JOURNAL OF PHARMA INSIGHTS AND RESEARCH ISSN NO. 3048-5428

RESEARCH ARTICLE

Evaluation of Anthelmintic Efficacy of *Ruta graveolens* Aqueous Extract

Ramakrishna S*[1], Narayana Murthy G[2], Pruthviganesh B[3], Likhitha K[4], Harish H. N[5]

[1] *Assistant Professor, Department of Pharmacognosy, National College of Pharmacy, Shivamogga, Karnataka, India.*
[2] *Principal and Professor, Department of Pharmacognosy, National College of Pharmacy, Shivamogga, Karnataka, India.*
[3] *UG Scholar, Department of Pharmacognosy National College of Pharmacy, Shivamogga, Karnataka, India.*
[4] *Lecturer, Department of Pharmacognosy, National College of Pharmacy, Shivamogga, Karnataka, India.*
[5] *PG scholar, Department of Pharmacognosy, National College of Pharmacy, Shivamogga, Karnataka, India.*

Publication history: Received on 22nd Feb 2025; Revised on 5th March 2025; Accepted on 6th March 2025

Article DOI: 10.69613/9w7pc936

Abstract: *Ruta graveolens*, commonly known as rue, has been utilized in traditional medicine systems for treating parasitic infections. The present study aimed to evaluate the anthelmintic potential of *R. graveolens* aqueous extract against gastrointestinal nematodes using *Lumbricus terrestris* as a model organism. The plant material was authenticated, processed, and extracted using water as a solvent through refluxation. Phytochemical screening showed the presence of various bioactive compounds including alkaloids, flavonoids, glycosides, tannins, and carbohydrates. The anthelmintic activity was assessed by monitoring paralysis and death times of earthworms exposed to different concentrations of the extract. At 25 mg/mL concentration, the aqueous extract demonstrated superior anthelmintic activity compared to the standard drug albendazole (10 mg/mL), with paralysis time of 90 seconds and death time of 190 seconds. In contrast, albendazole showed paralysis at 300 seconds and death at 1500 seconds. The extract's enhanced anthelmintic activity may be attributed to the presence of bioactive compounds including alkaloids, flavonoids, and phenolic substances. The findings signify the traditional use of *R. graveolens* in parasitic infections and suggest its potential as a natural anthelmintic agent. The study provides a scientific basis for further investigation into the isolation and characterization of active compounds responsible for the observed anthelmintic activity.

Keywords: *Ruta graveolens*; Anthelmintic activity; Phytochemical analysis; *Lumbricus terrestris*; Traditional medicine.

1. Introduction

Helminthic infections remain a significant global health concern, particularly in developing nations, affecting both humans and livestock. The continuous search for effective, natural anthelmintic agents has led to renewed interest in medicinal plants traditionally used for parasite control [1]. *Ruta graveolens* L., commonly known as rue, belongs to the family Rutaceae and represents one of the most significant species among the 40 members of genus Ruta. Native to Mediterranean regions, Southern Europe, and North Africa, *R. graveolens* has been cultivated worldwide for its medicinal properties [2]. The historical significance of this plant dates back to the fifth century BCE, documented in the works of Hippocrates and Dioscorides, and it has maintained its position in various traditional medicine systems including Ayurveda and Unani [3].

The plant is a perennial, herbaceous shrub growing to a height of 60-90 cm, characterized by alternate, compound leaves and yellow flowers blooming from June to August. *R. graveolens* demonstrates remarkable adaptability, thriving in well-drained soils under full sun to partial shade conditions [4]. Phytochemically, *R. graveolens* is rich in bioactive compounds including alkaloids, flavonoids, and coumarins. Notable compounds isolated from the plant include acridone alkaloids, furanoacridones such as arborinine and evoxanthine, and various quinolone alkaloids including graveoline [5]. The plant also contains significant amounts of essential oils, contributing to its characteristic strong aroma and potential biological activities [6].

Traditional applications of *R. graveolens* extend beyond its anthelmintic properties, encompassing treatments for anxiety, fever, dermatological conditions, and microbial infections [7]. The plant's strong aromatic properties have led to its use as a natural repellent against various pests, including snakes, making it a common feature in traditional home gardens [8]. The aim of this present research work was to evaluate the anthelmintic potential of *R. graveolens* aqueous extract, aiming to provide scientific validation for its traditional use in parasite control.

* Corresponding author: Ramakrishna S

Figure 1. Flowers and leaves of *Ruta graveolens*

2. Materials and Methods

2.1. Plant Material

Fresh leaves of *Ruta graveolens* were procured from the local market in Shivamogga, Karnataka, India. The plant material was authenticated at the Department of Botany, SRMC College of Applied Sciences, Shivamogga. A voucher specimen was deposited in the institutional herbarium for future reference [9].

2.2. Preparation of Extract

The collected leaves were thoroughly cleaned with water to remove debris and foreign matter, cut into small pieces, and shade-dried at room temperature (25 ± 2°C). The dried material was pulverized into a coarse powder using a mechanical grinder and stored in an airtight container until further use.

2.2.1. Extraction

The extraction was performed using the refluxation method as described by Forsyth [10]. Ten grams of dried *R. graveolens* powder underwent three consecutive 4-hour refluxation cycles, followed by two 2-hour cycles using distilled water as the solvent. The combined extracts were filtered and concentrated using a rotary evaporator under reduced pressure at 45°C. The final dried extract was weighed to calculate the percentage yield and stored at 4°C in an airtight container.

2.3. Phytochemical Tests

The following phytochemical tests were carried out on the aqueous extract to identify major classes of bioactive compounds [11].

2.3.1. Carbohydrates

Molisch's Test: 2 mL of extract was treated with 2 drops of alcoholic α-naphthol solution in a test tube. 2 mL of concentrated sulfuric acid was added carefully along the sides of the test tube. Formation of a violet ring at the junction indicated the presence of carbohydrates.

Benedict's Test: 5 mL of Benedict's reagent was added to 2 mL of extract. The mixture was heated in a boiling water bath for 5 minutes. A red-orange precipitate indicated the presence of reducing sugars.

2.3.2. Alkaloids

Mayer's Test: 2 mL of extract was treated with 2-3 drops of Mayer's reagent (potassium mercuric iodide solution). Formation of pale yellow precipitate confirmed alkaloid presence.

Hager's Test: 2 mL of extract was treated with few drops of Hager's reagent (saturated picric acid solution). A yellow precipitate indicated alkaloid presence.

Wagner's Test: 2 mL of extract was treated with Wagner's reagent (iodine in potassium iodide). Formation of brown/reddish precipitate indicated alkaloid presence.

2.3.3. Proteins

Biuret Test: 2 mL of extract was treated with 2 mL of 10% sodium hydroxide solution and 2-3 drops of 0.7% copper sulfate solution. Development of violet color indicated protein presence.

Ninhydrin Test: 2 mL of extract was boiled with 2 mL of 0.2% Ninhydrin solution. A violet color indicated the presence of amino acids and proteins.

2.3.4. Tannins

Ferric Chloride Test: 2 mL of extract was treated with 3-4 drops of 1% ferric chloride solution. A green-black coloration indicated the presence of tannins.

2.3.5. Flavonoids

Sodium Hydroxide Test: 2 mL of extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which became colorless on addition of dilute acid, indicated flavonoid presence.

Ferric Chloride Test: To 2 mL of extract, few drops of ferric chloride solution were added. Formation of blackish red color indicated flavonoid presence.

2.3.6. Glycosides

Keller-Kiliani Test: 2 mL of extract was treated with 1 mL of glacial acetic acid containing traces of ferric chloride and 1 mL of concentrated sulfuric acid. A brown ring at the interface indicated deoxy-sugar characteristic of cardenolides.

Legal's Test: To 2 mL of extract, 1 mL of pyridine and 1 mL of sodium nitroprusside were added. Formation of pink to red color indicated the presence of glycosides..

2.4. Anthelmintic Activity

Adult Indian earthworms (*Lumbricus terrestris*) of uniform size (8-10 cm length) were selected as the test organism due to their anatomical and physiological resemblance to intestinal roundworms of humans [12]. The anthelmintic activity was evaluated following the method described by Neeraj Choudhary et al. [13]. Test solutions were prepared in distilled water at concentrations of 25 mg/mL for the extract and 10 mg/mL for albendazole (standard drug). Six earthworms were placed in petri dishes containing 25 mL of test solutions. Distilled water served as the control. All solutions were freshly prepared before experimentation. The time taken for paralysis (marked by loss of movement) and death (marked by lack of response to external stimuli) of the worms was recorded. Each experiment was performed in triplicate.

3. Results and discussion

3.1. Extract Yield

The aqueous extraction of *R. graveolens* leaves yielded 1.6 g of dried extract from 10 g of plant material, representing a yield of 16% w/w. The extract appeared dark brown in colour with a characteristic aromatic odour.

Table 1. Yield of *Ruta graveolens* aqueous extract

Parameter	Value
Initial weight of dried plant material (g)	10.0
Weight of dried extract (g)	1.6
Percentage yield (% w/w)	16.0

3.2. Phytochemical Screening

Preliminary phytochemical screening of the aqueous extract revealed the presence of several bioactive compounds. The extract showed strong positive results for alkaloids, as evidenced by precipitate formation with all three reagents (Mayer's, Hager's, and

Wagner's). Flavonoids were confirmed by the development of yellow coloration with NaOH and a green-black color with $FeCl_3$. The presence of glycosides was indicated by the formation of a brown ring at the interface in the Keller-Kiliani test and a red color in Legal's test. Tannins were detected through the formation of a blue-black color with $FeCl_3$. Carbohydrates showed positive results in both Molisch's and Benedict's tests, while proteins showed weak positive results.

Table 2. Phytochemical screening of *R. graveolens* aqueous extract

Phytochemical constituent	Test performed	Result
Alkaloids	Mayer's test	+++
	Hager's test	+++
	Wagner's test	+++
Flavonoids	NaOH test	++
	$FeCl_3$ test	++
Glycosides	Keller-Kiliani test	++
	Legal's test	++
Tannins	$FeCl_3$ test	++
Carbohydrates	Molisch's test	++
	Benedict's test	++
Proteins	Biuret test	+
	Ninhydrin test	+

+++ = strongly positive; ++ = moderately positive;
+ = weakly positive; - = negative

3.3. Anthelmintic Activity

The aqueous extract of *R. graveolens* demonstrated significant anthelmintic activity against L. terrestris. At 25 mg/mL concentration, the extract induced paralysis at 90 ± 2 seconds and death at 190 ± 3 seconds. In comparison, the standard drug albendazole (10 mg/mL) showed paralysis time of 300 ± 5 seconds and death time of 1500 ± 10 seconds. The control group (distilled water) showed no paralysis or death of worms during the 24-hour observation period.

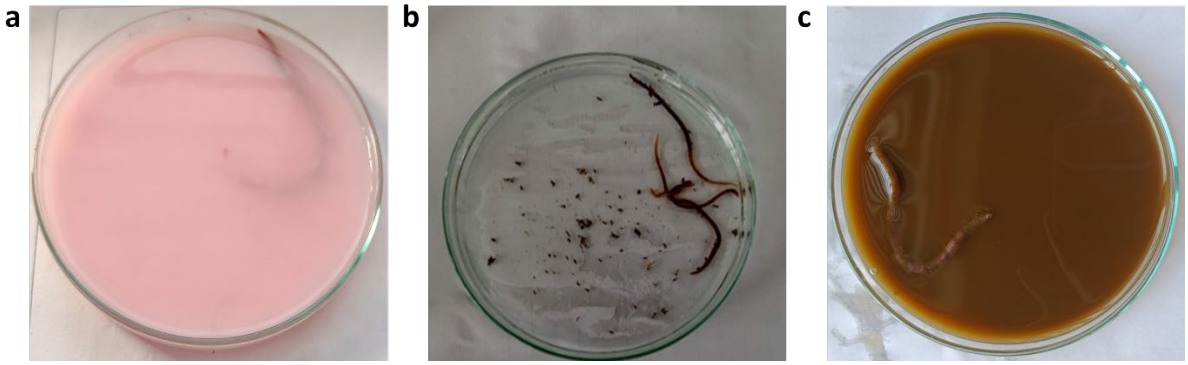

Figure 2. Evaluation of Anthelmintic activity with a. Albendazole 10 mg/mL Standard b. Control c. *R. graveolens* extract 25mg/mL

Table 3. Anthelmintic activity of *R. graveolens* aqueous extract against *Lumbricus terrestris*

Treatment	Concentration (mg/mL)	Time taken for paralysis (seconds)*	Time taken for death (seconds)*
R. graveolens extract	25	90 ± 2	190 ± 3
Albendazole (standard)	10	300 ± 5	1500 ± 10
Control (distilled water)	-	No paralysis	No death

*Values are expressed as mean ± SEM (n=6)

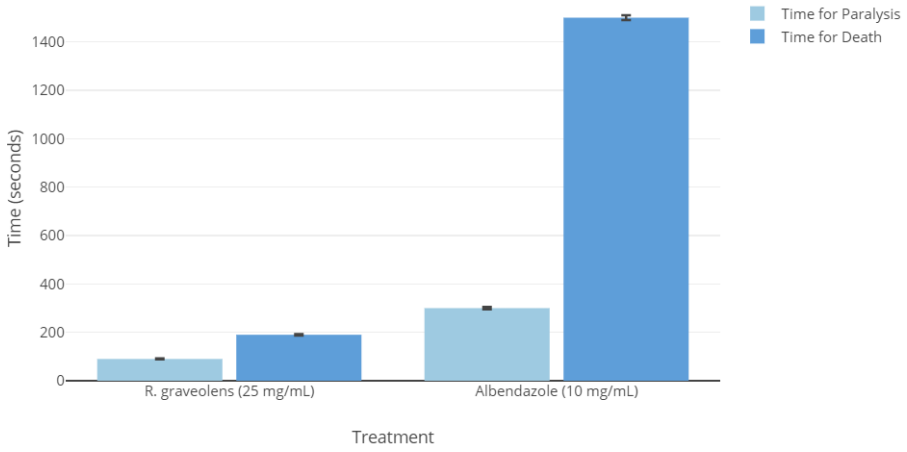

Figure 3. Results of anthelmintic activity of *R. graveolens* compared with Albendazole

3.4. Discussion

The superior anthelmintic activity of *R. graveolens* extract compared to albendazole suggests the presence of potent anthelmintic compounds. The rapid onset of paralysis and death might be attributed to the synergistic action of various bioactive compounds identified in the phytochemical screening. Alkaloids, which were strongly present in the extract, are known to cause paralysis in worms by interfering with neuromuscular function [14]. Flavonoids have been reported to exhibit anthelmintic properties through multiple mechanisms, including disruption of energy metabolism and interference with the nervous system of parasites [15]. The presence of tannins may contribute to the anthelmintic effect through their ability to bind to free proteins in the gastrointestinal tract of host animals or glycoprotein on the cuticle of the parasite [16]. This binding may impair key metabolic processes such as energy generation and nutrient absorption, leading to mortality of the parasites. The significantly lower paralysis and death times observed with the plant extract compared to albendazole indicate its potential as an alternative or complementary anthelmintic agent.

4. Conclusion

The present research work shows the applications of traditional plant *Ruta graveolens* through scientific evaluation. The aqueous extract demonstrated superior anthelmintic activity compared to the standard drug albendazole, which can be attributed to the presence of various bioactive compounds including alkaloids, flavonoids, and tannins. These results indicate that *R. graveolens* could serve as a promising source for the development of novel anthelmintic agents, though further studies focusing on isolation of active compounds, toxicity assessment, and *in vivo* efficacy are warranted.

Compliance with ethical standards

Acknowledgements

The authors are grateful to the Department of Pharmacognosy, National College of Pharmacy, Shivamogga, Karnataka, for providing the necessary facilities to conduct this research. We also thank the Department of Botany, SRMC College of Applied Sciences, Shivamogga for their assistance in plant authentication.

Conflict of interest statement

The authors declare that they have no conflict of interest in the publication of this research.

Statement of ethical approval

The experimental protocol was approved by the Institutional Animal Ethics Committee of National College of Pharmacy, Shivamogga (Approval number: NCPS/IAEC/2024/01). All procedures performed in this study involving animals were in accordance with the ethical standards of the institution and national research committee guidelines for the care and use of laboratory animals.

Statement of informed consent

Not applicable as this study did not involve human participants.

References

[1] Wink M. Medicinal plants: A source of anti-parasitic secondary metabolites. Molecules. 2012;17(11):12771-91.

[2] Pollio A, De Natale A, Appetiti E, Aliotta G, Touwaide A. Continuity and change in the Mediterranean medical tradition: Ruta spp. (rutaceae) in Hippocratic medicine and present practices. Journal of Ethnopharmacology. 2008;116(3):469-82.

[3] De Feo V, De Simone F, Senatore F. Traditional phytotherapy in the Peninsula Sorrentina, Campania, Southern Italy. Journal of Ethnopharmacology. 2002;82(2-3):127-43.

[4] Kong YC, Lau CP, Wat KH, Ng KH, But PP, Cheng KF, Waterman PG. Antifertility principle of *Ruta graveolens*. Planta Medica. 1989;55(2):176-8.

[5] Berberian DA, Freudenthal RI, Ralls JW, Johnson RP. Biological and chemical studies of *Ruta graveolens*, antifertility plant. Journal of Pharmaceutical Sciences. 1974;63(9):1396-8.

[6] Ratheesh M, Helen A. Anti-inflammatory activity of *Ruta graveolens* Linn on carrageenan induced paw edema in wistar male rats. African Journal of Biotechnology. 2007;6(10):1209-11.

[7] Ivanova A, Mikhova B, Najdenski H, Tsvetkova I, Kostova I. Antimicrobial and cytotoxic activity of *Ruta graveolens*. Fitoterapia. 2005;76(3-4):344-7.

[8] Zeichen R, Rey A, Argañaraz E, Bindstein E. Perinatal toxicology of *Ruta chalepensis* (Rutaceae) in mice. Journal of Ethnopharmacology. 2000;69(2):93-8.

[9] Malik F, Kumar A, Bhushan S, Khan S, Bhatia A. Reactive oxygen species generation and mitochondrial dysfunction in the apoptotic cell death of human myeloid leukemia HL-60 cells by a dietary compound withaferin A with concomitant protection by N-acetyl cysteine. Apoptosis. 2007;12(11):2115-33.

[10] Forsyth WGC. Cacao polyphenolic substances. III. Separation and estimation on paper chromatograms. Biochemical Journal. 1952;51(4):511-6.

[11] Harborne JB. Phytochemical methods: A guide to modern techniques of plant analysis. 3rd ed. London: Chapman and Hall; 1998.

[12] Vidyarthi RD. A textbook of zoology. 14th ed. New Delhi: S. Chand Publishing; 1977.

[13] Choudhary N, Khajuria V, Gillani ZH, Tandon VR, Arora E. Anthelmintic potential of *Oxalis corniculata*. Journal of Natural Products. 2014;7:26-30.

[14] Martin RJ. Modes of action of anthelmintic drugs. The Veterinary Journal. 1997;154(1):11-34.

[15] Sharma US, Sharma UK, Singh A, Sutar N, Singh PJ. *In vitro* anthelmintic activity of *Murraya koenigii* Linn. leaves extracts. International Journal of Pharma and Bio Sciences. 2010;1(3):1-4.

[16] Hoste H, Martinez-Ortiz-De-Montellano C, Manolaraki F, Brunet S, Ojeda-Robertos N, Fourquaux I, Torres-Acosta JF, Sandoval-Castro CA. Direct and indirect effects of bioactive tannin-rich tropical and temperate legumes against nematode infections. Veterinary Parasitology. 2012;186(1-2):18-27.