

**EVALUATION OF ANTI-HELMINTIC ACTIVITY OF ETHANOLIC EXTRACT OF
WHOLE PLANT OF *RUTA GRAVEOLENS* [EERG]**

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ABSTRACT

In Recent years there has been rapid increase in the infections caused by the helminthes and their development of resistance to most of the commercially available anthelmintics drugs. so, Plants clamied for possessing the anthelmintic property is getting attention these days. The traditionally clamied anthelmintics are sustainable and environmentally acceptable crude drugs. so, The present study is based on to evaluate the anthelmintic potential of ethanolic extract of *Ruta graveolens*.L whole plant by using Pheretima Posthuma as test worms (100mg/ml, 50mg/ml, 25mg/ml) of *Ruta graveolens*.L whole plant ethanolic extract were tested in the assay, which involved determination of time of paralysis (P) and time of death (D) of the worms it showed shortest time of paralysis (P=22 min) and death (D=41min) in 100mg/ml, while the time of paralysis and death will increase in 50mg/ml (P=25min) and D=47min) and in 25mg/ml concentration (P=31min) and (D=60min) respectively as compared to albendazole 10mg/ml used as standard reference (P=20min) and (D=43min) and normal saline as control. The results of present study indicated that the ethanolic extract *Ruta graveolens*.L (EERG) whole plant showed potential therapeutic significant paralysis and also caused death of worms. so it is concluded that the plant *Ruta graveolens*.L proved for its traditional claim as an effective anhelimintic herbal drug.

KEYWORDS: *Ruta graveolens*, anthelmintics, albendazole, Time of paralysis, time of death.

1. INTRODUCTION

Herbal medicine is used to treat many conditions, such as allergies, asthma, eczema, premenstrual syndrome, rheumatoid arthritis, fibromyalgia, migraine, menopausal symptoms, chronic fatigue, irritable bowel syndrome, and cancer, among others. It is best to take herbal supplements under the guidance of a trained provider.^[1]

Helminths are parasitic worms that feed on a living host to gain nourishment and protection, while causing poor

nutrient absorption, weakness and disease in thhost. These worms and larvae live in the small bowel and are referred to as intestinal parasites The following groups of worms are classed as helminths:

- Nematodes or roundworms
- Trematodes, which includes flukes or flatworms
- Cestodes or tapeworms
- Monogenans, also members of the flatworm phylum



Figure 1: Helminths.

CHARACTERISTICS OF HELMINTHS

Helminths all share a similar morphology and are multicellular organisms that are visible to the naked eye. The worms are usually caught through treading on contaminated soil in warm, humid countries that have poor sanitation and hygiene. If an infected person or animal has defecated on soil, helminth eggs present in their feces contaminate the soil. These eggs mature and hatch to produce larvae that grow into adult worms of up to 13 mm in length. These adult worms can penetrate human skin, which can happen if a person walks on contaminated soil. The worms then enter the bloodstream and migrate towards the lungs and also the throat where they are swallowed and transported to the gut.

Some of the features of the different groups of the helminths include:

- The trematodes or flatworms are flat, leaf-shaped and unsegmented. They are hermaphroditic, meaning they have the reproductive organs associated with both males and females.
- Nematodes are cylindrical and have lips, teeth and dentary plates. The worms are either male or female.

The cestodes or tapeworm is segmented and hermaphroditic. They have a sucker and a projecting, hooked rostellum.^[2]

Gastrointestinal parasite becomes a serious threat to the livestock production in the developing nation inspite of the development of anthelmintic resistances in the parasites of higher economical significance; chemotherapy is still used widely for the purpose of controlling the helminthes.^[3]

Development of resistance to most of the commercially available anthelmintics becomes a severe problem worldwide.^[4]

So, plants claimed for possessing the anthelmintic property is getting attention these days.^[5] Anthelmintic plants offer a traditional alternative to manufactured anthelmintics that is both sustainable and environmentally acceptable.^[6]

About the Plant under study

The most common medicinal plant of this family is *Ruta graveolens L.*, which is commonly known as „Rue" or Sudab or Sadab" in Hindi (Indian language). Although it is native to Europe, it is distributed throughout the world. It is an ornamental evergreen shrub of up to one meter tall and has considerable medicinal importance. More than 120 natural compounds mainly including acridone alkaloids, coumarines, essential oils, flavonoids, and furoquinolines have been found in the roots and aerial parts of this plant,⁷ and is the main source of Sunnocoumarins such as psoralen, xanthotoxin (8-methoxy-psoralen; 8-MOP) and sergaptin (5-methoxy-psoralen, 5-MOP).⁸ This plant is commonly cultivated in India and is commonly called as sudab or

sadab 2,4,9 Two species of *Ruta* (genus) are reported to grow in India of which *Ruta graveolens* (garden rue) is well known for its aromatic and medicinal uses⁴ In traditional system of medicine it is used as stimulant, emmenagogue, diuretic, and thornefacient.^[7]

MATERIALS AND METHODS

PLANT COLLECTION AND IDENTIFICATION

The crude *Ruta graveolens L.* whole plant was collected from Sri Venkateshwara University, Tirupathi, Andhra Pradesh, India. in the month of January, 2024. The plant was authenticated by plant taxonomist Dr. K. Madhava Chetty, Assistant Professor, Dept of Botany, Sri Venkateshwara University, Tirupati, AP, India.

The *Ruta graveolens L.* whole plants were cut into proper size and washed 3 times with drinking water then dried in shade with proper care. The dried plant material were blended into course powder and passed through sieve 60.^[8]



Fig No. 2: *Ruta Graveolens. Linn.*

4.2 PREPARATION OF EXTRACT

The course powder 500gm of *Ruta graveolens L.* whole plant was subjected to maceration and transferred to stoppered flask, and treated with pure ethanol 80% until the powder is fully immersed at room temperature. The flask was shaken every hour for the first 6 hours and then it was kept aside and again shaken after 24 hours from time to time to ensure better extraction. This process is repeated for 7 days, followed by exhaustive maceration for 5 days by using solvent methanol. The solvent was decanted and filtered with filter paper and recovered with the help of rotary vacuum evaporator. The extract EERF was dried under desiccator and stored in an air tight container. The final extract was then subjected to investigate the anti-helminthic activity.

COLLECTION OF WORMS

Earthworms *Pheretima posthuma* (Pheretimididae) have been used widely for the initial evaluation of anthelmintic activity, because of their easy availability. Indian adult earthworms (*Pheretima posthuma*) were collected and authenticated from the zoology department of Agriculture University, Hyderabad. The earthworms are initially washed with the tap water then washed with

normal saline to remove all filthy earthy matter. The earthworms of 3-5 cm in length and 0.1 - 0.2 cm in width were used for all the experimental protocol due to their anatomical and physiological resemblance with the intestinal roundworms parasites of human be.^[9]

MECHANISAM OF ACTION

As a vermicial, albendazole causes degenerative alterations in the intestinal cells of the worm by binding to the colchicine-sensitive site of β-tubulin, thus inhibiting its polymerization or assembly into microtubules (it binds much better to the β-tubulin of parasites than that of mammals Albendazole leads to impaired uptake of glucose by the larval and adult stages of the susceptible parasites, and depletes their glycogen stores. Albendazole also prevents the formation of spindle fibers needed for cell division, which in turn blocks egg production and development; existing eggs are prevented from hatching. Cell motility, maintenance of cell shape, and intracellular transport are also disrupted. At higher concentrations, it disrupts the helminths' metabolic pathways by inhibiting metabolic enzymes such as malate dehydrogenase and fumarate reductase, with inhibition of the latter leading to less energy produced by the Krebs cycle. Due to diminished ATP production, the parasite is immobilized and eventually dies.

Some parasites have evolved to have some resistance to albendazole by having a different set of acids comprising β-tubulin, decreasing the binding affinity of albendazole.^[10]

METHODES OF ANTI HELMINTIC ACTIVITY (EERG)

The anthelmintic activity was performed accordingly containing Five groups of approximately equal sized Indian earthworms consisting of six earthworms in each group were released into 50ml of desired formulation.

- Group -1 severed as control, receive only distilled water
- Group -2 served as test dose 25mg/ml ethanolic extract of *Ruta graveolens*
- Group -3– 4 are served as test doses 50mg/ml and 100mg/ml, ethanolic extract of *Ruta graveolens*
- Group -5 sereved as standard and received standard drug albendazole(10mg/ml).

Observations are made for the time taken to paralysis / death of individual worms do not receive even in distilled water. Death was concluded when the worms lose their motility followed with fading away of their body colour.

RESULTS ANTI HELMINTIC ACTIVITY OF RUTA GRAVEOLENS

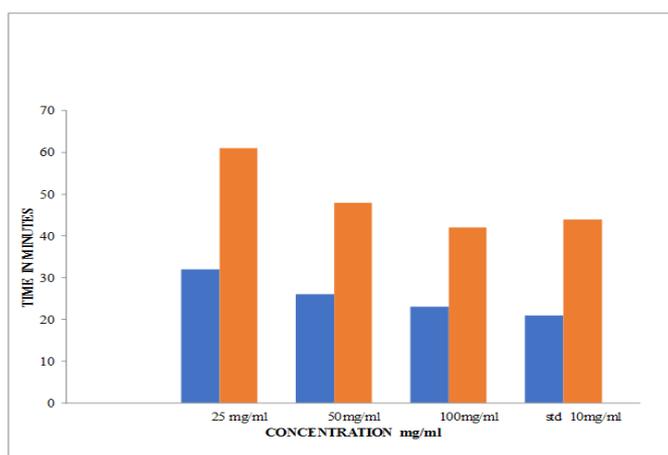
The qualitative phytochemical screening of *RUTA GRAVEOLENS L.* were done to detect the presence of various chemical constituents, the ethanolic extract of *RUTA GRAVEOLENS* gave positive test for various phytoconstituents such as Steroids, Flavanoids, Alkaloids, Tannins.

TABLE 1: Effects of Anti-Helmenthic Activity Of eerg Whole Plant of *Ruta Graveolens L.*

GROUPS	Conc mg/ml	Time of paralysis [P]	Time of Death [D]
Group 1 control	Normal saline	-	-
Group 2 EERG	25mg/ml	31±0.62min	60±0.42min
Group 3 EERG	50mg/ml	25±0.54min*	47±0.35min*
Group 4 EERG	100mg/ml	22±0.32min**	41±0.51min**
Group 5 standard Albendazole	10mg/ml	20±0.23min	43±0.22min

The result were expressed as mean ± SEM. Statistical analysis was carried out using one way ANOVA

followed by the student –t test. P<0.05 was considered statistically significant. [n=6].*= $P < 0.01$ ** $P < 0.001$.



Graph 1: Effects of Anti-Helmenthic Activity of eerg Whole Plant of *Ruta Graveolens L.*

The results of Anthelmintic activity showed that the ethanolic extract of *Ruta graveolens* exhibited good Anthelmintic activity in dose dependent manner by giving shortest time of paralysis and death of worms i.e., compared to standard Albendazole (concentration 10mg/ml) from the results it is observed that *Ruta graveolens* shown potent Anthelmintic activity from one observation we found that 100mg/ml EERG was concluded that paralysis was observed at 22 minutes and 41 minutes to bring death of worms which is comparable with that of Albendazole (10mg/ml)

As death of worms was observed at 43 minutes. The predominant effect of Albendazole on worms is to cause impaired uptake of glucose by the larval and adult stages of the susceptible parasites, and depletes their glycogen stores leading to paralysis of worms

6. DISCUSSION

Ruta graveolens belonging to family *rutaceae* was known to be a herbal remedy for pain, wound healing, fever, dysentery, urinary stones, intestinal infections but it did not claim to be commercial drug as it did not have any scientific proofs in this dissertation work. The Anthelmintic activity has been scientifically proved and compared to the standard drug Albendazole.

Ruta graveolens is commonly called as *Rue*, *common rue*, or *herb of grace* It belongs to a *rutaceae* family. It is an erect and woody herb, shrub found at the hedges of the forest and the hill sided, Commonly in tropical regions.

The collected dry plant of *Ruta graveolens* was authenticated by botanist Dr. K. Madhav Chetty and the later was subjected for extraction through cold maceration under standard laboratory conditions by using 80% ethyl alcohol and the percentage yield of extract is 14.2%.

By phytochemical investigation the plant *Ruta graveolens* contain Steroids, Flavonoids, Tannins, Glycosides.

In phytochemical screening as the chemical constituents like Flavanoids, saponins were identified may be responsible for the anthelmintic activity.

Albendazole leads to impaired uptake of glucose by the larval and adult stages of the susceptible parasites, and depletes their glycogen stores. That leads to paralysis of worms.

7. CONCLUSION

- *Ruta graveolens* is widely used herb in folk and Ayurvedic systems of medicine for various properties
- It belongs to the family *Rutaceae*
- By phytochemical investigation the plant *Ruta graveolens* contain Steroids, Flavonoids, Tannins, Glycosides.

- *Ruta graveolens* whole plant ethanolic extract has observed to possess significant Anthelmintic activity at all the test doses when compare to the standard Albendazole (10mg/ml)
- Hence, we confirm that the plants under study *Ruta graveolens* whole plant is a herb having alternate source of herbal anthelmintic drug and also further studies are needed to isolate a new active lead compound for suitable anthelmintic drug
- The above findings justified the traditional claim for the anthelmintics activity of this plant *Ruta graveolens* posses a good anthelmintic activity which is proven scientifically in a well systematic manner
- It contain the phytochemicals including alkaloids, coumarin, isoquinoline, terpenoids, methoxy-psoralen, volatile compounds, rutin, furoquinolines, and flavonoids. reported in pervious studies which may be responsible for the treatment of helmenths
- It is widely grown in different parts of the world, this herb has historically been in use since the ancient times
- *Ruta graveolens* L. (Rutaceae) is a medicinal plant widely used in the Mediterranean region to treat pain, dermatitis, rheumatism and other inflammatory diseases, but its use is limited by its potential toxicity.

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