, and skin disease.
Material and Methods

Plant collection
The aerial parts of *C. rutidosperma* collected from beribadh, Mirpur, Dhaka, Bangladesh. The time of collection was 1st July 2013. The specific identification of *C. rutidosperma* plant for the present study performed by the Botanist Bushra Khan, Scientific Officer, Bangladesh National Herbarium, Mirpur, and Dhaka, Bangladesh. A voucher specimen (DACB: 38625) deposited in the Herbarium for further reference.

Aqueous extract preparation
Powdered dried plants (250g) macerated with 900mL of double distilled water with occasional stirring at 25 ± 2°C for seven days. Further, the raw solvent extract filtered using a sterile cotton set inside Buchner funnel give the final yield of 5.6% (14g) crude extracts after solvent evaporation using rotary evaporator. In addition, the crude extract analyzed for the anthelmintic activity against *H. contortus*.

*Haemonchus contortus* collection
The live adult worm of *H. contortus* (Nematode) collected from local abattoirs. After cleaning, parasites stored in 0.9% phosphate-buffered saline (PBS) of pH 7.4 prepared with (8.01g NaCl, 0.20g KCl, 1.78g Na₂HPO₄ and 0.27g KH₂PO₄) in 1L of distilled water at 37°C. The research carried out according to the rules governing the use of laboratory animals as acceptable internationally and the experimental protocol approved by the Animal Ethics Committee, (SUB/IAEC/17.01) of State University of Bangladesh.

Drugs and Treatments
The anthelmintic activity performed using 0.9% normal saline water (Opsonin pharma Ltd. Bangladesh) and Albendazole (Eskayef Ltd. Bangladesh). *H. contortus* collected immediately after slaughter of fresh cattle, followed by washing and keeping in the PBS. The parasites divided into five groups consisting of six parasites in each. Extract at the concentrations of 25, 50, and 100 mg/mL whereas, reference standard albendazole prepared at the concentrations of 15 mg/mL.

Phytochemical screening
The ethanolic extract of *C. rutidosperma* was qualitatively investigated for alkaloids, flavonoids, tannins, glycosides, reducing sugar, saponins, and steroids presence according to the standard procedures.[11]

In vitro anthelmintic assay
The evaluation of anthelmintic activity of the aqueous extract *C. rutidosperma* (AECR) under in-vitro conditions against adult *H. contortus*. The Observations recorded for the time taken to paralysis and death of individually selected parasites. The time of paralysis recorded when no movement observed unless shaken vigorously.[12] The death time recorded after evaluating that the parasites did not move when shaken vigorously, dipped in warm water (50°C) or subjected to external stimuli. Anthelmintic activity of the plant leaves expressed as the time needed for paralysis and death of worms as compared to control whereas, Albendazole (15 mg/mL) used as reference standard.

Ethics approval and consent to participate
All the experimental mice were treated following the Ethical Principles and Guidelines for Scientific Experiments on Animals (1995) formulated by The Swiss Academy of Medical Sciences and the Swiss Academy of Sciences. The Institutional Animal Ethical Committee (SUB/IAEC/17.01) of State University of Bangladesh approved all experimental rules.

Statistical analysis
All the values expressed as mean±SEM. Student’s t-test was employed to estimate considerable differences between the test and control group. Statistical analysis was performed with Prism 7.0 (Graph Pad Software Inc., San Diego, CA). Results deemed as statistically significant when the p-value was less than (p< 0.001).

Results and Discussion
The medicinal plants are the blessed of nature. It used for curing different kinds of diseases during the time from the ancestor. *C. rutidosperma* is among one of those plants which enriched with its medicinal properties. In our recent phytochemical analysis, we confirmed that *C. rutidosperma* contains several types of phytochemicals which have anthelmintic activity against GI nematodes.

Our preliminary phytochemical investigation of AECR revealed that the presence of alkaloids, flavonoids, tannins, glycosides, reducing sugar, saponins, and steroids (Table 1). Previous studies previous studies mentioned that tannins are responsible for producing anthelmintic activities.[13] On the basis of the present study, the exact anthelmintic activity mechanism of AECR is difficult to explain. Preliminary phytochemical analysis of AECR also indicated the presence of steroids and flavonoids. Earlier study reported that flavonoids may produce anthelmintic activities due to their binding capability with the free proteins in the GI tract of the host animal.[14]

We observed in-vitro anthelmintic assay of AECR leaves against GI nematodes *H. contortus* at the doses of 25; 50; 100 mg/mL. The anthelmintic activity of AECR significantly (p< 0.001) paralyzed and caused death against worm at the doses of 50 and 100 mg/mL when compared with the control group whereas, AECR significantly (p< 0.01) paralyzed and caused death against worm at the doses of 25 mg/mL. Reference standard drug Albendazole (15 mg/mL) also showed the similar effects (p< 0.001) as AECR (50 and 100 mg/mL). The effect of AECR also dose-dependent manner.
The anthelmintic activity of AECR measured depending on the loss of movement or paralysis and complete destruction or death of live worm. In-vitro experiments concentrations of AECR at 25, 50, and 100 mg/mL doses exhibited paralysis at 41.63; 33.87 and 21.67 min whereas, death at 96.07; 87.08 and 63.21 min respectively, for H. contortus (Table 2). The reference standard drug Albendazole (15 mg/mL) exerts paralysis at 13.29 min and death at 51.63, for H. contortus. During the investigation, no paralyzed and death observed at the control group worm.

**Conclusion**

The present investigation clearly showed that C. rutidosperma leaves displayed favorable in-vitro anthelmintic activity. Though aqueous extracts of C. rutidosperma showed less activity on the survival of adult worm at different concentrations exhibited good paralysis time of parasites. Further investigations needed to identify the actual mechanism of action (M/A) and as well as to isolate bioactive compounds responsible for this biological activity.

**Acknowledgements**

The authors are grateful to Professor Dr. Md. Saiful Islam Pathan, Head, Department of Pharmacy, State University of Bangladesh for his permission to use the facilities of the Pharmacology Laboratory.

**Abbreviations**

AECR = Aqueous Extract of Cleome Rutidosperma  
H. contortus = Haemonchus contortus  
GI = Gastrointestinal

**References:**


www.rphsonline.com Research in Pharmacy and Health Sciences | Vol 4 | Issue 1 | Jan-Mar, 2018;415-418.
Shahed et al., Study of anthelmintic activity of Cleome rutidosperma

Table 1: Preliminary qualitative phytochemical screening of AECR.

<table>
<thead>
<tr>
<th>Phytochemical constituents</th>
<th>Tests</th>
<th>Inferences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Mayer’s test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Hager’s reagent test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Wagner’s reagent test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Dragendorff’s test</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Modified borntrager’s test</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Lead acetate test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Molisch’s test</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>Potassium Dichromate Test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Ferric Chloride Test</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>Frothing test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Foam test</td>
<td>+</td>
</tr>
<tr>
<td>Sterols</td>
<td>Libermann burchard’s test</td>
<td>+</td>
</tr>
<tr>
<td>Reducing sugar</td>
<td>Benedict’s test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Fehling’s Test</td>
<td>+</td>
</tr>
</tbody>
</table>

Key: + present

Table 2. In-vitro anthelmintic activity of AECR leaves on H. contortus.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/mL)</th>
<th>Time to paralysis (min)</th>
<th>Time to death (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.9% w/v NaCl</td>
<td>No Paralysis</td>
<td>No Death</td>
</tr>
<tr>
<td>Albendazole</td>
<td>15</td>
<td>13.29±1.09***</td>
<td>51.63±1.91***</td>
</tr>
<tr>
<td>AECR</td>
<td>25</td>
<td>41.63±1.70**</td>
<td>96.07±1.35**</td>
</tr>
<tr>
<td>AECR</td>
<td>50</td>
<td>33.87±1.96***</td>
<td>87.08±1.23***</td>
</tr>
<tr>
<td>AECR</td>
<td>100</td>
<td>21.67±0.90***</td>
<td>63.21±0.51***</td>
</tr>
</tbody>
</table>

Each value is expressed as the mean ± SEM (n =6), AECR = Aqueous extract of Cleome rutidosperma leaves. **p<0.01 and ***p<0.001 compared with the control group (Dunnett’s Test). Control worm remained healthy throughout the period of observation.

Figure 1: Cleome rutidosperma DC (Capparidaceae)

Figure 2: Haemonchus contortus (Nematodes)