Journal of International Research in Medical and Pharmaceutical Sciences

Volume 19, Issue 3, Page 1-8, 2024; Article no.JIRMEPS.12305
ISSN: 2395-4477 (P), ISSN: 2395-4485 (O)

Anthelmintic Screening of *Hylocereus polyrhizus* Rind Extracts

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.56557/jirmeps/2024/v19i38812

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.ikprress.org/review-history/12305

Original Research Article

Received: 04/06/2024
Accepted: 06/08/2024
Published: 09/08/2024

ABSTRACT

The purpose of this study was to evaluate the rind extract of *Hylocereus polyrhizus's* anthelmintic efficacy. Ethanol and water were utilized to extract the powdered rind of *H. polyrhizus*. The phytochemical screening of *H. polyrhizus* ethanol extract (HPEE) and water extract (HPAE) was done. The adult motility assay and resistance-modifying study were then used to screen for the plant's anthelmintic properties against *Eudrilus eugeniae*. Due to its morphological and physiological resemblance to the human intestinal roundworm, *Eudrilus eugeniae* was chosen in the investigation. On *E. eugeniae*, HPEE and HPAE demonstrated dose-dependent paralytic and fatal effects. The anthelminthic activity of albendazole was also assessed by HPEE and HPAE through resistance-modifying research and the adult worm (*E. eugeniae*) motility assay. The addition of different concentrations of HPEE and HPAE considerably ($p < 0.05$) increased the activity of

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Cite as: *Yesudanam, Sinchu, Aparna Raj A. S., Jothika J., R. S. Sreelakshmi, and Soniya M. Sunil. 2024. "Anthelmintic Screening of Hylocereus Polyrhizus Rind Extracts". Journal of International Research in Medical and Pharmaceutical Sciences 19 (3):1-8. https://doi.org/10.56557/jirmeps/2024/v19i38812.*

albendazole against *E. eugeniae*. Against *E. eugeniae*, they exhibited dose-dependent anthelmintic action. When compared to HPEE, HPAE exhibits the strongest activity. Albendazole's lethality and paralytic action were considerably increased by HPEE and HPAE. HPEE and HPAE underwent phytochemical screening, which identified the presence of coumarins, alkaloids, glycosides, tannins, and saponins. The findings of this investigation demonstrate the anthelmintic activity of *H. polyrhizus*.

Keywords: Hylocereus polyrhizus; anthelmintic activity; Eudrilus eugeniae; albendazole.

1. INTRODUCTION

When worms infest a certain area of the body, helminthiasis develops. Although their natural home is the digestive tract, these worms can occasionally burrow into other organs, such as the liver. Worldwide, helminthiasis is a problem, but it is more common in tropical and subtropical nations with high rates of poverty [1]. An estimated 1.5 billion people worldwide have one or more soil-transmitted helminth infections, according to the World Health Organization [2]. In Sub-Saharan Africa, about 25% of the population has one or more helminth infections, with a frequency of more than 70% in the majority of West African nations [3]. Out of all the helminths, nematodes are the most prevalent [4]. It is possible to have many helminth infections at the same time in places with high prevalence [5]. Each year, the neglected tropical diseases—of which helminth infections are one subtype—cause 57 million disability-adjusted life-years (DALYs) and around 534,000 fatalities [6]. It has been discovered that intestinal worms like *Haemonchus contortus* and *Ascaris lumbricoides eugeniae* have become resistant to levamisole, albendazole, and closantel [7]. In addition to causing anthelmintic resistance, the majority of medications used to treat these worms have typical side effects that include nausea, vomiting, stomach pain, and a drop in blood pressure in people [8]. Once more, the evidence of resistance in livestock [9], has raised concerns about anthelmintic resistance in people, posing a major threat to animal production in developing nations. As a result, additional strategies for helminth infestation control must be developed [10]. Among the natural goods being investigated for their anthelmintic qualities are medicinal herbs.

The exotic superfruit known as dragon fruit (*Hylocereus spp.*), which is a member of the Cactaceae family and is growing in popularity in both urban and rural regions due to its appealing colour, mouthwatering flavour, and high nutritional and therapeutic value, is found in India [11]. It can adapt to tropical and subtropical climates, both humid and semi-arid. The public greatly favours dragon fruit, also known as pitaya, a non-local fruit because of its effectiveness, advantages, and high nutritional content. The antioxidant content of dragon fruit is what makes it so effective. The amount of betacyanin pigment found in 100 g of dragon fruit peel was found to be 150.46 mg. Vitamins A, C, and E, alkaloids, terpenoids, flavonoids, thiamine, niacin, pyridoxine, cobalamin, phenolics, and beta-carotene are among the other substances found in dragon fruit peel. Many pharmacological effects, including anti-oxidant, anti-cancer, anti-diabetic, anti-fertility, anti-ulcer, cardioprotective, and neuroprotective properties, have been documented for it [12]. Furthermore, dragon fruit can be utilized as natural colorants, functional ingredients, edible films, active and ecologically friendly packaging, photoprotective product preparation, and additives in food and nutraceutical items [13]. Thus, the anthelmintic characteristics of *Hylocereus polyrhizus* were assessed in this study.

2. MATERIALS AND METHODS

2.1 Plant Sample Collection and Preparation

In March 2024, *H. polyrhizus* was obtained from Trivandrum Lulu hypermarket. Dr. E. A. Siril, a professor, the head of the botany department at the University of Kerala in Kariavattom, Trivandrum, recognized and verified the plant material. In the University of Kerala's Department of Botany's herbarium, there is a voucher specimen designated KUBH 11388. For authentication, we have prepared the herbarium by collecting the flowering stage of plant from the dragon fruit farm, Palode and then pressed and dried under pressure. The rind was chopped into little pieces after peeling, and it was left in the shade for seven days to dry. Care must be given while drying because under high temperature the phenolic compounds get decomposed. Using an

ultrafine pulveriser, the dehydrated samples were ground into powder, put in a sealed vial, and kept in a freezer at -18°C.

Preparation of Ethanolic and Aqueous extracts of *H. polyrhizus*:

a) Ethanolic extract – 140 grams of powdered *H. polyrhizus* rind plant material was weighed, suspended in 1L of ethanol, and shaken periodically for three days at room temperature. After filtering through Whatman filter paper number 1, the suspension was concentrated at lower pressure at a temperature range of 25 to 60 degrees Celsius. Until it was needed, the extract was stored in a refrigerator at 4°C. The ethanol extract's percentage yield was then determined to be 9.7% w/w and classified as HPEE.
b) Aqueous extract – 140 grams of powdered *H. polyrhizus* rind plant material was weighed, suspended in 1L of water, and shaken periodically for a week at room temperature. After filtering through Whatman filter paper number 1, the suspension was concentrated at lower pressure at a temperature range of 25 to 60 degrees Celsius. Until it was needed, the extract was stored in a refrigerator at 4°C. After that, the aqueous extract was given the designation HPAE, and 51.65% w/w was determined to be its percentage yield.

Phytochemical screening: Using conventional protocols, qualitative phytochemical screening of both *H. polyrhizus* extracts were carried out to identify the presence of secondary metabolites such as tannins, saponins, alkaloids, and flavonoids [14,15].

Test organism: The African nightcrawler, *Eudrilus eugeniae*, bears morphological and physiological similarities to *Ascaris lumbricoides*, a human intestinal roundworm. The adult *E. eugeniae* was obtained from the Kerala Agricultural University's (KAU) Department of Agronomy, College of Agriculture, Vellayani.

2.2 Determination of Anthelmintic Activities

Using the adult motility assay [16], the anthelmintic properties of *E. eugeniae* were ascertained. Nine concentrations of HPEE and HPAE were generated, namely 32, 16, 8, 4, 2, 1, 0.5, 0.25, and 0.125 mg/mL. Adult earthworms measuring between 5 and 7 cm in length were aseptically placed into petri dishes with the proper labels and various HPEE and HPAE concentrations. After then, the worms were watched for paralysis and death for a maximum of eight hours. It was believed that paralysis occurred when no portion moved, with the exception of the worms when they were shaken violently. The worms were deemed dead when they stopped moving in Ringer's lactate solution, did not reanimate when shook violently, and eventually lost all of their body colour. The albendazole concentrations in petri dishes for the positive control group were 10, 5, 2.5, 1.25, and 0.625 mg/mL. For each petri dish, three worms of roughly the same size were employed. Three copies of the aforementioned process were completed.

2.3 Determination of Resistance Modifying Activity of HPEE and HPAE against *E. eugeniae*

It was established how both *H. polyrhizus* extracts affected albendazole's ability to inhibit *E. eugeniae* growth. Albendazole stock solution (10 mg/mL) and two sub activity concentrations of HPEE and HPAE extract (0.25 and 0.125 mg/mL) were utilized. A series of dilutions were performed on the albendazole stock solution to get the following concentrations: 5, 2.5, 1.25, and 0.625 mg/mL. Each concentration was put in a volume of 20 ml into petri dishes with the proper labels. Three mature *E. eugeniae* worms, measuring from 5 to 7 cm in length, were put into each petri dish containing the appropriate amount of albendazole. For eight hours, this was monitored for paralysis and death. This was done three times [17].

2.4 Statistical Analysis

GraphPad Prism version 10.0 for Windows (Graph Pad Software Inc., San Diego, CA, USA) was used to analyse all of the data. The data collected for anthelmintic research were analysed using a one-way ANOVA and Dunnett's post hoc test.

3. RESULTS

3.1 Phytochemical Screening of HPEE and HPAE Extracts

The purpose of the qualitative phytochemical screening of *H. polyrhizus* was to identify potential phytochemicals in both extracts. There

were various components found in the ethanolic rind extract, including proteins, saponins, phenolic compounds, terpenoids, alkaloids, flavonoids, coumarins, carbohydrates, triterpenoids, and glycosides. Additionally, the aqueous extracts also contain these components (Table 1).

3.2 Determination of Anthelmintic Assay Using *E. eugeniae*

Adult motility assay: At the maximum dose of 32 mg/mL, HPAE caused the worms to become paralyzed and died in 5.67 ± 0.05 and 8.82 ± 0.06 minutes, respectively, while HPEE caused the same paralysis and death in 9.5 ± 0.11 and 14.2 ± 0.08 minutes. The worms were paralyzed and killed by HPAE at a concentration of at least 0.5 mg/mL in 252.5 ± 0.08 and 314.50 ± 0.12 minutes of exposure, whereas the worms were paralyzed and killed by HPEE in 311.8 ± 0.15 and 354.3 ± 0.12 minutes of exposure. Upon exposure, the worms were killed at 232 ± 0.05 and 130 ± 0.15 minutes, respectively, by albendazole (10 mg/mL). Albendazole paralyzed and killed the worms at 312 ± 0.08 and 347 ± 0.08 minutes of exposure, respectively, at the lowest dose of 1.25 mg/mL (Fig. 1).

Table 1. Phytochemical screening of *H. polyrhizus*

Sl. No.	Secondary metabolites	HPEE	HPAE
1	Tannins	++	++++
2	Alkaloids	++	++++
3	Saponins	+	+++
4	Glycosides	++	+++
5	Flavonoids	+++	++++
6	Coumarins	+++	++++
7	Carbohydrates	++++	++++
8	Triterpenoids	+++	++++
9	Proteins	++	+++
10	Phenolic compounds	+	+
11	Steroids	++	+++
12	Terpenoids	++	+++

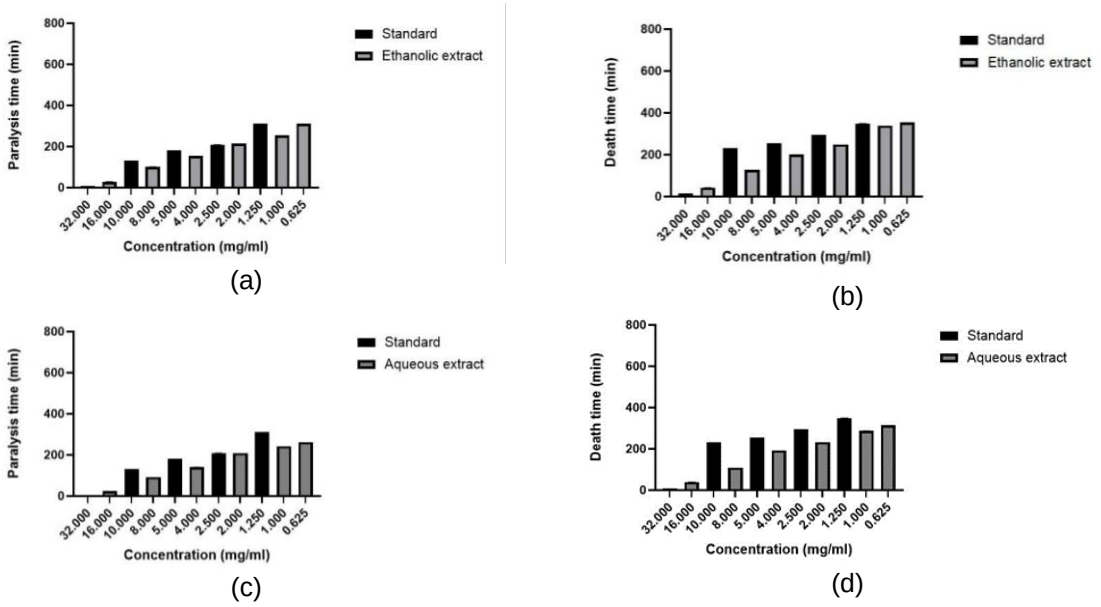

Fig. 1. Effect of HPEE and HPAE on albendazole on paralysis and death of *E. eugeniae*, (a) Paralysis time of HPEE, (b) Death time of HPEE, (c) Paralysis time of HPAE, (d) Death time of HPAE. No paralysis and death were observed after the maximum time of exposure (8 hours) in the negative control group (Ringer's lactate solution)

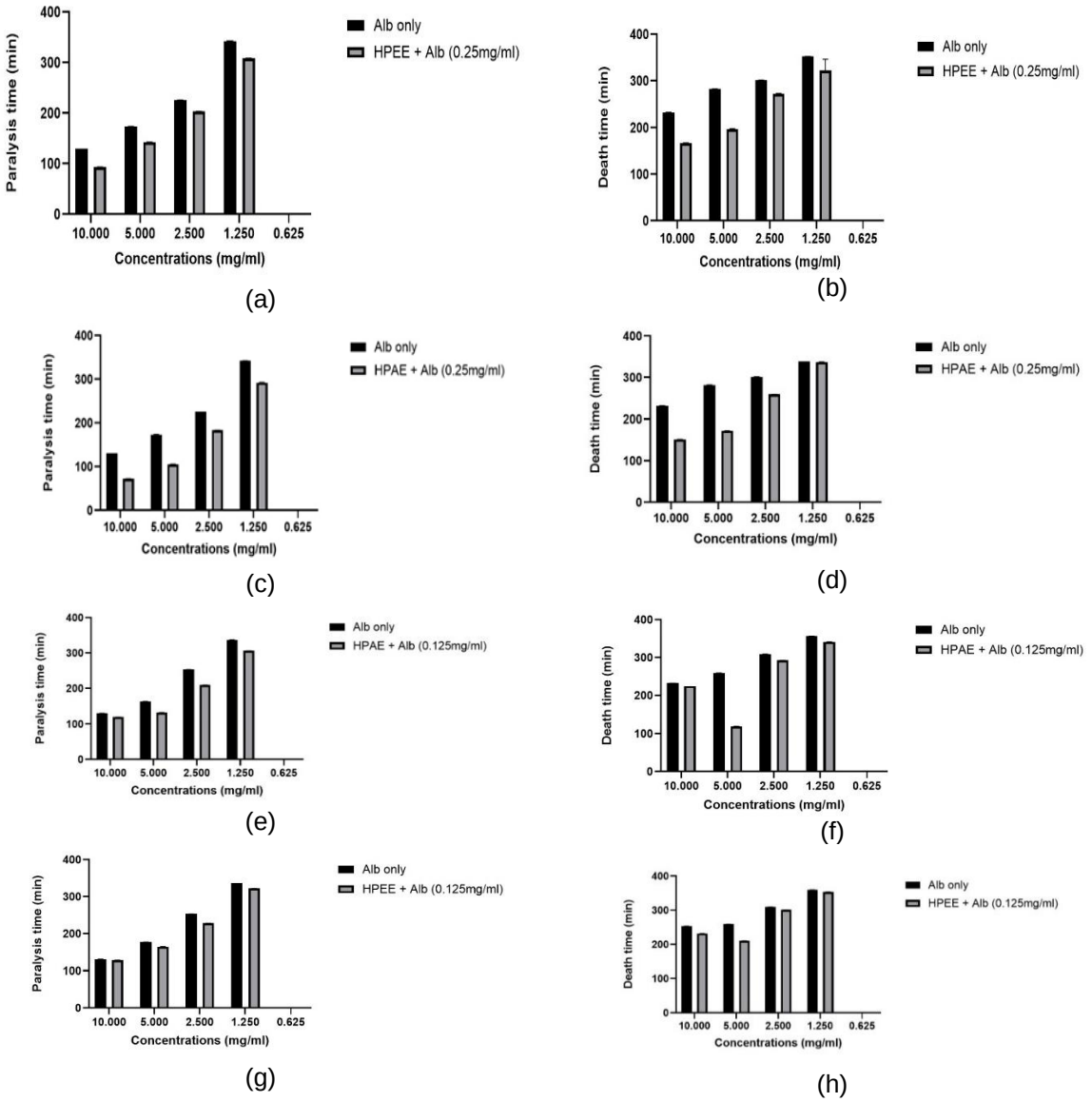

Fig. 2. Comparison of the effect of HPEE and HPAE on paralysis and death time of *E. eugeniae,* (a, b) Paralysis and death time of *E. eugeniae* of HPEE and Alb at 0.25 mg/mL, (c, d) Paralysis and death time of *E. eugeniae* of HPAE and Alb at 0.25 mg/mL, (e, f) Paralysis and death time of *E. eugeniae* of HPEE and Alb at 0.125 mg/mL, (g, h) Paralysis and death time of *E. eugeniae* of HPAE and Alb at 0.125 mg/mL $p < 0.05$ compared to control (one-way ANOVA followed by Dunnett's *post hoc* test)

3.3 Influence of HPEE and HPAE Extracts on Anthelmintic Activity of Albendazole

All the worms were rendered paralyzed by albendazole alone at a dose of 10 mg/mL in 130 ± 0.55 minutes. The paralysis period was significantly ($p < 0.05$) shortened when albendazole (10 mg/mL) and HPAE (0.25 mg/mL) were combined. After being exposed for 72.3 ± 0.31 minutes, the worms became paralyzed. When coupled with 0.25 mg/mL HPAE, similar results were seen at lower concentrations of albendazole (5,2.5, and 1.25 mg/mL). Comparatively speaking, HPEE (0.25 mg/mL) and albendazole (10 mg/mL) together also shortened the paralysis time to 93 ± 0.57 min. After 232 ± 1.51 minutes of exposure, all the worms were destroyed with albendazole (10 mg/mL). The combination of 0.25 mg/mL HPAE and 10 mg/mL albendazole resulted in a significant ($p < 0.05$) reduction in the death time.

After being exposed for 151.75 ± 0.07 minutes, the worms died. Lower albendazole concentrations (5, 2.5, and 1.25 mg/mL) in combination with 0.25 mg/mL HPAE produced similar results. The combination of albendazole (10 mg/mL) and HPEE (0.25 mg/mL) resulted in a shorter death time of 167 ± 0.88 min, which was less than the combination of HPAE (0.25 mg/mL) and albendazole (10 mg/mL).

In a significant ($p < 0.05$) decrease in paralysis time, the worms became incapacitated after 119.71 ± 0.12 minutes of exposure when albendazole (10 mg/mL) was coupled with 0.125 mg/mL HPAE. Lower dosages of albendazole (5 and 2.5 mg/mL) coupled with HPAE (0.125 mg/mL) produced similar results. After being exposed for 337 ± 1.20 minutes, the worms were all paralyzed by the lowest dosage of albendazole (1.25 mg/mL). However, there was a slight reduction in the paralysis time when it was paired with HPAE. Similarly, after 129 ± 1.00 minutes of exposure, the worms were paralyzed when HPEE (0.125m/mL) and albendazole (10 mg/ml) were combined. After 232.147 ± 0.11 minutes of exposure, all the worms were killed by albendazole (10 mg/mL) alone. However, the worms died after 224.95 ± 0.08 min of being exposed when coupled with HPAE (0.125 mg/mL), greatly ($p < 0.05$) shortening the death time. Comparable outcomes were also noted when albendazole (10 mg/mL) and HPEE (0.125 mg/mL) were combined; this resulted in a death time reduction of 253 ± 0.88 min, while it was still lower than that of HPAE.

3.4 Anthelmintic Activity of HPEE and HPAE Fractions against *E. eugeniae*

Against *E. eugeniae*, every fraction of HPEE and HPAE demonstrated concentration-dependent activity and were active. The aqueous extract has the greatest activity of paralysis and death among them all. Fig, 2 shows the results of a comparative investigation of different concentrations of HPEE and HPAE combined with albendazole and the reference medication.

4. DISCUSSION

Hylocereus. polyrhizus contains a variety of phytoconstituents, with a wide range of therapeutic effects. They include tannins, saponins, glycosides, alkaloids, flavonoids, coumarins, carbohydrates, triterpenoids, and glycosides were detected by phytochemical screening of both extracts (Table 1).

The anthelmintic activity of HPEE and HPAE could be attributed to the presence of saponins, glycosides, tannins, alkaloids, and other phytochemicals. Literature revealed has associated secondary metabolites with anthelmintic action, like saponins and alkaloids, have been found [18,19]. Antioxidant alkaloids decrease nitrate production, which has been shown to disrupt local homeostasis, an essential condition for the development of helminths [20]. As a result, helminth deaths may result from a decrease in nitrate formation by the extract's alkaloids. On *E. eugeniae*, HPEE and HPAE demonstrated dose-dependent paralytic and fatal effects.

It has been observed that plants extracts can either increase or decrease the effectiveness of anthelmintic drugs. The addition of different concentrations of HPEE and HPAE considerably ($p < 0.05$) increased the activity of albendazole against *E. eugeniae*. This could entail increased helminth integrity disruption, motility inhibition, and decreased worm uptake of glucose, which would ultimately cause the worm to become paralyzed and die. According to a related study, albendazole's increased anthelmintic activity may be caused by secondary metabolites in the extract, which boosted the drug's absorption into the body and ultimately raised its activity. When coupled with albendazole, HPEE and HPAE significantly reduced the paralytic and mortality effect of albendazole [21].

5. CONCLUSION

H. polyrhizus extracts with HPEE and HPAE have anthelmintic action against *E. eugeniae*. When compared to HPEE, HPAE exhibits the strongest activity between them. HPEE and HPAE considerably increased albendazole's paralytic action. The two extracts contain tannins, saponins, glycosides, alkaloids, flavonoids, coumarins, carbohydrates, triterpenoids, and glycosides, according to phytochemical screening.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

The authors are thankful to the management of Mar Dioscorus College of Pharmacy, for providing the necessary facilities, infrastructure, equipment and guidance for carrying out the research work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://prh.ikprress.org/review-history/12305