International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 16s,2025
https://theaspd.com/index.php

In vitro anthelmintic activity and phytochemical characterization of leaves of *Crassula ovata* against *Pheretima posthuma*

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Abstract*: This study set out to assess the anthelmintic properties of Crassula ovata leaf extracts, using Pheretima posthuma as a model organism. The investigation explored two solvent systems—hydroalcoholic and methanolic—at varying concentrations (10, 25, and 50 mg/ml). The effects were evaluated by recording the time taken for the worms to become paralyzed and subsequently die. Among the test conditions, the methanolic extract at 50 mg/ml demonstrated the highest efficacy, inducing paralysis in approximately 2.12 ± 0.16 minutes and death in 5.18 ± 0.9 minutes. In comparison, the hydroalcoholic extract at 50 mg/ml required significantly longer, with paralysis and death occurring at 12.02 ± 0.9 minutes and 14.56 ± 0.43 minutes respectively. These results were contrasted with albendazole (25 mg/ml), which served as the reference drug and produced paralysis and death at 2.24 ± 0.16 minutes and 6.21 ± 0.71 minutes. The results indicate a clear dose-dependent relationship, where higher extract concentrations yielded more rapid anthelmintic effects. Notably, the methanolic extract outperformed the hydroalcoholic counterpart in all test concentrations. Further phytochemical analysis revealed that the methanol extract contained higher levels of polyphenols and flavonoids, compounds often associated with anthelmintic activity. These constituents likely contribute to the plant's observed anthelmintic potential.*

Keywords*: Pheretima posthuma, Anthelmintic, Crassula ovata, polyphenols, flavonoids*

INTRODUCTION

The term helminths come from Greek and mean "worms." When people get infected by parasites, they are often categorized as *heirlooms* or *souvenirs*. *Heirloom* parasites are those passed down through generations, tracing back to human ancestors in Africa. On the other hand, *souvenirs* are parasites acquired through interactions with animals, shaped by factors like evolution, migration, and agricultural practices. [1,2,3] Helminthiasis refers to infections caused by parasitic worms such as pinworms, tapeworms, and roundworms. These parasites primarily inhabit the human gastrointestinal tract but can also migrate to organs like the liver. Infected individuals shed worm eggs through their feces, which can contaminate soil—especially in regions lacking proper sanitation. [4] One of the major challenges in treating these infections is the growing resistance of intestinal worms to existing anthelmintic medications. This resistance limits treatment effectiveness and underscores the urgent need for new and more effective therapeutic approaches.[5]

Crassula ovata commonly referred to as the jade plant, it belongs to the Crassulaceae family and is widely recognized as an ornamental species. These plants are particularly valued for their ability to withstand dry conditions, thanks to their thick, fleshy leaves and water-storing stems, making them ideal for low-maintenance gardens and xeriscaping. Over the centuries, *Crassula* has played a significant role in traditional medicine, horticulture, and decorative landscaping. [7,8] As a member of the Crassulaceae family, this evergreen shrub thrives in arid regions, featuring distinctive ovate leaves and a strong, tree-like growth structure. Originally from South Africa, *Crassula ovata* has been widely naturalized across different parts of the world, gaining popularity in indoor and outdoor gardening due to its resilience and minimal care requirements. [6] Crassula ovata leaves contains alkaloids, flavonoids, carbohydrates, tannins, phytosterols, terpenoids. It has been used for anti-diabetic, anti-oxidant and for anti-microbial. *Crassula ovata* is known for its wide-ranging medicinal properties and has shown promise in the treatment of various ailments. However, its leaves remain largely unexplored for anthelmintic potential. In light of this gap, the present study was designed to investigate the anthelmintic efficacy of *Crassula ovata* leaves extract.

MATERIAL AND METHOD:

Plant Material

The leaves of *Crassula ovata* was collected from local nursery from region Sudhowala of district Dehradun, of Uttarakhand State of India. Plant materials were authenticated by Dr. Harish Dutt, Department of botany, University of Jammu. Jammu and Kashmir, India.

Drugs and Chemicals

Albendazole, Ethanol, Methanol, Distilled water was of Analytical grade obtained from laboratory of Dev Bhoomi Uttarakhand University.

Preparation of Crude Extract

Leaves of *Crassula ovata* was taken and washed well with distilled water 2 to 3 times. After that it was kept under shading for about 20 days. Dried leaves were crushed using mortar and pestle and was further reduced to powder using electric grinder and was kept in air tight container.

For Methanolic extract: Through Maceration process, 50g of powder was macerated in 250 ml methanol in a conical flask for about 24 hours and repeated the same for three times.

For Hydro-alcohol extract: Through maceration process, 50g of powder was macerated in 250 ml of hydro-alcohol (i.e 80:20 ratio = 200ml ethanol was dissolved in 50ml of distilled water) and repeated the same for three times.

After the 24 hrs of maceration process, the solution was filtered and thus dried using water bath, Dried extract was thus used in order to perform anthelmintic activity.

A **B** **C** **D**

Figure 1: Maceration of Methanolic (A&B) and hydro-alcoholic extract (C&D)

Phytochemical Screening

1) Qualitative analysis:

The phytochemical screening was performed with standard procedures. [8,9] The primary focus of the study was to perform a phytochemical analysis of *Crassula ovata*, aiming to identify its key chemical constituents.

PHYTOCHEMICALS	TESTS	PROCEDURE	RESULT
Alkaloids [5,7,8,9,19,20]	Mayer's test	1ml of filtrate is treated with Mayer's reagent	Either white or creamy ppts. Indicates alkaloids presence.
	Dragendroff test. [7,25]	In 1 ml of filtrate 1 or 2 ml of dragendroff reagent is added.	Yellow precipitates appears then alkaloids are present.
Carbohydrates [23]	Molish test	In 1 ml of filtrate add few drops of Molish reagent.	Orange or red precipitate may

			indicate the presence of carbohydrates.
Saponins [20,24,28]	Foam test	In distilled water add 1 or 2 ml of extract and shake vigorously.	Persistent froth appears even when shaken vigorously.
Flavonoids [5]	Shinoda test	In 1 ml of extract 4 drops of Hcl is added and then add magnesium turning.	Magenta or pink colour indicates the presence of Flavonoids
Steroid and Triterpenoids [5,7,22,23]	Salkowski reaction	5 ml of extract + 2ml of Chloroform + 3 ml of Conc. H_2SO_4. Creating a layer.	Reddish brown tint indicates the presence of terpenoids.
Phenols [5,7,21]	Fecl3 test	1 or 2 drops $Fecl_3$ + small amount of extract.	Blue, Green, red or purple indicates the presence of phenols.

2) Quantitative analysis:

Total polyphenol content:

The total phenolic content of the samples was determined using the Folin–Ciocalteu reagent, following a slightly modified version of the protocol described by Pourmorad et al. [10,11] Gallic acid was used as a reference standard to construct a calibration curve [26], with concentrations ranging from 5 to 125 mg/L. For analysis, a 10 mg sample was dissolved in 10 mL of methanol (the same procedure was applied for the hydroalcoholic extract). From each standard and sample solution, 0.5 mL was combined with 2.5 mL of 50% Folin–Ciocalteu reagent and 2.5 mL of distilled water. After allowing the mixture to stand for five minutes, 2 mL of a 7.5% aqueous sodium carbonate solution was added. The resulting mixture was thoroughly mixed and left to incubate in the dark at room temperature for 15 minutes.

The absorbance of all prepared samples and standards was measured at 765 nm using a Cecil CE7410 UV-Vis spectrophotometer. Results were reported as milligrams of gallic acid equivalents (GAE) per 100 mg of dry leaf material. [11,27]

Total Flavonoid Content:

The total flavonoid content was measured using the aluminum chloride colorimetric method [26], with slight adaptations from the procedure developed by Chang et al. [11,12] Quercetin was used as the standard compound to construct the calibration curve. A 10 mg quantity of quercetin was dissolved in 96% ethanol and diluted to prepare concentrations of 2, 4, 6, 8, and 10 µg/mL.For the assay, 1 mL of each standard and test sample was mixed with 3 mL of 96% ethanol. To this mixture, 0.2 mL of 10% aluminium chloride solution, 0.2 mL of 1 M potassium acetate, and 5.6 mL of distilled water were added. The solutions were gently stirred and allowed to incubate at room temperature for 10 minutes.After incubation, the absorbance of each solution was recorded at 376 nm using a Cecil CE7410 UV-Visible spectrophotometer. A control solution, prepared without aluminum chloride, was used as the blank. Flavonoid content was then quantified and expressed as quercetin equivalents (QE) per 100 mg of plant extract, with values reported as the mean ± standard deviation for three replicates (n = 3). [11]

Earthworm Collection

The worms were collected from agriculture field of Dev Bhoomi Uttarakhand University, Dehradun. (UK) and were identified by zoologist of Dev Bhoomi Uttarakhand University, Dehradun. The earthworms (*Pheretima posthuma*) were thoroughly rinsed with normal saline to eliminate any residual fecal matter. Specimens measuring between 5 to 6 cm in length and 0.1 to 0.2 cm in width, with weights ranging from 0.8 to 3.04 grams, were selected for the study.

These earthworms were chosen due to their close anatomical and physiological resemblance to human intestinal roundworms, making them a suitable model for evaluating anthelmintic activity. [13,30,31,32,33]

Preparation and Test and Standard drug

Plant extracts were prepared at three concentrations: 10, 25, and 50 mg/mL. For the methanolic extract, 100 mg, 250 mg, and 500 mg of the crude extract were each dissolved in 10 mL of distilled water to achieve the respective concentrations. The hydroalcoholic extract was prepared using the same approach, with 100 mg, 250 mg, and 500 mg of crude extract each dissolved in 10 mL of distilled water to obtain final concentrations of 10, 25, and 50 mg/mL.

Distilled water served as the negative control, while albendazole was used as the standard reference drug for comparison in this study. [14]

Anthelmintic Activity

The anthelmintic assay for all selected plants was conducted in accordance with the standard protocol, with slight modifications. [5] Actively moving, uniform-sized worms were selected, placed in Petri dishes, and rinsed with water. The worms were organized into four main groups, with each group further subdivided into three smaller groups (10, 25, 50 mg/ml), containing three worms per subgroup.

a. Control group,

b. Methanolic extract - treated group.

c. Hydro-alcohol extract - treated group

d. Albendazole (standard) solution treated group. [15,16,28]

Same-sized worms were chosen and placed in Petri dishes having 10 mg/ml, 25 mg/ml, and 50 mg/ml concentrations of both Hydroalcoholic Extracts (HAE) and Methanolic Extracts (ME) of Crassula ovata. [15,17] Time taken to get paralyzed, and the death of the individual worm's readings were noted. [18]

Figure 2: Pictures [A]: Control group, [B]: Methanolic extracted group, [C]: Hydroalcoholic extract treated group, [D]: Albendazole (standard) treated group

RESULT

Preliminary phytochemical analysis of the extract indicated the presence of several bioactive compounds, including carbohydrates, polyphenols, tannins, flavonoids, alkaloids, saponins, and steroids as presented in Table 2. Leaves of *Crassula ovata* were subjected to extraction using methanol and a hydro-alcoholic mixture. The resulting extracts were analyzed for their anthelmintic potential and assessed for phenolic and total flavonoid content as represented in Table 3 and Table 4. Among the two, the methanolic extract exhibited the highest levels of both phenolic compounds and flavonoids, are presented in Table 5. This finding confirms the presence of these may contribute to its potential anthelmintic property as studies had shown that Tannins are responsible for anthelmintic activity [29].

The extract displayed a concentration-dependent effect on the test organisms, causing progressive paralysis—ranging from reduced motility to complete unresponsiveness to external stimuli—which ultimately led to the death of the earthworms. The anthelmintic effects of both methanolic and hydroalcoholic extracts of *Crassula ovata*, alongside the standard drug albendazole, are presented in Table1.

In the case of the methanolic extract, increasing concentrations (10, 25, and 50 mg/mL) resulted in a marked decrease in paralysis time (50.02 ± 0.21, 8.59 ± 0.40, and 2.12 ± 0.16 minutes) and death time (63.42 ± 0.13, 11.09 ± 0.20, and 5.18 ± 0.90 minutes, respectively) and for Hydro-alcohol the paralysis time (75.40 ± 0.60, 35.22 ± 0.17 and 12.02 ± 0.9 minutes) and death time (77.80 ± 0.57, 35.90±0.70 and 14.56 ± 0.43 minutes). At the highest concentration, the extract's performance was nearly comparable to that of albendazole (10mg/ml and 25 mg/ml), which, at 10 mg/mL, caused paralysis in 14.36 ± 0.21 and at 25 mg/ml in 2.24 ± 0.16 minutes respectively and death in 17.09 ± 0.12 for 10 mg/ml and 6.21 ± 0.71 minutes for 25 mg/ml.

No paralysis or mortality was observed in the control group within the 24-hour observation period.

S. No.	Group / Test substance	Concentration (mg/ml)	Time of Paralysis (minutes)	Time of Death (minutes)
1.	Control Group	-	-	-
2.	Albendazole (Standard)	10 mg	14.36±0.21	17.09±0.12
		25 mg	2.24±0.16	6.21±0.71
3.	Methanolic extract treated	10 mg	50.02±0.21	63.42±0.13
		25 mg	8.59±0.40	11.09±0.20
		50 mg	2.12±0.16	5.18±0.9
4.	Hydro-alcoholic extract treated	10 mg	75.40±0.60	77.80±0.57
		25 mg	35.22±0.17	35.90±0.70
		50 mg	12.02±0.9	14.56±0.43

Table 1: Invitro anthelmintic activity of leaves of *Crassula ovata* extract.

Phytochemicals	Tests	Methanolic	Hydro-Alcoholic
Alkaloids	Mayers test	Active	Active
	Dragendroffs test	Active	Non-active
Carbohydrates	Molish test	Active	Active
Saponins	Foam test	Active	Non-active
Flavonoids	Shinoda test	Active	Active
Steroid and Triterpenoids	Swalowski reaction	Active	Active
Phenols	Fecl3 test	Active	Active

Table 2: Qualitative Chemical composition analysis of methanolic and hydro-alcoholic extract of *Crassula ovata.*

Sample	Concentration (mg/ml)	Readings
01	17.2	0.0981
02	10.0	0.0466
03	13.0	0.0683
04	20.04	0.1208
05	7.9	0.0316
06	6.0	0.0181

Table 3: Absorbance of standard Gallic acid

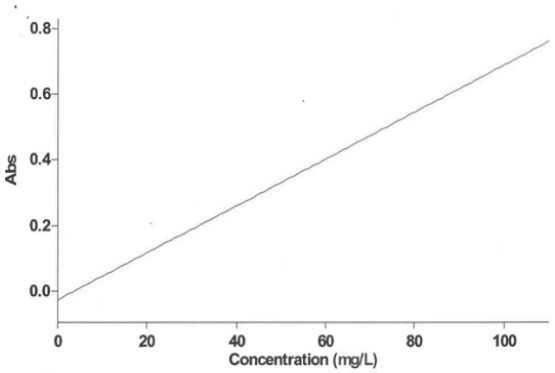

Figure 3: Standard Curve for Gallic acid

Sample	Concentration (mg/ml)	Readings
01	129.0	0.7853
02	5.0	0.0262
03	37.4	0.2244
04	5.0	0.0260
05	8.4	0.0469
06	5.5	0.0291

Table 4: Absorbance of standard quercetin.

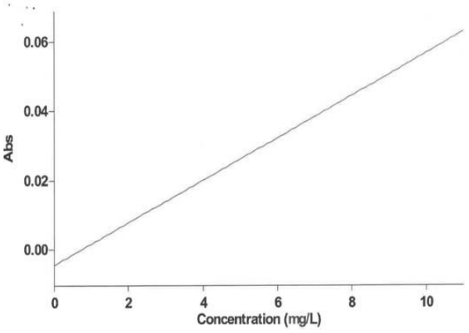

Figure 4: Standard curve for quercetin

PHYTOCEHMICALS	TESTS	METHANOLIC (mg GAE/g)	HYDRO-ALCOHOLIC (mg QE/g)
Total Polyphenols Content	Folin–Ciocalteu reagent	7.7	6.1
Total Flavonoid Content	Aluminium chloride colorimetric method	8.2	5.6

Table 5: Quantitative analysis of Methanolic and hydro-alcoholic extract of *Crassula ovata*.

CONCLUSION

The findings indicate that the methanolic extract of *Crassula ovata* leaves exhibits a stronger anthelmintic effect compared to its hydro-alcoholic counterpart. The primary objective of this study is to isolate and identify the specific chemical constituents within *Crassula ovata* extracts that contribute to their anthelmintic properties. Identifying these active compounds will help clarify their mechanisms of action and define their pharmacological profiles. Determining these chemical components is essential for several reasons. It will facilitate the creation of standardized formulations with reliable anthelmintic activity. Additionally, it may allow for the exploration of possible synergistic interactions among various compounds, potentially enhancing the extract's efficacy. Furthermore, a thorough evaluation of the safety and potential adverse effects of these extracts will be crucial for their use as anthelmintic treatments.
Ultimately, this research aims to advance our understanding of the pharmacological potential of *Crassula ovata* extracts and highlight their promise as effective and safe anthelmintic agents. These insights could

play a key role in the development of novel treatments for worm infections, addressing a significant global health challenge.

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