International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 15, Issue 1, 2023

Original Article

IN VITRO EVALUATION OF ANTHELMINTIC ACTIVITY OF AQUEOUS EXTRACT OF *ARDISIA COLORATA ROXB*. LEAVES IN ADULT EARTHWORMS

PRASENJIT SARKAR[1*], N. MEENA DEVI[2], U. DHARMARAJ MEETEI[3], GOBINATH V.[1]

[*]**Department of Pharmacology, Regional Institute of Medical Sciences, Imphal, Manipur, 795004**
[*]**Email: sarkar.prasenjit154@gmail.com**

Received: 13 Oct 2022, Revised and Accepted: 20 Dec 2022

ABSTRACT

Objective: The study aims to evaluate the anthelmintic activity of aqueous extract of *Ardisia colorata Roxb*. leaves (AQEAC) using adult earthworms (Pheretima posthuman).

Methods: The total of 24 adult earthworms were divided into four groups, with six worms in each group (n=6). The anthelmintic activity of AQEAC at two different doses (25 mg/ml and 50 mg/ml) was evaluated by assessing the time of paralysis (min) and time of death (min) of the earthworms. Albendazole (25 mg/ml) was used as standard and 2% gum acacia as control.

Results: The result showed that AQEAChad significant anthelmintic activity ($p<0.001$) in a dose-dependent manner but was less potent than the standard drug albendazole.

Conclusion: AQEAC demonstrated significant anthelmintic activity but was less potent than the standard drug albendazole. However, further studies with higher doses are required to evaluate the dose-dependent activity and to evaluate the exact mechanism responsible for anthelmintic activity.

Keywords: *Ardisia colorata roxb*., Anthelmintic activity.

DOI: https://dx.doi.org/10.22159/ijcpr.2023v15i1.2063 Journal homepage: https://innovareacademics.in/journals/index.php/ijcpr

INTRODUCTION

Helminthiasisisone of the most prevalent infections in humans, affectinga significant part of the population worldwide. In the developing countries like India, it is a substantial threat to public health and causes anemia, malnutrition, pneumonia, and eosinophilia [1]. According to WHO, 1.5 billion people worldwide are infected with soil-transmitted helminths (STHs) [2]. The STH sarethe infestation by a roundworm (*Ascaris lumbricoides*), whipworm (*Trichuris trichiura*), and hookworm (*Ancylostoma duodenale* and *Necator americanus*). They enter the human body from contaminated foodcontaining eggsof worms (faeco-oral), and some helminths like hookworms can penetrate the skin directly.

Anthelmintics are medicines that either kill (vermicide) or expel (vermifuge) infested helminths from the body [3]. They include the benzimidazole class of drugs, like albendazole and mebendazole, macrocyclic lactones like ivermectin, or pyrazinoisoquinoline derivatives like praziquantel [4]. However, after prolonged use of anthelmintics, the problems derived from developing resistance in helminths to various anthelmintic compounds and classes.

In recent years globally, herbal medicines are gaining more popularity and importance due to their safety, minimum side effects, efficacy, and cost-effectiveness in treating various diseases.

Ardisia colorata Roxb. belongs to the family of Myrsinaceae. This plant is considered one of the important medicinal plants in traditional medicine all over India, more precisely in the Northeastern part of India, for its antioxidants, anti-inflammatory, and anti-diabetic properties**.** It is also a folk medicine for liver disease, cough, and diarrhoea [5].

Phytochemical studies on chemical characterization of various parts of A. coloratarоxb. demonstrated the presence of bergenin, rapanone, and ilexol [5, 6].

However, so far, the anthelmintic activity of *Ardisia colorata Roxb.* has not been scientifically evaluated. Considering its use in gastrointestinal ailments by locals, the present study was taken to assess the anthelmintic effect of aqueous extract of leaves of *Ardisia colorata Roxb.* on adult earthworms (Pheretima posthuma).

MATERIALS AND METHODS

Setup

The study was performed in the Department of Pharmacology, Regional Institute of Medical Sciences, Imphal, Manipur.

Collection of plant materials

The plant was collected from the Lamphel market, Imphal west, Manipur, in August 2021. Further, the plat was authenticated by the Department of Botany, D. M. College, Imphal, with the ACC. No. 007.10 DMU.

Preparation of plant extract

The leaves of Ardisia colorata Roxb. were cleansed with tap water, shade-dried, and made into a coarse powder witha mixer grinder. Aqueous extract of leaves of Ardisia colorata Roxb. (AQEAC) was prepared by the soxhlet extraction method described by Meshram GG *et al.* [7]. Approximately 100 g of the powder was subjected to Soxhlet extraction, using 5 L of distilled water as solvents. The dark green, semi-solid extracts were obtained. The dried extract was scraped, weighed, and stored in an airtight container. The yield was 10.66%.

Worm collection and authentication

The study was conducted on adult earthworm *Pheretima posthuman*, as they were similar anatomically and physiologically to Ascaris lumbricoides. The earthworms were collected from the moist soil of the Lamphel area, Imphal west, Manipur. Worms were cleaned with normal saline (0.9%) to remove all fecal matter and mud. The earthworms of 5-7 cm in length and 0.4-0.5 cm width were used. The earthworms were authenticated by the Department of Life Sciences, Manipur University.

Experimental design

The anthelmintic assay was carried out based on the method described by Ajayieoba *et al.* with some modifications [8]. The earthworms were divided into four groups, with six worms in each group. In group 1, 2% gum acacia was used as the control. In group 2, the standard Albendazole 25 mg/ml suspension was used. In

group-3 and group-4, test drugs of AQEAC were used at a 25 mg/ml dose and 50 mg/ml, respectively. Test drug and standard drugs were dissolved in 2% gum acacia, and 10 ml of the desired formulation were poured into separate Petridishesunder room temperature; the worms were placed into the Petri dishes containing the extract or the standard drug as described below in table 1.

Evaluation of anthelmintic activity

The worms were kept under close observation. Time taken for paralysis (P) of the worms was noted during observation; no spontaneous movement was found except when shaken vigorously. Time taken for death (D) of worms were recorded when there was movement of the worms even after vigorous shaking or when dipped in warm water (50 °C), followed by gradual fading of their body color.

Statistical analysis

The data obtained were analyzed using one-way ANOVA followed by the Bonferroni test using SPSS version 25. Results were expressed in mean±SEM. $p<0.05$ was considered significant.

Table 1: Distribution of earthworms in different groups and their treatment

Groups	Treatment
Group-1 (Control)	2% gum acacia
Group-2 (Standard)	Albendazole suspension at a dose of 25 mg/ml
Group-3 (Test A)	AQEAC at a dose of 25 mg/ml
Group-4 (Test B)	AQEAC at a dose of 50 mg/ml

RESULTS

Phytochemical screening

A preliminary phytochemical study of an aqueous extract of *Ardisia colorata Roxb.* showed the presence of tannins, saponins, flavonoids, and alkaloids.

Anthelmintic activity

The anthelmintic activity was assessed by evaluating the time of paralysis (P) and time of death (D) in earthworms after applying standard drugs and different concentrations of the test drug.

Table 2 showed that the aqueous extract from leaves of *Ardisia colorata Roxb.* (AQEAC) had significant anthelmintic activity when compared with control ($p<0.001$). The standard drug albendazole at 25 mg/ml showed the best activity for the time of paralysis and death (31.33±1.45 and 38.50±1.57 min, respectively), while AQEAC (25 mg/ml) showed 82.83±1.58 and 89.00±1.77 min respectively and AQEAC (50 mg/ml) showed 47.67±2.04 and 54.67±2.11 min respectively for the time of paralysis and death. Moreover, a higher dose of AQEAC (50 mg/ml) showed significant ($p<0.001$) increased anthelmintic activity as depicted by the reduced time of paralysis and death (fig. 1). However, the extract at either dose was found to be less effective than the standard drug albendazole.

Table 2: Anthelmintic activity of AQEAC on adult earthworms

Group	Drugs	Time of paralysis (P) in min	Time of death (D) in min
1	Control (2% gum acacia)	-	-
2	Standard (Albendazole 25 mg/ml)	31.33±1.45^{a*}	38.50±1.57^{a*}
3	Test A (AQEAC 25 mg/ml)	82.83±1.58^{a*b*}	89.00±1.77^{a*b*}
4	Test B (AQEAC 50 mg/ml)	47.67±2.04^{a*b*}	54.67±2.11^{a*b*}

n=6 in each group, values are mean±SEM. Symbols a* and b* were used to compare with groups 1 and 2, respectively. *p<0.001

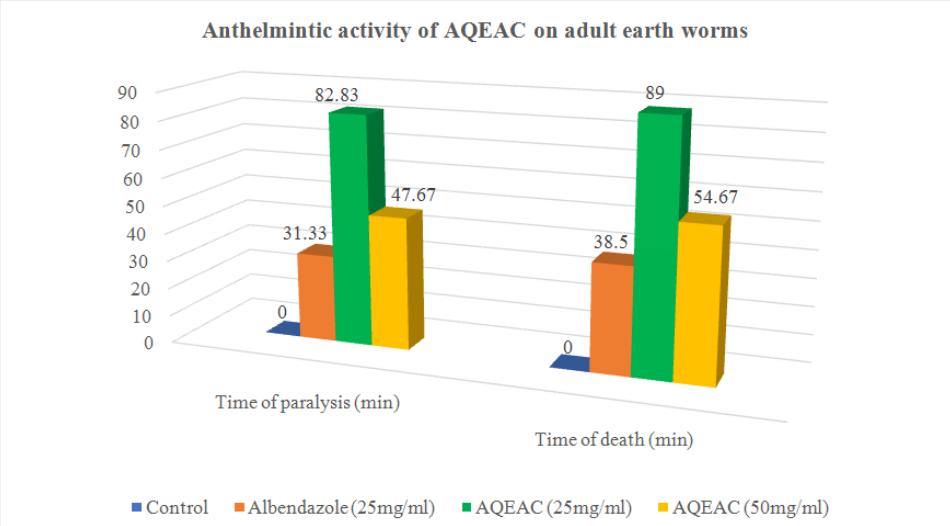

Fig. 1: Anthelmintic activity of AQEAC when compared to standard drug albendazole on time of paralysis and time of death

DISCUSSION

A similar study done by Datta S *et al.* by using the same standard drug, Albendazole and Centella asiatica Linn extract, shows results that are comparable with the present study [2].

A preliminary phytochemical study of the extract showed that tannins, saponins, flavonoids, and alkaloids are present. Tannins cause uncoupling of oxidative phosphorylation and bind to free proteins of host GIT, resulting death of the parasite. Saponin present

in the study causes stabilization of membrane permeability and formation of pores, resulting death of the host-parasite [4].

A conventional anthelmintic drug like, albendazole is effective and safe in infections caused by a parasite and are well-established standard medicine in anthelmintic studies. The aqueous extract of the plant is found to be more effective at a dose of 50 mg/ml, though the extract at either dose was found less effective than the standard medicine of albendazole.

As the plant is cheap and readily available in the local market, therefore leaves of this plant could be categorized under anthelmintic herbal drugs and could become a vital ingredient of the anthelmintic formulation.

CONCLUSION

The study demonstrated that an aqueous extract of *Ardisia colorata Roxb.* has significant anthelmintic activity but is less potent than the standard drug albendazole.

However, further studies with higher doses are required to evaluate the dose-dependent activity and the exact mechanism responsible for anthelmintic activity.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Patel PR, Raval BP, Karanth HA, Patel VR. Potent antitumor activity of Rubia cordifolia. Int J Phytomed. 2010 Jan 1;2(1).
2. Datta S, Ningthoujam G, Zosangpuii C, Shyamasakhi P, Meena N. *In vitro* evaluation of anthelmintic activity of ethanolic extract of Centella asiatica Linn. In Indian adult earthworms. Int J Curr Pharm Sci. 2021 Sep;15:39-41. doi: 10.22159/ijcpr.2021v13i5.1884.
3. Roy SD, Goswami R, Das S, Shil D, Baniya R, Haldar S. Pharmacognostic evaluation and anthelmintic activity of leaf and stem extract of Carica papaya. J Pharm Res. 2012 Sep;5(9):4763-6.
4. KantiSinha S. *In vitro* evaluation of the anthelmintic activity of ethanolic extract of leaves of toona ciliata m. roem on Indian earthworm. IJAR 2019;7(7):250-3. doi: 10.21474/IJAR01/9352.
5. Sanjeev S, Murthy MK, Sunita Devi M, Khushboo M, Renthlei Z, Ibrahim KS. Isolation, characterization, and therapeutic activity of bergenin from marlberry (Ardisia colorata Roxb.) leaf on diabetic testicular complications in Wistar albino rats. Environ Sci Pollut Res Int. 2019 Mar;26(7):7082-101. doi: 10.1007/s11356-019-04139-9, PMID 30648235.
6. Sumino M, Sekine T, Ruangrungsi N, Ikegami F. Ardisiphenols A-C, novel antioxidants from the fruits of Ardisia colorata. Chem Pharm Bull (Tokyo). 2001;49(12):1664-5. doi: 10.1248/cpb.49.1664, PMID 11767097.
7. Meshram GG, Kumar A, Rizvi W, Tripathi CD, Khan RA. Evaluation of the anti-inflammatory activity of the aqueous and ethanolic extracts of the leaves of Albizzia lebbeck in rats. J Tradit Complement Med. 2016 Apr 1;6(2):172-5. doi: 10.1016/j.jtcme.2014.11.038, PMID 27114941.
8. Ajaiyeoba EO, Onocha PA, Olarenwaju OT. *In vitro* anthelmintic properties of Buchholzia coriaceae and gynandropsis gynandra extracts. Pharm Biol. 2001 Jan 1;39(3):217-20. doi: 10.1076/phbi.39.3.217.5936.