e-ISSN : 2455-5258

Research in Pharmacy and Health Sciences

Research Article

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

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ABSTRACT

Background: *Cleome rutidosperma* is 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.

Received: 02-11- 2017

Revised: 02-12-2017

Accepted: 21-12-2017

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Email:
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Funding: Nil

Competing Interests: None

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 Hurhuria 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,[9] 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.[10] *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 ± 2ºC 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 Na_2HPO_4 and 0.27g KH_2PO_4) 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 anthelminthic 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.[15,16] *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

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Table 1: Preliminary qualitative phytochemical screening of AECR.

Phytochemical constituents	Tests	Inferences
Alkaloids	Mayer's test	+
	Hager's reagent test	+
	Wagner's reagent test	+
	Dragendorff's test	+
Glycosides	Modified borntrager's test	+
Flavonoids	Lead acetate test	+
	Molisch's test	+
Tannins	Potassium Dichromate Test	+
	Ferric Chloride Test	+
Saponins	Frothing test	+
	Foam test	+
Sterols	Libermann burchard's test	+
Reducing sugar	Benedict's test	+
	Fehling's Test	+

Key: + present

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

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

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)

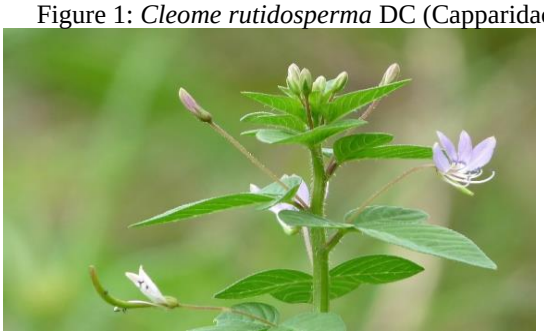

Figure 2: *Haemonchus contortus* (Nematodes)