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Indian Journal of Pharmacy and Pharmacology

Journal homepage: https://www.ijpp.org.in/

Original Research Article

Phytochemical screening, formulation and evaluation of *in-vitro* anthelmintic activity of syrup from ethanolic extract of *Cucumis melo* leaves

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Abstract

Background: Helminthiasis is a common parasitic infection caused by helminths such as tapeworms, flukes, and roundworms affecting both humans and animals. Anthelmintic drugs are widely used for treatment. However concerns regarding drug resistance and side effects have led to the search for natural alternatives.

Objectives: This study focuses on the phytochemical screening, formulation, and evaluation of anthelmintic activity of syrup prepared from the ethanolic extract of Cucumis melo leaves.

Methods: The ethanolic extract of *Cucumis melo* leaves was obtained using the maceration method. The anthelmintic activity was assessed using *Pheretima posthuma* (earthworms) at four different concentrations (25, 50, 75 and 100 mg/ml). Albendazole (20 mg/ml) served as the reference standard while saline was used as the control. The formulated syrup was assessed for a range of physicochemical characteristics such as visual appearance, pH level, density, specific gravity and viscosity.

Results: Phytochemical analysis of the ethanolic extract demonstrated the presence of alkaloids, flavonoids, saponins, tannins, terpenoids, phenolic substances and glycosides. The extract showed notable dose-dependent anthelmintic effects. At a concentration of 100 mg/ml, the extract caused paralysis and mortality in 10.24 and 20.08 minutes respectively closely resembling the effects of Albendazole (8.46 and 15.55 minutes). The physicochemical assessment validated the appropriateness of the prepared syrup.

Conclusion: The ethanolic extract of *Cucumis melo* leaves demonstrates promising anthelmintic activity and has potential for development as a cost effective natural therapeutic agent for treating parasitic infections.

Keywords: Helminthiasis, Roundworms, Cucumis melo, Pheretima posthuma, Albendazole.

Received: 30-03-2025; **Accepted:** 16-05-2025; **Available Online**: 19-06-2025

1. Introduction

The use of medicinal plants is essential in human healthcare, with almost 80% of the worldwide population relying on plant-derived remedies. Since ancient times, these plants have served as the cornerstone of traditional and ethnomedical practices across the world. Several ancient civilizations including the Chinese continue to use herbal remedies for treating various ailments. These practices encompass a wide range of healthcare systems such as folk or tribal medicine along with Chinese, Ayurvedic, Korean, Siddha, Japanese, Iranian, Unani and traditional African medicine.

The dicotyledonous plant *Cucumis melo* belonging to the *Cucurbitaceae* family is native to Persia (Iran) and Central Asia. It is widely cultivated across regions like Turkey, China and India. *Cucumis melo* comprises a variety of volatile compounds that are biosynthetically produced from fatty acids, carotenoids, amino acids and terpenes. Muskmelons provide a range of health advantages including antioxidant, antimicrobial, anti-inflammatory, anti-ulcer, anti-fertility, analgesic and liver-protective effects. They are abundant in vital minerals like potassium, calcium, magnesium and carotenoids. The leaves of muskmelons are large, rough, and dark green, with 5–7 lobes in an orbicular, ovate, or angled

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https://doi.org/10.18231/j.ijpp.2025.017

form. These dentate leaves measure 5–8 cm in diameter with a cordate base and are attached to simple tendrils on 4–10 cm long petioles. Muskmelon leaves are often mistaken for cucumber leaves but they are less sharply pointed and toothed.

Helminthiasis, frequently referred to as worm infestation, is a macro parasitic illness that impacts both humans and animals, where parasitic worms (helminths) infect different parts of the body. These parasites are generally categorized into tapeworms, flukes and roundworms. While they primarily reside in the gastrointestinal tract they can also invade other organs causing significant physiological damage. Anthelmintic activity refers to the ability of substances to treat or eliminate helminth infections by targeting specific mechanisms in the worms either by killing them or inhibiting their growth and reproduction. Anthelmintic medications are utilized to combat different types of parasitic worms such as roundworms, pinworms, whipworms, hookworms and tapeworms. Broad-spectrum anthelmintics are effective against both flatworms and nematodes.[1-7]

2. Materials and Methods

2.1. Plant collection and authentication

The leaves of *Cucumis melo* was collected from their natural habitats in Guruvareddiyur, Anthiyur (TK), Erode (DT), Tamil Nadu, in the month of June 2024.It was verified by Dr. M. Kalyanasundaram, Ph.D., Principal of J.K.K. Munirajah College of Agricultural Science, T.N.Palayam-638506, Gobi (TK), Erode(DT), Tamil Nadu, India.

2.2. Preparation of plant extracts

The plant extracts were obtained through the cold maceration technique. Exactly 50 grams of powdered *Cucumis melo* leaves were weighed and transferred to clean sterilized containers. The powdered material was extracted with 250 ml of ethanol. The containers were securely closed and kept at room temperature for 48 hours, with occasional gentle agitation to promote even extraction. Following the 48 hours period, the liquid extract was filtered and the leftover solid residue, referred to as the marc was pressed to extract any remaining liquid. The combined filtrates were concentrated using a distillation setup to produce the final concentrated extract which was collected and preserved in sterile containers for subsequent use.[8-10]

Table 1: Theoretical yield of ethanolic extract of *Cucumis melo*leaves

S. No	Solvent used	Theoretical yield (%w/w)
1.	Ethanol	6.5

3. Preparation of *Cucumic melo* Anthelmintic Syrup

66.7 gm of Sucrose was weighted and added to purified water and heated until it dissolved with occasional stirring. Sufficient boiling water was added to produce 100 ml. (**Table 2**)

Table 2: Formulation composition of simple syrup.

S. No	Name of the ingredient	Quantity
1.	Sucrose	66.7 gm
2.	Distilled Water	100 ml

1 gm of ethanolic extract of Cucumis melo was mixed with simple syrup IP and the total volume was adjusted to 100 ml. (**Table 3**)

Table 3: Formulation composition of ethanolic extract of *cucumis melo* leaves in anthelmintic syrup

S. No	Name of the ingredient	Quantity
1.	Ethanolic Extract of leaves of Cucumis melo	1 gm
2.	Simple Syrup	100 ml

4. Phytochemical Analysis

The ethanolic extract of Cucumis melo leaves underwent initial phytochemical analysis to identify the presence of different phytoconstituents as per the procedures detailed in Khandelwal 2008.

4.1. Anthelmintic activity of ethanolic extract of leaves of Cucumis meloon Pheretima posthuma

Indian adult earthworms acquired from a local vendor were rinsed with normal saline to eliminate fecal residues. According to the experimental protocol, earthworms measuring 8-10 cm in length and 0.3-0.4 cm in width were utilized. The assay was conducted on Indian adult earthworm (Pheretima posthuma) due to its anatomical and physiological similarities with the intestinal roundworm parasites found in humans. The easy availability of earthworms encourages their widespread use for initial in-vitro assessments of anthelmintic substances.

Ethanolic extract from the leaves of *Cucumis melo* was examined for its anthelmintic properties against earthworms. All test solutions and standard drug solutions were freshly prepared before initiating the experiment. Three groups of earthworms roughly equal in size were placed into 20 ml solutions of four different concentrations (25, 50, 75, 100 mg/ml) in Petri dishes containing aforementioned extract solutions. Albendazole (20 mg/ml) served as the reference standard, while saline acted as the control. The experimental design for the anthelmintic activity of ethanolic extract of *Cucumis melo*leaves on Pheretima posthuma is displayed in (**Table 3**). The time required for paralysis and death of the worms was determined. The time for paralysis was recorded when no movement was observed, except when the worms were shaken vigorously. The time for the worm's death was noted after confirming that they did not move even under vigorous shaking.

Table 4: Experimental design of Anthelmintic activity of ethanolic extract of leaves of *Cucumis melo* on *Pheretima posthuma*

S. No	Group
1	I (Normal Control)
2	II (Standard Albendazole 20mg/ml)
3	III (Test 1 - 25 mg/ml Ethanolic extract of leaves of *Cucumis melo*)
4	IV (Test 2 - 50 mg/ml Ethanolic extract of leaves of *Cucumis melo*)
5	V (Test 3 - 75 mg/ml Ethanolic extract of leaves of *Cucumis melo*)
6	VI (Test 4 - 100 mg/ml Ethanolic extract of leaves of *Cucumis melo*)

5. Evaluation of *Cucumis melo* Anthelmintic syrup

6.6.1. Physicochemical parameters

The *Cucumis melo* Anthelmintic syrupwas evaluated for various physicochemical parameters such as physical appearance (colour, odour, taste, pH).

A. Colour examination

5 ml of syrup was poured into watch glasses and positioned against a white backdrop under white tube lighting. Its color was examined visually.

B. Odour examination

2 ml of syrup was individually sniffed. The time gap between two sniffing sessions was maintained at 2 minutes to eliminate the influence of the earlier sniffing.

C. Taste examination

A small amount of syrup was sampled and assessed for its flavour on the taste receptors of the tongue.

D. Determination of pH

Deposited a precisely measured volume of 10 ml of the syrup into a 100 ml volumetric flask and added distilled water to bring the total volume to 100 ml. The mixture was sonicated for approximately 10 minutes. The pH was assessed using a digital pH meter.

6.6.2 Determination of density

To obtain a clean specific gravity bottle, rinse it two to three times with distilled water. If necessary, cleanse the bottle with an organic solvent such as acetone and allow it to dry. Measure the weight of the empty dry bottle with the capillary tube stopper (w1). Fill the bottle with distilled water and insert the stopper, removing any surplus liquid from the outer tube with tissue paper. Weigh the bottle containing distilled water on an analytical balance (w2). Next, fill the bottle with the unknown liquid and secure the stopper again wiping any excess liquid from the outside of the tube using tissue paper. Weigh the bottle containing the unknown liquid on an analytical balance (w3).Calculate the density of unknown liquid by using following formula,

$$\text{Density of unknown liquid (syrup)} = \frac{\text{Weight of unknown liquid(w3)}}{\text{Weight of distilled water(w2)}} \text{ X Density of distilled water}$$

6.6.3. Determination of specific gravity

To acquire a clean specific gravity bottle, rinse the bottle a minimum of two to three times using distilled water. If necessary wash the bottle with an organic solvent such as acetone and allow it to dry. Weigh the empty dry bottle along with the capillary tube stopper (w1). Fill the bottle with distilled water and insert the stopper; remove any excess liquid from the side tube with tissue paper (w2). Weigh the bottle with the stopper and water on an analytical balance (w2). Repeat the process for the liquid being tested by draining and drying the bottle as described in steps 4 to 6. Weigh the bottle with the stopper and the test liquid on the analytical balance (w3).

Calculate the specific gravity of unknown liquid by using following formula,

$$\text{Specific gravity of unknown liquid (syrup)} = \frac{\text{Weight of unknown liquid(w3)}}{\text{Weight of distilled water(w2)}}$$

6.6.4 Determination of viscosity

To use a clean Ostwald viscometer, rinse the bottle two to three times with distilled water. If necessary, cleanse the bottle with an organic solvent such as acetone and let it dry. Position the viscometer vertically on an appropriate stand. Pour water into the dry viscometer until it reaches mark G. Measure the time in seconds for water to flow from mark A to mark B. Perform step 3 at least three times to achieve a precise reading. Rinse the viscometer with the test liquid and then fill it to mark A determining the time it takes for the liquid to flow to mark B. Proceed with the measurement of liquid densities as outlined in the density determination experiment.[14-15]

6. Results

Ethanolic extract from *Cucumis melo*leaves underwent a series of chemical assays to identify phytoconstituents, with the findings presented in (**Table 5**).

Active phytochemicals including Alkaloids, Flavonoids, Saponins, Tannins, Terpenoids, Phenolic Compounds and Glycosides are recognized for their role in Anthelmintic activity.

Table 5: Phytochemical analysis of Ethanolic extract of *Cucumis melo* leaves

S. No	Phytochemical Test	Ethanolic extract
1.	Alkaloids	+
2.	Flavonoids	+
3.	Tannins	+
4.	Saponins	+
5.	Terpenoids	+
6.	Phenolics	+
7.	Glycosides	+
8.	Steroids	+
9.	Proteins	-
10	Carbohydrates	-

NOTE: (+) Present (-) Absent

7.1. Anthelmintic activity of ethanolic extract of leaves of Cucumis meloon Pheretima posthuma

Efficacy of the ethanolic extract from *Cucumis melo* leaves as an anthelmintic was assessed on *Pheretima posthuma*. The paralysis and mortality time of *Pheretima posthuma* were monitored. The time for paralysis was recorded when no movement was detected unless the worms were shaken vigorously. Death was determined when the worms ceased to be motile, accompanied by a diminishing of their body colours. The findings are displayed in **Table 6** and

Figure 1.

Table 6: Anthelmintic activity of ethanolic extract of leaves of *Cucumis melo* on *Pheretima posthuma*

S. No	Group	Paralysis time (Min)	Death time (Min)
1	I (Normal Control)	0	0
2	II (Standard Albendazole 20mg/ml)	8.46± 0.24	15.55± 0.35
3	III (Test 1 - 25 mg/ml Ethanolic extract of leaves of *Cucumis melo*)	19.27± 0.55	52.30±1.20
4	IV (Test 2 - 50 mg/ml Ethanolic extract of leaves of *Cucumis melo*)	16.05±0.48	45.12±0.90
5	V (Test 3 - 75 mg/ml Ethanolic extract of leaves of *Cucumis melo*)	13.12±0.35	30.24±0.65
6	VI (Test 4 - 100 mg/ml Ethanolic extract of leaves of *Cucumis melo*)	10.24±0.28	20.08±0.50

Values are expressed as Mean ± SEM, (n=3).

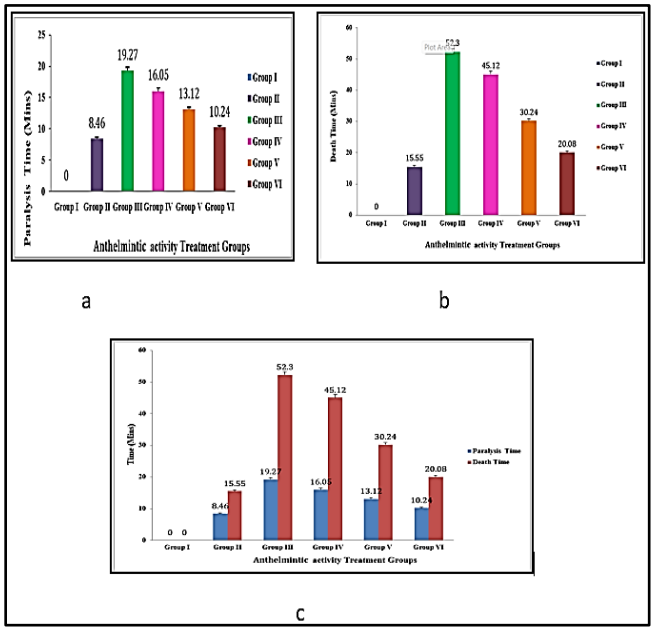

Figure 1: Effect of Anthelmintic activity of ethanolic extract of leaves of Cucumis melo on Pheretima posthuma **a;** Paralysis time Vs anthelmintic activity treatment groups **b;** Death Time Vs Anthelmintic activity treatment groups **c;** Paralysis time Vs death time.

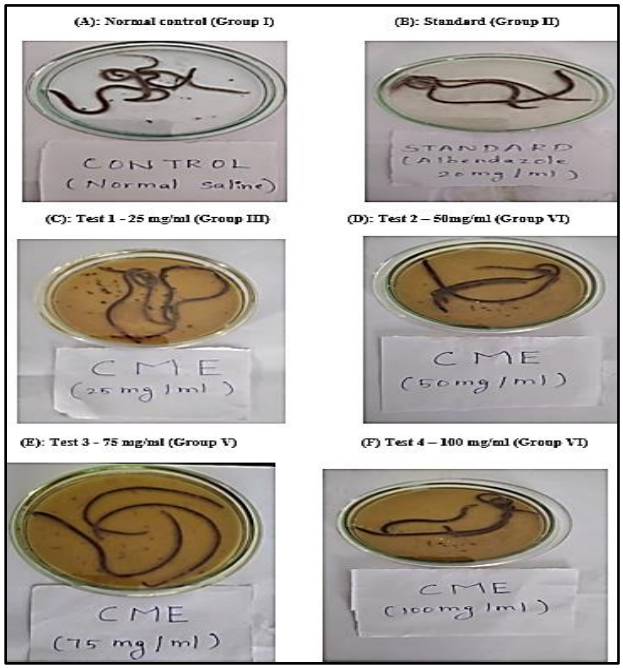

Figure 2: Photographic evaluation of Anthelmintic activity of ethanolic extract of leaves of *Cucumis melo* on *Pheretima posthuma*

7. Discussion

The research examined the effectiveness of the ethanolic extract of *Cucumis melo* leaves at different concentrations compared to a control (normal saline) and a reference drug (Albendazole). In the control group, there was no indication of paralysis or mortality among the worms demonstrating the

non-reactive nature of normal saline. The group treated with Albendazole at 20 mg/ml displayed marked anthelmintic effects, with paralysis noted at 8.46 minutes and mortality at 15.55 minutes.

Table 7: Physicochemical parameters *Cucumis melo* antihelmintic syrup

S. No	Physicochemical parameters	Observations
1.	Colour	Brown
2.	Odour	Aromatic
3.	Taste	Sweet
4.	pH	5.7
5.	Density	1.06 gm
6.	Specific gravity	0.5288 kg/m^3
7.	Viscosity	0.0583 poise

The ethanolic extract of *Cucumis melo*exhibited a dose-dependent anthelmintic effect. At a concentration of 25 mg/ml, paralysis occurred at 19.27 minutes, followed by death at 52.30 minutes. Raising the concentration to 50 mg/ml decreased the times for paralysis and death to 16.05 and 45.12 minutes, respectively. At 75 mg/ml, these times further diminished to 13.12 and 30.24 minutes. The highest concentration of 100 mg/ml produced the most remarkable activity with paralysis recorded at 10.24 minutes and death at 20.08 minutes, closely matching the effectiveness of Albendazole. (**Table 6** and **Figure 2**).

These results indicate that the ethanolic extract of *Cucumis melo*has potential anthelmintic qualities. The observed dose-dependent response implies that increased concentrations boost its effectiveness. The dose-dependent decrease in paralysis and mortality times suggests that the ethanolic extract contain active phytochemicals responsible for its anthelmintic properties. Prior research has identified substances such as flavonoids, alkaloids, saponins, tannins and phenolic compounds in *Cucumis melo*, which are recognized for their anthelmintic effects. These bioactive substances may disrupt the energy metabolism, neuromuscular function, or integrity of the worm cuticle, resulting in paralysis and subsequent death.

Although the outcomes are encouraging additional research is crucial to isolate and identify the particular active components contributing to the anthelmintic effects. Furthermore comprehensive studies on the mechanism of action, possible toxicity, and pharmacological safety are necessary to confirm the extract as a viable alternative to synthetic anthelmintic medications. This research underscores the potential of *Cucumis melo* as a natural source for creating new anthelmintic agents, presenting a sustainable and cost-efficient approach for addressing parasitic infections. However, while the extract showed encouraging results further investigations are needed to isolate and pinpoint the active phytochemicals responsible for the observed effects as well as to clarify their mechanisms of action.

7.3 Evaluation of leaves of Cucumis melo Antihelmintic syrup

Physicochemical parameters

Cucumis melo antihelmintic syrup was subjected to observe the various physicochemical parameters and results obtained are illustrated in **Table 7**.

Cucumis melo anthelmintic syrup was subjected to observed the various physicochemical parameters and results obtained are illustrated in **Table 7**. The colour, odour and taste of formulated syrup was found to be brown, aromatic odour and sweet in taste respectively and clear without particles. The leaves on *Cucumis melo* anthelmintic syrup showed good elegance. The leaves on *Cucumis melo* anthelmintic syrup evaluated for measurement of pH, specific gravity, density and viscosity. The leaves on *Cucumis melo* anthelmintic syrup was found to be pH 5.7, density 1.06gm, Specific gravity 0.5288kg/m3 and Viscosity 0.0583 poise. Physicochemical parameters of prepared syrup like colour, odour, taste, pH were satisfactory.

8. Conclusion

The present study evaluated the anthelmintic efficacy of ethanolic extracts from *Cucumis melo* leaves at various concentrations, comparing their effects to those of a control (normal saline) and a standard drug (Albendazole). The results demonstrated that the ethanolic extract exhibits significant, dose-dependent anthelmintic activity. In contrast, the control group showed no activity, while Albendazole produced rapid effects, with paralysis and mortality occurring at 8.46 and 15.55 minutes, respectively. The extract showed increasing effectiveness with higher concentrations; at 100 mg/ml, it induced paralysis and death at 10.24 and 20.08 minutes, respectively values comparable to those of Albendazole. This dose-dependent reduction in paralysis and death times suggests the presence of potent bioactive phytochemicals, such as flavonoids, alkaloids, saponins, tannins, and phenolic compounds. These findings highlight the potential of *Cucumis melo* as a promising natural source for developing new anthelmintic agents, offering a sustainable and cost-effective alternative for managing parasitic infections.

9. Acknowledgment

I sincerely express my gratitude to the management of JKK Munirajah Institute of Health Sciences College of Pharmacy for providing the necessary facilities and support to successfully carry out this research work.

10. Source of Funding

None.

11. Conflict of Interest

None.

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Cite this article: Nithya M, Perumal P, Abinath A, Aravindhan V, Eswaran A, Ruthnakumar S, Surendhar M. Phytochemical screening, formulation and evaluation of In-Vitro anthelmintic activity of syrup from ethanolic of Cucumis melo leaves. *Indian Journal Pharma Pharmacolgy.* 2025;12(1):108-113.