



RESEARCH ARTICLE

PHYTOCHEMICAL PROFILING AND IN VITRO EVALUATION OF THE ANTHELMINTIC ACTIVITY OF *PIMPINELLA HEYNEANA* (DC.) BENTH. FLOWERS

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ABSTRACT

Pimpinella heyneana (Dc.) Benth flowers extracts were subjected to in-vitro anthelmintic activity against the earthworm *Pheretima posthuma*. *Pimpinella heyneana* dried coarse flower powdered were extracted with hydro-alcohol 30:70 for 72h. The extracts were evaluated for Pharmacognostical, Physicochemical and Phytochemical evaluation with in-vitro Anthelmintic screening using various concentrations such as 10, 20, 40, 80 and 100 mg/ml, Albendazole 15 mg/ml were used as a reference standard, and distilled water was used as control. The results were expressed in paralysis and death time of the earthworms in min. The results obtained from the study indicate that ethyl acetate showed more significant anthelmintic property compared to other extracts and more effective than the standard. The results concluded that polyherbal extracts of Zingiber officinale rhizome and Punica granatum peels was more potent candidature as compared with standard drug albendazole.

Keywords: *Pimpinella heyneana* (Dc.) Benth flowers, anthelmintic, paralysis time, death time.

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INTRODUCTION

Helminthiasis is a widespread neglected tropical disease caused by parasitic worms, including nematodes, cestodes, and trematodes, affecting both humans and livestock [1-4]. It contributes to malnutrition, anaemia, impaired immunity, stunted growth, and significant economic losses due to reduced livestock productivity [5-7]. Although synthetic anthelmintic drugs such as albendazole, levamisole, and ivermectin are widely used, their prolonged and indiscriminate use has contributed to the development of drug resistance, adverse effects, high treatment costs, chemical residues in animal products, and limited accessibility in rural areas [8-9]. These limitations have created an urgent need for safe, affordable, environmentally friendly, and plant-based alternatives for helminth control [10-11]. Medicinal plants are valuable sources of anthelmintic compounds. Secondary metabolites such as tannins, flavonoids, alkaloids, terpenoids, and saponins may act

through multiple mechanisms to inhibit or eliminate parasites. Among these plants, *Pimpinella heyneana* (Family: Apiaceae), commonly known as Konda Jeelakarra, is an aromatic herb traditionally used in ethnomedicine [12]. It contains various bioactive phytochemicals, including quercetin, rutin, chlorogenic acid, phenylpropanoids, terpenoids, coumarins, and β -sitosterol, which have been associated with antioxidant, antimicrobial, nephroprotective, and in vitro anthelmintic activities. These reported pharmacological properties suggest that *P. heyneana* may be a potential natural source for further investigation as an anthelmintic agent [13].

2. MATERIALS AND METHODS

2.1 Study Design and Study Site

The present study was designed as a pilot experimental study using a controlled time-series approach. The study was conducted in 2026 in the Department of

Pharmacology, Vignan Institute of Pharmaceutical Sciences. The objective was to evaluate the in vitro anthelmintic activity of the hydroalcoholic flower extract of *Pimpinella heyneana* using *Pheretima posthuma* as the experimental worm model and albendazole as the reference drug.

2.2 Chemicals and Reagents

The chemicals and reagents used in the study included:

- Ethanol
- Methanol
- Normal saline solution (0.9% NaCl)
- Albendazole
- Distilled water

All chemicals and reagents used were of appropriate analytical grade and high purity.

2.3 Instruments and Apparatus

The following instruments and laboratory apparatus were used:

- Reflux apparatus
- Condenser
- Round-bottom flasks
- Beakers
- Petri dishes
- Electrical heating apparatus
- Other standard laboratory equipment required for extraction and pharmacognostical evaluation

2.4 Collection and Authentication of Plant Material

The flowers of *Pimpinella heyneana* were collected from the gardens of Vignan Institute of Pharmaceutical Sciences. The plant material was authenticated by Prof. K. Madhava Chetty, Botanist, Tirupati. Fresh flowers were selected for the study.

The adult Indian earthworms, *Pheretima posthuma*, used for the anthelmintic assay were collected from the Botanical Garden of Vignan Institute of Pharmaceutical Sciences.

2.5 Preparation of Hydroalcoholic Extract of *Pimpinella heyneana* Flowers

The yellow-coloured portions of the flowers of *Pimpinella heyneana* were separated and shade-dried at room temperature. The dried material was coarsely powdered, and 500 g of the powder was subjected to hot reflux extraction using a hydroalcoholic solvent system. The solvent consisted of ethanol and water in a 70:30 ratio. The extraction was carried out for approximately 8–9 hours, followed by condensation.

The extracted material was subsequently concentrated under reduced pressure using a suitable evaporation system to obtain a semisolid residue. The residue was further air-dried to obtain the final solid extract [14–18].



Figure 01: *Pimpinella heyneana* plant with flowers.

2.6 Pharmacognostical Studies

Pharmacognostical studies were carried out to establish the identity, authenticity, purity, and quality of the flowers of *Pimpinella heyneana*. The evaluation included morphological, microscopical, organoleptic, and physicochemical examinations in accordance with relevant WHO guidelines. These investigations were performed to establish preliminary quality-control parameters for the crude drug [19].

2.7 Preliminary Phytochemical Screening

Preliminary phytochemical screening was performed using standard qualitative chemical tests to detect the presence of major classes of phytoconstituents, including alkaloids, carbohydrates, phenols, flavonoids, proteins and amino acids, saponins, steroids, triterpenes, tannins, and glycosides [20].

2.8 Physicochemical Constants

Physicochemical evaluation of the shade-dried powdered flowers was performed according to relevant WHO guidelines. The parameters evaluated included total ash, acid-insoluble ash, water-soluble ash, loss on drying, water-soluble extractive value, alcohol-soluble extractive value, petroleum ether-soluble extractive value, and foaming index. These parameters were assessed to determine the quality, purity, and suitability of the plant material for extraction and standardization [21–22].

3. In Vitro Anthelmintic Activity of *Pimpinella heyneana* Flowers

The hydroalcoholic extract of *Pimpinella heyneana* flowers was evaluated for in vitro anthelmintic activity using adult Indian earthworms (*Pheretima posthuma*).



Figure 02: Adult Indian earthworms (*Pheretima posthuma*).

3.1 Evaluation of Anthelmintic Activity Using Earthworms

The anthelmintic activity was evaluated using a standard in vitro assay involving adult Indian earthworms. *Pheretima posthuma* was selected as the experimental model because of its anatomical and

physiological similarities to intestinal helminths and its suitability for preliminary in vitro anthelmintic screening.

3.2 Experimental Procedure

The anthelmintic activity was evaluated using adult *Pheretima posthuma* earthworms. Albendazole was used as the reference drug. The test solutions were prepared using normal saline as the diluent.

Albendazole was prepared at a concentration of 20 mg/mL. The hydroalcoholic extract of *Pimpinella heyneana* flowers was prepared at concentrations of 10, 20, 40, and 80 mg/mL.

The respective test solutions were transferred into appropriately labelled Petri dishes. Adult earthworms of approximately equal size (about 8 cm in length) were selected and placed in the respective Petri dishes at room temperature.

The time required for paralysis and the time required for death were recorded in minutes. Paralysis time was recorded when the worms showed no observable movement, except after vigorous shaking. Death was recorded when the worms showed no movement even after vigorous shaking and subsequent exposure to warm water at approximately 50 °C.

3.3 Experimental Groups

Table 01: Grouping of Earthworms for Evaluation of the Anthelmintic Activity of the Hydroalcoholic Extract of *Pimpinella heyneana* Flowers

Sl. No.	Experimental Group	Treatment
1	Group I – Control	1% distilled water/vehicle
2	Group II – Standard	Albendazole, 20 mg/mL
3	Group III – Test	Hydroalcoholic extract of <i>P. heyneana</i> flowers at 10, 20, 40, and 80 mg/mL



Figure 02: Adult Indian earthworms exposed to different concentrations of the test extract.

4. RESULTS

4.1 Physicochemical Evaluation

Table 02: Results of Physicochemical Parameter Assessment of *Pimpinella heyneana* Flowers

Physicochemical Parameter	Analytical Significance	Result (% w/w)
Total Ash	Indicates the total mineral content and possible	14.0

	extraneous inorganic matter	
Acid-Insoluble Ash	Indicates siliceous matter and possible sand contamination	4.0
Water-Soluble Ash	Indicates the water-soluble portion of total ash	2.0
Loss on Drying	Indicates residual moisture content	6.0
Water-Soluble Extractive	Indicates the amount of water-soluble constituents	10.0
Alcohol-Soluble Extractive	Indicates the amount of alcohol-soluble constituents	8.0
Petroleum Ether Extractive	Indicates the amount of non-polar, lipid-soluble constituents	1.0

Inference: Extractive-value determination is a simple and economical method for evaluating the quality of crude plant material and may assist in detecting exhausted or adulterated materials and selecting a suitable extraction solvent. In the present study, the alcohol-soluble extractive value was higher than the petroleum ether extractive value, indicating greater extraction of alcohol-soluble constituents.

4.2 Fluorescence Analysis

Table 03: Fluorescence Profile of *Pimpinella heyneana* Flower Powder

Reagent/Treatment	Visible Light	Short UV (254 nm)	Long UV (366 nm)	Diagnostic Observation
Dry flower powder, untreated	Pale cream to brownish white	Dull light green	Dark brown to black	Baseline fluorescence
Powder + distilled water	Creamy white	Light yellowish green	Black/no fluorescence	Solubilization of polar constituents
Powder + 1 N NaOH in water	Light yellow	Bright yellowish green	Dark green	Fluorescence suggestive of flavonoid-type constituents
Powder + 1 N NaOH in	Greenish	Deep	Vibrant	Fluorescence suggestive of

methanol	yellow	green	fluorescent green	phenolic/coumarin-type constituents
Powder + 1 N HCl	Light brownish white	Light green	Light brown	Acid-mediated changes in fluorescent constituents
Powder + 50% HNO ₃	Light reddish brown	Olive green	Deep dark brown	Chemical reaction with constituents
Powder + 50% H ₂ SO ₄	Dark brown	Dark greenish black	Pitch black	Carbonization/chemical degradation
Powder + 1% iodine solution	Faint blue-black	Blue-green	Deep dark blue	Suggestive of starch-containing material
Powder + 5% FeCl ₃	Dull dark green	Bluish green	Inky blackish green	Suggestive of phenolic/tannin-type constituents

Inference: The flower powder exhibited characteristic colour and fluorescence changes following treatment with different chemical reagents. These fluorescence characteristics may be useful as preliminary diagnostic parameters for the identification and quality assessment of *P. heyneana* flower powder. However, fluorescence reactions should be interpreted as supportive qualitative observations and should not be considered definitive identification of individual phytoconstituents.

4.3 Preliminary Phytochemical Screening

Table 04: Preliminary Phytochemical Screening of Different Extracts of *Pimpinella heyneana* Flowers

Phytochemical	Petroleum Ether	Chloroform	Ethyl Acetate	Ethanol	Aqueous
Alkaloids	–	+	+	++	+
Flavonoids	–	+	++	+++	++
Tannins	–	–	+	++	++
Saponins	–	–	+	++	+++
Glycosides	–	+	++	+++	++
Steroids	++	++	+	+	–
Terpenoids	++	++	+	+	–
Phenols	–	+	++	+++	++
Proteins	–	–	–	+	+
Carbohydrates	–	–	+	++	+++

Key: +++ = abundantly present; ++ = moderately present; + = present in trace amounts; – = absent.

Inference: Preliminary phytochemical screening indicated the presence of several classes of phytoconstituents in the different extracts of *P. heyneana* flowers. The ethanol extract showed abundant flavonoids, glycosides, and phenolic constituents, while the aqueous extract showed abundant saponins and carbohydrates. These qualitative findings provide preliminary evidence for the presence of secondary metabolites that may contribute to the biological activity of the plant extract.

4.4 In Vitro Anthelmintic Activity

Table 05: Anthelmintic Activity of the Hydroalcoholic Extract of *Pimpinella heyneana* Flowers

Experimental Group	Concentration (mg/mL)	Paralysis Time (min)	Death Time (min)
Control (Normal Saline)	—	No paralysis observed	No death observed
Standard (Albendazole)	20	15.42 ± 0.35	32.18 ± 0.64
Hydroalcoholic Extract	10	68.15 ± 1.22	94.30 ± 1.85
Hydroalcoholic Extract	20	42.50 ± 0.92	65.12 ± 1.10
Hydroalcoholic Extract	40	26.18 ± 0.74	48.25 ± 0.95
Hydroalcoholic Extract	80	18.35 ± 0.41	35.60 ± 0.52

Values are expressed as mean ± SEM (standard error of the mean), with n = 6 worms per treatment group.

4.5 Interpretation of Anthelmintic Activity

The hydroalcoholic extract demonstrated a concentration-dependent reduction in both paralysis time and death time. At 10 mg/mL, the extract produced paralysis at 68.15 ± 1.22 min and death at 94.30 ± 1.85 min. Increasing the concentration to 20, 40, and 80 mg/mL progressively reduced both parameters.

At 80 mg/mL, the extract produced paralysis at 18.35 ± 0.41 min and death at 35.60 ± 0.52 min. The reference drug, albendazole at 20 mg/mL, produced paralysis at 15.42 ± 0.35 min and death at 32.18 ± 0.64 min.

These findings indicate that the hydroalcoholic extract exhibited concentration-dependent anthelmintic activity under the experimental conditions used. At the highest concentration tested, the activity of the extract approached that observed with albendazole; however, the extract did not demonstrate identical activity based on the reported mean paralysis and death times.

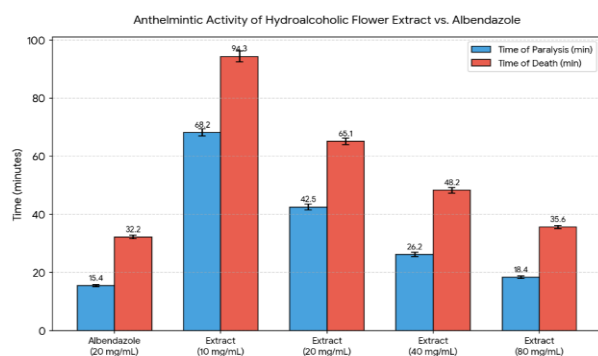


Figure 04: Bar diagram representing the in vitro anthelmintic activity of the hydroalcoholic extract of *Pimpinella heyneana* flowers.

5. CONCLUSION

The present study provides preliminary pharmacognostical, physicochemical, phytochemical, and in vitro pharmacological data on the flowers of *Pimpinella heyneana* (DC.) Benth.

- Quality-control parameters:** The morphological, organoleptic, fluorescence, and physicochemical characteristics established in the present study may serve as preliminary quality-control parameters for the identification and standardization of *P. heyneana* flowers.
- Phytochemical profile:** Preliminary phytochemical screening demonstrated the presence of several classes of phytoconstituents, particularly flavonoids, phenols, tannins, saponins, and glycosides, in the investigated extracts.
- In vitro anthelmintic activity:** The hydroalcoholic flower extract demonstrated concentration-dependent anthelmintic activity against *Pheretima posthuma*, as evidenced by reductions in paralysis and death times with increasing extract concentration. The highest concentration tested showed activity approaching that of the albendazole reference group under the conditions of this in vitro assay.

Overall, these findings support further investigation of *P. heyneana* flowers as a potential source of bioactive compounds with anthelmintic activity. However, the present findings are limited to an in vitro earthworm model and should not be interpreted as evidence of clinical efficacy or safety in humans or animals.

6. FUTURE DIRECTIONS

The following investigations are recommended to further characterize the anthelmintic potential of *P. heyneana*:

- Bioassay-guided isolation:** Fractionate the active extract using chromatographic techniques and identify the compounds responsible for the observed anthelmintic activity using appropriate spectroscopic and analytical methods.
- Mechanistic studies:** Investigate the possible mechanisms underlying the anthelmintic activity, including effects on the neuromuscular system,

tegument, energy metabolism, or other parasite-specific targets.

- In vivo efficacy and safety evaluation:** Conduct appropriately designed animal studies to evaluate pharmacokinetics, bioavailability, therapeutic efficacy, acute toxicity, and repeated-dose toxicity before considering further development.
- Standardization and formulation development:** Develop a standardized extract and investigate suitable pharmaceutical or veterinary dosage forms after establishing reproducible efficacy and safety profiles.
- Dose-response and statistical validation:** Conduct studies with adequate sample sizes and appropriate statistical analyses, including one-way ANOVA followed by a suitable post hoc multiple-comparison test where assumptions are satisfied.

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DECLARATION COMPETING INTEREST

Not Declared

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INFORM CONSENT AND ETHICAL CONSIDERATION

Not Applicable

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